VOLUME 9 JULY, 1942 NUMBER 2

PROCEEDINGS

of

# The Helminthological Society of Washington

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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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**North American monogenetic trematodes. V. The family Hexabothriidae, n. n. (Polystomatoidea).** EMMETT W. PRICE, U. S. Bureau of Animal Industry.

This paper is the fifth of a series dealing with monogenetic trematodes from the North American continent and of a general revision of the order Monogenea. The present section deals with a group of parasites living for the most part on the gills of sharks and skates, and previously assigned to the family Onchocotylidae. A review of the literature, however, reveals that this family name cannot be retained since the type genus *Onchocotyle* Diesing, 1850, is antedated by *Hexabothrium* Nordmann, 1840, and the new name Hexabothriidae is hereby proposed to replace Onchocotylidae Stiles and Hassall.[1] The organization of this paper is the same as for previous installments (Price, 1937, 1938, 1939a, and 1939b).

## Family HEXABOTHRIIDAE, new name

*Synonym.*—Onchocotylidae Stiles and Hassall, 1908.

*Diagnosis.*—Anterior haptor in form of a more or less well developed oral sucker, or of 2 latero-ventral bothria; posterior haptor rectangular or circular, bearing 6 suckers each provided with a large crescentic hook, and an appendix-like structure bearing a pair of terminal suckers and 1 to 3 pairs of hooks. Intestine consisting of 2 branches, usually with lateral and medial diverticula, uniting posteriorly and extending into haptor. Eyes usually absent (present in Diclybothriinae). Common genital aperture ventral, median; cirrus usually unarmed; testes numerous, postovarial. Ovary relatively large, tubular, tortuous; vaginae present, double. Parasites of fishes, usually selachians.

*Type genus.*—*Hexabothrium* Nordmann, 1840.

### *Key to subfamilies of Hexabothriidae*

1. Haptoral appendix bearing 3 pairs of large hooks ..... Diclybothriinae Price
   Haptoral appendix bearing 1 pair of small hooks ..... 2
2. Vaginae uniting to form a single duct before entering vitelline reservoir ..... Rajonchocotylinae, n. sf.
   Vaginae not uniting, entering transverse vitelline ducts separately ..... Hexabothriinae, n. n.

## Subfamily HEXABOTHRIINAE, new name

*Synonyms.*—Onchocotylinae Cerfontaine, 1899; Diaphorocotylinae Monticelli, 1903, in part.

*Diagnosis.*—Anterior haptor in form of oral sucker; posterior haptor more or less rectangular, with 6 suckers arranged in 2 parallel rows, and with relatively long appendix bearing near its tip a pair of strongly muscular suckers and a pair of small hooks. Roots of large haptoral hooks not narrowing abruptly before insertion of blade. Vaginae not uniting, but enter transverse vitelline ducts separately.

[1] This family name was inadvertently used in Part 4 of this series (Price, 1939, p. 80).

Eggs without meridional bands or ridges, with prolongation at one or both poles.

*Type genus.*—*Hexabothrium* Nordmann, 1840.

*Key to genera of Hexabothriinae*

1. Cirrus armed with spines ..... *Hexabothrium* Nordmann
   Cirrus unarmed ..... 2
2. Haptoral hooks not uniform ..... *Heteronchocotyle* Brooks
   Haptoral hooks uniform ..... 3
3. Vitellaria extending into haptoral appendix ..... *Neoerpocotyle* n. g.
   Vitellaria not extending into haptoral appendix ..... *Erpocotyle* Beneden and Hesse

Genus *Hexabothrium* Nordmann, 1840

*Synonyms.*—*Onchocotyle* Diesing, 1850; *Acanthonchocotyle* Cerfontaine, 1899.

*Diagnosis.*—Hooks of haptor proper about equal in size. Cirrus armed with spines. Vitellaria not extending into haptoral appendix. Egg with prolongation at one pole.

*Type species.*—*Hexabothrium appendiculatum* (Kuhn, 1829) Nordmann, 1840.

Nordmann (1840) proposed the genus *Hexabothrium* for *Polystoma appendiculatum* Kuhn and later Diesing (1850) erected the genus *Onchocotyle* for the same species. Later authors have accepted *Onchocotyle* Diesing as the correct name of the genus in spite of the fact that it is a stillborn synonym and a deliberate renaming of *Hexabothrium*. Since *Hexabothrium* clearly has priority over *Onchocotyle*, and is otherwise available, it must be revived even though this action may result in considerable confusion.

The distinguishing character of the type species of this genus, *H. appendiculatum* (Kuhn), was established by Cerfontaine (1899). This author had before him only a single specimen from the type host, and while it may be argued that there is no proof that the form studied by Cerfontaine was actually *H. appendiculatum*, it may be assumed to be the same. The original description of *H. appendiculatum* given by Kuhn (1829) shows little more than that the worm which he described and figured belonged to the family now under consideration; no characters of a specific nature were given. A later description of this species by Nordmann (1832), based on a part of Kuhn's original material, which had been placed at his disposal by Rudolphi, likewise failed to give details that would serve definitely to distinguish the species. It appears, therefore, that Cerfontaine's characterization of *H. appendiculatum* may be taken to establish the species since his material was from the same host and from the same general geographical area as that from which Kuhn obtained the original specimens. *Onchocotyle appendiculata* (=*H. appendiculatum*) has been reported from a number of hosts, but there is little evidence that the forms from these hosts are actually this species, the identifications apparently having been based on external similarities. In view of this, only the type host is listed in connection with this and other species mentioned in this paper unless there is reasonably good evidence to show that the identifications were correct.

The genus *Hexabothrium* contains 3 species as follows: *H. appendiculatum* (Kuhn, 1829),[2] from *Scyllium catulus; H. canicula* (Cerfontaine, 1899), from *S.*

[2] The reports of *H. appendiculatum* from "Raia batis" and "Raia clavata" by Scott (1901) and from the "picked dogfish" by Lebour (1908) do not refer to the species in question. The form reported by Scott, since it is from rays, is probably a *Rajonchocotyle*, although this cannot be determined with certainty from his illustration of the species. The form described and figured by Lebour is some species of *Erpocotyle* but owing to insufficient details it is impossible to determine its specific status.

*canicula*, and *S. stellare* (according to Guberlet, 1933); and *H. musteli* (MacCallum, 1931), from *Mustelus canis* (= *Cynias canis*). Of these 3 species only the last named occurs in North America.

*Hexabothrium musteli* (MacCallum, 1931), n. comb.
Figs. 1, A; 2, A; 3, A

*Synonyms.*—*Acanthonchocotyle musteli* MacCallum, 1931; *Onchocotyle musteli* (MacCallum, 1931) Dollfus, 1937.

*Description.*—Body exclusive of haptor about 1.8 mm long by 390 μ wide in equatorial region. Oral sucker 133 to 152 μ wide, with flaring margin. Haptor about 510 μ long, bearing the usual 6 suckers; haptoral appendix about 415 to 510 μ long. Large hooks of haptoral suckers slightly unequal in size, those of the 1st and 3rd pairs 240 to 255 μ long and those of 2nd pair 255 to 280 μ long; hooks of appendix about 57 μ long.[3] Pharynx about 40 μ in diameter. Genital aperture 285 to 325 μ from anterior end of body. Cirrus armed with curved spines about 12 μ long. Ovary in equatorial zone. Vaginal apertures 76 to 114 μ from genital opening; vaginal termini of peculiar structure, consisting of a small aperture surrounded by more or less heavily cuticularized tissue from which radiate rib-like bands of similar tissue. Vitellaria extending from pharyngeal region to near junction of haptor and body proper. Egg about 170 μ long, provided with long slender filament at posterior pole.

*Host.*—*Cynias canis* (Mitchill).

*Location.*—Gills.

*Distribution.*—United States (Woods Hole, Mass.).

*Specimens.*—U.S.N.M. Helm. Coll. No. 8131 (type) and 8132 (paratypes).

The specimens upon which this species was based were collected by the late Dr. G. A. MacCallum, July 31, 1913; they are poorly preserved and badly distorted due to faulty technique. The measurements given in the present paper are based upon 2 of the least distorted specimens. *Hexabothrium musteli* may easily be distinguished from the other 2 species of *Hexabothrium*, *H. appendiculatum* and *H. canicula*, by the shape of the cirrus spines, those of the former being curved and hook-like instead of spike-shaped as in the other 2 species.

Genus *Erpocotyle* Beneden and Hesse, 1863

*Synonyms.*—*Squalonchocotyle* Cerfontaine, 1899; *Erpetocotyle* Furhmann, 1928.

*Diagnosis.*—Cirrus unarmed. Vitellaria not extending into haptoral appendix. Egg with prolongation at each pole. Other characters as in *Hexabothrium*.

*Type species.*—*Erpocotyle laevis* Beneden and Hesse, 1863.

The genus *Erpocotyle* was proposed by Beneden and Hesse (1863) for a worm taken from the gills of *Mustelus laevis*. The original description and figures of *Erpocotyle laevis*, type of the genus, are inadequate for specific determination, but there appears to be no doubt that this species is congeneric with species placed in the genus *Squalonchocotyle* by Cerfontaine (1899).

In spite of the fact that *Erpocotyle laevis* cannot be identified from the original description, there is some evidence of a more or less circumstantial nature that

[3] In this and all other species described in this paper the measurements of the large hooks represent the distance from the tip of the blade to the base of the root, following a line passing approximately through the center and along the curve of the hook; in the case of the hooks from the haptoral appendix the measurements represent the distance in a straight line from the tip of the root to the height of the curve of the blade.

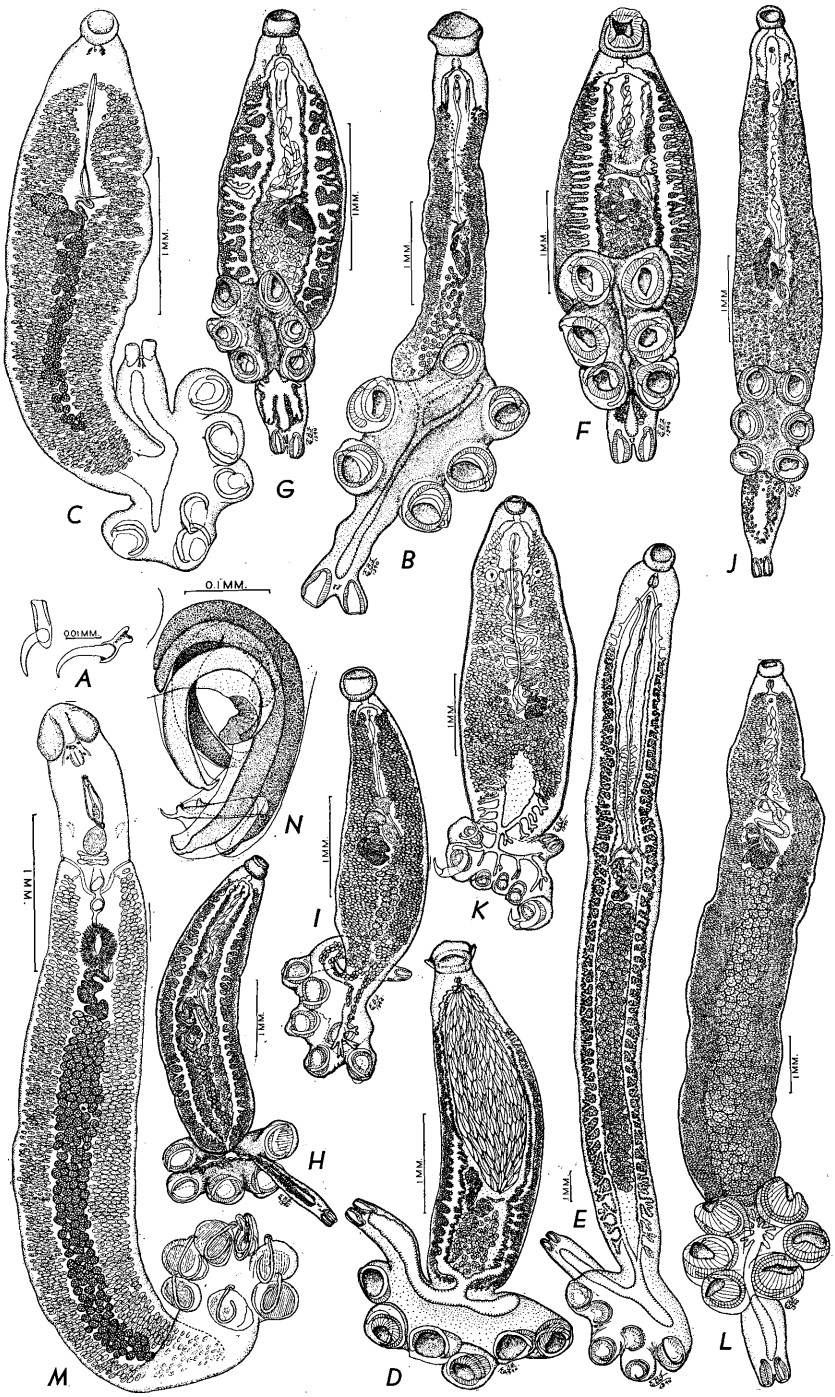


FIG. 1. A—*Hexabothrium musteli*, hooks from cirrus; B—*Erpocotyle sphyrnae;* C—*E. squali;* D—*E. macrohystera;* E—*E. somniosi;* F—*Neoerpocotyle maccallumi;* G—*N. ginglymostomae;* H—*N. microstoma;* I—*N. tiburonis;* J—*N. mavori;* K—*Heteronchocotyle hypoprioni;* L—*Rajonchocotyle wehri;* M and N—*Diclybothrium armatum* (B to M represent complete worms in ventral view; N represents tip of haptor of *D.*

makes it possible to establish the identity of the species and to revive *Erpocotyle* as a recognizable genus. Since *Erpocotyle laevis* was described from *Mustelus laevis,* and in view of the rather pronounced host specificity of the monogenetic trematodes, other species of hexabothriids from the type host must be examined to determine whether or not one of the later described forms might not be the same as the earlier species. There have been described in addition to *E. laevis* 2 species, *Squalonchocotyle vulgaris* and *S. catenulata,* that must be taken into consideration. The first of these, *S. vulgaris,* was described by Cerfontaine (1899) from *Mustelus vulgaris* and subsequently reported by Guberlet (1933) from *M. laevis.* Cerfontaine regarded *S. vulgaris* as the same species as that described by Thaer (1850) under the name of *Polystomum appendiculatum* Kuhn. However, an examination of Thaer's paper suggests that this author was dealing with a mixed species, since his illustrations show 2 types of eggs, one occurring in chains and the other singly. The other illustrations are of a worm which could not be the same as *S. vulgaris,* but correspond to the form described from *M. laevis* by Guberlet (1933) under the name of *S. catenulata.* Cerfontaine's description of *S. vulgaris* is of a worm having large haptoral hooks of a more or less distinctive type, vaginal openings almost marginal and at a level slightly posterior to the level of the genital aperture, and eggs provided with polar prolongations but not occurring in chains. A study of specimens of *S. vulgaris* available through the courtesy of the late Dr. J. E. Guberlet shows that the above-mentioned characters are specific.

An examination of specimens of *S. catenulata* shows that Guberlet's description of the form is quite complete and accurate except for the distribution of the vitellaria. In this species the vitelline follicles extend into the haptoral appendix in the same manner as shown by Thaer for his *Polystomum appendiculatum;* this is not true in the case of *S. vulgaris.* Apparently the distribution of vitelline follicles in various hexabothriids has been regarded by most taxonomists as a character of little importance since no particular stress has been placed on this point. Cerfontaine (1899) leaves the impression that the extent of the vitellaria is a variable character, the follicles extending into the haptor in some specimens and not in others. On the contrary, it has been the experience of the present writer that this character is quite dependable and apparently generic.

Assuming the validity of vitelline distribution as a character of taxonomic importance, the species from *Mustelus laevis* that most closely approaches *Erpocotyle laevis* Beneden and Hesse is *Squalonchocotyle vulgaris* Cerfontaine. The writer, therefore, regards these 2 species as identical, thereby making possible the utilization of *Erpocotyle* as a recognizable genus.

As present constituted, the following species may be included in the genus *Erpocotyle: E. abbreviata* (Olsson, 1876), n. comb., from *Acanthias vulgaris; E. antarctica* (Hughes, 1928), n. comb., from *Mustelus antarcticus; E. borealis* (Beneden, 1853), n. comb., from *Scymnus glacialis; E. canis* (Cerfontaine, 1899), n. comb. (syn. *Onchocotyle appendiculata* Beneden, 1858, nec Kuhn, 1829), from *Eugaleus galeus* (syn. *Galeus canis*); *E. dollfusi,* n. sp. (syn. *O. abbreviata,* form D of Dollfus, 1937),[4] from *Echinorhinus brucus; E. eugalei,* n. sp. (syn. *O. abbreviata,* form B of Dollfus, 1937),[4] from *Eugaleus galeus; E. galeorhini,* n. sp. (syn. *O. abbreviata,* form A of Dollfus, 1937),[4] from *Galeorhinus mustelus; E. laevis* Beneden and Hesse, 1863 (syns. *Polystomum appendiculatum* Thaer, 1850, nec Kuhn, 1829, in part; *Squalonchocotyle vulgaris* Cerfontaine, 1899), from *Mustelus laevis* and *M. vulgaris; E. macrohystera* n. sp. (syn. *Squalonchocotyle vulgaris* MacCallum, 1931, nec Cerfontaine, 1899), from *Carcharias milberti; E. somniosi* (Causey, 1926), n. comb., from *Somniosus microcephalus; E. sphyrnae* (MacCallum, 1931), n. comb., from *Sphyrna zygaena; E. spinacis* (Goto, 1894), n. comb., from *Spinax* sp.; *E. squali* (MacCallum, 1931), n. comb. (syn. *S. acanthi* MacCallum,

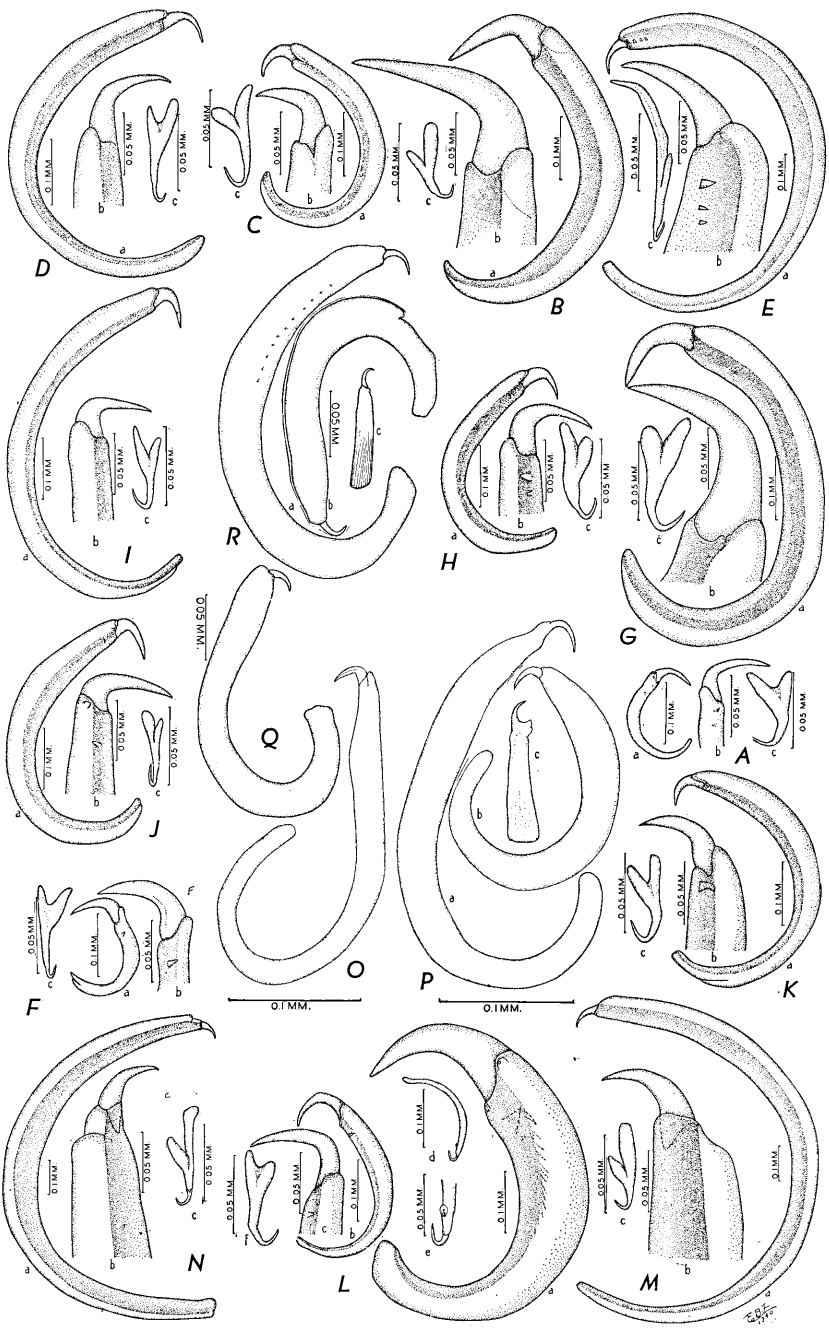


FIG. 2. Haptoral hooks of: A—*Hexabothrium musteli;* B—*Erpocotyle sphyrnae;* C—*E. squali;* D—*E. macrohystera;* E—*E. somniosi;* F—*E.* sp., from squid; G—*Neoerpocotyle maccallumi;* H—*N. ginglymostomae;* I—*N. microstoma;* J—*N. tiburonis;* K—*N. mavori;* L—*Heteronchocotyle hypoprioni;* M—*Rajonchocotyle laevis;* N—*R. wehri;* O—*Diclybothrium armatum,* from haptoral sucker; P—*D. armatum,* from haptoral appendix; Q—*D. hamulatum,* from haptoral sucker; R—*D. hamulatum,* from haptoral appendix. (Except for L, R & P, a = hook from haptoral sucker, b = tip of hook from haptoral sucker, c = hook from haptoral appendix; for L, a = largest of hooks from haptoral suckers, b = medium-sized hook from haptoral suckers, c = tip of medium-sized hook, d = small-sized hook from haptoral suckers, e = tip of small-sized hook, f = hook from haptoral appendix; for R & P, a = outer, posrly directed appendix hook, and c = inner appendix hook.)

