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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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Survival on Pasture of Free-living Stages of Some Common Gastrointestinal Nematodes of Sheep

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The survival of free-living stages of various species of sheep nematodes has been studied by many workers, both in the laboratory and on pasture and range. One of the most practical procedures employed for obtaining information on this subject is to test pastures, which have been naturally or experimentally contaminated with sheep parasite eggs, for larval survival by grazing parasite-free lambs thereon at regular intervals, and later examining them for parasites. Reliable information derived from such work has considerable practical application to the general problem of sheep nematode control. The purpose of this paper is to present results of a series of six experiments of this kind designed to study the effects of the varying weather conditions for an entire year.

LITERATURE SUMMARY

The published information on this subject is difficult to summarize concisely. Some reports concern one species of parasite, others several species and some do not clearly define the species. Varying experimental procedures have been employed in the laboratory and in the field, and different criteria have been used to determine larval viability after different exposures. Relatively few studies, however, have been reported which include (1) experiments conducted throughout the variable weather conditions of an entire year, and (2) regular testing of pastures for viable larvae at short intervals by the use of parasite-free lambs. Shorb (34) reported the most through study of this kind to date, but was able to make only a few tests of larval survival at relatively long, irregular intervals. Similar studies of more limited scope have been reported by Baker (1), Boughton and Hardy (2), Dikmans and Andrews (5), Doll and Hull (9, 10), Furman (12), Goldsby and Eveleth (13), Griffiths (15), Hawkins et al. (18), Kates (19), Ransom (27–30), Sarles (31, 32), Seghetti (33), Swales (38), and Todd et al. (42). As no recent reasonably complete survey of the literature has been made, the author does this on the basis of the species or genera of parasites in order of their normal location in the digestive tract of sheep. The first paragraph under each parasite is the author's summary of significant facts brought out in the reports detailed thereafter.

Nematodes of the Abomasum

Haemonchus contortus.—This species is one of the most pathogenic parasites of sheep and a more extensive literature is available on its preparasitic stages than on those of other species. Conflicting results have been reported by various

[1] The writer expresses his appreciation to Dr. G. Dikmans, Dr. D. A. Shorb, and Mr. J. T. Lucker for much helpful advice and criticism.

workers. However, evidence points to the conclusion that infective larvae of this species are unlikely to survive for more than six months on pasture or range regardless of the conditions of exposure, most of them being destroyed in less than six months. Preinfective larvae and eggs are more susceptible than infective larvae to adverse conditions. Warm weather and adequate precipitation favor optimum development and survival of the free-living stages.

Ransom (27–30) reported the first studies on this species under experimental and natural conditions. He concluded that pastures should be free of viable larvae after exposure of 1 year, that some larvae survive the winter in the Washington, D. C. area, and that eggs and preinfective larvae were speedily killed by freezing and drying, while infective larvae were highly resistant to these conditions. Bozicevich (3) reported that some infective larvae in soil survived a 7-month exposure at Washington, D. C., and that eggs, and first and second stage larvae in soil were killed by short exposures (few hours to several days) to freezing and subfreezing temperatures. Relatively long survival of *H. contortus* on pasture was reported by Baker (1) in New York and Boughton and Hardy (2) in Texas, 1 year in the former case and 22 months in the latter (three worms recovered from two test lambs). Mönnig (25) also reported long survival time of infective larvae exposed to various experimental conditions, and Guberlet (16) concluded, as a result of work in Oklahoma, that stomach worm larvae probably do not survive longer than 8 to 10 months under field conditions.

Other workers indicate somewhat shorter survival periods for stomach worm larvae. Dikmans and Andrews (5) and Kates (19) found only small numbers survive the winter on pastures at Beltsville, Md., while Sarles (31) obtained no survival on pasture in the same area after it had been free of parasitized sheep from November to June the following year. Doll and Hull (9, 10) in Kentucky observed no survival on a contaminated plot after one 3½-month and two 3-month exposures during the summer and only low survival in one experiment after a 3-month exposure. An experiment by Hawkins et al. (18) in Michigan resulted in a pasture testing free of *H. contortus* after being rested 2 months in late summer and fall. Swales (38) concluded that stomach worm larvae do not survive the winter in eastern Canada. Kauzal (22) stated that in Australia 1 to 2 months of continuous dry weather greatly reduces infection on pastures and may completely eradicate it. Later (23) he reported the recovery of infective larvae from inoculated grass plots over a period of 7½ weeks, during which the temperature and rainfall were favorable for survival (min. 40–50° F.; max. 60–65° F.; rainfall 2–3 inches); after 3-day exposure 80 percent of the larvae were recovered, and there was a gradual decline until at the end of 7½ weeks only 20 percent were recovered. Veglia (44) concluded, as a result of numerous experiments, that unfavorable conditions for larval survival were warm-dry and cold-dry weather, while warm-moist weather was most favorable both before and after deposition of eggs on pasture. Goldsby and Eveleth (13) reported no survival after winter exposure in North Dakota, but Seghetti (33) found low survival after winter exposure on the range in eastern Montana, and reported a rapid destruction of larvae during the summer.

In addition to the work already summarized, a series of experiments has been reported from Beltsville, Md. by Shorb (34–37) and Dinaburg (6, 7). Shorb (35) found no survival on pasture after exposures of 3½, 2½ and 2 months during the summer, and no survival on a pasture contaminated in November and December and tested in March of the next year and on a pasture contaminated in March and tested the following May. Other experiments of Shorb (35, 36) and Dinaburg (6, 7) were conducted by inoculating small outdoor grass plots with eggs and/or

larvae and thereafter making recoveries of infective larvae at intervals. They found eggs and preinfective larvae are rapidly killed or rendered non-viable by abnormally low or high temperatures, drying, and exposure to direct sunlight; conversely shade, presence of adequate moisture, and moderate temperatures favored longer survival Infective larvae were more resistant than preinfective stages. Dinaburg (6) observed that less than 1 percent of the inoculated larvae could be recovered from plots after slightly less than 3 months outdoors regardless of the season, and all viable larvae disappeared after somewhat longer periods, and later (7) reported that no development occurred when the mean maximum air temperature was below 65° F.

Gordon (14) confirmed the reliability of the "Dinaburg Line" of 65° F. under field conditions in Australia and concluded that simultaneous occurrence of warm weather and adequate rainfall was essential for production of clinical haemonchosis in sheep, observing that outbreaks seldom occur in winter rainfall areas, but were common in summer rainfall areas.

Ostertagia circumcincta and Ostertagia sp.—The information available indicates that larvae of these nematodes develop and survive better at lower mean temperatures than those of the large stomach worm. Most reports show that *Ostertagia* spp. occur in greater frequency and abundance in sheep where the mean annual temperatures are relatively low, or during the cooler seasons in warmer climates.

Furman (12) studied the effects of environmental factors upon the free-living stages of *O. circumcincta.* In laboratory experiments infective larvae survived more than 271 days and preinfective larvae 1 to 2 months at 5° C. Shorter survival periods were recorded when eggs and larvae were exposed to −6° C. Although infective larvae were highly resistant to temperature extremes, mortality increased progressively at 27° C., 37° C., and 45° C. Desiccation was particularly destructive. In field tests in California, larvae survived all summer on irrigated pasture of high moisture content, but were rapidly killed on non-irrigated pasture. More eggs developed to infective stages in the fall and winter than in summer. Morgan (26) reported that *O. circumcincta* larvae are killed by a ½-hour exposure at 45° C., and by 2-hour exposure in sunlight after desiccation, but survived up to 3 months in water. Dinaburg (8) observed that larvae were very resistant to sustained subfreezing temperatures during the winter months and the eggs survived and developed at lower temperatures than did those of *Cooperia curticei, Trichostrongylus* spp., and *Oesophagostomum columbianum.*

Other information generally supports the laboratory work on the free-living stages. Some survival (no data) on pasture in New York for 1 year was reported by Baker (1), and for the winter period in Maryland by Dikmans and Andrews (5). Doll and Hull (9, 10) recovered 17 *O. circumcincta* from lambs grazed on a pasture plot 2 to 3 months after it had been contaminated during the summer with a variety of sheep nematode eggs in dung; this was the largest number of any one species recovered. Fallis (11) in Ontario observed that 30 percent of the parasites recovered from lambs slaughtered in June were *Ostertagia* spp., which indicated good survival of larvae during the winter on pasture. Goldsby and Eveleth (13) reported survival of *Ostertagia* spp. in North Dakota from December to June, but their rather high numbers in two test lambs were probably due to the long exposure (4 months) to the experimental pasture. Griffiths (15) likewise tested sheep nematode survival over the winter months in Canada and found that sufficient *O. circumcincta* survived on pasture from October to May to cause infection in the spring, and Swales (38) reached the same conclusion for eastern Canada. Hawkins et al. (18) reported considerable survival of *Ostertagia* spp.

on a pasture in Michigan from September 1 to January 15. Kates (19) reported similar results in Maryland from October to May. Seghetti (33) observed that on range plots in eastern Montana *Ostertagia* spp. were quickly killed during the summer, but small numbers survived the winter. Shorb (34) found no survival during the summer in Maryland after exposures of 2, 2½, and 3½ months, and that only small numbers survived wintering on pasture. Threlkeld (40) observed that *O. circumcincta* larvae survive better in shade than in direct sunlight, that warm-dry weather for 76 days was lethal, and that they could survive temperatures as low as −10° F. under pasture conditions in Virginia.

Nematodes of the Small Intestine

Trichostrongylus spp.—Although one species of this genus, *T. axei*, is commonly found in the abomasum, the genus is here considered as a group under nematodes of the small intestine. From the published data it appears that the free-living stages react to favorable and unfavorable summer conditions in somewhat the same manner as those of *H. contortus*, but are more resistant to adverse conditions in the cooler months.

Mönnig (25) in South Africa reported the first detailed study of the effects of natural and laboratory conditions on the eggs and larvae of *Trichostrongylus* spp. Embryonated eggs resisted drying up to 15 months (?), survived in water 45 days, but were quickly killed by freezing unless sheltered. Infective larvae were viable after 10-day exposure to freezing, 8½ months of drying on a slide indoors, and 7 months in water indoors. Larvae in various soils in pots outdoors survived shorter periods exposed to direct sunlight than in shade. Zavadovskii and coworkers (45–48) in Russia reported studies on free-living stages of various species of the family Trichostrongylidae, mainly of the genus *Trichostrongylus*, but usually mixed with *Ostertagia* spp., so their results are of questionable value for either genus. However, in a winter experiment in the Moscow area they observed that 97 to 99 percent of Trichostrongylidae larvae perish during 4 months in the open and, after overwintering on pasture "are practically deprived of any invasive significance towards spring."

A significant study was reported by Taylor (39) in England. His observations showed that ovine trichostrongylid larvae, obtained from mixed cultures and exposed on grass plots in an open field beginning in September, had a very high mortality during the first few weeks, and the number of living larvae recovered from grass thereafter decreased gradually until none were found after 39 weeks of exposure. This work has recently been confirmed and extended by Croften (4), who employed in his studies *T. retortaeformis* from the rabbit. Small grass or clover plots were used and it was observed that most of the larvae occurred on that part of the herbage in which there was the least climatic change and the highest humidity; these conditions were found in the humus or "mat" and the lower portion of the grass blades. Some infective larvae died soon after exposure, but small numbers survived long periods, in cold weather as long as 20 weeks. A large proportion died when evaporation was high; in one case 95 percent were dead at the end of 4 weeks.

There are several reports on survival of *Trichostrongylus* spp. larvae on contaminated pastures as determined by grazing parasite-free lambs thereon after various exposure periods. Griffiths (15) observed some survival of *T. colubriformis* over the winter in Canada, while Swales (36) stated that survival over a similar period in eastern Canada was low or absent. Baker (1), however, in New York reported survival of 1 year on pasture, while Kates (19) observed only slight survival over the winter at Beltsville, Md. Dinaburg (8) confirmed the

work of Mönnig and Zavadovskii et al. in regard to the rapid destruction of preinfective stages upon exposure to winter weather conditions, concluding that low temperatures during December, January, and February at Beltsville, Md. were lethal. Hawkins et al. (18) in Michigan reported substantial survival after 4½-month exposure on pasture from September to January, while Shorb (34) observed only slight survival over the winter and no survival after 2, 2½, and 3½ months of summer exposure at Beltsville, Md. Seghetti (33) showed that the main acquisition of *Trichostrongylus* spp. by lambs on the range of eastern Montana occurred during relatively heavy rainfall in May and June, and that later in the summer, when the rainfall was less than 1 inch in 2 months, most of the free-living stages were killed by 10 days' exposure. Small numbers of larvae were able to survive the severe winter weather of eastern Montana. In contrast, Goldsby and Eveleth (13) reported a substantial survival over the winter on a pasture in the Red River Valley of North Dakota, but their test lambs were allowed to graze the rested pasture for 4 months and many of the parasites recovered at autopsy probably were the result of recontamination of the pasture rather than the residual survival over winter.

Cooperia spp.—Survival of the preparasitic stages of this genus has been little studied, but it is possible to conclude from available data that for *C. curticei*, in particular, the survival time, regardless of the season of the year, is relatively short, as in the case of *Haemonchus*. Dinaburg (8) and Kates (19) found no survival on pasture over the winter at Beltsville, Md., while Shorb (34) reported no survival during the summer in different experiments after 2, 2½, and 3½ months in the same area. Swales (38) reported similar results for the winter period in eastern Canada. The report of Baker (1) that *C. curticei* survived for a year on a New York State pasture is at variance with other reports.

Nematodirus spp.—The free-living stages of these nematodes differ from others under consideration in that larval development is completed within the egg membranes or shell before hatching. Infective larvae hatch only under favorable conditions, retaining the sheaths of both the first and second moults. Hence, these larvae are well protected from adverse conditions both during development and after hatching. Eggs and larvae are particularly resistant to low temperatures and desiccation.