1931), from *Squalus acanthias; E. striata* (Miller, 1927), from *Squalus suckleyi;* and *E. torpedinis,* n. sp. (syn. *S. abbreviata,* form C of Dollfus, 1937),[4] from *Torpedo marmorata.* The North American representatives of this genus are *E. macrohystera, E. somniosi, E. sphyrnae, E. squali,* and *E. striata.*

*Erpocotyle sphyrnae* (MacCallum, 1931), n. comb.
Figs. 1, B; 2, B; 3, B

*Synonym.—Squalonchocotyle sphyrnae* MacCallum, 1931.

*Description.*—Body slender, 3.9 to 5.1 mm long by 680 to 765 μ wide. Oral sucker 630 to 680 μ in diameter, weakly muscular and not lined with tubercles. Haptor rectangular, about 1.7 mm long by 1 mm wide, with suckers about 425 to 510 μ in diameter; haptoral appendix 1.2 to 1.3 mm long by 425 to 500 μ wide, with suckers about 255 to 290 μ long by 170 to 250 μ wide. Hooks of haptoral suckers equal in size, about 700 to 800 μ long; hooks of appendix 45 to 50 μ long. Pharynx 114 μ long by 95 μ wide; intestinal tract not observed except in haptor where it consists of a simple anteriorly directed cecum and a simple cecum extending into haptoral appendix. Genital aperture 600 to 680 μ from anterior end of body; testes relatively numerous, exact number not ascertainable, confined to postovarial region. Ovary in equatorial zone; vaginal apertures in fields of intestinal branches, at same level as genital aperture. Vitellaria extending from about 250 μ distal to genital aperture to posterior end of body proper. Eggs about 180 μ long by 50 μ wide, with polar prolongations of varying lengths.

*Host.—Sphyrna zygaena* (Linnaeus).

*Location.*—Gills.

*Distribution.*—United States (Woods Hole, Mass.).

*Specimens.*—U.S.N.M. Helm. Coll. No. 8138 (type) and 8137 (paratypes).

The available material of this species consisted of 3 slides of 2 specimens each; they had been collected by the late Dr. G. A. MacCallum at Woods Hole, Mass., on July 22 and August 1, 1913. All of the specimens were more or less distorted by excessive flattening and many details of structure could not be satisfactorily studied. Perhaps the most distinctive feature of this form is the large haptoral hooks, the blades of which are relatively much longer with respect to total length than in related species.

*Erpocotyle squali* (MacCallum, 1931), n. comb.
Figs. 1, C; 2, C; 3, C

*Synonyms.—Squalonchocotyle squali* MacCallum, 1931; *S. acanthi* MacCallum, 1931.

*Description.*—Body relatively robust, 3.4 to 7 mm long by 765 to 935 μ wide. Oral sucker 220 to 228 μ in diameter, without tubercles. Haptor rectangular, 1.3 mm long, with suckers about 225 μ in diameter; haptoral appendix 765 to 935 μ long by 255 to 340 μ wide, with terminal suckers 123 to 152 μ long by 95 to 114 μ wide. Hooks of haptoral suckers slightly unequal, those of anterior 2 pairs 500 to 650 μ long and those of posterior pair 430 to 600 μ long; hooks of appendix

[4] The trematodes which Dollfus (1937) referred to *Squalonchocotyle abbreviata,* forms A, B, C, and D, and which are here named *Erpocotyle galeorhini, E. eugalei, E. torpedinis* and *E. dollfusi,* respectively, are apparently separate and distinct from *E. abbreviata* (Olsson). Aside from host affiliations, all of these forms show more or less distinct differences from *E. abbreviata.* There are marked differences in body size and in the large haptoral hooks in "forma A"; hook differences, vaginal orifices in extraintestinal fields and posterior to genital aperture, and eggs in chains in "forma B"; hook differences, short vaginae, and vaginal orifices posterior to genital aperture in "forma C"; and marked difference in body size, and club-shaped vaginal termini in "forma D."

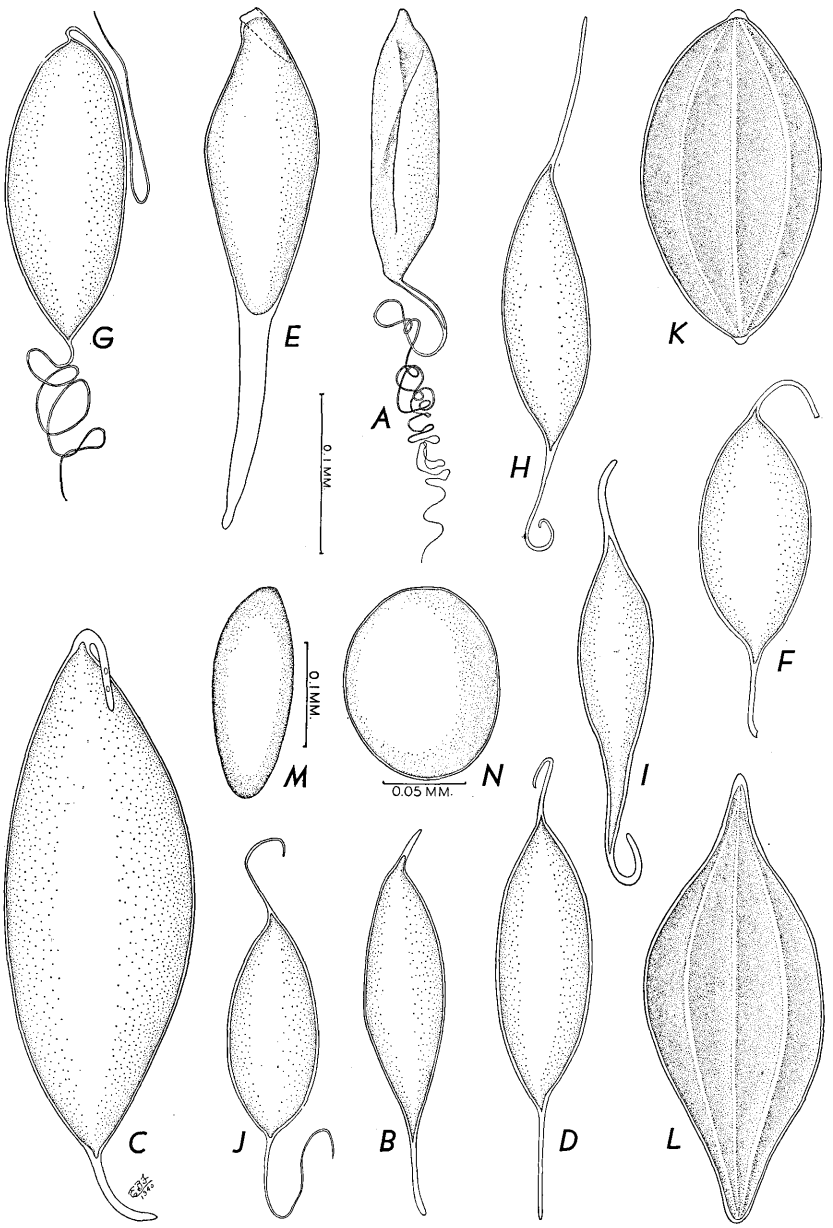


FIG. 3. Eggs of: A—*Hexabothrium musteli;* B—*Erpocotyle sphyrnae;* C—*E. squali;* D—*E. macrohystera;* E—*E. somniosi;* F—*Neoerpocotyle maccallumi;* G—*N. ginglymostomae;* H—*N. microstoma;* I—*N. tiburonis;* J—*N. mavori;* K—*Rajonchocotyle laevis;* L—*R. wehri;* M—*Diclybothrium armatum;* N—*D. hamulatum.* (All figures except M & N drawn to same scale.)

72 μ long. Pharynx about 75 μ in diameter; intestinal tract not observable except in haptor where it is of the usual type. Genital aperture 425 to 475 μ from anterior end of body. Testes about 60 in number, occupying a narrow field in central portion of postovarial part of body proper. Ovary slightly preequatorial, of usual shape. Vaginal apertures and vaginae not observed, a little posterior to level of genital aperture, "about halfway out to the margin of the body," according to MacCallum. Vitellaria dense, extending from slightly distal to level of genital aperture to near posterior end of body proper. Eggs ovoidal, 285 to 340 μ long by 95 to 130 μ wide, with short polar prolongations.

*Host.*—*Squalus acanthias* Linnaeus.

*Location.*—Gills.

*Distribution.*—United States (Woods Hole, Mass.).

*Specimens.*—U.S.N.M. Helm. Coll. No. 8133 (type), 8134 (paratypes), and 8135 (cotypes of *S. acanthi*).

In 1931, the late Dr. G. A. MacCallum described as *Squalonchocotyle squali* and *S. acanthi* some specimens which he collected from *Squalus acanthias* at Woods Hole, Mass. The material consists of 3 lots totaling 13 specimens and was collected on August 6, 1913, July 20, 1922, and August 20, 1922, those obtained on July 20, 1922, being the ones on which he based his description of *S. acanthi.* All of the specimens were not in the best of condition, consequently some details of structure could not be made out. A comparison of the specimens of *S. acanthi,* which were the least distorted, with those of *S. squali* (= *E. squali*) showed no differences that could not be accounted for on the basis of technique employed in their preservation and staining, and they are regarded as representing a single species. *Erpocotyle squali* appears to be most closely related to *E. striata* (Miller), except that in the latter species the testes are fewer in number than in the former, and the egg does not possess the longitudinal ridges reported by Miller (1927) for *E. striata.*

*Erpocotyle macrohystera,* n. sp.
Figs. 1, D; 2, D; 3, D

*Synonym.*—*Squalonchocotyle vulgaris* MacCallum, 1931, nec Cerfontaine, 1899.

*Description.*—Body proper relatively broad, 3.8 to 4.6 mm long by 946 μ to 1 mm wide. Oral sucker poorly muscular, 460 to 527 μ wide, with membranous margin, lined with numerous minute tubercles. Haptor rectangular, 1.7 to 2.5 mm long by 1 to 1.2 mm wide, with suckers 425 to 645 μ in diameter, which are lined with tubercles similar to those of oral sucker; haptoral appendix 1 mm long by 400 to 510 μ wide, with terminal suckers 255 μ long by 85 μ wide. Hooks of haptoral suckers 700 to 760 μ long; hooks of appendix 76 μ long. Pharynx about 75 μ in diameter; intestinal branches with short median and lateral diverticula. Genital aperture 510 to 595 μ from anterior end of body; testes relatively few, about 50 in number, occupying interintestinal field in posterior third of body proper. Ovary of usual shape, at junction of posterior and middle body thirds. Vaginal apertures posterior to level of genital opening and lying close to medial margin of intestinal branches. Vitellaria extending from some distance distal to intestinal bifurcation to posterior end of body proper. Uterus voluminous. Eggs fusiform, 190 to 210 μ long by 50 to 57 μ wide, with relatively short polar prolongations.

*Host.*—*Carcharias milberti* Valenciennes.

*Location.*—Gills.

*Distribution.*—United States (Woods Hole, Mass.).

*Specimens.*—U.S.N.M. Helm. Coll. No. 8138 (cotypes).

This species, based on 9 specimens collected August 3, 1916, was described by MacCallum (1931) as *Squalonchocotyle vulgaris* Cerfontaine. A comparison of this

material with the description of *S. vulgaris,* however, shows that MacCallum was in error and that the species is actually quite distinct from all other species of the genus, and may be distinguished from them by the enormously distended uterus, as well as by the interintestinal position of the vaginal apertures and the post-equatorial location of the ovary.

*Erpocotyle somniosi* (Causey, 1926), n. comb.

This species was described by Causey (1926) as *Onchocotyle somniosi,* and was based on specimens obtained at Excursion Inlet, Alaska, from the gills of *Somniosus microcephalus* (Bloch and Schneider). Through the courtesy of Prof. David Causey the type specimens were available for examination; these show that the original description of this form is quite complete and a redescription is unnecessary. However, for the purpose of comparison with other species considered in this paper, figures of the complete worm (Fig. 1, E), haptoral hooks (Fig. 2, E) and the egg (Fig. 3, E) are given. As pointed out by Causey, *E. somniosi* is very similar to *E. borealis,* which was described by Beneden (1853). So far as is possible to ascertain from Beneden's description and figures of *E. borealis,* the principal difference between the 2 species is in the nature of the large haptoral hooks. In *E. somniosi* the blade or claw is inserted into the root of the hook in much the same manner as in members of the genus *Rajonchocotyle,* while in *E. borealis* the insertion of the blade is more or less typical of species of *Erpocotyle.*

*Erpocotyle striata* (Miller, 1927), n. comb.

*Erpocotyle striata* was described as *Onchocotyle striata* by Miller (1927) from specimens obtained from *Squalus suckleyi* (Girard) at Friday Harbor, Washington. Specimens made available through the courtesy of the late Prof. J. E. Guberlet, University of Washington, Seattle, Washington, show that nothing can be added to the original description. Apparently this species may be distinguished from other species of the genus by the egg, which, according to Miller, is striated in much the same manner as in species of *Rajonchocotyle.* It has not been possible to check this point, however, since in the specimens available the eggs were so collapsed that no details of structure could be made out.

*Erpocotyle* sp.

One immature specimen of hexabothriid (U.S.N.M. Helm. Coll. No. 36722) was found in the collection of the late Dr. G. A. MacCallum; it was labelled "From a squid" and had been collected at Woods Hole, Mass., August 10, 1926. This specimen offers nothing in the way of characters that would permit more than a broad generic determination. The hooks of the haptoral suckers (Fig. 2, F) appear quite distinctive in that the blades are enormous as compared with the roots; however, as the roots of the hooks seem to increase greatly in size as the worms become mature, the relative size of the blades in this specimen may be of no significance.

*Neoerpocotyle,* new genus

*Synonym.*—*Squalonchocotyle* Cerfontaine, 1899, in part.

*Diagnosis.*—Vitellaria extending into haptoral appendix. Other characters as in *Erpocotyle.*

*Type species.*—*Neoerpocotyle maccallumi,* n. sp.

The following species are included in the genus *Neoerpocotyle: N. catenulata* (Guberlet, 1933), n. comb. (syn. *Polystomum appendiculatum* Thaer, 1850, nec Kuhn, 1829, in part), from *Mustelus laevis* and *M. vulgaris; N. grisea* (Cerfontaine, 1899), n. comb. (syn. *Onchocotyle appendiculata* Taschenberg, 1879, nec

Kuhn, 1829), from *Hexanchus griseus; N. ginglymostomae* (Brooks, 1934), n. comb., from *Ginglymostoma cirratum; N. maccallumi,* n. sp., from *Carcharias limbatus; N. mavori* (Linton, 1940), n. comb., from *?Morone americana; N. microstoma* (Brooks, 1934), n. comb., from *Sphyrna zygaena;* and *N. tiburonis* (Brooks, 1934), n. comb., from *Sphyrna tiburo.* With the exception of *N. catenulata* (Guberlet) and *N. grisea* (Cerf.), all of the above species are from North American hosts.

*Neoerpocotyle maccallumi,* n. sp.
Figs. 1, F; 2, G; 3, F

*Synonym.—Squalonchocotyle canis* MacCallum, 1931, nec Cerfontaine, 1899.

*Description.*—Body robust, 3.5 to 4.08 mm long by 1.1 to 1.5 mm wide. Oral sucker 554 to 765 μ in diameter, with delicate membranous margin and with cavity lined with small tubercles. Haptor rectangular, 1.5 to 1.8 mm long, with suckers 425 to 510 μ in diameter and lined with small tubercles similar to those in oral cavity; appendix 1.02 mm long by 425 to 560 μ wide, with terminal suckers 340 μ long by 136 to 170 μ wide. Hooks of haptoral suckers about equal, 900 to 1,200 μ long; hooks of appendix about 76 μ long. Pharynx 80 μ in diameter; esophagus very short. Intestinal branches with prominent lateral and inconspicuous median diverticula; haptoral and appendicular ceca simple. Genital aperture about 850 to 935 μ from anterior end of body. Testes small, number not ascertainable with certainty but approximately 40 to 50. Ovary convoluted, in equatorial zone; seminal receptacle prominent, in ovarial zone. Vaginal apertures in fields of intestinal branches and at same level as genital opening; vaginae voluminous, sinuous. Vitellaria in lateral fields except for a relatively wide dorsal band of follicles anterior to transverse vitelline duct, extending from level of genital aperture to posterior end of body proper and continuing into haptoral appendix. Eggs oval, 152 μ long by 76 μ wide, occurring in chains.

*Host.—Carcharias limbatus* Müller and Henle.

*Location.*—Gills.

*Distribution.*—United States (Woods Hole, Mass.).

*Specimens.*—U.S.N.M. Helm. Coll. No. 8140 (type) and 8139 (paratypes).

This species was described as *Squalonchocotyle canis* Cerfontaine by MacCallum (1931) and was based on specimens collected at Woods Hole, Mass., August 23, 1923, and September 1, 1925. A comparison of these specimens, 5 in number, with Cerfontaine's description of *Squalonchocotyle canis* (=*Erpocotyle canis*) revealed a number of differences which are sufficient for considering them as belonging to a new genus and species.

*Neoerpocotyle maccallumi* may be distinguished from all other species of *Neoerpocotyle* on the basis of host, presence of a distinct dorsal band of vitelline follicles immediately anterior to the transverse vitelline duct, and the failure of the vitelline follicles in the haptoral appendix to reach the end of the cecum.

*Neoerpocotyle ginglymostomae* (Brooks, 1934), n. comb.

This species was described under the name of *Squalonchocotyle ginglymostomae* by Brooks (1934) from 2 specimens collected by Dr. H. W. Manter from the gills of the nurse shark, *Ginglymostoma cirratum* (Bonnaterre), at Tortugas, Florida. Both specimens (U.S.N.M. No. 8810 (type) and the paratype from Manter's collection) were available to the writer. A study of this material showed that Brooks failed to observe that the vitelline follicles followed the course of the haptoral cecum into the appendix and that the haptoral suckers were lined with small tubercles similar to those occurring in the cavity of the oral sucker, otherwise his description is quite accurate. For comparison with other North American species,

illustrations of the complete worm, hooks and egg have been prepared and included in this paper (Fig. 1, G; Fig. 2, H; Fig. 3, G). Aside from the fact that this species is extremely small, it may easily be distinguished from all others by the peculiar branching of the appendicular cecum.

*Neoerpocotyle microstoma* (Brooks, 1934), n. comb.

This species was based on specimens from the hammerhead shark, *Sphyrna zygaena* (Linnaeus), which were collected at Beaufort, N. C., by Dr. H. W. Manter. A study of the type (U.S.N.M. No. 8811) and of the incomplete paratype from Manter's collection shows that little can be added to the original description. As in the case of *N. ginglymostomae,* the vitelline follicles follow the course of the intestine into the haptor and appendix (Fig. 1, H), a point overlooked by Brooks. Although difficult to make out in the limited material available, the haptoral suckers appear to be lined with small tubercles similar to those occurring in the oral cavity. While closely resembling *N. tiburonis* (Brooks) from a closely related host, this species may be distinguished by the small constricted oral sucker, relative size and structure of the haptoral hooks (Fig. 2, I) and by the egg (Fig. 3, H).

*Neoerpocotyle tiburonis* (Brooks, 1934), n. comb.

*Neoerpocotyle tiburonis* (syn. *Squalonchocotyle tiburonis*) was based on a single adult (U.S.N.M. No. 8812) and 7 immature specimens collected by Dr. H. W. Manter from the gills of a bonnet shark, *Sphyrna tiburo* (Linnaeus), at Tortugas, Florida. All of the specimens were available for examination and a study of this material shows that this species is very close to *N. microstoma* from *Sphyrna zygaena;* both possess small tubercles in the haptoral suckers as well as in the oral cavity, vitellaria extending into the haptor and haptoral appendix, and intestinal ceca which branch in the haptor proper but not in the haptoral appendix. *N. tiburonis* may be distinguished from *S. microstoma,* however, on the basis of host affinities and by the comparatively large oral sucker. A comparison of these 2 species may be made by reference to figures 1, 2, and 3.

*Neoerpocotyle mavori* (Linton, 1940), n. comb.

*Neoerpocotyle mavori* (syn. *Onchocotyle mavori*) was described by Linton (1940) from specimens (U.S.N.M. No. 8155 (type) and 8412 (paratypes)) which had been collected from the "bottom of an aquarium in which were a number of white perch (*Morone americana*), from Tashmoo Pond, Martha's Vineyard, Mass." As Linton has pointed out, there is doubt as to the perch being the host since all hexabothriids so far described have been found only on selachians. A study of the specimens shows that the original description is adequate and a redescription unnecessary. *N. mavori* differs from all other species of the genus in being more slender and having the vitellaria commencing a considerable distance posterior to the level of the genital aperture (Fig. 1, J); other differences, particularly in the hooks and the egg, may be observed from the figures given in figures 2 and 3.

Genus *Heteronchocotyle* Brooks, 1934

*Diagnosis.*—Haptoral hooks markedly dissimilar, 2 hooks very large, 1 intermediate, and 3 small. Vitellaria not extending into haptor or haptoral appendix. Other characters similar to those of *Erpocotyle.*

*Type and only species.—Heteronchocotyle hypoprioni* Brooks, 1934.

*Heteronchocotyle hypoprioni* Brooks, 1934

This species was based on specimens collected by Dr. H. W. Manter from the gills of the yellow shark, *Hypoprion brevirostris* Poey, at Tortugas, Florida. An

examination of the type (U.S.N.M. No. 8809) and of paratypes kindly loaned by Dr. Manter shows that in general the species is well described except for a few details. In the original description the position of the testes is given as "the intercrural space immediately posterior to the ovary." This is mainly true, but some testes were also found to extend anteriorly into the ovarial zone (Fig. 1, K). The writer has not been able to confirm Brooks' statement that the seminal receptacle is "unique since, instead of being an outpocketing from the oviduct, it appears as an enlargement of the oviduct itself." So far as can be determined from whole mounts and cross sections—no frontal or sagittal sections available—the seminal receptacle seems to differ from that in other hexabothriids only in being more or less spiral instead of vesicular. This species is extremely interesting because of the unusual variation in size and lack of pairing of the large haptoral hooks (Fig. 2, L).

RAJONCHOCOTYLINAE, n. subfam.

*Diagnosis.*—Haptor circular; ovary in preequatorial part of body. Vaginae uniting to form a single duct before joining vitelline reservoir. Eggs with meridional bands or ridges, without polar filaments.

*Type genus.*—*Rajonchocotyle* Cerfontaine, 1899.

*Key to Genera of Rajonchocotylinae*

Vitellaria extending into haptoral appendix ..... *Rajonchocotyloides* Price
Vitellaria not extending into haptoral appendix ..... *Rajonchocotyle* Cerfontaine

Genus *Rajonchocotyle* Cerfontaine, 1899

*Diagnosis.*—Vitellaria not extending into haptoral appendix. Other characters those of subfamily.

*Type species.*—*Rajonchocotyle batis* Cerfontaine, 1899.

The genus *Rajonchocotyle* contains 7 species as follows: *R. alba* Cerfontaine, 1899, from *Raja alba; R. batis* Cerfontaine, 1899 (syn. *Onchocotyle appendiculata* Olsson, 1867, nec Kuhn, 1829), from *Raja batis; R. kenojei* Yamaguti (1938), from *Raja kenojei; R. laevis,* n. sp., from *Raja laevis; R. ovata* Guberlet, 1937, from *Raja binoculata; R. prenanti* (Saint Remy, 1890) Cerfontaine, 1899 (syn. *O. borealis* Stossich, 1885, nec Beneden, 1853), from *Raja oxyrhynchus;* and *R. wehri,* n. sp., from *Raja stellulata.* Only 3 of these species, *R. laevis, R. ovata,* and *R. wehri,* are known to occur on North American hosts.

*Rajonchocotyle laevis,* n. sp.
Figs. 2, M; 3, K

*Description.*—Body about 11 mm long by 1.2 mm wide. Oral sucker relatively well developed, 185 μ in diameter. Haptor about 2 mm wide, bearing the usual 6 suckers each about 935 μ in diameter; haptoral appendix about 3 mm long by 765 μ wide, with terminal suckers about 360 μ long by 300 μ wide. Hooks of haptoral suckers slightly unequal, those of anterior and middle pairs about 1600 μ long and those of posterior pair about 1500 μ long; hooks of appendix about 60 μ long. Pharynx spherical, 85 μ in diameter; intestinal tract not observable except for the simple cecum extending into the haptoral appendix. Genital aperture about 1 mm from anterior end of body; testes about 90 in number, confined to postovarial part of body. Ovary convoluted, situated about one-third of body length from anterior end; vaginal apertures not observed. Vitellaria extending from level of genital aperture to posterior end of body proper. Eggs 200 to 225 μ long by 87 to 115 μ wide, with small knob at each pole and with 7 meridional ridges.

*Host.*—*Raja laevis* Mitchill.

*Location.*—Gills.

*Distribution.*—United States (Woods Hole, Mass.).

*Specimens.*—U.S.N.M. Helm. Coll. No. 36721 (cotypes).

This species is based upon a single slide containing one complete but mutilated specimen and a fragment of another, which had been collected by the late Dr. G. A. MacCallum at Woods Hole, Mass., on August 22, 1916. Owing to the mutilated condition of the specimens most of the measurements given above are approximate. This species seems to resemble most closely *R. alba* Cerfontaine but differs from that species in the shape of the blades of the large haptoral hooks, shape of the appendicular hooks and larger egg.

*Rajonchocotyle wehri*, n. sp.

Figs. 1, L; 2, M; 3, L

*Description.*—Body 10 to 11 mm long by 1.7 to 2 mm wide, sides more or less parallel. Oral sucker well developed, 425 μ in diameter. Haptor 2.5 to 3 mm wide, bearing the usual 6 suckers measuring about 850 μ in diameter; haptoral appendix about 1.6 mm long by about 950 μ wide, with terminal suckers 400 μ long by 220 μ wide. Hooks of haptoral suckers about 1450 μ long; hooks of appendix 72 μ long. Pharynx 115 μ in diameter. Intestinal tract not observable except in haptor and appendix; in haptor proper the intestine is ramified in a manner typical of the genus, the appendicular cecum being simple. Genital aperture 680 μ from anterior end of body; testes very numerous, number not ascertainable. Ovary convoluted, situated about one-third of body length from anterior end; vaginal apertures not observed. Vitellaria extending from level of intestinal bifurcation to posterior end of body proper. Eggs fusiform, 285 to 300 μ long by 105 to 120 μ wide, with 7 meridional ridges.

*Host.*—*Raja stellulata* Jordan and Gilbert.

*Location.*—Gills.

*Distribution.*—United States (Friday Harbor, Wash.).

*Specimens.*—U.S.N.M. Helm. Coll. Nos. 36719 (type) and 36720 (paratypes).

The specimens, 6 in number, on which the above description is based were collected by Dr. E. E. Wehr at Friday Harbor, Wash., July 3, 1927. This species may easily be distinguished from all others of the genus by the size and shape of the egg and by the large haptoral hooks.

*Rajonchocotyle ovata* Guberlet, 1937

This species has not been described in detail. The only information concerning it is the following statement by Guberlet (1937): "Una especie aparentemente no descrita de *Rajonchotyle* [sic], denominada *R. ovata*, n. sp., se encuentro con frecuencia sobre las branquias de *Raja binoculata* (Girard); este tremátodo se distingue de otros del mismo género, por su gran tamano y huevos con costillas promientes. Las observaciones sobre esta forma no son suficientes para determinar su distribución a lo largo de la costa." The illustrations of "*Rajonchocotyle* sp." given by Guberlet (1937) apparently refer to this species and from the shape and size of the egg, *R. ovata* may be regarded as a valid species. So far as may be determined from the available information, *R. ovata* resembles *R. wehri*, n. sp., from the same locality; however the size of the egg—about 60 μ long, according to figure—and the difference in host make it unlikely that the 2 forms are identical.

Genus *Rajonchocotyloides* Price, 1940

*Diagnosis.*—Vitellaria extending into haptoral appendix. Other characters those of subfamily.

*Type species.*—*Rajonchocotyloides emarginata* (Olsson, 1876) Price, 1940.

This genus at present contains only the type species which was described by Olsson (1876) and redescribed by Price (1940); it occurs on the gills of *Raja clavata* in Europe.

Subfamily DICLYBOTHRIINAE Price, 1936

*Diagnosis.*—Anterior haptor in form of ventral bothria; posterior haptor with 6 muscular suckers arranged in lateral rows, each sucker with a large hook, and with a posterior, lobe-like projection bearing 1 pair of small suckers and 3 pairs of large hooks, those of 2 pairs similar to hooks of suckers on haptor proper. Eyes present.

*Type genus.*—*Diclybothrium* F. S. Leuckart, 1835.

Genus *Diclybothrium* F. S. Leuckart, 1835

*Synonyms.*—*Diclibothrium* Leuckart, 1836, *Diplobothrium* Leuckart, 1842 (= *Diclybothrium* renamed).

*Diagnosis.*—Characters of subfamily.

*Type species.*—*Diclybothrium armatum* F. S. Leuckart, 1835.

The genus *Diclybothrium* contains 2 species, namely, *D. armatum* Leuckart, 1835, from *Acipenser rostratus, A. ruthenus, A. stellatus, A. baeri,* and *A. fulvescens,* and *D. hamulatum* (Simer, 1929), from *Polyodon spathula.*

*Diclybothrium armatum* F. S. Leuckart, 1835
Figs. 1, M & N; 2, O & P; 3, M

*Synonyms.*—*Diclibothrium armatum* Leuckart, 1836; *Diklibothrium crassicaudatum* Leuckart, in Kollar, 1836; *Diplobothrium armatum* (Leuckart, 1835) Leuckart, 1842; *Hexacotyle elegans* Nordmann, 1840; *Polystoma* (*Hexacotyle*) *armatum* (Leuckart, 1835) Dujardin, 1845; *?Erpocotyle circularis* Linstow, 1904. *?Diclibothrium circularis* (Linstow, 1904) Skwortzoff, 1928.

*Description.*—Body slender, 2.5 to 13 mm long by 225 μ to 1.1 mm wide, elliptical in cross section. Anterior haptor in form of 2 ventral, oval bothria, 80 to 150 μ wide. Posterior haptor circular to rectangular, 320 μ to 1 mm long by 320 to 800 μ wide, bearing 6 sessile or subsessile muscular suckers, each provided with a large hook, and with a more or less triangular posterior appendix 225 to 320 μ long by 120 to 240 μ wide, bearing 1 pair of relatively small muscular suckers and 3 pairs of large hooks. Suckers of haptor proper striated, 204 to 368 μ in diameter; hook 400 to 470 μ long. Suckers of haptoral appendix about 60 μ long; outer pair of large hooks similar in form to those in suckers of haptor proper, 440 to 540 μ long, directed posteriorly and ventrally; hooks of second pair similar in form to those of outer pair, 340 to 350 μ long, directed posteriorly and dorsally; those of third pair straight, more or less taenioid, 96 to 120 μ long, near tip of lobe and directed ventrally or anteriorly. Oral aperture ventral, 136 to 400 μ from anterior end of body; pharynx oval, 64 to 100 μ long by 64 to 96 μ wide; esophagus and intestine not discernible in available specimens. Brain antero-dorsal to pharynx; eyes present, 2 pairs. Excretory pores dorsal and lateral, at level of posterior end of cirrus. Genital aperture median, 240 to 680 μ from anterior end of body. Cirrus muscular, 160 to 320 μ long by 50 to 160 μ wide, tip evertible and armed with short, thickly-set spines extending backward from tip for a distance of about 40 μ; seminal vesicle piriform, 96 to 240 μ long by 80 to 176 μ wide, connected to base of cirrus by narrow isthmus. Testes very numerous (300 or more), small, postovarial. Ovary tubular, greatly convoluted, median, about one-fourth of body length from anterior end. Vitellaria lateral, extending from near level of vaginal apertures to near posterior end of body proper. Seminal

receptacle elongate, about 200 μ long, to left of anterior portion of ovarian mass. Vaginae present, openings slightly dorsal, 450 μ to 1.5 mm from anterior end of body. Ootype prominent, preovarial, surrounded by dense mass of unicellular glands. Uterus relatively long, slender, sinuous, in median field. Eggs 208 to 224 μ long by 88 to 140 μ wide, without polar prolongations.

*Host.*—*Acipenser fulvescens* Rafinesque.

*Location.*—Gills.

*Distribution.*—United States (New York Aquarium; Black Lake, Michigan; and Canada (St. Lawrence River, near Iroquois, Ontario).

*Specimens.*—U.S.N.M. Helm. Coll. 35287, 35584, and 35118.

Three specific names have so far been assigned to worms from the gills of sturgeons in Europe and America. Leuckart (1835) described as *Diclybothrium armatum* a form from the gills of *Acipenser rostratus*, and Kollar (1836) reported this form under the name of *Diklibothrium crassicaudatum* Leuckart from *A. stellatus*. Later Leuckart (1842) redescribed the form under the name of *Diplobothrium armatum*, the generic name being changed from *Diclybothrium* to *Diplobothrium*. Linstow (1904) described a similar form from *A. ruthenus*, which he named *Erpocotyle circularis* and which was later placed in the genus *Diclibothrium* by Skwortzoff (1928); a considerably detailed description of *D. circularis* (Linstow) from Russian sturgeons, was given by the latter author. Gill trematodes have been reported from *Acipenser rubicundus* (=*A. fulvescens*) in Canada under the name *Diplobothrium armatum* by Stafford (1904) and by Cooper (1915).

It is not possible to determine with certainty whether all of the species of *Diclybothrium* reported from sturgeons are the same or represent distinct forms. The descriptions of *D. armatum* by Leuckart (1842) and that of *Erpocotyle circularis* (=*D. circularis*) by Linstow (1904) are inadequate, as certain details of structure have been omitted. Furthermore, it is not absolutely certain from Skwortzoff's (1928) description of *D. circularis* that this author was dealing with the same species as that described by Linstow since there is a wide difference in egg size (220 μ by 180 μ, according to Linstow and 110 to 140 μ long, according to Skwortzoff). In view of the fact that the egg size of *D. circularis* as given by Linstow is within the size range given in this paper for the species occurring on American sturgeons, the writer is inclined to regard *D. circularis* Linstow as the same as *D. armatum* Leuckart. However, it is admitted that the evidence for this synonymy is not particularly strong, and the only means of settling the question is a restudy of specimens from the European sturgeons, particular attention being given to the morphology of the hooks.

Specimens of *Diclybothrium* available to the writer consisted of a number of individuals collected from *Acipenser rubicundus* (=*A. fulvescens*) in 1911 and 1912 by the late Dr. G. A. MacCallum at the New York Aquarium, 3 from *A. fulvescens* collected July 10, 1927, by A. McIntosh in Lake Huron, 2 loaned by Dr. H. W. Stunkard, New York University, labelled "*Diplobothrium armatum* Leuck., ex gills sturgeon, St. Lawrence River, 6–11–15," and 2 loaned by Dr. Sewell H. Hopkins, Texas A. and M. College. The latter specimens apparently are from the same lot as those in Stunkard's collection and represent a portion of the material upon which Cooper (1915) based his report of the species from Canada.

### *Diclybothrium hamulatum* (Simer, 1929)

This species was described as *Diplobothrium hamulatum* by Simer (1929) from specimens obtained from the gills of the paddle fish, *Polyodon spathula* (Wahlbaum). For the most part Simer's description of this form is adequate and a redescription is unnecessary. From a study of specimens from the above host (not available at the time *Diplobothrium hamulatum* was declared by Price (1936) to

be a synonym of *Diclybothrium armatum*) which were kindly placed at the writer's disposal by Dr. Sewell H. Hopkins, Texas A. and M. College, the characters which appear to be distinctive are the haptoral hooks (Fig. 2, Q & R), the relative number of testes, and the shape and size of the egg (Fig. 2, N). In *D. hamulatum* the hooks of the haptoral suckers are more robust than in *D. armatum;* slight differences are also noted in the appendicular hooks. The number of testes are only about one-half that for *D. armatum,* and the egg is round instead of oval and is much smaller than that of the latter species.