Fallis (11) observed that eggs of *N. spathiger* were not killed after exposure for 2 weeks at −10° C. Zviagintzev (50) reported that eggs of *N. helvetianus* are very resistant to freezing and desiccation, while infective larvae resist desiccation better than freezing. Relatively good survival of *Nematodirus* spp. on pasture or range has been reported by Dikmans and Andrews (5) and Kates (19) (Beltsville, Md.), Goldsby and Eveleth (13) (North Dakota), Griffiths (15) (Canada), Hawkins et al. (18) (Michigan), Swales (38) (Canada), and Seghetti (33) Montana). Little evidence is available on the effects of summer conditions on eggs and larvae, but it appears that they are less resistant to warm than to cold weather. Zavadovskii and Zviagintzev (49) studied the seasonal fluctuations in *Nematodirus* spp. in a llama and concluded that invasion of larvae is greatest in the spring. Other similar data in the literature generally show that heavy infections of *Nematodirus* usually appear in lambs in spring and summer, declining in fall and winter. However, heavy infections may develop at other seasons when conditions are favorable.

Bunostomum trigonocephalum.—Little information is available on the survival of preparasitic stages of this parasite, but from the fact that it is seldom found in large numbers in sheep except under very favorable conditions, such as described by Habermann (17), and that its larvae are destroyed by winter ex-

posure on pasture [Kates (19), Swales (38), Goldsby and Eveleth (13)], by summer exposure on pasture [Shorb (34)], and its eggs by freezing [Fallis (11)], it is reasonable to conclude that generally the preparasitic stages do not survive long on pasture under most climatic conditions and grazing practices. The report of Baker (1) on survival on pasture for one year is at variance with the above reports. (Note: this parasite was not present in our parasitized sheep.)

Nematodes of the Large Intestine

Oesophagostomum spp.—Of the two species of nodular worms of sheep, *O. columbianum* is more pathogenic and generally is found more frequently and in larger numbers than *O. venulosum;* most of the studies on larval survival in this genus definitely or probably concern the former species. Free-living stages of nodular worms have been shown by various workers to be the least resistant of the important pathogenic species of sheep nematodes to the effects of exposure to weather on pasture, regardless of season.

No survival over the winter on pasture has been reported by Dinaburg (8), Goldsby and Eveleth (13), Kates (19), Kauzal (22), Ransom (30), Sarles (32), and Swales (38) for such diverse climatic regions as Australia, eastern Canada, Maryland, and North Dakota. Furthermore, no survival was reported by Hawkins et al. (18) in Michigan after an exposure of 3½ months in late summer and fall, by Shorb (34) in Maryland, and Doll and Hull (9, 10) in Kentucky for exposure periods of from 2 to 3½ months in summer.

Chabertia ovina.—Recently Threlkeld (41) reported a test of pasture survival of this species in Virginia. A small paddock was contaminated with eggs by a single infected sheep and thereafter tested by grazing thereon parasite-free lambs. It was stated that larvae survived exposures of slightly more than 2 months, 5½ months, and 8½ months; the latter two periods included the winter months. Because of the procedure employed, this report needs confirmation. No other work of significance has been done, but from our knowledge of the infrequent occurrence of heavy infections of this species in sheep generally, the reaction of its larvae to environmental conditions may be similar to that of *B. trigonocephalum,* requiring particularly favorable conditions for development and survival. (Note: this parasite was not present in our parasitized sheep.)

Trichuris ovis.—This parasite differs from others mentioned in that sheep acquire infections by ingestion of infective, embryonated eggs rather than hatched, infective larvae. Thus, throughout the pasture phase it has the added protection of the egg membranes or shell. Studies on the human whipworm, *T. trichiura,* have shown that moisture, warm weather, and shade favor development and survival of the eggs and, although similar studies have not been made with *T. ovis,* they probably react similarly. The information available on the sheep whipworm is found in reports of tests of contaminated pastures by use of parasite-free lambs. Some survival during variable periods of winter and summer has been reported by various workers already cited. (Note: this parasite was not present in significant numbers in our parasitized sheep.)

EXPERIMENTAL PROCEDURES

Six one-quarter acre pastures, known to be free of viable free-living stages of sheep parasites, were contaminated with nematode eggs of various species by permitting heavily parasitized sheep to graze thereon for four days in five experiments and for six days in one experiment. Minimum estimates of the numbers of eggs of each genus or species of nematode deposited on each pasture were determined by differential, direct centrifugal flotation counts on fecal samples pooled

from all the contaminating sheep. The principal details of this egg count method have been previously described by Kates (20) and Kates and Shorb (21). Fecal samples were taken in equal quantities from all parasitized sheep at the beginning and end of the pasture contamination period, and the average number of eggs per gram of pooled feces was determined for each parasite. This figure was multiplied by the estimated minimum grams of feces deposited by all the parasitized sheep on the pastures for the four or six day period. The resulting totals, which are given in tables 2 and 3, are rough minimum estimates of the total eggs of each nematode parasite deposited on the pastures. The droppings were evenly distributed over the quarter-acre pastures, insuring uniform distribution of infective larvae.

Eleven species of nematodes, belonging to six genera, were present in the parasitized sheep. Two genera were represented by one species each, i.e. *Haemonchus contortus* and *Cooperia curticei*, while two or more species of the other four genera were present. Nematodes of the genus *Trichostrongylus* were mainly *T. colubriformis* with small numbers of *T. axei* and *T. vitrinus;* those of the genus *Oesophagostomum* were mainly *O. columbianum* with lesser numbers of *O. venulosum;* those of the genus *Ostertagia* were mainly *O. circumcincta* with lesser numbers of *O. trifurcata,* and those of the genus *Nematodirus* were mainly *N. spathiger* with only small numbers of *N. filicollis.*

TABLE 1.—*Monthly summary of weather data for the two winter periods.*

Month	Temperature, °F.		Precipitation (inches)	Month	Temperature, °F.		Precipitation (inches)
	Max.	Min.			Max.	Min.	
December	70	16	3.44	October	78	23	10.13
January	61	−4	1.64	November	76	18	2.07
February	53	8	2.21	December	46	−8	3.97
March	76	22	5.44	January	67	11	1.62
April	91	26	0.66	February	68	0	3.52
				March	74	4	4.12
				April	82	19	2.74

When the pastures were contaminated with parasite eggs, each was subdivided by fencing into seven equal strips or plots (approximately 1/28 acre, or 1500 square feet), and these subdivisions were tested for viable infective larvae in the following manner. Two weeks after the parasitized sheep were removed from the pastures, and thereafter at two-week intervals, two lambs, free of parasites except for light infections of *Strongyloides* and coccidia and of approximately similar age and weight, were grazed on each subdivision for one week. Exceptions with regard to the time intervals mentioned are noted in Tables 2 and 3. In all tests the grass on the plots was uniformly and thoroughly cropped by the lambs. Not all subdivisions of each pasture were tested because some experiments continued into the winter and little forage was present. Except for experiment 4, begun in May, one subdivision in each pasture was tested for the effect of overwintering on the larvae by testing one plot on each pasture the May following contamination. In these tests the grazing period for the lambs was two weeks instead of one.

Each pair of lambs, after grazing the desired subdivision, was placed in a clean concrete-floored pen and autopsied 5 weeks thereafter. This holding period allowed sufficient time for development of the parasites, which facilitated their recovery and identification. The holding pens were thoroughly washed daily to

TABLE 2.—*Results of three experiments begun in August, September, and October.*

Nematode[a] genera	Estimated minimum number of eggs deposited on pasture	Nematodes recovered postmortem from 2 lambs exposed to 1/7 of pasture for indicated periods							Total nematodes recovered from all lambs
		Sept. 2–9	Sept. 16–23	Sept. 30–Oct. 7	Oct. 14–21	Oct. 28–Nov. 4	Nov. 25–Dec. 2	Following May 5–19	
EXPERIMENT 1. Pasture A (¼ acre) grazed by 14 parasitized sheep August 15–19.									
Haemonchus	62,440,000	12,060	406	167	216	9	1	106	12,965
Oesophagostomum	252,000	8	0	1	1	0	0	0	10
Trichostrongylus	2,016,000	126	30	37	288	283	48	2	820
Ostertagia	756,000	120	9	1	37	12	0	12	191
Cooperia	756,000	78	33	66	86	60	2	0	325
Nematodirus	1,176,000	138	27	429	2,976	1,507	756	267	6,100
Totals	67,396,000	12,530	511	701	3,604	1,871	807	387	20,411
EXPERIMENT 2. Pasture B (¼ acre) grazed by 13 parasitized sheep September 15–19									
Haemonchus	197,484,000	.....	.....	1	14	0	3	0	18
Oesophagostomum	756,000	.....	.....	0	0	0	0	0	0
Trichostrongylus	4,704,000	.....	.....	0	18	89	159	7	273
Ostertagia	1,932,000	.....	.....	0	6	21	4	474	505
Cooperia	1,764,000	.....	.....	0	0	2	0	0	2
Nematodirus	588,000	.....	.....	0	59	195	87	119	460
Totals	207,816,000	.....		1	97	307	253	600	1,258
EXPERIMENT 3. Pasture C (¼ acre) grazed by 15 parasitized sheep October 15–19.									
Haemonchus	187,992,000	.....	.....	.....	.....	0	12	30	42
Oesophagostomum	840,000	.....	.....	.....	.....	0	0	0	0
Trichostrongylus	6,972,000	.....	.....	.....	.....	0	9	6	15
Ostertagia	2,184,000	.....	.....	.....	.....	0	0	1,294	1,294
Cooperia	3,276,000	.....	.....	.....	.....	0	0	0	0
Nematodirus	756,000	.....	.....	.....	.....	6	14	181	201
Totals	202,020,000	.....	.....	.....	.....	6	35	1,511	1,552

[a] See text for species of parasites.

TABLE 3.—*Results of three experiments begun in May, June, and July of the year after those in Table 2.*

Nematode genera	Estimated minimum number of eggs deposited on pasture	Nematodes recovered postmortem from 2 lambs exposed to 1/7 of pasture for indicated periods								Total nematodes recovered from all lambs
		June 3–10	June 16–23	June 30 to July 7	July 14–21	July 28 to Aug. 4	Aug. 25 to Sept. 1	Sept. 22–29	Following May 13–26	
EXPERIMENT 4. Pasture D (¼ acre) grazed by 22 parasitized sheep May 15–19.										
Haemonchus	551,832,000	4,339	4,450	9,067	5,184	5,830	1,922	27	.....	30,822
Oesophagostomum	33,936,000	20	39	155	73	6	0	0	.....	293
Trichostrongylus	16,616,000	79	1,783	2,526	1,528	317	180	2	.....	6,415
Ostertagia	1,624,000	36	89	286	127	57	35	0	.....	630
Cooperia	7,897,000	66	600	1,771	358	217	190	6	.....	3,408
Nematodirus[a]	.....	7	26	30	8	3	0	3	.....	77
Totals	611,905,000	4,547	7,187	13,835	7,278	6,433	2,327	38	.....	41,645
EXPERIMENT 5. Pasture E (¼ acre) grazed by 21 parasitized sheep June 16–20.										
Haemonchus	237,400,000	.....	.....	2,468	1,331	480	279	12	0	4,570
Oesophagostomum	9,240,000	.....	.....	25	5	0	0	0	0	30
Trichostrongylus	9,430,000	.....	.....	126	125	160	18	0	0	429
Ostertagia	4,800,000	.....	.....	16	14	26	0	0	0	56
Cooperia	5,650,000	.....	.....	32	65	36	21	5	0	159
Nematodirus	3,160,000	.....	.....	10	47	163	7	4	0	229
Totals	269,680,000	.....	.....	2,677	1,587	865	323	21	0	5,473
EXPERIMENT 6. Pasture F (¼ acre) grazed by 19 parasitized sheep July 14–20.										
Haemonchus	175,080,000	.....	.....	.....	4,993	2,295	675	84	0	8,047
Oesophagostomum	16,488,000	.....	.....	.....	181	22	2	3	0	208
Trichostrongylus	26,544,000	.....	.....	.....	1,648	675	287	104	0	2,714
Ostertagia	5,760,000	.....	.....	.....	9	18	7	9	0	43
Cooperia	13,248,000	.....	.....	.....	1,845	541	81	71	0	2,538
Nematodirus	1,392,000	.....	.....	.....	50	46	35	62	9	202
Totals	238,512,000	.....	.....	.....	8,726	3,597	1,087	333	9	13,752

[a] Eggs present, but in numbers too small to count by method employed.

prevent further infection in case any parasites reached maturity before autopsies were made. The number and species of parasites obtained from the lambs in successive tests were regarded as indicative of the relative numbers and species of infective larvae developing and surviving on the pastures up to the time of the test.

CLIMATIC CONDITIONS DURING EXPERIMENTS

Although these experiments were conducted over a period of almost two years, they may be considered to represent a period of about one full year, from May to May. Experiments 1 to 3 were begun in August, September, and October, respectively, of one year, and experiments 4 to 6 in May, June, and July, respectively, of the next year in order to have enough parasite-free lambs available to complete

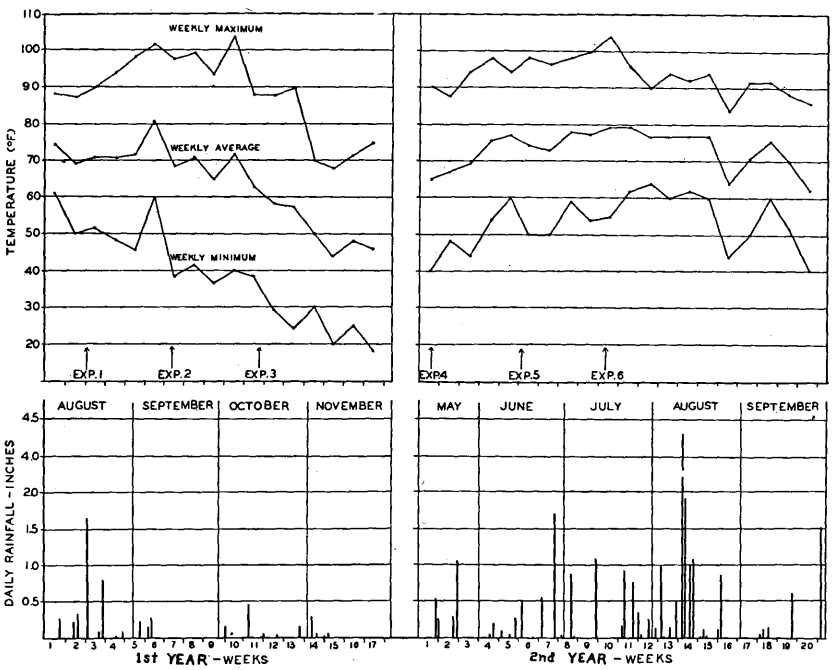

FIG. 1. Rainfall and temperature data for test periods covered in experiments 1 to 6 except for winter weather data, which are summarized on a monthly basis in Table 1. Arrows indicate the beginning of the 6 successive experiments.

testing the pastures. Throughout the period daily precipitation and air-shade temperature data were recorded near the pastures. These are summarized for the main grazing periods in Fig. 1 and for the two winter periods in Table 1. Climatic conditions, before and after the initiation of each experiment, varied considerably. The short period of pasture contamination began about the middle of each month as indicated.