In differentiating *Diplobothrium hamulatum* from *D. armatum,* Simer (1929) stated: "No European material is available for restudy, but from Leuchart's [sic] description it is evident that *D. hamulatum* differs from *D. armatum* in the possession of 4 instead of 2 posterior spines and in having non-stalked, non-ciliated lateral suckers rather than those of the stalked, ciliated type." A casual examination of Leuckart's figures of *D. armatum,* however, shows 4 posterior hooks (spines) and not 2 as Simer stated. As a matter of fact the species has 6 posterior hooks, and it is quite evident from Simer's figure of *D. hamulatum* that these were seen by him but he failed to interpret them properly. As to the ciliated versus nonciliated condition of the "lateral suckers," it is certain, in spite of the fact that Leuckart makes a statement to the effect that the suckers are ciliated, that what he observed was the serrated outline of the suckers as viewed in optical section.

Simer regarded the form from *Polyodon spathula* as identical with that occurring on the American sturgeon, *Acipenser rubicundus,* basing his conclusion on an examination of specimens in the collection of Dr. George R. LaRue, University of Michigan, which had been obtained at Black Lake, Michigan. Specimens in the collection of Mr. Allen McIntosh collected at Black Lake, Michigan, and probably part of the material referred to by Simer, have been examined and found to correspond in all respects to specimens from *A. rubicundus* collected elsewhere and distinct from the form from *P. spathula.*

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**Early natural infections of suckling pigs with helminth parasites.** JOHN S. ANDREWS and JAMES W. CONNELLY, U. S. Bureau of Animal Industry.

It is well known that pigs farrowed and kept under insanitary conditions may become infected with parasites early in life. However, very little specific information is available as to how soon after birth such infections may be acquired. Spindler (1937, Proc. Helminth. Soc. Wash. 4(2): 62–63) recovered migrating ascarid and kidney worm larvae, immature threadworms, and encysted nodular worm larvae from the bodies of pigs necropsied 3 to 11 days after birth.

To obtain further information on how soon after birth pigs may become infected with worms, the writers made post-mortem examinations of 17 suckling pigs from the swine herd of the Georgia Coastal Plains Experiment Station, Tifton, Georgia. These pigs were from litters that had been farrowed under conditions of partial sanitation; some that were examined had been killed by the sows, and others had been culled from the herd and killed. The ages of the pigs varied from a few hours to 66 days.

METHOD OF MAKING POST-MORTEM EXAMINATIONS

Each portion of the alimentary tract was opened separately and the contents washed into a separate container, sedimented and examined for worm parasites with the aid of a dissecting microscope. Also, the mucosa was scraped with a scalpel and the material thus obtained was examined microscopically for worms. Livers and lungs were cut into small pieces or finely ground by passing through a food chopper and baermannized separately. In addition, the body muscle tissue of 15 of the pigs was finely ground and baermannized.

FINDINGS

A mature female *Strongyloides ransomi* was recovered from the small intestine of a 4-day-old pig that had been farrowed October 1, 1941; the uteri of this worm were filled with eggs each containing an active larva in the incurved embryo stage; no eggs were found in the contents of the large intestine. Since under ordinary conditions the prepatent period of *S. ransomi* as determined by fecal examination is from 6½ to 7 days, it is considered that the infection encountered may have been acquired the day the pig was born.

A live, exsheathed, third-stage larva of *Stephanurus dentatus*, was recovered from a Baermann apparatus containing the ground-up muscle tissue of a 12-day-old pig (farrowed September 11, 1941).

Eggs of *Oesophagostomum* sp. were found in the feces of a pig 42 days old. Since the prepatent period of *Oesophagostomum* is from 5 to 7 weeks it is considered that this parasite was acquired the day the pig was born. No worms were recovered from the lungs and livers of the pigs examined.

Observations herein reported demonstrate that even under conditions of partial sanitation pigs may acquire infections with *Strongyloides*, *Stephanurus*, and *Oesophagostomum* within a few hours to a few days after birth. The observations indicate that any practice of confining sows and litters in restricted quarters may contribute to worm infection of the pigs by compelling the sow to urinate and defecate where the pigs may come in contact with the infective stages of parasites and point to the desirability of ample range for sows and litters as a parasite control measure.

**Observations on fatalities in sheep caused primarily by heavy natural infections with the stomach worm, *Haemonchus contortus*.** KENNETH C. KATES, U. S. Bureau of Animal Industry.

During the latter part of 1941 6 sheep (5 lambs born early in 1941 and 1 old ewe) from a group of 30 maintained as a heavily parasitized flock at the Zoological Division, United States Department of Agriculture Beltsville Research Center, Beltsville, Md., died or were destroyed *in extremis*. The symptoms shown by these 6 sheep were severe anemia, manifested by the pallor of the visible mucous membranes, marked physical weakness, progressive loss of weight, and loss of appetite or anorexia. Although some emaciation was noticeable in all cases, it was more marked in those sheep dying later in the year, that is, in those in which the infection was of long standing. Examination of the blood showed a marked decrease in red blood cells. Haematocrit readings on 2 of the 6 sheep showed 8.5 per cent and 8 per cent packed red cells, respectively.

Fecal examinations showed the presence of numerous *H. contortus* eggs and very small numbers of eggs of other nematodes.

At necropsy the 6 sheep showed evidence of a severe anemia similar to that described by Fourie (1931, 17th Rpt. Dir. Vet. Serv., Dept. Agr., Union So. Africa, 2: 495–572) in fatal cases of experimental *H. contortus* infection.

As little information is available in the literature concerning the actual numbers of nematodes present in sheep dying of natural parasitic infections, careful post-mortem examinations were made to determine the numbers of the various species of nematodes present. Death in each case was diagnosed primarily as due to haemonchosis, and the 6 cases, therefore, are very similar. The purpose of this report is to present specific data on the actual numbers of parasites recovered post mortem from sheep dying primarily as a result of naturally acquired stomach worm infections.

## ORIGIN AND MAINTENANCE OF PARASITIZED ANIMALS

In early May 1941 about 20 cull ewes and bucks from the breeding flock of the Zoological Division were placed on a quarter-acre pasture known to be contaminated with larvae of various nematodes of sheep. After the pasture had been well grazed, the sheep were given adequate quantities of alfalfa twice daily in order to maintain the flock in good health. Adequate quantities of water were available to the sheep at all times. On August 1, 1941, 10 heavily parasitized lambs, weighing from 50 to 60 pounds each, obtained from 2 nearby Maryland farms, were placed with the 20 cull sheep. This flock was employed from time to time thereafter for short periods to infect other pastures, after which the sheep were returned to the original quarter acre pasture.

Of the original 20 cull sheep, 1 (40–U) died in September, while 5 of the 10 lambs died as shown in table 1, namely, 1 in August, 1 in September, 1 in October, and 2 in December. Three of the lambs developed a severe diarrhea shortly before death.

## RESULTS OF POST-MORTEM EXAMINATIONS

The general condition and appearance of the internal organs of the 6 sheep were essentially similar. The presence of a severe anemia caused the kidneys, liver, and other organs and tissues to be pale, and the blood in all cases was thin, pale, and watery. Some edema was present in all cases, and the omental and mesenteric fat had practically disappeared. No lesions complicating the picture of the severe anemia, caused by heavy parasitism, were found.

Upon careful examination of the alimentary tract, the most striking feature was the mass of *H. contortus* in the fourth stomach (Fig. 1), and the brownish chocolate color of the abomasal contents, the latter, according to Fourie (*loc. cit.*),

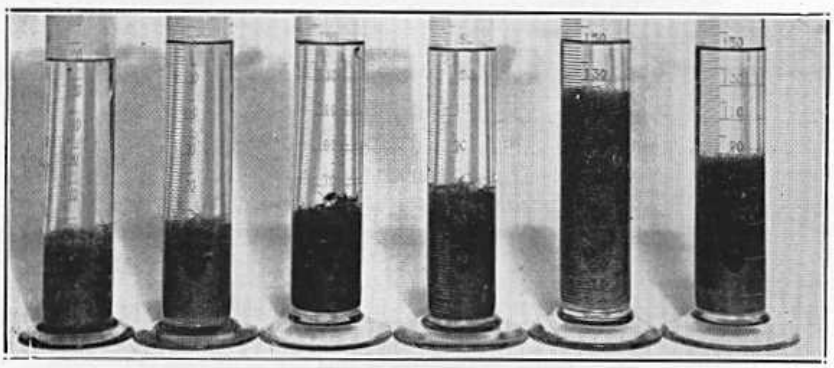

FIG. 1. Volume of *Haemonchus contortus* recovered at necropsy from (left to right) lambs 483, 482, 485, 481, 490, and ewe 40–U. The last graduate on right contains the largest number of worms and was obtained from ewe 40–U. These worms sedimented to give less volume than the smaller number of worms from lamb 490 in the adjacent graduate.

being the result of hemorrhage caused by the worms. As very little food material was present in the abomasum, the worms were obtained practically free from debris merely by washing the abomasal contents in a large crystallizing dish. In the case of those animals which had been dead several hours before they were examined, many specimens of *H. contortus* were found in the small intestine, where they had migrated from the fourth stomach.

In table 1, the data on the 6 sheep including the worms recovered post mortem are recorded. The table is so arranged as to correspond to figure 1 showing the stomach worms recovered.

TABLE 1.—*Necropsy and other data on sheep and lambs dying of naturally acquired H. contortus infections*

| | Sheep and lamb numbers | | | | | |
|---|---|---|---|---|---|---|
| | Lamb 483 | Lamb 482 | Lamb 485 | Lamb 481 | Lamb 490 | Ewe 40–U |
| Date of death | 12/30/41 | 12/23/41 | 10/6/41 | 9/4/41 | 8/2/41 | 9/29/41 |
| Weight at death (lbs.) | 35 | 36 | 50 | 60 | 60 | 75 |
| Haematocrit reading prior to death | 8.5 | 8 | ..... | ..... | ..... | ..... |
| Species and number of parasites recovered at necropsy | | | | | | |
| *Haemonchus contortus*[a] | 3,309 | 3,792 | 8,000 | 10,000 | 14,520 | 17,850 |
| *Ostertagia circumcincta* | 60 | 90 | 50 | 200 | 320 | 900 |
| *Trichostrongylus* sp.[b] | 800 | 2,050 | 500 | 500 | 500 | 85 |
| *Cooperia curticei* | 150 | 725 | 100 | 200 | 145 | 75 |
| *Strongyloides papillosus* | 500 | 200 | 100 | 200 | 275 | 300 |
| *Nematodirus spathiger* | 85 | 1,192 | 14 | 350 | 24 | 176 |
| *Nematodirus filicollis* | 49 | 108 | ..... | 50 | 6 | 24 |
| *Bunostomum trigonocephalum* | 44 | 11 | ..... | 2 | ..... | 5 |
| *Oesophagostomum columbianum* | 117 | 126 | 51 | 8 | 50 | 225 |
| *Trichuris ovis* | 47 | 47 | 47 | 55 | 40 | 6 |
| *Moniezia expansa* | 3 | 7 | 1 | ..... | ..... | 4 |
| Totals | 5,164 | 8,348 | 8,863 | 11,565 | 15,880 | 19,650 |

[a] The numbers of *H. contortus* recovered at necropsy are shown in the same order in figure 1.

[b] Approximately 95 per cent of the trichostrongyles were *T. colubriformis;* only small numbers of *T. axei* and *T. vitrinis* were found.

The largest number of worms recovered were from the cull ewe (40–U) which died on September 29, 1941. Of the 19,650 helminth parasites present in the alimentary tract of this ewe, 17,850 were *H. contortus.* The number of worms of other species present was not large enough to be of more than minor significance.

In the case of the 5 lambs it is interesting to note that the longer the survival period the smaller the number of *H. contortus* recovered at necropsy, the estimated numbers being 14,520, 10,000, 8,000, 3,792, and 3,309, respectively, for the 5 lambs dying between August 2, 1941, and December 30, 1941. There was a correspondingly similar decrease in the number of *Ostertagia circumcincta,* although in no case was this species present in large numbers. In one case (lamb 482) there were present relatively large numbers of *Trichostrongylus colubriformis* and *Nematodirus spathiger,* which may have contributed to the death of this lamb. It is believed, however, that the 3,792 *H. contortus* recovered in this case were the primary cause of death.

Although 10 heavily parasitized lambs were obtained from 2 nearby Maryland farms on August 1, 1942, only 5 died during the succeeding 7 months. The remaining 5 lambs continued in very poor condition for several months and their level of parasitism gradually declined, as determined by fecal examinations, and reached very low levels by March 1942.

SUMMARY

1. Ante-mortem and post-mortem observations are reported on 1 ewe and 5 lambs dying primarily of heavy natural infections with *H. contortus*.

2. The clinical symptoms exhibited by the sheep were those of a severe progressive anemia commonly associated with severe haemonchosis.

3. The largest number of *H. contortus* (17,850) recovered at necropsy was from the largest sheep (40–U) which weighed 75 pounds at the time of death.

4. The number of *H. contortus* recovered from the 5 lambs varied from 14,520 to 3,309, the smaller number being correlated with the relatively greater duration of the infection.

5. With the possible exception of one case (lamb 482), the presence of other species of nematodes did not appear to complicate the general clinical and pathological picture of fatal haemonchosis.

**On the survival of the preparasitic stages of the cattle lungworm on pastures.**

DALE A. PORTER, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama.

In the absence of an efficient anthelmintic for the removal of the cattle lungworm, *Dictyocaulaus viviparus*, the control of this parasite must be based largely on management practices designed to prevent exposure of the host. One question in management of a herd involves the length of time that the larvae survive on pastures in the absence of an infected host. An experiment was reported recently in which yearling cattle failed to become infected with lungworms after grazing on a pasture contaminated with lungworm larvae nearly 5 months previously. (Porter *et al.*, 1941, Jour. Parasitol., 27(Suppl.): 22). It is the purpose of this note to record the results of some experiments in which calves did not acquire lungworms when grazed on pastures vacated by infected calves only 6 to 7 weeks previously.

The first experiment was conducted at a local dairy where purebred Jersey calves were being raised for sale and for selective replacement of the milking herd. The calves were kept in a small shed having access to one-half acre of carpet and Bermuda grass. This pasture was well drained and received no drainage from adjacent fields. On July 29, 1938, when the observations were begun, the pasture contained 17 calves from 2 weeks to 6 months old. All were in very poor condition as a result of early infection with coccidia, gastrointestinal nematodes (*Cooperia punctata, Nematodirus helvetianus, Ostertagia ostertagi,* and *Trichostrongylus colubriformis*), and lungworms. The latter parasites were diagnosed by recovery of first stage larvae from the feces of 6 out of 13 calves over 2 months old. On August 8, 1938, all calves were moved to a small pasture similar to their former quarters. Five of the herd died or were killed in extremis, 2 in mid-August, and 1 each in September, October and November. Three of these animals harbored lungworms, 2 having been diagnosed as positive while on the original pasture. The third calf, 10 days old when moved to new quarters, was passing lungworm larvae a month later, and harbored mature lungworms when it was destroyed at 2 months of age.

On September 20, 43 days after the original pasture was vacated, it was divided by a fence into equal areas, and a 1-month-old calf, raised free of parasites, was introduced into one of the areas. Two similar calves were added to the same area

on the 49th and 56th day, respectively, after the pasture was vacated. These calves remained on this area for 1 month. The first calf was killed and examined at the end of this time. The second calf was stabled for 8 days additional, and the third for an additional period of 24 days, before necropsy. All 3 calves failed to pass lungworm larvae during their lives and were free of lungworms at necropsy.

The second half of the pasture remained vacant until one calf was introduced on January 3, and a second calf on January 10, 1939, 148 and 155 days, respectively, after the original infected animals had been removed. Daily fecal examinations for 6 weeks followed by necropsy of the animals revealed no evidence of lungworm infection. However, 3 gastrointestinal nematodes (*C. punctata, N. helvetianus,* and *O. ostertagi*), were recovered from the host animals in both tests, indicating that the preparasitic stages of these parasites were more resistant than those of the lungworm. *Trichostrongylus colubriformis,* also found in the original herd, was not recovered from the test calves. During the shortest period the pasture was vacant (Aug. 8 to Sept. 20), 3.1 inches of rainfall were recorded and the temperature varied from 15.5° to 40° C. (60°–104° F.). Rainfall during August and September was 3.28 and 2.02 inches, respectively. These represent, respectively, 1.4 and 1.16 inches below the average. The average monthly temperature was somewhat above normal, the highest temperature of the year (40° C. or 104° F.) being recorded on August 25, during the second week the pasture was vacant.[1]

Data are available on only a few of the calves that were born after the original herd was moved to new quarters, but it is definitely known that some of the calves became infected with lungworms from the older infected calves on the new pasture during the period of the experiment described in this paper. Two calves born the second week of August were passing lungworm larvae on October 19, 2 months later. One calf born August 26, and another born October 4, were found to be infected with lungworms when fecal examinations were made when these animals were 2 months old. Late in October the calves in this herd were allowed to range over about 20 acres of pasture during the day and were confined to their barn and small pasture only at night. Animals born into this herd after this time have remained free from lungworms up to the present (June 1942).

The second experiment was conducted on the property of the Regional Laboratory. Two plots, each one-eighth acre in size, were fenced off on a pasture that had been used exclusively for horses for several years. Plot 1 was on a well drained slope, the ground being sparsely covered with carpet grass. Plot 2 was located in a slight depression so that a small area had been sufficiently moist in the early spring to insure a lush growth of carpet grass and clover. Feces containing first-stage larvae of the cattle lungworm were collected daily from an infected calf and equal portions were distributed on each plot. It was estimated that approximately 2,500,000 larvae were distributed on each plot between April 15 and May 2, 1941. On May 9, 7 days later, a 9-month-old calf was placed on each plot. These calves were allowed to graze, without being given any supplemental feed, for the next 2 weeks, after which they were returned to the laboratory barn. Daily fecal examinations were made for the next 6 weeks, and tri-weekly examinations were continued for an additional 6-week period. Larvae of the cattle lungworm appeared in the faces of the calf from plot 1 on June 13, 35 days after the first, and 21 days after the last exposure; the larvae continued to pass for the next week after which none was observed. The period required for development of the parasite in this calf is in agreement with that recorded by the writer previously (Porter, 1941, Jour. Tenn. Acad. Sci., **6**: 359). The calf from plot 2, however, never passed lungworm larvae

[1] Recorded observations for Auburn, Ala., were obtained from Climatological Data, Alabama Section, U. S. Weather Bureau, Vol. XLII, 1938, and Vol. XLVII, 1941.

or showed any indications of having acquired these parasites. Failure of this animal to become infected may have been due to the abundance of grass on the plot which made it unnecessary to graze intensively and close to the ground as was the case in plot 1. The conditions for development and survival of the preparasitic stages on plot 2 should have been equal to if not more favorable than those on plot 1 because of the protection afforded by the vegetation.

On June 20, 1941, 48 days after the plots had been contaminated with lungworm larvae, a 5-months-old calf was placed on each plot. These animals were allowed to graze until July 11, and then returned to the barn. Both animals remained negative for lungworm larvae so far as could be judged by daily fecal examinations. The calf from plot 1 did not harbor lungworms when necropsied on July 30, and fecal examinations for the next 2 months failed to reveal any evidence of lungworms in the calf from plot 2. During the period from May 2 to May 9 there was 0.82 inch of rainfall and the temperature varied from 11.6° to 31.1° C. (53°–88° F.); during the longer period (May 2 to June 20) 2.88 inches of rainfall were recorded and the temperature varied from 8.3° to 36.1° C. (47°–97° F.). Actually the average monthly temperatures during this experiment were only slightly above normal, whereas rainfall was quite deficient. During May the rainfall was 2.9 inches below normal, this being the driest month of the year.

The results indicate that, under conditions of the experiment, (1) lungworms were transmitted readily from one calf to another when these animals were crowded together on well drained and comparatively dry pastures, a condition which was remedied when the animals were given greater range; and that (2) during warm, dry weather the infection persisted on well drained pastures for at least 1 week but not for 6 to 7 weeks after the parasitized host was removed.

**Death of pigs associated with the presence in the heart tissue of larvae of *Strongyloides ransomi*.** L. A. SPINDLER and C. H. HILL, U. S. Bureau of Animal Industry.

In investigations now being carried out at the Beltsville Research Center to ascertain the effects on young pigs of infections with *Strongyloides ransomi*, a number of the test host animals died suddenly within 2 weeks or more after infection. In each case live and/or dead third-stage larvae of *Strongyloides* were found in the heart, in the regions of the auriculoventricular node and bundle. In the case of one pig the gross lesions consisted chiefly of petechial hemorrhages containing larvae, and a fibrinous adhesive pericarditis. In the case of two other pigs that died several weeks after a natural infection there were anemic infarcts in the heart muscle which in some cases appeared to be associated with the presence of emboli in the arterioles adjacent to the lesions; these emboli appeared to be composed of dead larvae. In the case of another pig that died approximately 2½ weeks after experimental infection no gross lesions other than a few petechiae containing larvae were observed, but active third-stage larvae were found in press preparations of small pieces of myocardial tissue; the larvae in question appeared to be located between the muscle fibers. By means of a technique previously used for examination of large quantities of host tissue (Spindler, 1937, Proc. Helminth. Soc. Wash. 4(2): 62–63), a total of 552 live, third-stage larvae were recovered from the myocardium.

A thrombus firmly adherent to the endocardium was present in one or both ventricles of each animal that died. This indicates that the endocardium may have been injured by the larvae with resultant clot formation.

Preliminary observations of the heart sounds of infected pigs indicated that a condition of partial heart block occurred after infection; this may have been the result of injury by migrating larvae to the auriculoventricular conducting system.

The observations herein reported have a bearing on both veterinary and human

medicine. Intestinal threadworms are prevalent in practically all species of food animals. On the farm young pigs often die without any apparent cause. In light of the observations herein reported there is a possibility that *Strongyloides* may under certain conditions be responsible for the sudden death of pigs.

*Strongyloides stercoralis,* the species that parasitizes man, is prevalent in some parts of the world. In view of the fact that *Strongyloides* larvae invade the myocardium of pigs, and that partial heart block may follow such infection, the possibility that larvae of *S. stercoralis* also may get into the myocardium of infected persons and cause similar injury should be kept in mind.

**Studies on oxyuriasis. XXVI. Resistance of white rats on a vitamin A-deficient diet to experimental infection with *Enterobius vermicularis*.** MYRNA F. JONES and M. O. NOLAN, National Institute of Health.

In order to facilitate biological studies of the human pinworm, feeding experiments were conducted in an effort to develop *Enterobius vermicularis* in laboratory animals. Cram (unpublished data) fed embryonated eggs of *E. vermicularis* to monkeys, dogs, guinea pigs, and mice. Negative results were obtained with the monkeys and dogs. Active larvae of *E. vermicularis* were recovered from the stomach and duodenum of the guinea pigs and from the small intestine of the mice within short periods after feeding; also, live and dead larvae were recovered from the large intestine of the mice after slightly longer periods. However, it was never possible to establish infection in these animals. In an attempt to determine whether or not a dietary deficiency would influence the development of the worms in an abnormal host, the present authors worked with vitamin A-deficient rats.

The rats used in these experiments were bred from Wistar albino stock in the Animal Breeding Unit, National Institute of Health. All of the parent rats and their offspring were free of infection with the rat and mouse pinworms, *Syphacia obvelata* and *Aspiculuris tetraptera,* as determined by fecal examinations. In most instances, as a check on the fecal examination, one rat from each litter was sacrificed as soon as the litter was weaned, the intestinal tract was examined and in all cases found negative for the parasites.

Both test and control rats were weaned at the age of 3 to 4 weeks. The test animals were then fed a vitamin A-free diet consisting of: Casein (A-free), 18 per cent; Osborne-Mendel salts, 4 per cent; dried Brewer's yeast (Vita-food), 5 per cent; Wesson oil, 10 per cent; rice flour, 63 per cent. Five drops of Drisdol (40,000 U.S.P. vitamin D units per gram) were added to each 1000 grams of the mixture. All but 12 of the control rats were fed this same diet, supplemented with 15 units per day of pro-vitamin A in the form of carotene; these 12 control rats were given a normal diet of Purina Rabbit Chow.

Characteristic symptoms associated with vitamin A deficiency were commonly observed in the test animals as early as 5 weeks. In many of the animals showing no clinical manifestation of the deficiency, as well as in those showing symptoms, pus in the sublingual glands was observed on necropsy.

Our greatest difficulty was encountered in obtaining the necessary pinworm eggs for feeding purposes. We had to rely upon the cooperation of infected persons or parents of infected children, and it was not always possible to obtain the material at the time desired. Usually, the pinworms were collected from an infected individual in the early part of the night by the use of the NIH swab and the worms were then refrigerated until morning. Eggs recovered from the worms were placed in a small amount of distilled water and were incubated at 37° C. until ring and a half embryos had developed. They were then kept moist at a temperature of 5° C. until used. Before feeding them to the rats a random sample

TABLE 1

| Time after feeding ova to rats (days) | – A rats positive | Litter control rats positive |
|---|---|---|
| Less than 1 | 1 | 0 |
| 1 | 0 | 1 |
| 2 | 2 | 1 |
| 3 | 2 | 3 |
| 4 | 0 | 0 |
| 5 | 3 | 1 |
| 6 | 1 | 1 |
| 7 | 1 | 1 |
| Total | 10 | 8 |

from each lot of eggs was tested for viability by the usual method of hatching active larvae in artificial gastric juice. For the most part, the eggs were 1½ to 2½ days old when fed to the rats; some were 3½ to 7 days old, and a few lots were slightly over 2 weeks old. The viability of these ova seemed unaffected by cold storage at 5° C. Eggs from each lot of material were fed to animals on the A-deficient ration and to control rats at the same time and both groups of animals were held for the same number of days before being chloroformed and examined.

Various methods to recover the pinworm larvae from the intestinal tract were tried and it was found that larvae were recovered most efficiently and easily by using a small Baermann apparatus, consisting of an 80-mesh screen fitted into the top of a 400-cc funnel. The intestinal tract was divided into portions, as follows: Small intestine, upper ⅔ and lower ⅓; cecum, 2 parts; and colon. The contents of each portion, mixed thoroughly with warm physiological saline, were poured into the apparatus and allowed to stand in an incubator room at 37° C. for approximately 5 hours before the sediment was drawn off and examined.

More than 100 rats were handled in the experiments but many of the animals developed increasingly severe symptoms of vitamin A deficiency at a time when pinworm material was not available and could therefore not be used. The number of rats (representing 11 litters) that were carried successfully through the experiments was 76; of these, 36 were on an A-deficient diet and 40 were litter control animals.

Our results indicate that a vitamin A-deficient diet did not increase the susceptibility of this host to *E. vermicularis* infection. Larvae were recovered from 10 test and 8 control rats up to 7 days after feeding them embryonated ova (see

TABLE 2

| Time on – A diet before feeding ova to rats (weeks) | No. of positive rats | No. of negative rats | Time on – A diet before feeding ova to rats (weeks) | No. of positive rats | No. of negative rats |
|---|---|---|---|---|---|
| 1 | 1 | 5 | 8 | 1 | 1 |
| 2 | 2 | 4 | 9 | 0 | 1 |
| 3 | 1 | 0 | 10 | 0 | 1 |
| 4 | 0 | 1 | 11 | 2 | 1 |
| 5 | 2 | 2 | 12 | 0 | 0 |
| 6 | 1 | 4 | 13 | 0 | 2 |
| 7 | 0 | 4 | | | |
| | | | Total | 10 | 26 |

Table 1); no larvae or adult worms were recovered from the rats after longer intervals.

In both test and control animals, pinworm larvae were found to be more numerous and active in the lower third of the small intestine. In 7 rats (6 test and 1 control) a small number of larvae, most of them inactive or dead, were recovered from the cecum 2 to 6 days after infection, but in no case were larvae found in the colon.

We found also that there was no increase in the susceptibility of the test rats to pinworm infection as a result of the progressive physical deterioration resulting from continued A deficiency (see Table 2).

Our failure to establish *E. vermicularis* infection in the white rat would seem to indicate that in this abnormal host susceptibility to the parasite is not increased by a vitamin A deficiency.

**New host-parasite records.** G. DIKMANS, U. S. Bureau of Animal Industry.

During the year 1941 the Zoological Division of the U. S. Bureau of Animal Industry received for identification several specimens of helminth parasites which had been collected from both domestic and wild ruminants. Among these the following constitute new host records:

| *Parasite* | *New host* | *Locality* | *Collector* |
|---|---|---|---|
| *Ostertagia bisonis* | *Bos taurus* (Cattle) | Montana | F. H. Wilkins |
| *Ostertagia grühneri*<br>*Ostertagia occidentalis* | *Ovis canadensis* (Mountain sheep) | Idaho | E. R. Quortrop |
| *Ostertagia marshalli*<br>*Ostertagia occidentalis*<br>*Protostrongylus rushi*<br>*Thysanosoma actinioides* | *Oreamnos americanus* (Mountain goat) | Idaho | O. J. Hummon |

*Ostertagia bisonis* was originally described by Chapin (1925, Jour. Agr. Res. 30(7): 677–681) as a parasite of the fourth stomach of the American bison, *Bison bison,* from Wainwright, Alberta, Canada, and has not been previously reported as a parasite of cattle. *Ostertagia grühneri* was reported in 1931 by Skrjabin as a parasite of the abomasum of reindeer, *Rangifer tarandus,* in the Archangel district of U.S.S.R. and of "northern deer" on the coast of the Sea of Okhotsh. It has also been found in reindeer in Alaska. *Osteragia occidentalis* was first reported and described by Ransom (1911, U. S. Bur. Anim. Indus. Bull. 127) as a parasite of sheep. None of these roundworms has previously been reported as parasites of the mountain sheep, *Ovis canadensis.* So far as the writer has been able to determine only one nematode, namely, *Skrjabinema oreamni* has previously been reported as a parasite of the mountain goat, *Oreamnos americanus.* This nematode was described by Swales (1934, Canad. Jour. Res. **10**: 527–532) who stated that the animal from which the specimens of *Skrjabinema* were obtained also harbored many stomach worms, but these were not further identified.

In addition to the nematodes listed in the table the writer identified as *Skrjabinema* sp. some nematodes collected from the viscera of a deer sent to the Zoological Division from Boise, Idaho, by Dr. T. B. Murray in 1929. The only other hosts from which *Skrjabinema* has been previously reported in North America are the domestic goat, *Capra hircus,* by Schwartz (1927, N. Amer. Vet. 8(10): 22–23), and the mountain goat, *Oreamnos americanus,* by Swales (*loc. cit.*). While the specific name of the deer from which these specimens of *Skrjabinema* were collected was not given by the sender, it appears reasonable to assume that it was the mule deer, *Odocoileus hemionus,* because this is the most common species of deer occurring in that area.

**A new nematode, *Skrjabinema parva* (Nematoda: Oxyuroidea), from deer.**

G. DIKMANS, U. S. Bureau of Animal Industry.

The genus *Skrjabinema* was created by Vereshchagin (1926, Trudy Gosud. Inst. Exper. Vet. 2(2): 20–32; 1927, Deut. Tierärztl. Wchnschr. 35(28): 455–456) for some roundworm parasites collected by Skrjabin in Leningrad from sheep originating in Turkestan and described by him as *Oxyuris ovis*. This worm has since been reported as a parasite of goats in Asiatic Russia (Turkestan), the United States (Nebraska, Massachusetts, Maryland), England and Austria, of *Gazella subgutturosa* in Turkestan and of the steenbuck, *Rhaphiceros campestris*, in South Africa. Skrjabin and Mitzkewitsch (1930, Deut. Tierärztl. Wchnschr. 38(12): 183–185) described as a second species, *Skrjabinema tarandi*, some nematodes collected from reindeer, *Rangifer tarandus*, in the Archangel district of the U.S.S.R. Böhm and Gebauer (1930, Ztschr. Parasitenk. 2(5): 589–594) added a third species, *Skrjabinema rupicaprae*, from the chamois, *Rupicapra rupicapra* and Mönnig (1932, 18th Rpt. Dir. Vet. Serv. and Anim. Indus. Union So. Africa, pp. 153–172) described 2 additional species, *S. africana* from the steenbuck, *Rhaphiceros campestris*, and *S. alata* from sheep, *Ovis aries*. Swales (1934, Canad. Jour. Res. 10: 527–531) reported *S. oreamni* as a parasite of the mountain goat, *Oreamnos americanus*, and made *S. tarandi* a synonym of *S. ovis*. At the present time, therefore, 5 species of *Skrjabinema* have been described from the following hosts and localities:

| *Species* | *Hosts* | *Locality* |
|---|---|---|
| *Skrjabinema ovis* | Sheep (*Ovis aries*); goat (*Capra hircus*); *Gazella subgutturosa*; steenbuck (*Rhaphiceros campestris*) | Asiatic Russia (Turkestan) Turkestan and U. S.; England and Turkestan; South Africa |
| *Skrjabinema rupicaprae* | Chamois (*Rupicapra rupicapra*) | Austria |
| *Skrjabinema africana* | Steenbuck (*Rhaphiceros campestris*) | South Africa |
| *Skrjabinema alata* | Sheep (*Ovis aries*) | South Africa |
| *Skrjabinema oreamni* | Mountain goat (*Oreamnos americanus*) | Canada |

The following diagnosis of the genus *Skrjabinema* is taken in part from Mönnig (*loc. cit.*): Oxyuridae of small size. Cuticle inflated around the anterior end. Body provided with lateral alae for the greater part of its length. Two lateral and four submedian head papillae present. Mouth opening surrounded by 3 lips, each lip divided into 2 lobes by an anchor-shaped structure with the flukes or hooks of the anchor extending into the lobes of the lip. Each lip bears on its inner surface a pair of tooth-like plates which project towards the center of the mouth opening. Interlabia present. Excretory pore behind level of esophagus. Vulva in anterior half of body. Tail of male with small lateral alae supported by 2 pairs of large, thickened ray-like papillae, one pair preanal and the other postanal and ending in an acute point. The tail bears a number of small papillae. A single spicule and a gubernaculum are present.

Mönnig (1932, *loc. cit.*) divided the genus into 2 subgenera, *Skrjabinema* and *Chilocrypta*, the extent to which the mouth wall surrounds the lips being the basis upon which the division was made. In the subgenus *Skrjabinema*, the lips project beyond the mouth wall, whereas in the subgenus *Chilocrypta* the lips are completely surrounded and hidden by the mouth wall as indicated by the name of the subgenus. *Skrjabinema ovis* and *S. oreamni* are included in the subgenus *Skrjabinema*; the other species, namely, *S. alata*, *S. africana*, and *S. rupicaprae* belonging to the subgenus *Chilocrypta*.

TABLE 1.—*Body measurements of the species of Skrjabinema*

| | Subgenus Chilocrypta | | | Subgenus Skrjabinema | | |
|---|---|---|---|---|---|---|
| | *S. alata* | *S. africana* | *S. rupicaprae* | *S. parva* | *S. ovis* | *S. oreamni* |
| | *mm* | *mm* | *mm* | *mm* | *mm* | *mm* |
| Length | 4.61 –5.16 | 5.12 –5.8 | 5.5 –5.6 | 3.5 –4 | 6.6 –8 | 9.5 –10.5 |
| Breadth, vulvar region | 0.31 | 0.29 –0.38 | 0.36 –0.4 | 0.22 –0.32 | 0.4 –0.57 | 0.45 – 0.55 |
| Breadth, head | 0.82 | 0.075 | 0.06 –0.068 | 0.064 | ..... | 0.093 |
| Excretory pore | 0.824–0.915 | 1.28 –1.37 | 1.00 –1.14 | ..... | 1.5 –1.7 | ..... |
| Nerve ring | 0.169 | 0.15 –0.169 | ..... | ..... | ..... | 0.240 |
| Oesophagus, total length | 0.662 | 0.413–0.515 | 0.48 –0.59 | 0.375–0.4 | 0.72 –1.13 | 0.650– 0.720 |
| Bulb, length | 0.15 | 0.154–0.165 | 0.120–0.150 | 0.125–0.143 | 0.170–0.245 | 0.188– 0.211 |
| Bulb, breadth | 0.146–0.150 | 0.146–0.150 | 0.108–0.128 | ..... | ..... | 0.196– 0.219 |
| Vulva, from head end | 1.24 –1.5 | 1.88 –2.1 | 1.75 –2.00 | 1.40 –1.65 | 2.00 –2.80 | 2.74 – 3.17 |
| Tail | 0.95 –1.00 | 0.55 –0.59 | 0.56 –0.68 | 0.38 –0.425 | 0.97 –1.23 | 1.48 – 1.70 |
| Lateral alae, breadth at vulva | 0.034 | 0.015 | ..... | 0.018–0.02 | 0.016–0.033 | ..... |
| Lateral alae, end from posterior extremity | 0.4 –0.53 | 0.11 –0.13 | ..... | 0.1 –0.175 | ..... | ..... |
| Eggs, length | 0.052–0.058 | ..... | 0.059–0.067 | 0.070 | 0.043–0.063 | 0.053– 0.057 |
| Eggs, breadth | 0.030–0.032 | ..... | 0.032–0.040 | 0.043–0.048 | 0.027–0.034 | 0.030– 0.035 |

*Skrjabinema parva*, n. sp. (Fig. 1.)

*Description.*—Male unknown. Female from 3.5 to 4 mm long and varying in width from 0.22 to 0.32 mm in the region of the vulva. Width of head about 0.065 mm. Mouth wall not completely surrounding the lips which are directed inwardly. Total length of esophagus, including the bulb, 0.375 to 0.4 mm; bulb nearly round, 0.125 to 0.145 mm in diameter. Vulva 1.4 to 1.65 mm from anterior

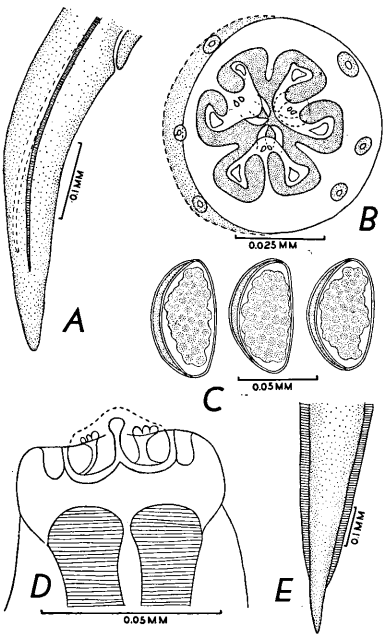


FIG. 1. *Skrjabinema parva.* A—Tail end of female. B—"En face" view of head. C—Eggs. D—Head end, dorsal view. E—Tail end showing termination of lateral alae.

end. Tail 0.380 to 0.425 mm long. Lateral alae 0.018 to 0.02 mm wide in region of vulva; left ala extending about 0.1 mm and the right ala to about 0.125 mm from the tip of the tail. Eggs 0.070 mm long by 0.045 mm wide.

*Host.*—Deer, probably *Odocoileus hemionus.*

*Location.*—Large intestine.

*Locality.*—Boise, Idaho.

*Specimens.*—U.S.N.M. Helm. Coll. Nos. 43680 (type) and 43681 (paratypes).