August of the first year was slightly cooler than normal for the area in which the experiments were conducted with about normal precipitation, some rain being recorded each week of the month. Conversely, September was an atypical month climatically, characterized by unusually high temperatures and drought after the first week. The subnormal precipitation continued through October and November accompanied by the usual decline in temperature. The first winter minimum tem-

perature was – 4° F., and the precipitation did not deviate markedly from normal except for April when only 0.66 inch was recorded. Snow cover, when present, seldom lasted more than a week at a time and there were long periods with no snow on the pastures. The weather in May of both years, when the final tests for survival over the winter were made, was typically mild and moist.

The period from May through September of the second year was uniformly warm and moist, the rainfall being evenly distributed throughout and about normal, except for August, when it was excessive. No extended period of drought occurred as in the previous fall. In experiments 1, 4, and 6 considerable rain fell shortly before, during, or shortly after pasture contamination, and was accompanied by relatively warm weather. In experiment 2, however, drought and high temperatures prevailed during pasture contamination, while cooler weather with very low rainfall characterized the analogous period in experiment 3. For the second winter period unusually high rainfall was recorded in October and about normal for the other months; the lowest recorded temperature was – 8° F. Snow cover during this winter was never great and did not last long on the pastures. Frequent freezing and thawing are characteristic of the winters in Maryland, as can be ascertained from the maximum-minimum temperatures summarized for the winter months in Table 1.

RESULTS

General Considerations

This series of experiments differs from similar studies previously reported in the literature in that (a) minimum estimates were made of the numbers of nematode eggs, by genera, deposited on the pastures; (b) reasonably heavy, natural contamination of the pastures with nematode eggs was accomplished over a short period; and (c) frequent and comparable testing of these pastures was carried out over an extended period for viable infective larvae by grazing on equal subdivisions thereof matched pairs of parasite-free lambs. By this procedure, it was possible to determine the relative availability of viable infective larvae on the pastures to highly susceptible test animals at well defined and regular intervals after pasture contamination. Furthermore, by initiation of a new experiment each month of the normal grazing season (May to October inclusive) it was possible to observe the effects on larval survival of annual and seasonal variations in local climatic conditions.

As shown in Tables 2 and 3, the majority of all eggs deposited were those of *H. contortus*, but large numbers of eggs of other genera, consisting of ten additional species, were also present. One general feature of the data is the great disparity between the minimum estimates of total number of eggs deposited on the pastures and total numbers of parasites recovered from all test lambs in each experiment. Although the estimated number of eggs deposited on the six pastures varied roughly from 67 million to 612 million, the number of parasites recovered from all test lambs in each experiment varied from only 1,258 to 41,645. These data, however, do not present a true picture of the maximum development of viable larvae at any one time, because successive tests for larval survival were made at different intervals after pasture contamination and all subdivisions of each pasture were not tested in all experiments. Therefore, only the one test, of the possible maximum of seven in each experiment, showing the maximum recovery of worms can be considered indicative of the maximum development of viable infective larvae. Furthermore, all viable larvae on the plots obviously were not ingested by the animals even though the grass was closely and uniformly cropped in all tests, and all ingested larvae obviously did not develop in the lambs.

Larval Survival by Genera

Haemonchus (contortus).—The numbers of *Haemonchus* eggs deposited on the experimental pastures far exceeded those of the other five genera. Experiment 1 (Table 2) shows the destructive effects of high temperature and drought on the infective larvae. From the time of pasture contamination (Aug. 15 to 19) to the first test (Sept. 2 to 9) conditions favorable for development of infective larvae occurred and the pair of test lambs contained 12,060 *Haemonchus* on autopsy. However, beginning with Sept. 7, and extending into October, no rain fell (Fig. 1, 1st year) and the average weekly maximum temperatures were higher than in August. This change in climatic conditions was immediately reflected in the abrupt decline in number of worms recovered in succeeding biweekly tests. In the five tests from mid-September to late November the worms recovered decreased from 406 to 1. The recovery of 106 *Haemonchus* in the test the following May show that when conditions are favorable for development of infective larvae at the time of pasture contamination, a small number can survive the winter at Beltsville, Md., even though extremely adverse conditions may intervene.

Experiments 2 and 3 show the destructive effect of both warm-dry and cool-dry weather on the developing free-living stages of *Haemonchus*. In experiment 2 the pasture was contaminated in mid-September, midway in a month-long period of drought accompanied by high temperatures. Only one *Haemonchus* was recovered in the first pasture test, few or none in later tests and the test the following May was negative. In experiment 3, begun in mid-October, most of the infective larvae were either killed or their development prevented by the cool-dry weather, but a small number survived until the following May, when 30 worms were recovered.

Experiments 4, 5, and 6 were conducted during the spring, summer, and early fall. Climatically this period was generally favorable as can be seen in Fig. 1 (2nd year), and this is reflected in the survival results shown in Table 3. In experiment 4, begun in mid-May, relatively good survival of *Haemonchus* occurred through the last test in August, some 3½ months after pasture contamination. Maximum recovery of worms in one test was over 9,000, in the first week of July. An abrupt drop occurred in the final test in late September, only 27 worms being recorded in comparison with almost 2,000 about three weeks earlier. This reduction in viable larvae, indicated by this final test, was probably caused by a combination of the adverse effect of the below normal precipitation during the first three weeks of September, and ageing of the larvae on pasture.

The destructive effect of high summer temperature alone is indicated by the results in experiments 5 and 6, in which the pasture was contaminated in mid-June and mid-July, respectively, when high maximum temperatures were recorded. Although the decline in recovery of infective larvae followed the same trend as in comparable tests in experiment 4, it was more rapid up to the late August tests. It appears, therefore, that high summer temperatures allowed fewer preinfective larvae to reach the infective, more resistant stage in experiments 5 and 6 than in experiment 4. In the last mentioned experiment, development of the eggs and larvae took place mainly in the somewhat cooler period in May and early June. The fact that in experiments 4, 5, and 6 there was a low survival of worms in the late September tests compared with those made previously, indicates the more injurious effect of the adverse September weather than that of mere ageing of the larvae, all three experiments showing similar low recovery of worms although there was a difference in exposure time of from one to two months.

Experiments 5 and 6 also show that pastures contaminated with large numbers of *Haemonchus* eggs in June and July are completely free of viable larvae, under

the conditions of these experiments, by May of the next year, the majority of larvae being destroyed before the onset of winter. However, experiments 1 and 3 show that small numbers of larvae may survive the winter, at least until the following May, when pasture contamination occurs in late summer and fall.

Trichostrongylus (colubriformis, axei, vitrinus).—Of the three species represented in this genus, *T. colubriformis* was recovered in greatest numbers. Experiments 1 and 2 show that preparasitic stages of *Trichostrongylus* are more resistant to warm-dry and cool-dry weather than those of *Haemonchus.* The maximum recovery of *Trichostrongylus* was obtained in experiment 1 about 2 months after pasture contamination, with both warm-dry and cool-dry periods intervening, whereas the maximum recovery of *Haemonchus* was obtained after only less than a month's exposure on pasture, before adverse weather conditions occurred. In experiment 2 the maximum recovery of *Trichostrongylus* was obtained about $2\frac{1}{2}$ months after pasture contamination.

The spring and summer experiments 4, 5, and 6 show about the same pattern of reduction of viable *Trichostrongylus* larvae as for *Haemonchus,* but a higher proportionate survival of the former. The data for survival over the winter of these two genera are similar in all experiments.

Generally, therefore, the preparasitic stages of this genus react similarly to *Haemonchus* to most climatic conditions, but differ mainly in having a higher survival rate during the grazing season regardless of conditions of exposure.

Oesophagostomum (columbianum, venulosum).—The preparasitic stages of this genus were the least resistant to weather conditions on pasture. There was no evidence in any experiment that free-living stages survived overwintering on pasture. Viable larvae never appeared on pasture, as far as could be determined by the testing method, in experiments 2 and 3, which were begun in September and October, respectively, and decreased rapidly in the other four experiments, particularly experiment 1. The maximum period any larvae survived was approximately 10 weeks in experiment 4, an exposure period from late spring to midsummer characterized by relatively favorable moisture and temperature conditions. The majority are destroyed by much shorter exposures than this regardless of weather conditions.

It should be noted that the recovery of *Oesophagostomum* in test lambs, as recorded in Tables 2 and 3, represents only worms recovered from the large intestines. When worms were present, small numbers of nodules were also observed in some cases, but considering the light infections and the five-week holding period before autopsy, to allow adequate time for the majority of worms to leave the nodules, it was felt that the few nodules present could be safely disregarded in the survival data.

Ostertagia (circumcincta, trifurcata).—The data on this genus confirm the reports of other workers that optimum development and survival on pasture occur in the cool seasons or periods of the year and that both the eggs and larvae are relatively resistant to winter exposure even when minimum temperatures considerably below freezing occur for more or less extended periods. Conversely, development and survival are adversely affected by exposure to summer conditions when high temperatures and rapid evaporation occur.

In experiment 1 the pasture was contaminated in mid-August and the highest survival of *Ostertagia* was obtained in the first test, September 2 to 9. This short exposure period in late August and early September was characterized by somewhat lower maximum temperatures and higher rainfall than in the remainder of September, as shown in Fig. 1. Further tests of the pasture in September, October, and November resulted in a much lower survival, a probable result of ex-

posure of the already developed infective larvae to the high maximum temperatures and drought which prevailed during most of September. Only 12 *Ostertagia* were recovered from the lambs of the test made the following May, indicating, in comparison with the results of the May tests in experiments 2 and 3, that most of the free-living stages were destroyed in experiment 1 by the adverse conditions of the previous autumn.

Experiments 2 and 3, begun in mid-September and mid-October, respectively, show the relatively marked resistance to cold weather of *Ostertagia* on pasture by the recovery of 474 and 1,294 worms, respectively, from lambs in May. These results indicate much better survival over the winter than occurred under the most optimum warm weather and much shorter exposure periods in experiments 1, 4, 5, and 6. In experiment 3, infective *Ostertagia* larvae did not appear on this pasture at least until after the early part of December and likely not until the following spring. It is probable, therefore, that the pasture stages in this experiment overwintered as eggs rather than as free larvae.

In experiments 5 and 6, begun in June and July, respectively, and progressing through the summer, survival was the lowest, but became slightly higher in experiment 4, begun in mid-May, possibly because the mean temperature was lower at time of pasture contamination and shortly thereafter than in experiments 5 and 6. Although the proportionate survival of *Ostertagia* on pasture appears low during the summer in comparison with the winter season, experiments 4, 5, and 6 show that small numbers of viable larvae may survive the remainder of the summer and early fall when eggs are deposited in May, June, or July, but none are likely to carry over until the next spring, as shown by experiments 5 and 6. Therefore, overwintering of large numbers of viable free-living stages resulted from egg deposition the previous fall. It is concluded, that for the Beltsville, Md. area the mortality rate of *Ostertagia* on pasture is relatively low when cool or cold weather prevails and is much higher during warm weather.

Cooperia (curticei).—This species generally presented a survival pattern on pasture similar to that of *Haemonchus* and *Oesophagostomum,* particularly in regard to the lethal effect of overwintering, as no evidence was obtained of survival over the winter in any of the experiments, regardless of the time of pasture contamination. Survival of *Cooperia* during spring and summer was proportionately slightly higher than of *Haemonchus* and *Oesophagostomum,* as shown in experiments 4, 5, and 6, Table 3. Warm-moist weather (Fig. 1, second year) offers the most favorable conditions for development and survival, as shown particularly in experiments 4 and 6. The adverse effect of relatively cool-dry weather is clearly indicated in experiments 2 and 3; only two *Cooperia* were recovered in all test lambs in the former and none in the latter experiment. On pastures contaminated in May, June, and July only a small number of viable larvae remained by the last week in September, the majority being killed by summer exposures of $4\frac{1}{2}$, $3\frac{1}{3}$, and $2\frac{1}{2}$ months, respectively, in these experiments.

Nematodirus (spathiger, filicollis).—The survival pattern of *Nematodirus* follows closely that of *Ostertagia* in that survival is highest in the cool or cold months, and is adversely affected by summer conditions when high temperatures occur, but to a lesser extent than for *Ostertagia;* this is particularly noticeable in the data of experiments 5 and 6.

In experiment 1 the largest number of *Nematodirus* was recovered from a pair of test lambs grazed on a pasture subdivision two months after the mid-August contamination period. The favorable rainfall and temperatures for the first three weeks after pasture contamination probably allowed most of the viable eggs to develop into infective larvae but not to hatch, while the immediately succeeding

3½-week period of drought probably inhibited hatching but did not cause larval destruction on a large scale. Thereafter, the rainfall of the first two weeks of October, though small, was sufficient to cause a large number of infective larvae to hatch, and thus become available to the grazing test lambs.

Experiments 2 and 3, begun in September and October, show relatively excellent survival the following May, the proportionate numbers of *Nematodirus* recovered from the test lambs at this time in comparison to number of eggs deposited being exceeded only by Ostertagia. Proportionately more *Nematodirus* than *Ostertagia* were recovered in May in experiment 1, a fact indicative that the September period of drought and high temperature had a more lethal effect on the pasture stages of the latter genus than of the former.

Experiments 4 and 5 show that small numbers of *Nematodirus* may survive from May or June to the latter part of September, and from July to the next May, as shown in experiment 6. However, the proportionate survival under the optimum summer conditions of experiments 5 and 6 is less than for the autumn and winter periods of experiments 1, 2, and 3. It is reasonable to conclude that large numbers of *Nematodirus* are not more often found in sheep on pasture and range because of their low egg productivity, rather than of the lack of resistance of their pasture stages to rigorous climatic conditions.

DISCUSSION

An excellent summary of the relation of climate to worm parasitism in livestock has been published by Lucker (24) and need not be repeated in detail here. Also the literature on sheep nematode larval survival has been surveyed in some detail in another section of this present paper, and only certain general aspects of the relation of parasite larval survival under natural conditions to the problem of sheep parasite control will be discussed in this section.

If sheep production is to be profitable, use of all practical measures for control of internal parasites is axiomatic. In order that they may be successfully applied at the least possible cost, a thorough knowledge of the biology and geographical distribution of the various parasite species is necessary, so that proper management and treatment procedures, both for prevention of infection and destruction of parasites in sheep, may be effectively carried out.

Although reasonably efficient anthelmintic drugs are now available for the destruction, or removal from the host, of some of the more pathogenic species, not only are they expensive but also the parasites may, and often do, produce serious injury or disease before the presence of the parasites is detected and medication begun. The ideal approach to the sheep parasite control problem is prevention of infection by natural or biological means, if possible, which entails little or no cost to the producer.

Procedures for control of internal parasites depend on many factors. When a relatively large area of range or pasture is availble per sheep grazed thereon, parasites can be controlled effectively in most cases by proper pasture rotation, which takes into consideration the destructive effects of climatic conditions upon the free-living stages. However, when pastures are overstocked, emphasis must be placed on medication rather than on biological or natural control measures, but the latter should be employed as much as possible to reduce the cost of medication.

It should be emphasized that parasite control measures cannot be recommended with any certainty of effective results unless accurate information is available on the species of sheep parasites present in different geographical areas, and in specific flocks in these areas, and their seasonal distribution. Furthermore, effective control measures may be more difficult to carry out in some areas than in

others, because the species complex of internal parasites varies somewhat, both qualitatively and quantitatively, in different climatic and geographical regions. If the known facts of sheep parasite control are properly applied, it should be possible to raise sheep profitably with only minimum risks of losses from internal parasites, and incurring only nominal costs for parasite control.

Because of the great importance of the sheep industry to the economy of Australia, extensive surveys and other work have been conducted by Gordon (14) and others to develop standard procedures for sheep parasite control for the different climatic areas of that country which support large sheep populations. The recommendations in his paper are based not only on scientific knowledge of the biology of the various parasite species, but also on the seasonal distribution of parasites in sheep throughout the principal sheep raising areas of Australia. The recommendations made for sheep parasite control for various regions of Australia cannot necessarily be applied in other regions of the world with different climatic conditions, as Australia is a subtropical country not usually subjected to winter frost and generally has a low annual rainfall, while the weather in other regions of the world where sheep are raised may differ markedly from this. Other parasite control plans must be devised for these other areas to take advantage of the killing effect on the free-living stages of subfreezing temperatures, variable periods of drought, high summer temperatures, etc.

In Table 4 an attempt has been made to summarize the effects of different

TABLE 4.—*Generalized summary of the relative effect of variable weather on survival of free-living stages of some gastrointestinal nematodes of sheep on pasture and range; based upon the literature and results reported herein.*

Nematode genera	Survival under indicated weather types[a]		
	Optimum[b]	Intermediate	Minimum or None
Oesophagostomum	Warm-moist	Cool-moist	Warm-dry, cool-dry, overwinter
Haemonchus	do	do	do
Cooperia	do	Cool-moist, warm-dry	Cool-dry, overwinter
Trichostrongylus	Warm-moist, cool-moist	Cool-dry, warm-dry	Overwinter
Ostertagia	Cool-moist, overwinter	Warm-moist, cool-dry	Warm-dry
Nematodirus	Cool-moist, cool-dry, overwinter	Warm-moist, warm-dry	

[a] Weather types:

Warm-moist: Summer weather—high maximum temperature—adequate rainfall.

Warm-dry: Summer weather—high maximum temperatures—low rainfall—droughts.

Cool-moist: Early spring or late fall weather—moderate temperatures—adequate rainfall.

Cool-dry: Early spring or late fall weather—moderate temperatures—low rainfall—droughts.

Overwinter: Winters—subfreezing temperatures common—precipitation, rain or snow.

[b] Survival categories:

Optimum: Many larvae surviving two or more months during the grazing season or overwinter.

Intermediate: Many larvae surviving longer than one month but less than two months during grazing season.

Minimum: Few or no viable larvae remaining after (a) exposure of one month or less during grazing season, or (b) overwinter.

general weather types on the free-living stages of the six sheep nematode genera concerning which we now have considerable reliable information. This table may be used as a guide in recommending pasture rotation schedules when seasonal weather conditions and anticipated parasite burdens in sheep are reasonably well known. Free-living stages of *Oesophagostomum, Haemonchus, Cooperia,* and probably *Bunostomum* and *Chabertia* do not survive the winter on pasture in significant numbers in areas where subfreezing temperatures are common. Therefore, in such areas pastures rested over the winter will be relatively free of these parasites in the spring. By proper anthelmintic treatment of the ewes in the spring before they and their lambs are placed on the rested pastures, clinical parasitism in the flock during the grazing season will be considerably delayed or even absent. By adding to this procedure proper pasture rotation during the grazing season, it is possible to reduce medication to a minimum and greatly reduce the cost of sheep parasite control.

On the other hand, in areas where *Ostertagia* and *Nematodirus* are common, killing of the pasture stages by subfreezing weather cannot be depended upon as an effective control measure because of the high resistance of the larvae of these parasites to low temperatures. More reliance must be placed on medication and pasture rotation during the grazing season.

The low resistance to drought of pasture stages of most internal parasites of sheep can be used to good advantage in a well planned program of control. Larvae of *Oesophagostomum, Haemonchus, Chabertia, Bunostomum,* and, to a lesser extent, *Cooperia* and *Trichostrongylus* are rendered nonviable by exposure to drought and high temperatures on pasture and range. Of course, it is not always predictable when droughts will occur, but when they do occur for one or more weeks, pastures already extensively grazed and probably heavily contaminated with parasite eggs and larvae, should be rested as long as possible.

Recommendations of pasture and range specialists (43) for the proper maintenance, use, and improvement of grasslands are generally good procedures for sheep parasite control. Overgrazing or continuous grazing should be avoided. Moderate to light grazing and frequent movement of stock to new or rested pasture or range not only are beneficial to development of good turf and forage but also tend to reduce numbers of viable parasite larvae. It is not known how much of the increased returns to stockmen, from following pasture and range improvement policies, are the result of improved forage and how much are the result of reduction of parasitism in the stock.

SUMMARY

1. Of all the parasites studied in the experiments reported herein, free-living stages of *Oesophagostomum* and *Haemonchus* are the least resistant to weather effects on pasture. Good development and survival occur only when the weather is continuously warm and moist.

2. Free-living stages of *Cooperia* and *Trichostrongylus* are slightly more resistant on pasture to all climatic conditions than the aforementioned genera.

3. Pasture stages of *Ostertagia* are highly resistant to cold weather, survive well exposure over the winter on pasture in Maryland, and have low resistance to drought and high temperatures.

4. The free-living stages of *Nematodirus* are the most resistant to the entire annual complex of climatic conditions. Reasonably long exposures to drought, and high and low temperatures have relatively little effect on the eggs and larvae. Only *Ostertagia* compares favorably with *Nematodirus* in regard to survival over the winter months.

5. During spring and summer, when there are no significant periods of drought, even when high temperatures are common, considerable numbers of larvae of most of the common nematode parasites of sheep will survive exposures on pasture as long as three to three and one half months, with the possible exception of *Oesophagostomum.*

6. Information now available indicates that the free-living stages of the common genera of gastrointestinal nematodes of sheep rank as follows in regard to their resistance to pasture and range conditions, beginning with the least resistant: *Oesophagostomum, Haemonchus, Cooperia, Chabertia, Bunostomum, Trichuris, Trichostrongylus, Ostertagia, Nematodirus.*

7. Pastures contaminated with eggs during spring, summer, and fall and then rested over the winter until the following spring, in regions where subfreezing temperatures occur, are likely to be free or almost free of viable larvae of the following: *Oesophagostomum, Haemonchus, Cooperia, Trichostrongylus,* and probably *Chabertia* and *Bunostomum.*

8. Resting pastures over the winter will not markedly reduce the numbers of viable larvae of *Ostertagia* and *Nematodirus* in regions subject to subfreezing or higher temperatures. The most injurious effects on the free-living stages of these parasites occur during periods of drought and high temperatures in summer.

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Relative Susceptibility of Various Species of Earthworms to the Larvae of *Capillaria annulata* (Molin, 1858) Cram, 1926[1]

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INTRODUCTION

It has been demonstrated that earthworms serve as true intermediate hosts to a number of species of nematode parasites of vertebrates and that they may serve as transport hosts to at least one species. In all of these instances, more than one species of earthworm has been shown to serve in this capacity, yet there is comparatively little information on the relative importance of the various species in the transmission of these parasites. Such information would be a worth-while addition to what is already known concerning invertebrate immunity and might also have practical application if a choice must be made between having earth-

[1] The author wishes to thank Dr. Clay G. Huff, formerly professor in the Department of Bacteriology and Parasitology, University of Chicago, and now with the Naval Medical Research Institute, Bethesda, Maryland, for his interest and suggestions during the course of the experiments and in the preparation of the manuscript. Thanks are also extended to Dr. T. H. Eaton. Jr., of Southwestern College, Winfield, Kansas, for assistance in identifying some of the earthworms used in this work.

worms because of their beneficial effects in the building of soil and not having them, or certain species of them, because of the role they play in the transmission of disease-producing organisms.

The present report describes attempts to obtain such information, using as the parasite, *Capillaria annulata*, a crop worm of chickens, turkeys, and other gallinaceous birds.

C. annulata occurs in the esophagus of its definitive host and is usually found only in the dilated portion of this organ. It is not a parasite of the lumen but is deeply imbedded in the mucosa, where it forms, through its movements, numerous tortuous burrows. According to Cram (1936) the males range in length from 10 to 37 mm., and in width from 52 to 80 μ; females range in length from 25 to 80 mm., and in width from 77 to 120 μ.

Larval forms of *C. annulata* which occur in the earthworm have been described by Wehr (1936). They are about 200 μ long and possess a stylet, capillary esophagus with a cell body, and a bifurcated tail end. The writer has observed several hundred of these larvae in histological sections of an experimentally infected earthworm and practically all of them were found in the longitudinal muscles.

Despite the fact that *C. annulata* was first described almost a century ago and has a cosmopolitan distribution, little was known of its life cycle until 1936, when Wehr discovered that earthworms were essential to the transmission of this nematode. Wehr's report concerning this fact was preceded by numerous attempts both by him and by Cram (1936) to bring about infection without an intermediate host, and such attempts have been made more recently by Zucchero (1942), without success.

Wehr was successful in transmitting *C. annulata* with two species of earthworms, *Allolobophora caliginosa* and *Eisenia foetida*, and since that time no new intermediate hosts have been reported.

METHODS

The studies described in this report involved collecting the ova of the parasite, providing conditions suitable for their embryonation, collecting and identifying the earthworms, inoculating them and storing them long enough for the larvae to develop to the infective stage, feeding the earthworms to chickens, and examining the chickens for the adult parasites. In addition, cut and stained sections were made of some of the earthworms, and a few were dissected.

Ova were collected from both naturally and experimentally infected chickens. In the former, the esophagus was removed and searched for the ova-filled burrows left by the female worms. To recover the ova, it was necessary only to remove the mucosa from the remainder of the esophageal wall and, with the aid of a dissecting microscope and needles, to tease the ova out of the burrows. In the experimentally infected birds, the feces were sedimented in water and the ova recovered with a saturated solution of sodium chloride. This latter procedure would be unsafe for use on naturally infected birds, because of the danger of encountering ova of other species of capillarids that may be present in the intestine.

The ova of *C. annulata* are in the unsegmented state when first recovered, and they must be held in a suitable medium to permit segmentation and embryonation to take place. In these experiments, the ova were held in a shallow layer of tap water at room temperature, the cultures being aerated at frequent intervals by shaking. With this method, embryonation was usually complete in 30 to 40 days. The cultures were then stored at a temperature of 2 to 7° C. until they were used for inoculating earthworms.

Identification of the earthworms was made on the basis of external characters, using the key published by Eaton (1942). His nomenclature is used throughout

this report. In so far as possible, collections of the earthworms were made at various places, in order to take advantage of the use of more than one strain of each species. The species and the localities in which they were collected were as follows: *Lumbricus terrestris*, Chicago and La Grange, Illinois; *Allolobophora caliginosa trapezoides*, Arcola, Illinois, and Beltsville, Maryland; *A. caliginosa typica*, Fox Lake, La Grange, and Palos Park, Illinois; *A. longa*, Chicago, Illinois; and *Eisenia foetida*, Chicago, Illinois. Thus, there were four species, and, of one of these, two varieties.

The earthworms were usually stored in the laboratory in soil collected at the same time and place as the earthworms. If soil from another source was used, it was first sterilized in an autoclave, in order to destroy any helminths or ova that might be present. As a rule, most of the earthworms survived the holding period, but occasionally deaths occurred from unknown causes.

Inoculation of the earthworms was accomplished by injecting embryonated ova into the oral cavity with a capillary pipette. This pipette was marked in such a way that the same volume of inoculum was given to each earthworm of a series, the approximate number of ova per inoculum having been determined by a dilution count. Prior to the time the ova were used in inoculations, they were tested for viability by an *in vitro* procedure described by Morehouse (1944). This investigator observed that when the filtered digestive fluid of an earthworm is added to viable ova of *Capillaria caudinflata*, the contained larvae become active and many of them break out of the egg within a few minutes. The writer has observed a similar phenomenon with respect to the ova of *C. annulata* and has used the test extensively in the present work.