As may be seen from table 1 which, so far as the species *S. alata, S. africana,* and *S. rupicaprae* are concerned, is taken from Mönnig 1932 (*loc. cit.*), *Skrjabinema parva* resembles these 3 species in general measurements but differs from them in that the mouth wall does not completely surround the lips. This character places this nematode in the subgenus *Skrjabinema* and it may be differentiated from the other members of this subgenus, namely, *Skrjabinema ovis* and *S. oreamni,* by its notably smaller size and by the fact that while the lips do project above the mouth wall they are far less prominent than in either of the 2 above mentioned species.

**Inoculations with *Trichomonas foetus* (Protozoa) in white rats and mice.**[1]

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There have been a limited number of published experiments on the inoculation

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Project No. 622–V; Trichomoniasis and other reproductive diseases of cattle; B. A. Beach (In charge), C. A. Herrick and W. Wisnicky, co-operators.

of white rats and mice with *Trichomonas foetus*. From the available literature conclusive results cannot be made. The purpose of this paper is to present research on inoculations with *T. foetus* in 495 white rats and 600 white mice. With this large number of experimental animals, certain conclusions may be drawn.

Futamura (1935) found the mouse more susceptible to intraperitoneal injections of *T. foetus* than guinea pigs. Fatal results often occurred or the trichomonad persisted as a uterine infection for 6 months. According to Morisita (1939), Tamaki and Taise (1936) isolated *T. foetus* bacteria-free by intraperitoneal injections into white mice. Zeetti (1940) found that young rats of either sex died from intraperitoneal injections of pure cultures or infectious trichomonad material from cows.

Trussell and McNutt (1941) inoculated 6 white mice intraperitoneally with ½ cc of *T. foetus* culture. Two were killed on each of the next 3 days. No trichomonads were recovered from the peritoneal cavity. Lwoff and Nicolau (1935) and Nicolau and Lwoff (1935) produced fatal encephalomyelitis in rats and mice by subdural or intracranial inoculations of *T. foetus*. Slight infections were also obtained by intraperitoneal injections in rats. Trussell and McNutt (1941) failed to infect white mice by oral inoculations of *T. foetus*.

Approximately 500 white rats and 600 white mice were used in the following experiments. These animals were obtained through the courtesy of the Department of Zoology, University of Wisconsin. Injected materials consisted of the following solutions: (1) Living *T. foetus* centrifuged and washed 3 times and suspended in 0.7 per cent saline in a concentration of 10 million per cc, (2) liquid portion of 72-hour *T. foetus* cultures composed of buffered saline-citrate solution with 5 per cent bovine serum. The concentration was approximately 2 million living organisms per cc, (3) trichomonad pyometra fluid from an infected cow with a count of 1½ million living organisms per cc, (4) autoclaved supernatant fluid of 72-hour *T. foetus* cultures with the organisms previously removed by centrifuging, (5) liquid portion of unused sterile culture material. Solutions 4 and 5 were used for controls.

*Experiment 1, to demonstrate the effects of intraperitoneal inoculations of T. foetus in white rats.*—One hundred rats in 4 groups of 25 each were injected with 1, 2, 4, and 5 cc respectively of solution 1. The animals showed no ill effects and the average maximum time that motile trichomonads could be recovered from the peritoneal cavity was 12 hours (Table 1).

TABLE 1.—*The effects of intraperitoneal injections of Trichomonas foetus in white rats.*

| | Amount | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 cc | | | | 2 cc | | | | 3 cc | | | | 5 cc | | | |
| | A[a] | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Solution 1 | 25 | 0 | 0 | 12 | 25 | 0 | 0 | 14 | 25 | 0 | 0 | 11 | 25 | 0 | 0 | 12 |
| Solution 2 | 25 | 0 | 0 | 18 | 25 | 0 | 0 | 21 | 25 | 0 | 0 | 19 | 35 | 0 | 0 | 28 |
| Solution 3 | 10 | 0 | 0 | 11 | 10 | 0 | 0 | 9 | 10 | 0 | 0 | 13 | 10 | 0 | 0 | 8 |
| Solution 4 | 10 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| Solution 5 | 25 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 25 | 0 | 0 | 0 |

[a] A, number of animals injected; B, number of animals that died; C, per cent mortality; D, average time in hours trichomonads could be recovered.

One hundred and ten rats were injected with solution 2 in 1, 2, 3, and 5 cc amounts, 25 rats each in the first 3 groups and 35 rats in the last group. The animals showed no effects and the average maximum time motile trichomonads were

recovered from the peritoneal cavity was 21 hours. Forty rats were injected with solution 3 in groups of 10 each with 1, 2, 3, and 5 cc respectively. Motile trichomonads could be recovered up to 10 hours while the animals showed no apparent effects. Two sets of controls inoculated with solutions 4 and 5 were negative and showed no detrimental effects on the animals (Table 1). Another series of 30 rats were injected intraperitoneally with 5 cc of *T. foetus* culture and 5 were sacrificed every 6 hours. Motile trichomonads were not recovered after 23 hours. Thus, intraperitoneal injections of *T. foetus* in varying amounts failed to produce trichomonad infections in white rats as could be detected by direct smears or cultures.

*Experiment 2, to demonstrate the effects of vaginal inoculations of T. foetus in white rats.*—Fifty nonpregnant white rats were inoculated intravaginally with 3 to 5 cc of solution 2. Twenty-five animals used as controls were inoculated with 3 to 5 cc of solution 5. The following day, and for 3 consecutive days vaginal smears were made with negative results. No animal showed motile trichomonads 24 hours after inoculation. One month later all animals were examined again with negative results. Ten other nonpregnant rats were inoculated with 3 cc of solution 3 but the results were also negative. Thus, the results show that as a general rule white rats are refractory to trichomonad infection by vaginal inoculation of *T. foetus*.

Subcutaneous inoculations of 15 white rats failed to produce the infection. Intravenous, intramuscular, intracranial, intrauterine and oral inoculations were not attempted.

*Experiment 3, to demonstrate the effects of intraperitoneal inoculations of T. foetus in white mice.*—White mice were injected with solution 1 in 1, 2, 3, and 5 cc amounts, 25 mice in the first group and 35 mice each in the last 3 groups. Four mice in the first group died, 8 in the second, 9 in the third, and 10 in the fourth. The results are summarized in table 2. Solution 2 showed a slightly higher mor-

TABLE 2.—*The effects of intraperitoneal injections of Trichomonas foetus in white mice.*

| | Amount | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 cc | | | | 2 cc | | | | 3 cc | | | | 5 cc | | | |
| | A[a] | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Solution 1 | 25 | 4 | 16 | 24 | 35 | 8 | 22 | 20 | 35 | 9 | 25 | 18 | 35 | 10 | 28 | 20 |
| Solution 2 | 25 | 8 | 32 | 34 | 35 | 12 | 32 | 30 | 35 | 10 | 28 | 29 | 35 | 17 | 48 | 32 |
| Solution 3 | 25 | 1 | 4 | 12 | 15 | 3 | 20 | 8 | 10 | 2 | 20 | 18 | 10 | 5 | 50 | 19 |
| Solution 4 | 25 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 20 | 3 | 15 | 0 |
| Solution 5 | 25 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 25 | 1 | 4 | 0 |

[a] A, number of animals injected; B, number of animals that died; C, per cent modtality; D, average time in hours trichomonads could be recovered.

tality rate than solution 1, while the results of solution 3 cannot be ascertained accurately because of the presence of various bacteria. The controls were negative for mortality except in the group that received 5 cc of solutions 4 and 5. The average length of time in which motile trichomonads could be recovered was higher in mice than in rats and definite multiplication took place during the first 18 hours. Usually, after 18 hours the trichomonads were not capable of living in the new environment or the resistance mechanism of the host overcame infection. Thus, it appears that white mice are more susceptible to intraperitoneal injections of *T. foetus* than white rats.

*Experiment 4, to demonstrate the effects of vaginal inoculations of T. foetus in*

*white mice.*—One hundred nonpregnant mice were inoculated intravaginally with 2 cc of solution 2. Twenty five animals used as controls were inoculated with 2 cc of solution 5. The following day and for 4 consecutive days vaginal smears were made with negative results. No animal showed motile trichomonads 24 hours after inoculation. One month later all animals were examined again with negative results. Twenty other nonpregnant mice were inoculated with 2 cc of solution 3 but results were negative. The results appear to be that white mice are not susceptible to trichomonad infection by vaginal inoculations of *T. foetus.*

SUMMARY

Four hundred and twenty white rats divided into several groups were inoculated intraperitoneally with washed *T. foetus* from culture material suspended in saline with a concentration of 10 million living organisms per cc, liquid portion of 72-hour *T. foetus* cultures composed of buffered saline-citrate solution with 5 per cent bovine serum; the concentration was approximately 2 million living organisms per cc, and trichomonad pyometra material from an infected cow with a count of 1½ million living organisms per cc. Infections could not be demonstrated. Mortality did not occur. Sixty white rats were refractory to vaginal inoculations. Subcutaneous injections in 15 white rats also failed. White mice were refractory to vaginal inoculations but in 480 white mice injected intraperitoneally mortality in some instances, as high as 50 per cent, is recorded. Multiplication of *T. foetus* occurred during the first 18 hours with subsequent rapid decline of the parasite.

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**The occurrence of *Bartonella* in cases of anaplasmosis and in apparently normal cattle.** JOHN C. LOTZE and GEORGE W. BOWMAN, U. S. Bureau of Animal Industry.

In a previous paper, Lotze and Yiengst (1942, Amer. Jour. Vet. Research, in press) reported the presence of "Bartonella-like" structure in the erythrocytes of cattle infected with *Anaplasma marginale.* The failure to detect these structures in all cases of anaplasmosis studied was taken as evidence that there was no direct connection between these forms and the so-called anaplasms. Further study on the "Bartonella-like" structures has shown that they also occur in cattle free of anaplasmosis and are similar to *Bartonella bovis* Donatien and Lestoquard (1934, Bull. Soc. Path. Exot. 27(7): 652–654) (syn. *Bartonella sergenti* Adler and Ellenbogen (1934, Jour. Comp. Path. & Therap. 47(3): 219–221)).

The parasites (Fig. 1), which resemble the "bacilliform" or paired "cocciform" structures found in *Bartonella* of the rat, were first detected in 1939 at the Station of the Zoological Division, Beltsville Research Center, during the "incubation" period in a calf infected with *Anaplasma.* Since that time, the parasites have been found occasionally at this station in other bovines affected with anaplasmosis. Although the height of the *Bartonella* infection usually occurred in the hosts

2 to 4 days previous to the time anaplasms were first found, the parasites were occasionally found only after the onset of anaplasmosis, in which cases both *Anaplasma* and *Bartonella* were sometimes found in the same erythrocyte. The presence of *Bartonella* in an anaplasmosis-free calf was detected soon after splenectomy and this case served as proof that the structures identified as *Bartonella* were in no way concerned with the developmental cycle of the anaplasm.

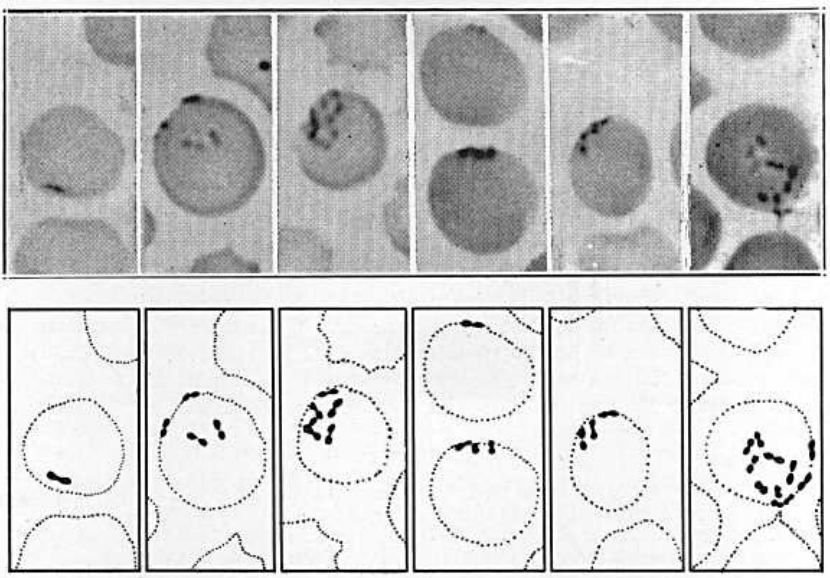

FIG. 1. Photomicrographs of bovine erythrocytes containing *Bartonella* and schematic representation of the same.

The importance of *Bartonella* in cattle is not clearly understood and it is conceivable that this parasite may be of consequence under certain conditions. In the cases of *Bartonella* infections studied, less than 0.1 per cent of the erythrocytes were parasitized by this organism at any one time. A flare up of *Bartonella* in the incubation period of anaplasmosis seems to indicate, however, that the presence of the etiological agent of anaplasmosis probably interferes temporarily with the host's defense mechanism against *Bartonella*.

**Earthworms as possible intermediate hosts of *Capillaria caudinflata* of the chicken and turkey.** REX W. ALLEN and EVERETT E. WEHR, U. S. Bureau of Animal Industry.

In order to obtain eggs of *Capillaria annulata* for experimental purposes, 12 earthworms (species undetermined) were fed on March 19, 1942, to each of 10 1-month-old chicks. The earthworms were collected from soil over which a number of turkeys known to be infected with *C. annulata* and *Syngamus trachea* were ranging. Feces of these young chicks were collected on March 30, April 4, and April 18, 1942, and examined for *Capillaria* eggs; a few were found on the last-mentioned date. It is possible that eggs may have been present in the feces earlier than April 18, as there was an interval of 2 weeks between this date and that of the previous examination.

Several of the eggs recovered from the feces of the chicks were slightly smaller (55.1 to 58 mm) than those recorded for the eggs of *C. annulata*. In order to

determine whether these birds were infected with a species of *Capillaria* other than *C. annulata*, 2 of the chicks were destroyed on April 23, 1942. The crops of these birds were negative for *Capillaria annulata*, but several specimens of another species of *Capillaria*, namely, *C. caudinflata*, were found in the duodenal region of the small intestine. Two turkeys, taken from the same lot from which the infected earthworms were collected were examined and found to harbor in the duodenal region of the intestines pure infections of *C. caudinflata*. This report constitutes the first record of this nematode from the turkey in the United States.

The male of *C. caudinflata* is about 12 to 17 mm long and may be readily distinguished from the males of other species of *Capillaria* occurring in domestic fowls by the presence of a lateral cuticular swelling on each side of the body just anterior to the cloacal aperture, and by a bursa-like membrane, bearing laterally 2 thick-set rays, posterior to the cloaca. The female worm, which reaches a length of about 27 mm, has the vulva located near the tip of a tubular or trumpet-shaped projection. In tap water the eggs of *Capillaria caudinflata* required from 2 to 4 weeks to embryonate at room temperature.

Strongly suspecting that the feeding of the earthworms may have been responsible for the presence of the *C. caudinflata* in the intestines of the young chicks, a more critical test was conducted in order to determine the possible rôle of earthworms in the transmission of this intestinal roundworm. A large number of earthworms (probably *Helodrilus caliginosus*) were collected from the previously mentioned turkey pen, thoroughly washed in running tap water to remove all debris adhering to the external surface of their bodies and fed, on April 27, 1942, to 4 1-month-old turkey poults and 2 5-day-old chicks. A few additional earthworms were fed to these birds on May 6, 1942. Since embryonated eggs of *C. caudinflata* were not available to feed to other birds, it was not possible to conduct a controlled experiment. After fecal examinations on May 27, 1942, had disclosed that 3 of the turkeys were passing capillarid eggs, 2 turkeys and 1 chick were killed and examined. The crops of both turkeys were infected with *Capillaria annulata* and the intestine of 1 with *Capillaria caudinflata*, 15 mature females and 2 mature males of the later species being recovered from the duodenum of one bird; no worms were found in the chick. The 2 remaining turkeys and 1 chick were killed and examined on June 2, 1942. A few specimens of *Capillaria caudinflata* were found in the intestine of all 3 birds; *C. annulata* was present in the crops of only the 2 turkeys.

From the foregoing it may be concluded that the young turkeys and chickens became infected with *Capillaria caudinflata* as a result of having been fed earthworms. However, it was not determined whether earthworms serve as a mechanical carrier of the eggs or larvae of *C. caudinflata*, or whether they are essential intermediate hosts.

**The occurrence in the United States of the turkey ascarid, *Ascaridia dissimilis*, and observations on its life history.** EVERETT E. WEHR, Zoological Division, U. S. Bureau of Animal Industry.

In 1928 Herbert L. Stoddard, of Thomasville, Georgia, submitted for identification to the Zoological Division, U. S. Bureau of Animal Industry, a number of internal parasites collected from the wild turkey in Georgia. Included in this collection were a few specimens of *Ascaridia dissimilis*, a nematode described by Vigueras (1931, Nota sobre algunos helmintos de *Meleagris gallopavo*, encontrados en Cuba, con descripcion de una nueva especie. (2) pp., 1 pl., figs. 1–3, Habana) from the small intestine of the domestic turkey in Cuba. Recently, additional specimens of this roundworm were collected by the writer from the wild turkey in Pennsylvania. The present report constitutes the first record of this species in wild turkeys in this country.

*Ascaridia dissimilis* is slightly smaller than the chicken ascarid, *Ascaridia galli*, but may be readily differentiated from the latter by the more median position of the fourth pair of ventral papillae of the male. In *A. galli*, these papillae are widely separated, each being situated somewhat posterior and slightly internal to each member of the second pair of lateral papillae, while in *A. dissimilis* the fourth pair of papillae are very close together and are located near the median line just posterior to the cloaca (Wehr, 1940, Jour. Parasitol. 26: 373–375).

Material for life history investigations was secured through the courtesy of Dr. Vigueras who kindly supplied several specimens of mature females obtained from the domestic turkey in Cuba. The uteri were removed from the adult specimens and the eggs embryonated in tap water at room temperature. Approximately 2 weeks were required for the eggs to become infective.

Large numbers of embryonated eggs were fed on April 10, 1941, to each of 6 3-week-old turkey poults. One bird killed on May 16 contained 20 partly mature worms in the small intestine. A number of immature worms were observed in the feces of another bird on May 29. On June 14 the remaining 5 birds were killed and a total of 8 immature worms was found in their small intestines.

In a second experiment, large numbers of infective eggs were fed on July 22, 1941, to each of 6 1-month-old turkeys. These birds were killed and examined for worms on the following dates: No. 1, July 24; No. 2, July 29; No. 3, August 6; No. 4, August 21; No. 5, September 10; and No. 6, September 27. No worms were recovered from the small intestines of the birds killed on the 2nd, 7th, and 15th days following the administration of the infective eggs. However, 4 immature worms were recovered from the bird killed on the 30th day, 1 large immature female was taken from the bird killed on the 50th day, and 2 apparently mature male worms were removed from the small intestine of the bird killed on the 67th day following administration of the embryonated eggs.

The data here presented demonstrate that *Ascaridia dissimilis* is transmissible through the ingestion of infective eggs, 2 months or longer being required for the worms to mature in the host. In these respects the development of *A. dissimilis* is similar to that of *A. galli*. It may also be concluded from the data presented that the domestic turkey is a potential host of *A. dissimilis* and that precautions should be taken to prevent the possible transfer of this parasite from the wild to the domestic turkey under range conditions.

**A second introduced rat mite becomes annoying to man.** H. E. EWING, U. S. Bureau of Entomology and Plant Quarantine.

When in 1923 the writer published the first records of the occurrence in the United States of the tropical rat mite, *Liponyssus bacoti* (Hirst), there was also reported in the same article (1923) a single record of the occurrence in this country of another introduced rat mite, *Allodermanyssus sanguineus* (Hirst). This record was based on the identification of 5 female specimens taken many years earlier, on June 30, 1909, in the District of Columbia. During the entire period from 1909 to 1938 only a single record of the occurrence of this mite in the United States was added. However, since the beginning of 1938 5 lots have come to the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture, and according to reports the mite has not only become annoying to man but may possibly cause a rash. This is indicated by a letter received recently from an exterminating company in New York City, under date of May 21, 1941, which reads in part as follows:

"Aside from a purely academic interest in the animal, I have a problem to decide: namely, to whom do they belong, the landlord or the tenant. The tenant originally complained of bed bugs, although a careful examination of the bed, etc.,

revealed no indication of them. Nevertheless, the apartment was thoroughly treated, and the tenant complained the following day that the condition had not been eliminated and produced the specimen I sent to you as evidence. The tenant also claims that the 'bed bug' caused a rash.''

This mite was described from Egypt in 1914 under the generic name *Dermanyssus*. It is in reality similar to *Dermanyssus gallinae* (Degeer), the common chicken mite, but may be distinguished from the latter since the female of *Allodermanyssus sanguineus* has 2 dorsal plates on the body instead of 1. Furthermore, the posterior of these 2 dorsal plates is very small, being not over one-tenth as large as the anterior plate.

Up to the present, 7 lots of *Allodermanyssus sanguineus* have been identified from the United States, all but 1 coming from the Atlantic seaboard. Data for these lots are as follows:

Arizona: Tucson, March 3, 1938, several specimens on *Mus musculus*, collected by R. A. Flock.

District of Columbia: June 30, 1909, 5 specimens taken on a desk by Mr. Dewey; February 14, 1939, 1 female on man, taken by R. T. Sullivan.

Georgia: Taken in New York, N. Y., in 1939, on merchandise from Georgia and sent in by C. L. Fluke.

New York: New York City, 1940, on candy in pastry department of a hotel, sent in by Sameth Exterminating Company; New York City, May 1941, in apartment, ''caused a rash,'' sent in with letter of May 21, 1941, by Jack Benmosche, of the Evins Exterminating Company.

Pennsylvania: Philadelphia, July 9, 1941, in basement of apartment, sent in by J. E. Sameth, Western Exterminating Company.

Very little is known concerning this mite abroad. When Hirst described the species, he had 3 lots of specimens from *Mus rattus*, 10 specimens from *Arvicanthis niloticus* in houses, and 1 specimen from *Acomys cahirinus*. All these records were from Egypt. The spread of this species in the United States will be watched with interest.

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**Studies on the saline requirements of *Neoechinorhynchus emydis*.** Bro. D. ALPHONSUS GETTIER, F. S. C.[1]

While relatively much work has been done on the maintenance *in vitro* of nematodes, and while at least some information on this subject is available for trematodes and cestodes, (cf. review of Hoeppli, Feng and Chu, 1938) no data at all are at hand for Acanthocephala. The present investigation, undertaken in order to provide a basis for further work along this line, deals with the saline requirements of *Nochinorhynchus emydis* somewhat in the same manner as the larvae of *Ascaris suum* were investigated by Fenwick (1939).

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

## MATERIAL AND TECHNIQUE

The worms used in this study were adults of *Neoechinorhynchus emydis*[2] isolated from the intestine of the Cumberland terrapin, *Pseudemys elegans*. The parasites were removed from the intestine with a pair of forceps and placed in a Petri dish filled with physiological salt solution. They were cleaned and each individual was checked for its normal appearance. The worms were then washed with those solutions in which they were to be kept in the subsequent experiments and were then put in test tubes containing 15 cc of the respective solutions. The tubes were corked and kept in a dark room maintained at a rather constant temperature of 20° C.

The worms were checked every day. They were poured into a watch glass and observed in regard to their motility. When no spontaneous movements were seen they were stimulated by touching with a pair of forceps. A worm giving no response to this stimulation and showing a certain characteristic stiffening of the body was regarded as dead and was eliminated. The solutions in the test tubes were renewed when signs of contamination appeared.

The various salt solutions used are mentioned under Results.

## RESULTS

The first series were designed in order to determine the optimal molecular concentration. Simple sodium chloride solutions were used. They were prepared at one-tenth per cent interval in the range from 0.3 per cent to 0.8 per cent and at one-half per cent interval in the range from 1 per cent to 4 per cent NaCl. It became at once obvious that solutions above 2 per cent were lethal. The most suitable concentration was found to lie between 0.5 per cent and 0.7 per cent. In this, the maximum life span of an individual was 20 days and the average life span of a series about 13 days. The optimal molecular concentration is therefore somewhat hypotonic and this is in agreement with Stoll's (1940) findings on *Haemonchus contortus* larvae.

In the next series the influence of various cations were studied. Solutions isotonic to 0.5 per cent NaCl were made up with $CaCl_2$, $MgCl_2$, and KCl respectively. The results are summarized in table 1.

TABLE 1.—*Survival of Neoechinorhynchus emydis in solutions of various cations, isotonic to a 0.5 per cent NaCl solution*

| | Life span in days | |
|---|---|---|
| | Average for series | Maximum individual survival |
| NaCl | 12.8 | 17.0 |
| $CaCl_2$ | 8.0 | 14.0 |
| $MgCl_2$ | 2.5 | 3.8 |
| $KCl_2$ | 1.7 | 2.8 |

It is obvious that the Mg and K ions were extremely toxic, whereas the Ca ion proved to be relatively harmless, though still less so than the Na ion.

Table 2 summarizes experiments in which small amounts of Ca were added to a 0.5 per cent NaCl solution. It appears that this addition exerted a decided beneficial influence, both the average and individual survival were longer. This must be attributed to the well known antagonistic action of the Na and Ca ions;

[2] The writer is indebted to J. T. Lucker, U. S. Dept. of Agriculture for confirmation of the identification.

TABLE 2.—*Survival of Neoechinorhynchus emydis in 0.5 per cent NaCl solutions to which small amounts of $CaCl_2$ were added*

| Percentages of $CaCl_2$ added to 0.5% NaCl solution | Life span in days | |
|---|---|---|
| | Average for series | Maximum individual survival |
| 0.00 | 13.8 | 20.0 |
| 0.01 | 18.3 | 28.0 |
| 0.02 | 20.3 | 25.0 |
| 0.05 | 17.0 | 22.5 |
| 0.10 | 17.9 | 26.0 |

the molecular concentration was not changed by these additions to such an extent as to influence the results.

Similar experiments were performed adding KCl, and $MgCl_2$ in amounts corresponding to the above mentioned $CaCl_2$ concentration to a 0.5 per cent NaCl solution. These additions seemed to exert no influence, neither beneficial nor harmful, upon the length of survival *in vitro*.

In a last series the influence of various Ringer solutions, diluted so as to be isotonic with the 0.5 per cent control NaCl solution, was studied. These experiments are summarized in table 3. The viability of the worms in this series was

TABLE 3.—*Survival of Neoechinorhynchus emydis in various modified Ringer solutions diluted so as to be isotonic to a 0.5 per cent NaCl solution*

| | Life span in days | |
|---|---|---|
| | Average for series | Maximum individual survival |
| Control (0.5% NaCl) | 6.5 | 10.5 |
| Ringer (frog) | 11.5 | 18.0 |
| Ringer—Locke | 12.0 | 22.5 |
| Ringer—Tyrode | 14.4 | 21.0 |
| Ringer—Dale | 13.5 | 25.5 |
| Buffered Ringer (C.L.E.) | 10.5 | 17.0 |

less than that of the parasites used in the preceding series as judged from the shorter survival of the control specimens in pure NaCl solutions. It appears quite clear, however, that all the Ringer solutions favored the survival. This again will have to be attributed to an antagonistic action of the various ions present in these solutions. On the basis of the above mentioned experiments one might assume that it too was due to the Ca ion.

## CONCLUSIONS

1. The most suitable molecular concentration for the survival of *Neoechinorhynchus emydis* lies between 0.5 and 0.7 per cent NaCl.

2. The toxic action of isotonic solutions of various cations is as follows: $Na < Ca < Mg < K$.

3. The addition of small amounts of $CaCl_2$ decreased the toxicity of the Na ion, the Mg and K ions were of no influence in this respect.

4. Similarly, the survival was longer in various modified Ringer solutions diluted to be isotonic to a 0.5 per cent NaCl solution, than in a pure 0.5 per cent NaCl solution.

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## The efficacy of vapor-heat treatments of narcissus bulbs, variety Triumph, for control of the bulb or stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, and the tolerance to this treatment of narcissus bulbs, variety King Alfred.

B. G. CHITWOOD, U. S. Bureau of Plant Industry, and F. S. BLANTON, U. S. Bureau of Entomology and Plant Quarantine.

### INTRODUCTION

The bulb or stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, is a serious pest of narcissus, at least in the eastern part of the United States, and most growers in this area take precautions to prevent the spread of this malady. Field grown narcissus bulbs usually receive an annual inspection during the growing season. When infected bulbs are scattered throughout a planting this fact is noted and the bulbs receive a thermal treatment after the fall harvest. When infected bulbs occur in isolated spots, these and adjoining bulbs are usually removed from the field. Thermal treatment every second to fourth year is usually adequate for the control of this disease. The frequency with which treatments are made depends upon efficacy of the treatment. Most growers who force their own bulbs in the greenhouse during the winter months treat the forced bulbs during the following summer. Due to the crowding of forced bulbs the disease commonly becomes widespread at this time. The forced bulbs are usually grown in the field 2 to 3 years before they are again forced. With such a program the treatment should be adequate to prevent widespread reoccurrence of the disease between forcing dates. Thermal treatments as discussed here refer to vapor heat and hot water with or without the combination of presoak and the use of formaldehyde either in the treating water or in the presoak.

The vapor-heat treatment of narcissus for the control of narcissus bulb pests was discussed by Latta (1939, U. S. Dept. Agr. Tech. Bull. 672). He stated that "... these tests have indicated a vapor-heat treatment of 8 hours at 110–111° F. is very effective as a control of this nematode, providing the treatments are made within a reasonable time after lifting or digging the bulbs, before the formation of resistant quiescent stage of the nematode which develops as the storage season progresses." Previously, Spruijt and Blanton (1932, Jour. Econ. Ent. 26(3): 613–620) had reported on the efficacy of some vapor-heat treatments, and the writers (1941, Jour. Wash. Acad. Sci. 31(7): 296–308) have recorded the results obtained with this type of treatment at the Babylon, N. Y., Laboratory.

In all of the previous work with vapor heat, as well as the earlier work on hot water, examinations were made immediately after treatment, the bulbs being macerated, soaked, and 100 of the most viable specimens of nematodes removed for further observations. The individual examinations in the vapor-heat tests were based on single bulbs. Thus the observations indicate the *minimum* numbers of individual nematode survivals from the population of a given bulb. Though individual bulb populations were commonly estimated at 10,000 nematodes, the selection and study of 100 probable viables could not be considered as indicating the total survivals in the whole population. The results (individual survivors) could be considered as a conservative estimate of the percentage revival of the stages present. Later investigations have shown that delayed examinations commonly result in more

revivals than early examinations. This is believed to be due to the development of younger stages of the nematode that would be overlooked by early examination of sieved material. However, it was a fault in experimental procedure which could not be foreseen at the time.

In this paper the writers desire to report on the results of a vapor-heat treatment, and some treatments combining vapor heat with a 2-hour presoak of the bulbs in water containing formaldehyde (0.5 per cent formalin).

Latta's work (1939) was conducted on the West Coast. His unpublished reports show that vapor-heat treatment for 8 hours at 110° F. with no presoak gave, on 9 dates between date of digging and 21 days thereafter, 100 per cent nemic mortality in 4 samples and 97 to 99 per cent nemic mortality in 5 samples; 4 out of 9 bulbs were completely cured of nematode disease by this treatment. A similar treatment preceded by a 2-hour aqueous presoak resulted in 100 per cent nemic mortality in 9 out of 9 bulbs. The latter treatment would appear to show promise as a means of curing bulbs of nematode disease but the small number of bulbs used would permit a predicted efficacy of only 70 per cent based on binomial distribution. As will be seen later (Table 1) the writers obtained an observed efficacy of 69 per cent (8 bulbs not cured out of 25 infected) in later experiments at this temperature and duration. The results are in substantial agreement when allowance is made for chance distribution.

The East Coast investigations of Spruijt and Blanton (1932, *loc. cit.*) and Chitwood and Blanton (1941, *loc. cit.*) on vapor heat were based on single bulb samples, at each temperature and duration and can only be considered exploratory.

## EFFICACY OF TREATMENTS

*Methods.*—The treatments were applied between August 27 and 31, 1940. The bulbs used to test nemic mortality were of the variety Triumph and measured 8 to 14 cm in circumference. Timing of the treatments was begun when the interior of the bulbs reached the desired temperature; this period was 1 hour and 10 minutes to 1 hour and 45 minutes. There were 56 bulbs used in each treatment, selected as diseased on the basis of pretreatment inspection. These bulbs were stored in trays and examined individually for nematodes 3 to 4 months after the treatment.

*Results.*—The results of the various treatments are given in table 1.

It is notable that in this experiment as in previous experiments (Chitwood and Blanton 1941, *loc. cit.*) the nematodes were found in fewer bulbs after the more severe treatments than after the less severe treatments and the controls. This is due to the fact that living nematodes crawl out of macerated bulb tissue and are more likely to be observed than dead nematodes which are seen only if they happen to be at the surface of bulb fragments and are washed off. Since the bulbs for the various treatments and control were selected at random it would be reasonable to assume that 31 bulbs in each sample contained living nematodes prior to treatment. Chitwood and Blanton (1941, *loc. cit.*) have concluded that the efficacy of the experimental treatment should be better than 90 per cent before recommendation for narcissus bulb treatment. Applying the formulae of binomial distribution the prediction of an efficacy of better than 90 per cent requires a sample containing a minimum of 29 bulbs in which all of the nematodes have been killed and no bulbs in which nematodes remain alive.

Assuming that nematodes were present in 31 of 56 bulbs examined after each treatment, any treatment in which no living nematodes were observed would be recommendable with odds of 19 : 1 that the efficacy is better than 90 per cent. The lot of "recommendable" treatments would include the following: VHF[1] 6

[1] VHF = Vapor-heat-formalin.

TABLE 1.—*Bulb-nematode infections found in 56 narcissus bulbs, variety Triumph, 3 to 4 months after each of several vapor-heat treatments preceded by a 2-hour presoak in water containing formaldehyde (0.5 per cent formalin); corresponding weight increase of King Alfred bulbs. Babylon, N. Y., 1940*

| Treatment | | Efficacy tests | | Tolerance | |
|---|---|---|---|---|---|
| Temperature | Duration | Bulbs infected with living or dead nematodes | Bulbs infected with living nematodes | Increase in weight | Rot found at harvest |
| °F. | *Hours* | *Number* | *Number* | *Per cent* | *Per cent* |
| 110 | 6 | 25 | 11 | 129 | 2 |
| 110 | 8 | 25 | 9 | 133 | 1 |
| 110 | 8[a] | 25 | 8 | 115 | 3 |
| 112 | 6 | 25 | 1 | 114 | 6 |
| 112 | 8 | 29 | 6 | 100 | 0 |
| 114 | 6 | 20 | 0 | 75[c] | 5 |
| 114 | 8 | 14 | 0 | 61[c] | 1 |
| 116 | 3 | 12 | 0 | 74[c] | 1 |
| 116 | 4 | 17 | 0 | 73[c] | 1 |
| 118 | 1 | 24 | 10 | 104 | 5 |
| 118 | 2 | 22 | 0 | 81[c] | 2 |
| 118 | 3 | 20 | 0 | 73[c] | 1 |
| 120 | 1 | 13 | 1 | 95 | 5 |
| 120 | 2 | 16 | 0 | 83[c] | 0 |
| No heat treatment | | 31 | 31 | 137 | 9 |
| 110 | 4[b] | ..... | ..... | 115[d] | 1 |

[a] Without presoak.
[b] Hot water-formalin (0.5% formalin).
[c] Possibly recommendable from standpoint of nemic control.
[d] Recommendable from standpoint of nemic control.

hours at 114°, VHF 8 hours at 114°, VHF 3 hours at 116°, VHF 4 hours at 116°, VHF 2 hours at 118°, VHF 3 hours at 118°, and VHF 2 hours at 120°. The following treatments are much too poor for consideration: VH[2] 8 hours at 110°, VHF 6 and 8 hours at 110°, VHF 6 and 8 hours at 112°, and VHF 1 hour at 118° (all temperatures in °F.).

The more cautious interpretation of the above data assumes nothing as to the number of infected bulbs but only credits bulbs as cured when dead nematodes are observed. On that basis no single treatment taken alone is adequate on which to base a recommendation. However, it is permissible to add records of treatments of lesser severity to those of greater severity. Using this method the records of vapor-heat-formalin-presoak treatments of 114° F. for 6 and those for 8 hours may be combined giving a record of 0–34. Vapor heat at 114° F. for 8 hours with a formalin presoak would, therefore, be recommendable with odds of 19 : 1 that the efficacy is better than 91 per cent. Other treatments that could also be considered recommendable on this basis are vapor heat 4 hours at 116°, 3 hours at 118° and 2 hours at 120° F., all with a formalin presoak.

### TOLERANCE OF TREATMENTS

At the same time the nema-infected bulbs were treated, 100 noninfected King Alfred bulbs were included in order to test the tolerance to the 16 treatments, including a hot-water-formalin treatment at 110° F. for 4 hours, a control, and 14 vapor-heat treatments. After treatment the bulbs were dipped in 2 per cent

[2] VH = Vapor heat.

ethyl mercury chloride as a precautionary measure against basal rot. The bulbs were weighed individually and planted in so-called dutch beds, the position of each bed being assigned at random in the field. In August of the following season the bulbs were dug and after proper curing were again weighed and the mean percentage increase in weight was calculated. The results are tabulated in table 1.

From these results it will be noted that all of the treatments showed some reduction in the weight increase as compared with that of the control. Those vapor-heat treatments for which no living nematodes were found in the efficacy tests (weight increases suffixed with superior *c*, Table 1) show appreciable difference from the control. Of these, the treatments causing the least damage, 118° and 120° F. for 2 hours, showed 50 to 52 per cent less increase in weight than the control. Those treatments for which living nematodes were recorded showed damage varying from 4 to 38 per cent reduction in the weight increase. On the other hand, the hot-water-formalin treatments of 4 hours at 110° F. gave the same percentage increase as the vapor-heat treatment for 8 hours at 110° F., namely 115 per cent. It will be seen from these results that vapor-heat at a given temperature and duration causes less damage than hot water-formalin but the extent of injury of vapor heat to the bulbs, as the temperature and duration is increased, is more drastic than is warranted by the increase in nemic control.

Incidental to the tolerance records as indicated by weight increase, records were also kept as to the percentage of basal rot in the stocks. These results are tabulated in table 1. The untreated control had the highest percentage of basal rot showing that none of the treatments as conducted contributed to the spread of the disease in this stock.

## DISCUSSION

Since a hot water-formalin treatment (0.5 per cent formalin) at 110° F. for 4 hours has been shown under Long Island conditions to have an efficacy of better than 95 per cent (Chitwood, Haasis and Blanton, 1941, Proc. Helminth. Soc. Wash. 8(2): 44–50), it is obvious that vapor heat either alone or in combination with formalin requires more severe temperatures in order to provide comparable efficacies.

The tolerance of narcissus bulbs to hot water-formalin for 4 hours at 110° F. is equivalent to that of vapor heat for 8 hours at the same temperature while the nematode control is much more efficacious. To obtain an efficacy equivalent to that of hot water-formalin, one would have to use vapor-heat treatments at much higher temperatures, causing a greater injury to the bulbs.