Following inoculation, the earthworms were returned to the containers of soil, where they were held for a period of time considered sufficient for the development of the larvae to a stage infective to the definitive host. They were then fed to chickens. The chickens were then held for a sufficiently long time for any *C. annulata* larvae present to develop to the adult stage. In order to examine the chickens for these adult stages, the esophagi were removed, opened, and pressed between heavy glass slides. These were examined with a dissecting microscope, using the medium power. If the parasites were few in number and well isolated, they could be counted while they were still in the mucosa. In a few cases, however, removal of the parasites was necessary in order to obtain an accurate count. This was carried out by peeling off the mucosa and extracting the worms with dissecting needles.

Histological sections of earthworms were made in the following manner: After the earthworms were held in containers of moist paper for a few days in order to remove the soil and grit from the digestive tract, they were extended and nacotized in water to which 95 percent alcohol was added, drop by drop, until the concentration reached 10 percent. When the specimens no longer responded to external stimuli, they were removed from the alcohol and placed in Bouin's fixative. Portions of these earthworms were subsequently imbedded in paraffin and serially sectioned, using a thickness of 10 μ. Staining was accomplished with Bullard's hematoxylin and eosin.

A few of the earthworms were dissected to determine the extent of infection. They were placed in physiologic saline and cut into small pieces with a scissors. The pieces were then teased apart and macerated with dissecting needles to free as many larvae as possible. However, because of the exceedingly small size of the larvae, it was necessary to give the earthworm tissues further treatment by digesting them in artificial gastric juice. This material consisted of a mixture of 0.75 g. of pepsin, 0.9 g. of sodium chloride, 300 cc. of tap water, and 3 cc. of hydrochloric acid. The process was carried out at about 37° C., with constant agitation.

Digestion was usually complete in about one hour. The material remaining after digestion was concentrated by centrifugation, and this, as well as the sediment from the original macerations of the earthworms, was examined.

EXPERIMENTS

Series 1

The first series of experiments was concerned primarily with attempts to demonstrate the larvae in histological sections of experimentally infected earthworms. As a partial confirmation of the identity of such larvae, earthworms which had been inoculated at the same time and with the same culture of ova as the sectioned ones were fed to chickens. In this series, each earthworm was inoculated with about 400 ova.

In the transmission test, a period of 30 days was allowed for the parasites to develop in the earthworms to a stage infective to chickens. The chickens were 30 days old at the time the earthworms were fed to them. The species, strain, and number of earthworms, and the number of chickens receiving them, were as follows: *L. terrestris,* Chicago strain, 3 to each of 2 chickens; *A. caliginosa typica,* Fox Lake strain, 3 to each of 2 chickens; and *E. foetida,* 4 to each of 2 chickens. Control earthworms were fed as follows: *L. terrestris,* 4 to 1 chicken; *A. caliginosa,* 6 to 1 chicken; and *E. foetida,* 6 to 1 chicken.

The chickens were examined 24 days after having been fed the earthworms, and none was found to be infected. The histological sections likewise showed no capillarids. A total of 516 sections were examined, and these consisted of 5- and 10-day-old infections of all 3 species of earthworms.

Series 2

This series involved the dissection of experimentally infected earthworms, each of which had received several hundred embryonated ova 70 to 90 days prior to dissection. They included 1 *L. terrestris,* Chicago strain, 8 *A. caliginosa typica,* Palos Park strain; and 6 *E. foetida.*

The total numbers of larvae recovered were, respectively, 100 (estimated), 1, and 2. Dissection of the same number of control earthworms of each species did not reveal any larvae.

Series 3

Most of the earthworms in this series were used in attempts to transmit the infection to the definitive host; a few were dissected. The transmission tests involved *L. terrestris,* La Grange strain; *A. caliginosa trapezoides,* Arcola strain; *A. caliginosa typica,* Palos Park strain; and *E. foetida.* Each earthworm was inoculated with about 450 ova. The time between the inoculations and the feeding of the earthworms to the chickens was either 35 or 51 days. In the 35-day group, 6 earthworms of each species were fed to a half-grown chicken; in the 51-day group, 4 of each species were fed to a half-grown chicken. Four control earthworms of each species were likewise fed to a half-grown chicken.

The chickens were examined after 21 days. The numbers of parasites found were as follows: 35-day group: *L. terrestris,* 34; *A. caliginosa trapezoides,* 5; *A. caliginosa typica,* 1; and *E. foetida,* 0. In the 51-day group, only the chicken to which had been fed *L. terrestris* became infected, and it harbored 5 of the parasites. None of the birds fed control earthworms became infected.

Dissections were made of 2 earthworms of each of the species *L. terrestris, A. caliginosa trapezoides,* and *E. foetida* from 43 to 50 days after inoculation. The total numbers of larvae found were, respectively, 7, 6, and 0. No controls were

available for *L. terrestris*, but 2 *A. caliginosa trapezoides* were dissected and found to be free from infection.

Series 4

Transmission experiments and dissections were also carried out in this series, which involved *L. terrestris*, Chicago strain; *A. longa* and *A. caliginosa typica*, Palos Park strain; and *E. foetida*. The procedure was similar to that used in Series 3, with the following exceptions: Each inoculated earthworm received about 1,000 ova, and the numbers of earthworms fed to the chickens were 4 for each of the species *L. terrestris* and *E. foetida* and 3 for each of the other 3 species. A developmental period of 34 days was allowed in the earthworms. Controls of the species *A. caliginosa typica* and *E. foetida* were not fed to chickens in this series, since other members of these groups had been previously found to be uninfected.

Only the chicken which had been fed inoculated *L. terrestris* became infected, and it harbored 7 worms. No larvae were found in the dissection of 2 inoculated earthworms of each of the species.

TABLE 1.—*Numbers of* CAPILLARIA ANNULATA *larvae recovered in the dissection of experimentally infected earthworms.*

Species of Earthworm	Source	No. Dissected	Total No. Larvae[a]
L. terrestris	Chicago, Ill	3	100[b]
L. terrestris	La Grange, Ill.	2	7
A. caliginosa trapezoides	Arcola, Ill.	2	6
A. caliginosa typica	Palos Park, Ill.	10	1
A. longa	Chicago, Ill.	2	0
E. foetida	Chicago, Ill.	10	2

[a] Controls were uniformly negative (see text).
[b] Estimated.

Series 5

This series involved only the 2 strains of *A. caliginosa, typica* and *trapezoides*. The former was the La Grange strain and the latter the Maryland strain. The number of eggs per inoculum was about 1000. Five earthworms of each variety were inoculated, and, 35 days later, were fed to a half-grown chicken. Two additional chickens received 5 control earthworms of each variety. Twenty-three days later the chicken which had been fed inoculated *A. caliginosa trapezoides* harbored 3 *C. annulata*, while the chicken which had been fed the other variety harbored 1. The two control chickens were found to be free from the parasites.

Series 6

This series was carried out with the same two varieties used in Series 5. Also, the inoculum was the same as in that series. The developmental period in the earthworm was slightly longer (43 days). Only 2 inoculated earthworms of each variety were fed to chickens, both of which were free from infection 21 days later.

DISCUSSION

Inspection of the pertinent data, which are given in Tables 1 and 2, shows that of the various species and varieties used in these experiments, *L. terrestris* was the most efficient transmitter, and it yielded more larvae on dissection than any of the

other species. This is of interest because *B. terrestris* has not previously been reported as an intermediate host for *C. annulata*. Next in importance was *A. caliginosa trapezoides*, followed by *A. caliginosa typica*. No infections were transmitted with either *A. longa* or *E. foetida*, although larvae were found in dissections of the latter species.

Only a small number of *A. longa* were available for use in these experiments. Therefore, the lack of any indication of infection in them in this work is of little significance.

That *E. foetida* can transmit *C. annulata* has been demonstrated by Wehr (1936) and by the writer (unpublished data). However, there was evidence in both of these demonstrations that *E. foetida* is somewhat refractory to infection as compared with *A. caliginosa*, and this may partially explain the failure of *E. foetida* to transmit infections in the present experiments. Also of interest in this connection is the fact that Morehouse (1944) was unable to transmit *C. caudiflata*, an intestinal capillarid of birds, with *E. foetida* or *L. terrestris*, but he was able to transmit it with *A. caliginosa*. However, Wehr and Allen (1945) showed that

TABLE 2.—*Numbers of adult* CAPILLARIA ANNULATA *recovered from chickens fed experimentally infected earthworms of various species, varieties, and strains.*

Species of Earthworm	Source	No. Fed to Chickens	No. Chickens Fed	No. Chickens Infected	Total No.[a] of Parasites
L. terrestris	Chicago, Ill.	10	3	1	7
L. terrestris	La. Grange, Ill.	10	2	2	39[b]
A. caliginosa trapezoides	Arcola, Ill.	10	2	1	5
A. caliginosa trapezoides	Beltsville, Md.	7	2	1	3
A. caliginosa typica	Fox Lake, Ill.	6	2	0	0
A. caliginosa typica	Palos Park, Ill.	13	3	1	1
A. caliginosa typica	La Grange, Ill.	7	2	1	1
A. longa	Chicago, Ill.	3	1	0	0
E. foetida	Chicago, Ill.	22	5	0	0

[a] Controls were uniformly negative (see text).
[b] The two infected chickens harbored 34 and 5 parasites.

E. foetida could transmit *C. caudinflata*, and, as pointed out above, *L. terrestris* has been found to be more susceptible to *C. annulata* than any of the other species used in the present work.

It was mentioned in the introduction to this paper that there is little information available concerning the comparative susceptibility of the various species of earthworms to the parasites they transmit. The work of Clapham (1934) with *Syngamus trachea*, a nematode parasite of birds, reveals some interesting facts with respect to earthworm transmission. It should be pointed out that the parasite-intermediate host relationship in the case of *S. trachea* differs from that of *C. annulata* in that the earthworm is not an obligatory intermediate host. Clapham demonstrated conclusively that *E. foetida* was more important in the transmission of *S. trachea* than was *L. terrestris*, as revealed by the number of mature parasites in birds which had been fed experimentally infected earthworms of these two species. However, in Clapham's work, the earthworms were not inoculated but

were placed in soil containing the parasite ova. Such a method of infection depends on the voluntary ingestion of the soil by the earthworms, and may introduce a variable due to a difference in the feeding habits of the two species of earthworms. It would be interesting to know whether such differences actually exist.

As for the results reported in the present paper, it seems questionable whether the differences between the various species and varieties are significant as reflected by the number of larvae found in dissections and the number of parasites transmitted. While the differences are quite marked, and are uniform in that the different strains of the individual species exhibited susceptibilities of a similar order, it is felt that definite conclusions should await more extensive experiments, in which infections of a greater intensity are obtained. The infection in these experiments were of unusually low intensity, and there was an indication that such would be the case, because at no time did a high percentage of the larvae hatch from the ova in the *in vitro* viability tests. This may mean that only a few were released from the ova in the digestive tracts of the earthworms. It is probable that this factor, as well as factors concerned with the immunity of the intermediate and definitive hosts, was responsible for the low intensity of the infections.

SUMMARY

1. Experimens were conducted to determine the relative susceptibility of various species and varieties of earthworms to the larvae of *Capillaria annulata*. They were *Lumbricus terrestris, Allolobophora caliginosa trapezoides, A. caliginosa typica, A. longa* and *Eisenia foetida.*

2. The data, although limited, seem to indicate that *L. terrestris* was the most susceptible, with *A. caliginosa trapezoides, A. caliginosa typica,* and *E. foetida* following, in that order. There was no evidence that an infection was produced in *A. longa.*

3. *L. terrestris* is reported for the first time as an intermediate host for *Capillaria annulata.*

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The Effects, *in Vitro*, of Certain Antibiotics on the Growth of *Trichomonas foetus*

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INTRODUCTION

The investigation of the effects of antibiotic drugs upon the parasitic protozoa has been, for the most part, rather haphazard. As new antibiotics become available, drugs and organisms are quickly tested *in vitro* and, if the results are promising, then *in vivo* examinations are made. In the field of medical bacteriology, the antibiotics have been and are undergoing exhaustive study, and much is being learned about the mode of action of those drugs upon the pathogenic bacteria. The fruit of this work should be a pattern of broad, general physiological rules from which accurate predictions can be made, and which will enable biologists, searching for new drugs, and chemists, synthesizing new drugs, to reach their goals via predictable paths. This investigation is a minor contribution toward this same state for medical protozoology.

Trichomonas foetus was chosen as the test organism for this study because it was a fairly representative flagellate, could be obtained in pure culture, and could be grown easily in a number of previously described media. Also, it was known to be sensitive to at least some antibiotics. Williams and Plastridge (1946) found that clavacin, gramicidin and actinomycin were toxic to *T. foetus* when added in concentrations sufficient to control the growth of bacteria in mixed cultures. Morgan and Campbell (1946), in testing 350 compounds for possible trichomonacidal effects, found that penicillin in a concentration of 8.0% killed *T. foetus* in one minute, and that tyrothricin in a concentration of 0.03% also killed *T. foetus* in one minute.

The purpose of this research is to investigate the physiological effects of certain efficacious antibiotics on *T. foetus* with the eventual goal of learning more about the mode of action of the antibiotics in general upon the parasitic protozoa.

MATERIALS AND METHODS

Culture Media.—The stock cultures of *T. foetus* were maintained as described by Cole (1947), on a modification of Schneider's (1942) citrate medium. The experimental organisms were grown in a fluid medium suggested by Plastridge (1943). It is essentially a beef-infusion with added nutrients, heat-inactivated bovine serum, and a trace of agar.

Organisms Employed.—The trichomonad used in this study was *Trichomonas foetus*[2], strain BR, originally isolated by Morgan and Wisnicky (1942) from a cow suffering from a trichomonad pyometra and since kept in pure culture on the modified Schneider's medium.

Dilution and Distribution of the Drugs.—Most of the drugs were obtained in sterile vials, while a few were obtained in bulk form. In the case of the former,

[1] A contribution from the Department of Zoology and Botany, The Catholic University of America, Washington, D. C. This paper, prepared under the direction of Dr. E. G. S. Baker, as Major Professor, and Mr. William F. Simpson and Dr. Benjamin G. Chitwood, as readers, is based on the author's dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The complete numerical tables and other charts and graphs may be found in the original thesis which has been microcarded and is available from the author.

[2] The culture was supplied by Dr. Banner Bill Morgan, University of Wisconsin, Madison, Wisconsin.

the diluting was accomplished in the original sterile vial; in the latter, diluted doses were sterilized by filtering through a Seitz filter. Experimental medium was employed as the solvent. One ml. portions were transferred to tubes containing 9.0 ml. of medium, each tube thus contained 10.0 ml. of medium with the desired concentration of the drug.

Conditions of Growth.—Experimental tubes were inoculated with sufficient stock culture to give an initial population of approximately 250,000 active trichomonads per ml. This was accomplished by the addition of 0.4–0.6 ml. of the stock culture, which at 48 hours (in the logarithmic phase) had a population of 5,000,000 to 6,000,000 trichomonads per ml. Every procedure, with the exception of the counting, was performed aseptically. Bacterial contamination was tested for daily by inoculating loopfuls of the culture into nutrient broth and thioglycollate medium. At the end of every series, stained films were examined. Any contaminated cultures were discarded and the work repeated. All cultures were incubated at 37° C ± 2° C in uniform 18 × 150 mm. Pyrex tubes.

Determination of Population Density.—The population counts were made with a Levy counting chamber. After thorough rotation of the culture tubes, 0.5 ml. samples were withdrawn and were added to 5.0 ml. of a 5.0% solution of formaldehyde. To this volume was added 4.0 ml. of physiological saline. After mixing thoroughly, a sample was withdrawn and pipetted under the cover glass of the counting chamber. A count was made of all the organisms in each of the four 1.0 sq. mm. sections and the average of these four readings was taken. The count per ml. was obtained by multiplying this figure by the total correction factor, 100,000. The average of the counts of both sides of the hemocytometer was taken as the final count. All experiments were performed in duplicate and 14 samples were counted for each determination. The figures obtained agree to within ± 5.0%, exceeding that number no more than once in twenty times.

At the time 0.5 ml. samples were withdrawn from the culture tubes for counting, a separate portion was examined under the microscope. If approximately half the organisms were not motile, the culture was considered "non-motile" and counting was discontinued. However, where cultures are referred to as "sterile," no motile organisms were observed.

Drugs Used.—The drugs[3] used in this study were 1. Streptomycin, 2. Bacitracin, 3. Aureomycin, 4. Borrelidin, 5. Polymyxin A (Aerosporin), 6. Polymyxin B, 7. Polymyxin D (B-71), 8. Circulin (Antibiotic Q-19), 9. Chloramphenicol (Chloromycetin), 10. Acti-dione, 11. Aureotracin and 12. Terramycin.

Sugar Determinations.—The Hagedorn and Jensen (1923) iodometric method as described in Gradwohl (1935) was used for the determination of glucose, but certain modifications were employed. Instead of the standard inoculum of 250,000 motile organisms per ml., an inoculum of 1,000,000 per ml. was employed so that the peak population would be reached in about 24 hours (as compared with 48 hours for the smaller inoculum) and would be slightly higher. These conditions, a higher peak in a shorter time, were more favorable for the study of the glucose consumption. Another modification was the introduction of yeast digestion controls. Since the method depends on reduction of the ferric ion to the ferrous ion, false results caused by reducing substances other than glucose must be eliminated. The following control tests were used: medium being tested plus yeast to determine the amount of reducing substance other than glucose in the medium; yeast plus a solution of known sugar to test the efficiency of the yeast

[3] All the drugs used in this study, with the exception of Acti-dione, were supplied by Dr. Henry Welch, Chief, Antibiotics Division, Food and Drug Administration, Washington, D. C. The Acti-dione was obtained from Dr. Alma J. Whiffen, Antibiotics Research Department, The Upjohn Company, Kalamazoo, Mich.

in digesting sugar; yeast plus distilled water to determine the amount, if any, of reducing substances present in the yeast itself. All sugar determinations were made in duplicate on duplicate cultures; the tests were made at 37° C., and bacteriological sterility was tested as each tube became negative. Results from contaminated tubes are not included in the data presented.

Miscellaneous Equipment.—Surface tension measurements were made with a DuNouy tensiometer. Optical density readings were taken with a Lumetron Photoelectric Colorimeter. Determinations of pH were made with a Beckman pH meter.

RESULTS AND DISCUSSION

The first phase in this study was the screening of available and selected antibiotics against *T. foetus*. It was necessary to establish, somewhat empirically, standards by which the drugs under examination could be evaluated. A standard inoculum of 250,000 active trichomonads per ml. was employed throughout the course of the study, unless otherwise stated, and the "end point" was defined as the lowest concentration of the drug in the presence of which the standard inoculum was rendered sterile within the 24 hour examination period. Any drug whose end point was 50 micrograms or less per ml. of culture medium was considered promising enough to warrant further study.

TABLE 1.—*Results of the screening of 12 antibiotics against* T. FOETUS

Antibiotic	Number of trials	End point in μg/ml.
Streptomycin	12	1000–2000
Bacitracin	22	No inhibition.
Aureomycin	7	Impure 25 Pure 100
Polymyxin A	3	50–75
Polymyxin B	3	100
Polymyxin D (B71)	4	1200
Circulin (Antibiotic Q-19)	3	250
Chloramphenicol (Chloromycetin)	3	500
Aureotracin	3	No end point. Ineffective at maximum solubility-20 μg/ml.
Acti-dione	3	500–750
Borrelidin	3	5
Terramycin	3	500–1000

Of all the antibiotics known, only 12 were chosen for investigation. Many are rare and exist only as laboratory curiosities. Many are so toxic that one might as well investigate the therapeutic possibilities of sodium cyanide. Still others are soluble only in alcohol or other organic solvents and this would serve to introduce an additional factor into an already complex nutritive picture. The 12 antibiotics chosen are water soluble, many of them are in commercial production, and, with one exception, are fairly non-toxic. The results of the screening are shown in Table 1. The results obtained with streptomycin, circulin, chloramphenicol, aureotracin, acti-dione and terramycin did not warrant further investigation. Borrelidin, although giving a very low end point, turned out to be too toxic to continue studying. Its toxic qualities were learned after the drug had been screened, and the results have been tabulated and reported for general interest.

Aureomycin.—With the exception of the aforementioned borrelidin, aureomycin proved to be the most effective of all the drugs tested. No chemical information is available on this important new antibiotic other than that it is a yellow

crystalline, amphoteric substance, stable in solution at pH 2.5, but not at pH 8.5. Its empirical formula is known and is given as C-51.84, H-5.24, N-5.46, Cl-13.27 and O-24.19 by Broschard and others (1949). However, its antibacterial, pharmacological and therapeutic properties have been described in detail (Ann. N. Y. Acad. Sci. 51: 175–342. 1948) and there is ample clinical evidence on record attesting its effectiveness, particularly in human rickettsial diseases.

Aureomycin was effective against *T. foetus* in concentrations down to and including 25 μg/ml., despite the fact that both blood and serum, which are normal constituents of the test medium, exert an antagonistic effect on its activity, as shown by Chandler and Bliss (1948).

Cailleau (1934) has shown that *T. foetus* can attack the following carbohydrates with good to fair acidification of the basal medium resulting: glucose, galactose, lactose, levulose, maltose, saccharose, raffinose, inuline, dextrine and amidon solution. In order to ascertain whether or not aureomycin would have the same effect on *T. foetus* in the presence of sugars other than glocose, other sugars were tested by adding them to the base medium in the same percent concentration as glucose and examining the resultant growth curves. It was necessary to determine which sugars could be substituted for glucose and the result of this study showed that maltose, raffinose, sucrose and d-mannose were equally as good as glucose; that melezitose, d-galactose and melibiose were satisfactory but not as good as glucose. The cultures lasted as long as the glucose control, but reached approximately 50% of its peak population. With none of the previously listed sugars was the activity of aureomycin against *T. foetus* altered in any way; neither enhanced nor inhibited; the same growth picture and end point were obtained in each case.

Some sugars were found to be quite inferior to glucose in supporting growth of *T. foetus* in that very low population maxima were obtained and in some cases the growth curve was of very short duration, $\frac{1}{2}$ to $\frac{2}{3}$ as long as the glucose control. Cellobiose, l-xylose, d-xylose, l-arabinose, rhamnose and d-mannitol were in this group.

A very interesting set of curves was obtained with the medium containing trehalose. Although the peak population reached was only about 55% of the control and was attained 48 hours after the control peak had been reached, the culture lasted almost twice as long. Calculations of the area under both curves and of the number of organism-hours supported reveals that trehalose is slightly superior to glucose. Trehalose is a non-reducing glucose-alpha-glucoside with a 1,1 linkage and this chemical configuration might well be the answer to the character of the growth curve obtained; namely, that *T. foetus* causes trehalose to be hydrolyzed very slowly, thereby releasing glucose molecules in slow but steady numbers upon which the organism can thrive. The number of moles available at any time may not be sufficient for the optimum growth of *T. foetus*, which would explain the low peak attained. If we may assume that the organism has a more specific need for carbohydrates than a general protein-sparing action, a slower hydrolysis of trehalose would explain the longevity of the culture, the effect being one of prolonging and rationing, as it were, the existing carbohydrate supply. This, of course, is strictly conjecture but the explanation, at the moment, seems logical.

Next, the glucose consumption of the organism was determined. Andrews and von Brand (1938) proved, by direct quantitative methods, that *T. foetus* consumes glucose, as had been previously inferred by other investigators, and that the rate of glucose consumption was not uniform throughout the course of a cultural cycle. It was high (averaging over 350 mgms. per billion in 24 hours) while the average number of trichomonads per 24 hours was increasing, and it

was low (averaging under 200 mgms. per billion in 24 hours) while the average number of trichomonads per 24 hours was decreasing. The results obtained in these experiments agree with the aforementioned findings of Andrews and von Brand except for the fact that higher values were obtained, especially in the actively increasing phase of the growth cycle. During the first 24 hours when the population was increasing, the glucose consumption was 557 mgms. per billion, and during the second 24 hours, when the population was decreasing, the glucose consumption was 207 mgms. per billion. These differences may be explained by the fact that a different strain of *T. foetus* was used in this study, that the composition of the medium was different, being much richer in peptone, beef infusion, etc., and that the concentration of glucose was much higher than in the previously mentioned work of Andrews and von Brand.

At the same time these tests were made on the control, similar assays were made on a set of cultures exposed to a sub-inhibiting dose of aureomycin, 10–15 μg/ml. The results of this series of tests show that aureomycin does not affect

TABLE 2.—*Sugar consumption of* T. FOETUS

	Average no. organisms per first 24 hrs. in millions/ml.	Mgms glucose consumed per billion organisms per first 24 hours	Average no. organisms per second 24 hrs. in millions/ml.	Mgms. glucose consumed per billion organisms per second 24 hours
Control				
Mean	2.40	557	3.64	207
Std. Dev.	0.24	71	0.45	51
Aureomycin				
Mean	1.96	503	3.25	226
Std. Dev.	0.31	63	0.84	54
Bacitracin				
Mean	2.04	396	4.20	373
Std. Dev.	0.30	54	0.34	58

the glucose consumption of *T. foetus*. During the first 24 hours, the consumption was 503 mgms. per billion as compared with 557 mgms. for the control, and for the next 24 hours the consumption was 226 mgms. per billion as compared with 207 mgms. The total consumption for the two 24 hour periods was 764 mgms. per billion for the control as against 729 mgms. per billion for the aureomycin-exposed organisms. These results are close enough, considering the error of the assay, to show that aureomycin has no effect on the glucose consumption of *T. foetus*. The results of the sugar consumption tests are summarized in Table 2.

It was observed that after exposure to aureomycin in sub-inhibiting doses, the organisms became broader and rounder. A 48-hour culture was examined after exposure to a sub-inhibiting dose and measurements were made of the organisms. They measured 16.3 $\mu \times 11.7$ μ as compared with 16.1 $\mu \times 6.7$ μ for the controls. This swelling could have been caused by interference with membrane permeability, resulting in the accumulation of water, or the aureomycin might have interfered, in some unknown manner, with the ability of the organisms to divide at the normal rate. An amount of this 48-hour exposed culture, sufficient to yield an initial count of 500,000 organisms per ml., was transferred to a new tube of medium, and the same was done for a 48-hour control. Readings were taken with a photoelectric colorimeter using distilled water for the zero point and uninoculated medium for the blank. The blank reading (in units of optical

density) was 2.1, the reading obtained with the control tube was 2.3, while the value of the tube containing swollen, aureomycin-exposed organisms was 2.5. If the swelling was due to the accumulation of water, no such appreciable difference in optical density should have been observed. This difference could only have been caused by the presence of additional "protoplasm" per organism and this hints strongly that the organisms were, in some manner, prevented from dividing. Chandler and Bliss (1948) have shown that aureomycin is bacteriostatic rather than bactericidal in its effect and it is possible that this holds true for its effect on *T. foetus.*

Enough of the 48-hour culture of aureomycin-exposed organisms was transferred to a tube of fresh medium to make a final concentration of 500,000 per ml., and the same procedure was followed with a control tube. In the low concentrations used, the aureomycin held at 37° C was completely inactivated after 48 hours, and if any trace was left, it would have been highly diluted in the transfer. So it can be assumed that the organisms which were aureomycin-exposed and which exhibited the swelling phenomenon were now present in an aureomycin-free environment. Counts were made every 24 hours. Th aureomycin-exposed organisms showed a steeper slope during the first 24 hours than the control. This would seem to indicate that the trichomonads were prevented from dividing and that after transfer to an aureomycin-free environment, they divided very quickly,—having been ready to do so for some time previously, and then return to a normal cycle and growth rate. Fisher and Stern (1942) have studied the effect of ethyl carbamate and urethane on oxygen consumption and cell multiplication in yeast. They hold that total respiration of the yeast is made up of several components that are not equally sensitive to urethane; that the component which is most sensitive to urethane is also the component which furnishes the energy for cell division. Gale and co-workers (1948) have shown that penicillin does, with certain bacteria, block the assimilation of glutamic acid. Sub-sterilizing doses of aureomycin have not interfered with glucose consumption but have inhibited, somewhat, at least, cell division. This would suggest some system of multiple, parallel metabolic paths, all of which must function to permit both synthesis and division. On the other hand, Nickerson (1948) suggests that cell division in micro-organisms is under the control of a unit enzymatic mechanism. Assuming this to be true for *T. foetus,* the action of aureomycin may be that of simply blocking, neutralizing or destroying such an enzyme.