Heat penetrates the bulbs rapidly in hot water, the entire load of bulbs receiving approximately the same treatment, whereas heat penetrates slowly in vapor treatments. In experimental lots, such as those reported in this manuscript, the disadvantage of vapor heat is not so pronounced. In large quantity lots, however, it requires a great deal more time to bring some of the bulbs up to the desired temperature. Bulbs on top of the stacks will, as a rule, reach the temperature sooner than those in the lower part of the stacks. Likewise bulbs in the center of heavily loaded trays will not reach the desired temperature as quickly as those on the outer surfaces. Such faults require an excessive duration of treatment for some bulbs in order to give adequate treatment to others. It has also been noted that two separate treatments may react differently. In other words, a cold spot may be noted in a portion of the treating room in one treatment but the succeeding treatment will have a cold spot in some other part of the treating room. Blanton (unpublished manuscript) states: "In vapor heat, some parts of the load heat more rapidly than others, and reach treatment temperature sometime before the mass temperature rises to that point. Disregarding the approach period the dura-

tion of the treatment in vapor heat at 110° F. should be approximately twice that of hot water for most pests.''

In tests such as those reported here the timing is begun after the center of all test bulbs has reached the desired temperature. Therefore, there is no apparent reason why vapor-heat treatments of this type are not as effective from the standpoint of heat penetration as hot-water treatments. Neither hot-water nor vapor-heat treatments except in combination with formalin have been found effective. The use of formalin presoak with vapor heat was an attempt to accomplish the same results as those accomplished with hot water-formalin. It would appear that the failure of vapor heat to duplicate hot water-formalin in these circumstances may have been due to the dissipation of the formalin as a result of the application of vapor heat.

## SUMMARY AND CONCLUSIONS

The present paper reports the results of a vapor-heat treatment and some treatments combining vapor heat with a 2-hour presoak of narcissus bulbs in water containing formaldehyde (0.5 per cent formalin), in an attempt to kill the bulb or stem nematode *Ditylenchus dipsaci* (Kühn) Filipjev. Narcissus bulbs, variety Triumph, were treated between Aug. 27 and 31, 1940. Timing of the treatments was begun when the center of the bulbs had reached the desired temperature. All bulbs were examined individually 3 to 4 months after the treatment. Of the series of treatments conducted only the following could be recommended for control of the nematodes: 114° F. for 8 hours; 116° F. for 4 hours; 118° F. for 3 hours and 120° F. for 2 hours, all of which are preceded by a formalin presoak. In former work it was shown that a treatment of hot water containing 0.5 per cent formalin at 110° F. for 4 hours has an efficacy of better than 95 per cent. The tolerance of narcissus bulbs to this latter treatment is much greater than it is to any of the vapor-heat treatments in which no living nematodes were observed.

**A description of *Aphelenchoides besseyi*, n. sp., the summer-dwarf nematode of strawberries, with comments on the identity of *Aphelenchoides subtenuis* (Cobb, 1926) and *Aphelenchoides hodsoni* Goodey, 1935.** J. R. CHRISTIE, U. S. Bureau of Plant Industry Station, Beltsville, Md.

In previous papers (Christie and Crossman, 1935, 1936; Christie, 1938) it was pointed out that spring dwarf and summer dwarf of strawberry plants are two different diseases caused by two different nematodes. Data were presented demonstrating that these two nematodes show rather conspicuous differences in physiology and behavior, for example, optimum temperature for development, thermal death point, and ability to grow on culture media. It has been stated, however (Christie and Crossman, 1935) that these two nematodes appeared to be morphologically identical and this statement has not been modified although it is erroneous.

The spring-dwarf nematode and the summer-dwarf nematode are not morphologically identical. As a matter of fact, the summer-dwarf nematode can be more easily differentiated than can any of the other closely related plant-parasitic species with the possible exception of *Aphelenchoides subtenuis* (Cobb, 1926).

This brings up the question: which, if either, of these two strawberry-bud nematodes is identical with the one causing red-plant and cauliflower disease in England? Mr. R. N. Swanton of the University of Reading, England, kindly sent the writer preserved specimens of nematodes that he identified as ''from 'cauliflower' strawberry plants as found in the south of England.'' These specimens definitely were not identical with the summer-dwarf nematode but the writer was unable to detect any morphological differences distinguishing them from the spring-

dwarf nematode. If either of the strawberry-bud nematodes that occur in the United States is identical with the one that occurs in England it is the spring-dwarf nematode. In view of this fact the summer-dwarf nematode is apparently an unnamed species. For the summer-dwarf nematode the writer proposes the name *Aphelenchoides besseyi*, n. sp. This name is proposed in recognition of the fact that Dr. E. A. Bessey appears to have been the first to associate this nematode with the disease that it causes.

*Aphelenchoides besseyi*, n. sp.

*Measurements.*—The following measurements are based on 10 specimens of each sex. ♀: Length, 660–750 μ; width, 17–22 μ; esophagus, 64–68 μ; tail, 36–42 μ; α, 32–42; β, 10.2–11.4; γ, 17–21; V, 68–70%. ♂: Length, 540–620 μ; width, 14–17 μ; esophagus, 63–66 μ; tail, 34–37 μ; α, 36–39; β, 8.6–8.8; γ, 15–17.

*Diagnosis.*—An *Aphelenchoides* most closely resembling *A. fragariae* (Ritzema Bos, 1891) and related plant-parasitic species, but differentiated from them by the following characters: Postvulvar uterine sac short, narrow, inconspicuous, usually extending less than one-third distance from vulva to anus, rarely containing spermatozoa; ovary wide, showing several developing ova in a single cross section through its middle region; excretory pore slightly anterior to nerve ring. The two sexes occur in about equal numbers.

*Type host.*—The cultivated strawberry, *Fragaria* hybrids.

*Type locality.*—Southeastern United States (The specimens used for this description were collected at Willard, N. C.).

*Comparisons and affinities.*—Probably *A. besseyi* has closer affinities with certain populations of what now passes under the name *A. parietinus* (Bastian, 1865) than it has with the other plant-parasitic species of the genus though it appears to resemble the latter more closely morphologically. The stylet of *A. besseyi* with its moderately well-developed basal swellings together with the characters set forth in the above diagnosis readily distinguish this species from *A. parietinus* (Bastian) as conceived by most authors, though possibly not from all the numerous variants. However, until the confines of *A. parietinus, sensu stricto,* have been more definitely established and the variants studied and their status defined it is impossible to differentiate between this and other species in a precise and satisfactory manner.

The postvulvar uterine sac serves to differentiate *A. besseyi* from the other plant-parasitic species of the genus, viz., *A. fragariae, A. olesistus* (Ritzema Bos, 1893), *A. ritzema-bosi* (Schwartz, 1911), *A. ribes* (Taylor, 1917), and *A. subtenuis.* In *A. besseyi* this structure is narrow, inconspicuous, usually extends less than one-third of the distance from vulva to anus, and rarely contains spermatozoa whereas in the other species noted above it is wider, more conspicuous, usually extends two-thirds or more of the distance from vulva to anus, and usually contains spermatozoa. A further aid in distinguishing *A. besseyi* is the comparatively short ovary that, in its middle region, shows several developing ova in a given cross section whereas in the other species (with the possible exception of *A. ritzema-bosi*) the developing ova are arranged tandem. The excretory pore of *A. besseyi* is slightly anterior to the nerve ring in which respect this species resembles *A. olesistus* and *A. subtenuis* but differs from *A. fragariae, A. ritzema-bosi,* and *A. ribes.* Furthermore, *A. besseyi* is a noticeably shorter nematode than *A. fragariae.* Based on 10 adult specimens of each sex these two species compare as follows: ♀ ♀: Length, *besseyi* 660–750 μ, *fragariae* 720–860 μ; width, *besseyi* 17–22 μ, *fragariae* 15–21 μ; α, *besseyi* 32–42, *fragariae* 40–46. ♂ ♂: Length, *besseyi* 540–620 μ, *fragariae* 640–790 μ; width, *besseyi* 14–17, *fragariae* 15–18; α, *besseyi* 36–39, *fragariae* 37–46.

The name *Aphelenchus subtenuis* was proposed by Cobb (1926) for a nematode that he found infecting narcissus bulbs grown, presumably, in the southeastern

United States. The species was subsequently transferred to the genus *Aphelenchoides*. The name *Aphelenchoides hodsoni* was proposed by Goodey (1935) for a nematode found infecting a narcissus plant (bulb and leaves) grown in the Isles of Scilly. Cobb published no figure and his meager description is wholly inadequate in a group the species of which are as difficult to differentiate as are the plant-parasitic *Aphelenchoides*. Goodey recognized the possibility that his specimens might be identical with those described by Cobb but, lacking the means to prove this, was left with no choice other than to describe his material as a new species.

From time to time during the past few years the writer has received from the states of Virginia and North Carolina narcissus plants infected with a nematode obviously identical with the one described by Goodey. By searching the files of the Division of Nematology the writer was fortunate in finding Cobb's original notes that included, besides a copy of the original manuscript as published, additional notes and several drawings. These drawings correspond in every respect with Goodey's description and figures. They show clearly the characteristic shape of the tail of the female with its blunt rounded terminus and short spike-like projection. Both Cobb and Goodey were dealing with the same species and *A. hodsoni* is a synonym of *A. subtenuis*.

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**The influence of chrysanthemum propagating methods on dissemination of the foliar nematode.** J. R. CHRISTIE, U. S. Bureau of Plant Industry Station, Beltsville, Md.

The commercial propagation of hardy chrysanthemums is done largely in greenhouses during winter. One method is to break the lateral shoots from the old crown and root them in sand. Another method is to bring the old crown into growth and take cuttings from the tips of the new growth. Cuttings of this kind will be referred to in this paper as top-cuttings. The former method is quick and easy and, with varieties that produce a large number of lateral shoots, it is possible to get a greater increase than by the latter method. Where a maximum increase in stock is desired both methods may be employed.

Propagation by top-cuttings is sometimes recommended as a means of controlling the chrysanthemum foliar nematode, *Aphelenchoides ritzema-bosi* (Schwartz, 1911). It is the opinion of those who recommend the practice that in stock propagated by this method the incidence of infection is likely to be much less than in stock propagated from lateral shoots. During the winter of 1941–42 two experiments were conducted the primary object of which was to test the validity of this contention. It is the purpose of this paper to report the results of these experiments and to record certain supplementary observations on the behavior of the parasite.

PROCEDURE

In an experimental field plot of chrysanthemums all the plants heavily infected

with the foliar nematode were selected and marked during September, 1941. This group of plants, 17 in number, was used in the following experiments and was made up of 2 varieties, Autumn Lights and Yellow Fellow. Autumn Lights is a hardy Korean hybrid that makes a bushy plant of moderate height and develops a large number of lateral shoots. Yellow Fellow is taller, less hardy, and produces fewer lateral shoots. It is field grown in some localities for cut flowers.

The 17 experimental plants were dug the latter part of December and brought into the greenhouse. The lateral shoots were removed, rooted in sand, potted, and grown on the bench until the first part of April. By this procedure a total of 164 plants was secured.

After the lateral shoots had been removed the crowns were potted and, when they had made sufficient growth, top-cuttings were taken. No stem was cut lower than about 4 inches from the surface of the soil in the pot. These cuttings also were rooted in sand, potted, and grown on the bench until the first part of April. By this procedure a total of 97 plants was secured.

The mother plants were allowed to continue growth and, as noted below, were subsequently used for observations on nematode distribution. The same mother plants were used throughout with a few exceptions; for example, certain plants did not provide any lateral shoots or did not make sufficient growth to permit taking top-cuttings.

The plants originating from lateral shoots were at all times kept separated from those originating from top-cuttings to preclude any possibility of the nematode's spreading from one group to the other. Special precautions were not taken to prevent spread from plant to plant within the 2 groups, the procedure being about the same as that usually practiced by growers. However, the possibility of such spread was no greater in one group than in the other.

During the first part of April, 1942, all plants were examined for the foliar nematode. These examinations were made by using the so-called Baermann apparatus. This simple and useful device consists of a glass funnel of appropriate size on the stem of which is inserted a short piece of rubber tubing closed with a pinch cock. The entire plant was cut off close to the soil, placed in a funnel, and submerged in tepid water. After 2 hours the pinch cock was opened and a suitable amount of fluid drawn into a Syracuse dish for examination. As the chrysanthemum foliar nematode has a natural tendency to migrate when the plant is wet, this method works exceptionally well and the possibility of overlooking a light infection, while not necessarily precluded, is far less than by any other method of examination known to the writer.

After the top-cuttings had been taken, as noted above, the mother plants were allowed to continue growth which, by early April, had reached a height of from about 7 to 10 inches. An attempt was made to secure information regarding the distribution of the foliar nematode on these mother plants by the following procedure.

For each plant all growth was cut off 4 inches above the surface of the soil and placed in a funnel. Then all the remaining lower growth was cut off close to the soil and placed in a second funnel. After soaking for 2 hours, 15 cc of fluid was drawn from each funnel and a determination made of the number of nematodes contained therein. Where the number did not exceed a few hundred it was determined by actual count, in other cases those in an aliquot part were counted and the total computed. The resulting numbers indicate the relative abundance of the nematodes on the top part of a plant as compared to their abundance on the bottom part of the plant but do not necessarily represent the total nematode population of the plant.

RESULTS

The results of the experiments are shown in tables 1 and 2.

When examined to determine the distribution of the parasites (Table 2), it will be noted that 3 of the mother plants were apparently uninfected (i.e., Nos. 74, 80, and 105) although 75 per cent of the lateral shoots previously secured from these same plants were infected (Table 1). Either these plants subsequently lost their infections or the nematodes were confined to the lateral shoots and were removed with them. The latter explanation seems the more probable.

TABLE 1.—*Number of nematode-infected plants in each of the two groups, one group propagated from lateral shoots and the other from top-cuttings, both from the same crowns*

| Designation and variety of crown | No. of plants from lateral shoots | | No. of plants from top-cuttings | |
|---|---|---|---|---|
| | Infected | Not infected | Infected | Not infected |
| #74 Autumn Lights | 13 | 9 | ..... | ..... |
| #80 " " | 25 | 0 | 0 | 16 |
| #81 " " | 14 | 5 | ..... | ..... |
| #98 " " | 6 | 2 | 2 | 9 |
| #105 " " | 15 | 8 | 0 | 16 |
| #141 " " | 8 | 0 | 0 | 8 |
| #82 Yellow Fellow | 1 | 3 | 0 | 5 |
| #101 " " | 17 | 0 | 0 | 4 |
| #103 " " | 3 | 4 | 0 | 8 |
| #228 " " | 4 | 3 | 0 | 3 |
| #247 " " | 0 | 1 | 0 | 2 |
| #286 " " | 5 | 2 | 0 | 2 |
| #291 " " | 3 | 4 | 0 | 1 |
| #356 " " | 2 | 0 | 0 | 4 |
| #374 " " | ..... | ..... | 0 | 3 |
| #398 " " | 5 | 2 | 0 | 13 |
| Totals | 121 | 43 | 2 | 95 |

TABLE 2.—*Number of foliar nematodes found on bottom part of mother plant up to a height of 4 inches and number found on top part of plant above 4 inches as revealed by comparable sampling (see text)*

| Designation and variety of crown | Number of nematodes | |
|---|---|---|
| | Bottoms | Tops |
| #74 Autumn Lights | 0 | 0 |
| #80 " " | 0 | 0 |
| #81 " " | 124 | 1 |
| #98 " " | 12 | 0 |
| #105 " " | 0 | 0 |
| #82 Yellow Fellow | 9 | 0 |
| #101 " " | 153 | 0 |
| #103 " " | 1191 | 0 |
| #228 " " | 256 | 0 |
| #247 " " | 629 | 0 |
| #258 " " | 2211 | 3 |
| #291 " " | 4674 | 0 |
| #356 " " | 849 | 0 |
| #374 " " | 433 | 0 |
| #398 " " | 1850 | 0 |

In December, when the crowns were brought into the greenhouse, the nematodes were located, for the most part, in the buds that occur around the base of the plant or in the growing points that terminate the lateral shoots. The latter position seems to be a favored location and nearly 74 per cent of the plants propagated from lateral shoots were infected (Table 1). When the crowns were potted and placed on the greenhouse bench the new growth came largely from uninfected buds, shoots from infected ones usually remaining stunted and growing very little. However, when infected buds managed to overcome this handicap, because of greater vigor, lighter infection or some other reason, and made rapid growth, the parasites seemed unable to maintain themselves in the growing point and this terminal infection was lost. The writer concludes that a combination of these 2 factors accounted for the lack of nematodes in the upper parts of the plants. The parasites were not confined to basal buds throughout the time the plants were growing in the greenhouse as some of the lower leaves eventually became infected. This infection of the foilage was never extensive but, nevertheless, was largely responsible for the high nematode counts shown in table 2.

After the cuttings from lateral shoots had been in sand for about 2 weeks many of the infected ones had developed conspicuous symptoms. The terminal bud looked unhealthy and the young, developing leaves surrounding it were small, distorted, thickened, and abnormally dark in color. Growth had been retarded and these plants were smaller than the others. In other words, the plants exhibited typical bud-nematode symptoms similar to those of strawberry plants infected with one of the strawberry-dwarf nematodes. This condition was temporary, however, and after a few weeks the plants had largely outgrown it and were not noticeably different from the others. At the time these plants were given their final examination the nematodes were not in the terminal growing points but in buds around the bases or in the lower leaves. The plants propagated by top-cuttings developed no recognizable symptoms.

## CONCLUSIONS

The results of these observations and experiments strongly support the view that propagation by top-cuttings is a recommendable procedure for control of the chrysanthemum foliar nematode and that a high degree of control can be achieved if care and judgment is exercised in taking the cuttings. Where this method is feasible it offers the grower an alternative to hot-water treatment. To what extent efficacy will be affected if the lateral shoots are not removed from the crown and if top-cuttings are taken from them also, after they have grown to suitable height, is a question to which these experiments do not provide an answer. Propagation by the removal and rooting of lateral shoots favors the perpetuation and dissemination of the parasite and, when practiced year after year, is a factor contributing to accumulative injury.

## MINUTES

*Two Hundred Twenty-first to Two Hundred Twenty-eighth Meetings*

The 221st meeting was held October 17, 1941. The following officers were elected: President, A. O. Foster, Vice President, M. P. Sarles, Recording Secretary, K. C. Kates, Corresponding Secretary and Treasurer, Edna M. Buhrer. The following were elected to membership: Drs. L. E. Swanson, C. M. Haensler, and P. C. Underwood. Papers were presented by Lawrence, Getz and Steiner.

The 222nd meeting was held November 24, 1941. Papers were presented by Farr, Coatney, Sarles, and Price.

The 223rd meeting was held December 17, 1941. Dr. E. W. Price was elected the Society's representative in the Washington Academy of Sciences. The following were elected to membership: Drs. William R. Jones, Vernon D. Chadwick,

Roy L. Mayhew, and Louis Olivier. Papers were presented by Courtney, Chitwood, and Olivier.

The 224th meeting was held January 21, 1942. Papers were presented by Graham and Dinaburg.

The 225th meeting was held February 18, 1942. Papers were presented by Reinhard, Otto, Schwartz, and Shorb.

The 226th meeting was held March 18, 1942. Papers were presented by Shorb, Kates, and Lucker.

The 227th meeting was held April 15, 1942. Papers were presented by von Brand, Dikmans, Habermann, Christensen, Hammon, and Bartlett.

The 228th meeting was held in conjunction with the annual picnic, May 9, 1942, at th U. S. Horticultural Station Log Lodge. The following officers were elected: President, John T. Lucker; Vice President, K. C. Kates; Recording Secretary, Doys A. Shorb; Corresponding Secretary and Treasurer, Edna M. Buhrer.

K. C. KATES
*Recording Secretary*

CONTENTS