In the earlier experiments, a fluffy light-yellow, easily soluble and relatively impure[4] type of aureomycin was used and it was with this drug that the 25 μg/ml. end point was obtained. Later, a recrystallized, dark-yellow, less soluble and pure product was used, but in this case the end point was somewhat under 100 μg/ml. Welch, Randall and Price (1947) in *in vivo* studies, demonstrated that a "factor" existed in the non-penicillin residue of amorphous penicillin which had no activity itself but which enhanced the activity of the drug against certain bacteria under test. Cole (1947) demonstrated the same effect, *in vitro,* against *T. foetus.* The possibility existed that an enhancement factor might be present in the impurities of aureomycin. An amount of aureomycin was added to a tube in the same concentration as the end point, namely, 25 μg/ml. This aureomycin was of the impure type and had been previously heat-inactivated. It had no effect on the standard inoculum. This showed, of course, that the degradation products and breakdown products of aureomycin have no effect on *T. foetus.* If, however, these degradation products contain a "factor" that enhances the activity of the aureomycin, then adding them to the pure drug should enhance its

[4] About 70% pure, the remainder being degradation and breakdown products.

activity by decreasing the end point from 100 μg/ml. to 25 μg/ml. Upon performing this experiment, though, it was found that the activity was not enhanced. The impure aureomycin either contained no "factor," or else it was destroyed by heat when the drug was heat-inactivated. At the present time, there is no aureomycinase or other means of inactivating this antibiotic without the use of heat, so the possibility of an enhancement factor must remain unanswered.

Bacitracin.—An interesting set of data was obtained from the bacitracin-exposed trichomonads. Not only were the peak populations higher by as much as 44%, but the life of the cultures was extended by from 48 to 72 hours.

It is clear, from the multitude of data collected, that the bacitracin effect is the same regardless of original inoculum; the optimum stimulating concentration is 5–6 mg./ml. The effect is not simply that of a growth stimulant. The observed effect could have been caused by one or both of the following: lowering the surface tension of the surrounding medium thereby making food supplies more completely available for consumption, or the use of the bacitracin or some portion of it as an additional metabolite.

The source organism of bacitracin is an as yet unidentified bacillus originally isolated from the infected tissue of a bone fracture case and reported by Johnson, Anker and Meleney (1945). The antibiotic is found in the filtrate of static cultures of this organism and can be extracted with n-butanol. That baci-

TABLE 3.—*Effect of various surface active agents on the base medium as compared with bacitracin*

Solutions tested	Surface tension in dynes/cm.	Survival
Medium control	50.8	Normal
Bacitracin	42.1	Normal
Tween 20	38.6	None
Tween 60	43.6	Fair
Tween 80	43.9	Fair
Sodium taurocholate	42.8	None
Tri-sodium phosphate	49.1	None

tracin has polypeptide character is evident from recently reported countercurrent distribution and starch column chromatographic studies by Barry, Gregory and Craig (1948). The major component demonstrable in the material examined by the former procedure, contained 83% of the solids and all of the biological activity; chromatography of the hydrolysate of this portion revealed the following amino acid composition (grams per 100 grams of substrate): phenylalanine, 11; leucine, 9; isoleucine, 22; glutamic acid, 10; aspartic acid, 17; lysine, 9; histidine, 10; cystine, 14. Definitely absent were methionine, valine, threonine, serine, proline and arginine.

In addition, it is known that bacitracin is a very effective surface active agent. Just how effective was not apparent until it was tested against several other known surface active agents. The latter were added to separate tubes of medium in final concentrations of 6 mg./ml., the optimum concentration for the bacitracin effect. However, at this concentration, all of the agents tested were quite toxic. The results are shown in Table 3.

It can readily be seen that bacitracin is a better surface active agent than any of the others tested, with the exception of Tween 20[5]. In order to establish or eliminate the effect of surface tension alone as the causative agent of the

[5] The Tweens used in this work were kindly supplied by the Atlas Powder Company, Wilmington 99, Delaware.

observed phenomenon of higher peak and longer duration, the following experiment was performed: dilutions were made of all the surface active agents tested in order to determine the highest concentrations in which the growth of the trichomonads was not inhibited. After this was determined, measurements were made to determine just how much the surface tension was lowered in each case. Finally, for each agent, a determination was made of the amount of bacitracin needed to produce a corresponding reduction in surface tension. The results are shown in Table 4. Comparisons were made between the growth curves obtained in the presence of this amount of surface active agent and in the presence of the amount of bacitracin found necessary to give the same surface effect. In no case did the surface active agents produce the picture caused by the bacitracin. In all cases, the cultures containing the surface active agents followed the controls as previously determined. This demonstrates that surface action alone is not the cause of the enhanced growth curve.

TABLE 4.—*Comparison of the concentrations of bacitracin and surface active agent needed in order to lower surface tension an equivalent amount*

Compound used	Highest conc. in which *T. foetus* grows as well as in the control	Surface tension in dynes/cm.	Amount of bacitracin necessary to lower surface tension an equivalent amount
Medium control	.....	51.5	.....
Tween 20	0.2 mg./ml.	42.2	0.54 mg./ml.
Tween 60	0.2 mg./ml.	47.4	0.12 mg./ml.
Tween 80	0.6 mg./ml.	45.5	0.18 mg./ml.
Sodium taurocholate	0.3 mg./ml.	45.5	0.18 mg./ml.
Tri-sodium phosphate	1.5 mg./ml.	50.1	0.05 mg./ml.

Determination of the glucose consumption by bacitracin-exposed organisms produced the following results: during the first 24 hours, the consumption was 396 mgms. per billion and during the second 24 hours, the consumption was 373 mgms. per billion. At face value, they are different from the control. However, the two sets of results cannot be evaluated by 24-hour periods because for the first 24 hours, the rate of growth of the bacitracin-exposed trichomonads is slightly depressed, probably due to a concentration effect caused by the addition of the relatively large dose of 6 mg./ml. During the second 24 hours when the control culture is declining, the bacitracin-exposed group is still actively increasing to its peak, which is higher and comes later than the control. The first 24-hour period represents the entire logarithmic phase of the control but only half of the logarithmic phase of the bacitracin-exposed trichomonads. A truer comparison would be the total amount of glucose consumed for the 48 hours. When these figures are examined, it is found that the total glucose consumption of the control is 764 mgms. per billion and for the bacitracin-exposed is 769 mgms. per billion. From these data it may be concluded that bacitracin probably does not change the glucose consumption of *T. foetus,* and this is further proof that surface tension alone is not the causative agent of the observed effect of bacitracin. If lowering of surface tension was significant, the existing food supply would be made more completely available to *T. foetus* and it might be expected that the glucose consumption would be increased. This is not the case. Also, if the food supply was consumed at a faster rate, then the cultural cycle should be a shorter one, instead of the observed longer one. The only logical conclusion is that bacitracin primarily supplies additional metabolite to the organisms. In view of the fact that the antibiotic is a polypeptide by nature, this view would not seem too unreasonable.

That the bacitracin is not simply a growth stimulant is further shown by the following consideration. With food and other environmental conditions equal, a growth stimulant causes a culture to present a different growth curve picture; namely, a steeper slope and shorter duration than the control. This, as shown, is not the case. Rather there is a slight depression during the first 24 hours of the cycle. The reason for this is probably a concentration effect, in that the addition of 6 mgms. per ml. of bacitracin, which is about 80% polypeptide, increases the amino acid content of the medium by about 50%.

Finally, substitution of the amino acids known to be present in the bacitracin molecule in concentrations equivalent to that resulting from the addition of 6 mg./ml. of bacitracin resulted in approximately the same growth curve picture, namely, higher peak and longer duration.

Polymyxin.—The most useful information, from the standpoint of immediate practical application, was the observation that *T. foetus* is selectively sensitive to polymyxins A and B, with end points of 50–100 μg/ml.; while it is relatively resistant to polymyxin D, the end point being 1000–1250 μg/ml. These polymyxin fractions show relatively the same bacterial spectrum, and are usually distinguished from each other only by amino acid hydrolysis and identification. The tenfold difference in sensitivity that *T. foetus* shows between fractions A and B, and fraction D, suggests that *T. foetus* provides the basis for a simple method of identifying polymyxin D.

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SUMMARY

1. Twelve antibiotics were screened against *T. foetus* for possible activity.

2. Aureomycin was found to affect *T. foetus* in concentrations down to and including 25 μg/ml., rendering the culture sterile within 24 hours.

3. Aureomycin does not affect the glucose consumption of *T. foetus.*

4. Evidence of the existence of qualitatively different fractions of total metabolism is suggested.

5. Impure aureomycin was shown to have an activity level higher than that for pure aureomycin. The possibility exists that an enhancement factor may be present in the impurities similar in action to that shown for penicillin.

6. Maltose, raffinose, sucrose and d-mannose were shown to be equally as good as glucose for the culturing of *T. foetus.*

7. The substitution of trehalose for glucose extended the life of the culture considerably but did not allow for equal peak populations with glucose.

8. Bacitracin added to cultures of *T. foetus* caused the growth cycle to attain higher peaks and to last longer than the controls. Surface tension alone was eliminated as the cause for this effect. The evidence that bacitracin can be used by *T. foetus* as a metabolite is strong.

9. *T. foetus* was shown to be selectively sensitive to polymyxins A and B, and relatively resistant to polymyxin D; the difference being tenfold. It is suggested that *T. foetus* be used as a differential test organism for the identification of the polymyxins.

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Some Digenetic Trematodes of Marine Fishes of Bermuda

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The material on which this report is based was collected by Dr. Franklin D. Barker during the summers of 1910 and 1912 at the Bermuda Biological Station. The collection was presented to the University of Nebraska by the General Biological Supply House, Inc. From the collection, two Monogenea, *Microcotyloides incisa* (Linton, 1910) Fujii, 1944, and *Echinopelma bermudae* Raecke, 1945; and five digenetic trematodes, *Pseudolepidapedon balistis* Manter, 1940, *Dollfustrema gravidum* Manter, 1940, *Lepidapedon nicolli* Manter, 1934, *Sterrhurus fusiformis* (Lühe, 1901) Looss, 1907, and *Dermadena lactophrysi* Manter, 1946, have been reported (Fujii, 1944; Raecke, 1944, 1945; Manter, 1946, 1947). The "*Lepidapedon nicolli*" of Manter (1947) is named as a new species below.

Linton (1907) reported 26 species of sexually mature Digenea from Bermuda, but many were poorly described and some of them not identified beyond "*Distomum* species." From the Barker collection 32 species were identified, of which 22 are here reported for the first time from Bermuda. Three are considered to be new species.

[1] Studies from the Department of Zoology, University of Nebraska. No. 248. The work was done under the direction of Dr. H. W. Manter.

A study of the scattered descriptions of the species of *Lepidapedon* led to the inclusion of a key to the 12 species in that genus.

The Barker material was preserved in alcohol and glycerine in vials sealed in jars. Much of it was in poor or only fair condition. A numbered label in each vial included the common name of the host, the organ infected, and usually the name of the killing solution. Unfortunately, no further records of hosts could be obtained. There is, therefore, some uncertainty regarding the exact species of host involved, and for this reason the common names of the hosts are stated first followed by the probable scientific names.

Holotype specimens have been deposited at the United States National Museum.

ASPIDOGASTREA

Family ASPIDOGASTRIDAE Baer, 1827

1. *Lobatostoma ringens* (Linton, 1905) Eckmann, 1932. Host: Bluefish, probably *Pomatomus saltatrix* (L.).

GASTEROSTOMATA

Family BUCEPHALIDAE Poche, 1907

2. *Prosorhynchus pacificus* Manter, 1940. New synonym: *Prosorhynchus atlanticus* Manter, 1940. Host: Red rockfish, probably *Sebastopyr ruberrimus* (Cramer).

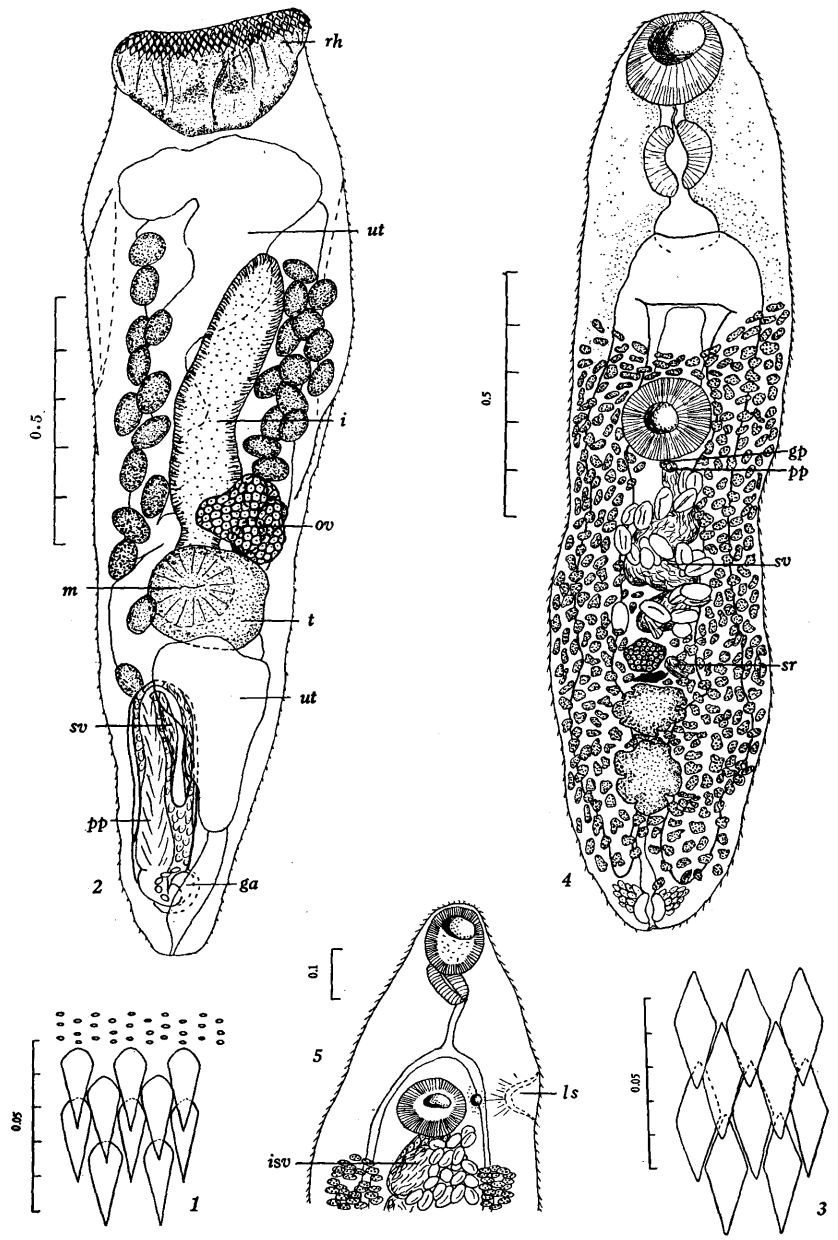
In the original descriptions of both *Prosorhynchus pacificus* and *P. atlanticus*, Manter (1940 and 1940a) noted the great similarity between the two species, differentiating them upon the basis of egg size "27 to 34 by 14 to 22 μ as compared with 24 to 27 by 12 to 17 μ" and the thicker and darker egg shell of *P. atlanticus*. The Barker Collection contained five specimens, four ranging between the limits established for the two species, and one larger than either, measuring 2.081 by 0.314 mm. with a rhynchus 0.380 by 0.204 mm. A comparison of the two species together with my five specimens reveals no specific difference. All of the measurements overlap or form a continuous series, and the anatomical characteristics show no differences when the individual variations referred to in the descriptions are considered. It will be noted that the lengths of the eggs form a continuous series (24 to 27 and 27 to 34 μ), while the widths overlap (14 to 22 and 12 to 17 μ). The eggs of the five specimens from Bermuda tend to vary in size within a specimen as does the width of the shell. *P. atlanticus* Manter, 1940 is considered a synonym of *P. pacificus* Manter, 1940.

3. *Dollfustrema gravidum* Manter, 1940. (Fig. 1) Host: Moray, probably *Gymnothorax moringa* (Cuvier). Location: Intestine.

The collection contained but one specimen belonging to this species. Together with the following species, it raised so many questions that a restudy of the type specimen of *D. gravidum* seemed desirable. It was promptly lent by Dr. E. W. Price from the Helminthological Collection of the United States National Museum.

A restudy of the type specimen and the paratypes (one on the same slide as the type, the other two from the personal collection of Dr. H. W. Manter) revealed that *D. gravidum* possesses a rhynchus provided with four alternating rows of relatively large slender spines approximately three times as long as wide, averaging 24 by 8 μ, which overlap about ⅓ their length, and about six rows of very small, numerous, blunt, knob-like projections (Fig. 1).

The type specimen did possess three gonads in tandem arrangement with the ovary between the testes. The paratype on the same slide had only one testis lying posterior to the ovary at the level of the pharynx. The remaining speci-



FIGS. 1–5. Trematodes of marine fishes of Bermuda. 1—*Dollfustrema gravidum* from moray, hooks of rhynchus (drawn to scale). 2—*D. macracanthum* from moray (dorsal view). 3—*D. macracanthum,* hooks of rhynchus (drawn to scale). 4—*Postporus mycteropercae* from permit (ventral view). 5—*Lepidapedon trachinoti* from permit (showing depressed lateral sucker-like organ). All figures (1–9), except those of the spines of *Dollfustrema,* were drawn with the aid of a camera lucida. The projected scale has a value in millimeters indicated on each figure. Abbreviations are as follows: *ga,* genital atrium; *gp,* genital pore; *i,* intestine; *isv,* internal seminal vesicle; *ls,* lateral sucker-like organ; *m,* mouth; *ov,* ovary; *pp,* pars prostatica; *rh,* rhynchus; *sr,* seminal receptacle; *sv,* seminal vesicle; *t,* testis; *ut,* uterus.

mens were so filled with eggs that all the gonads were obscured. It is probable that the gonads degenerate with age.

In all other respects the specimens agree with the original description.

4. *Dollfustrema macracanthum* n. sp. (Figs. 2–3) Host: Moray, *Gymnothorax moringa* (Cuvier). Location: Intestine.

Eighty specimens present a continuous series in body size, 0.328 to 1.927 by 0.182 to 0.569 mm. with no conspicuous breaks. The smaller specimens (those less than 0.800 mm. long) tend to be ovoid, the length usually less than twice the width, while the larger specimens are more elongate being over three times as long as wide. The smaller specimens are completely filled with eggs so that no internal organs are visible and sectioning was unsuccessful; the spines of the anterior sucker are difficult to observe except in three or four specimens which are definitely *D. macracanthum,* and it is assumed that the others are also *D. macracanthum.*

The description of the species, therefore, is based upon the eleven larger specimens, and particularly the holotype.

Description.—Body elongated, approximately three times as long as wide, 0.803 to 1.927 by 0.241 to 0.569 mm.; widest near anterior end; anterior end bluntly rounded or truncate, posterior end more pointed. Rhynchus well developed, flattened, consisting of alternating longitudinal muscles and glandular regions; surmounted by four alternating rows of large spines. Cephalic spines numerous, much larger than body spines (30 to 34 by 12 μ as compared with the 12 μ length of body spines), more or less rhomboid, overlapping ⅓ their length or less; forming a ring around the rhynchus. Body spines directed posteriorly; slightly curved.

Mouth in posterior half of middle third of body length. Pharynx rounded, 0.102 to 0.182 mm. in diameter; esophagus short; intestine saccular, 0.438 to 0.694 mm. long, extending anteriodorsad ¾ or more the distance to the rhynchus, but always a bit short of that organ.

Gonads in middle third of body; ovary anterior at level of esophagus and initial part of intestine, medial or to the left, 0.161 to 0.219 by 0.131 to 0.182 mm., subglobular to subtriangular, somewhat longer than wide; single testis immediately dorsal to or to right of pharynx, subglobular, 0.197 to 0.219 by 0.146 to 0.241 mm. Vitelline follicles usually longer than wide, 0.073 to 0.110 by 0.058 to 0.073 mm.; from base of cirrus sac to just anterior to intestine (those of smaller specimens tending to arc but never meeting); 17 follicles on right, 14 on left. The uterus is chiefly ventral except for a more dorsal posterior portion near the cirrus sac. It completely fills smaller specimens and more or less fills larger ones. Eggs ovoid, 19 to 27 by 14 to 20 μ with only a few, possibly abnormal, examples of each extreme of the measurements, usually 24 to 26 by 17 μ, thick shelled (2.5 μ), yellowish in color. Cirrus sac 0.365 to 0.511 by 0.124 to 0.168 mm., thick walled (13 to 15 μ), to the left, extending almost to pharynx. Seminal vesicle over half the length of cirrus sac, followed by colorless connecting tube which loops anteriorly to pars prostatica; genital atrium medium sized; genital pore near posterior end of body.

Excretory system not completely observed. Excretory pore terminal, preceded by a rather narrow tube, the extent of which could not be determined.

Comparisons.—The genus *Dollfustrema* contains two other species, *D. vaneyi* (Shen, 1930) Eckmann, 1932, and *D. gravidum* Manter, 1940. *D. macracanthum* differs from the first in possessing four rows of spines on the rhynchus, in the lateral distribution of the vitellaria, the greater length of the intestine, and the relatively longer cirrus sac. *D. vaneyi* was described from immature specimens and neither egg size nor uterine extent is known.

D. macracanthum is very closely related to *D. gravidum*. Both have four rows of spines on the rhynchus and a body filled with thick-shelled, ovoid eggs averaging 24 by 17 μ. The vitellaria are lateral and the length of the intestine is over half the body length with a mouth in the posterior part of the middle third of the body length. *D. macracanthum* differs from *D. gravidum* in its comparatively well developed rhynchus bearing spines which are heavier and more rhomboid in shape. The cirrus sac is approximately twice the length of the one in *D. gravidum*. The vitellaria are definitely follicular—not tending to become compact—and the intestine, which never reaches the rhynchus or bends posteriorly at its anterior extremity, has no swellings.

PROSOSTOMATA

Family PRONOCEPHALIDAE Looss, 1902

5. *Barisomum erubescens* Linton, 1910. Host: Angelfish, possibly *Holacanthus* sp. or *Pomacanthus* sp.

Family MEGASOLENIDAE Skrjabin, 1942

6. *Hapladena ovalis* (Linton, 1910) Manter, 1947. Host: Parrotfish, family Sparisomidae or Scaridae. Location: Intestine.

Family LEPOCREADIIDAE Odhner, 1905

7. *Lepocreadium trulla* (Linton, 1907) Linton, 1910. Host: Yellowtail, probably *Ocyurus chrysurus* (Bloch). Location: Intestine.

All 20 specimens except one are smaller than those recorded by Linton. The following measurements include lower limits of size: length 0.584 to 1.140 mm.; maximum width 0.336 to 0.730 mm.; oral sucker 0.119 to 0.150 by 0.099 to 0.140 mm.; pharynx 0.088 to 0.120 by 0.073 to 0.110 mm.; acetabulum 0.090 to 0.140 by 0.095 to 0.150 mm.; eggs are, as previously reported, 42 to 48 by 27 to 31 μ.

8. *Postporus mycteropercae* (Manter, 1947) Manter, 1949. (Fig. 4) Host: Hind, *Epinephelus* sp., probably *E. adscensionis* (Osbeck) or *E. guttatus* (L.). Location: Intestine.

My specimens are smaller than the type material, ranging from 1.110 to 1.745 mm. All other measurements are proportionately smaller. The sucker ratio ranges from 1: 0.70 to 0.84. The eggs are slightly shorter, ranging from 50 to 53 μ in length as compared with 58 to 61 μ. Fishes of the genus *Mycteroperca* are the only previously recorded hosts for this trematode.

Six additional specimens might possibly represent a different, unnamed species. Collected from the permit,[2] the trematodes closely resemble *P. mycteropercae,* the chief difference being the character of the prostatic vesicle. Rather than being "relatively long, extending some distance posterior to the acetabulum" as in *P. mycteropercae,* it is short, extending dorsally from the genital pore but not posteriorly. (Fig. 4.) Other differences noted are the normally wider ceca with distinctive transverse origin and the irregular border of the testes.

9. *Lepidapedon trachinoti* n. sp. (Figs. 5–7) Host: Permit,[2] probably either *Trachinotus goodei* Jordan and Evermann, or *T. falcatus* (L.).

Description (based upon 14 specimens from a total of 55).—Body flattened; elongated; usually widest in testicular zone, but sometimes equally wide at esophageal level and indenting slightly between the two levels; usually more bluntly rounded at posterior extremity; 1.445 to 2.270 by 0.445 to 0.664 mm. Body en-

[2] The original tags seemed to be labeled "Hermit." Dr. Samuel F. Hildebrand of the United States National Museum suggested that this fish probably is the permit, since there is no fish known as the hermit fish. Permit = *Trachinotus* sp., probably *T. goodei* Jordan and Evermann or possibly *T. falcatus* (L.).

tirely spined; spines of dorsal surface and sides are short (2 to 3 μ long), straight, directed posteriorly; spines of ventral surface curved into an S-shape, are much longer (22 to 26 μ not considering curvature). Oral sucker subterminal, very nearly round, 0.102 to 0.161 by 0.110 to 0.153 mm.; ventral sucker very nearly round, 0.102 to 0.182 by 0.131 to 0.168 mm.; sucker ratio from 1: 1.05 to 1.35. Forebody 0.270 to 0.482 mm., or about ¼ body length. Sucker-like structure on the left margin of body, lateral to acetabulum and genital pore; indicated only by medianly directed muscle strands in some specimens, but a definite depression in others (Figs. 5 and 6). Prepharynx usually very indistinct, often appearing absent, but in a few specimens it can be observed as very short, directed posteriolaterally; pharynx oblong, 0.066 to 0.073 by 0.044 to 0.073 mm., directed posteriomedianly so that the esophagus lies on the median line; esophagus 0.058 to 0.110 mm. long (0.146 mm. in one specimen), slender, often slightly curved, usually somewhat longer than pharynx; intestinal bifurcation about ¾ distance between pharynx and acetabulum; ceca narrow, about 0.015 to 0.022 mm. wide, approximately ¼ body width from the sides, ending blindly near posterior end of body. In one specimen the right cecum extended only ¾ body length. Genital pore near ventral sucker, sinistral, lying in the region between middle and anterior border of the ventral sucker. Testes usually with irregular border, but occasionally smooth; intercecal, more or less tandem with anterior testis usually lying slightly to the left; both occupying anterior part of posterior ⅓ of body length; anterior testis may be subglobular, but usually wider than long, 0.095 to 0.219 by 0.139 to 0.292 mm.; posterior testis larger, usually subglobular, 0.110 to 0.219 by 0.161 to 0.328 mm. Posttesticular space 0.212 to 0.620 mm., or about ¼ body length. Cirrus sac club-shaped, extending from genital pore to anterior level of the vitellaria; internal seminal vesicle oval, occupying the posterior ½ to ⅔ of cirrus sac; short pars prostatica and weak cirrus occupy anterior ⅓ to ½ of cirrus sac; external seminal vesicle as wide as the internal seminal vesicle, convoluted into slightly more than S-shape with three or four bends, surrounded by a membrane and relatively few glandular cells, extending anteriorly ¼ to ⅓ the length of the internal seminal vesicle then bending backward to extend about as far posterior to it into the vitelline region. Ovary elongated laterally, border indented with a tendency toward lobation, 0.051 to 0.124 by 0.073 to 0.204 mm.; seminal receptacle oval, dorsal to ovary, to the left of median line, lying between (or overlapping slightly) the ovary and anterior testis; yolk reservoir subglobular lying posterior and slightly dorsal to ovary, very nearly median, appoximately ¼ the size of the ovary (0.051 by 0.088 mm. in one specimen). Vitellaria extend from beyond the posterior ends of the ceca to a level about ⅓ the distance between acetabulum and ovary; dorsal, ventral and lateral to ceca; confluent posterior to testes in some specimens, partially invading the intercecal zone in some, but distinctly separated into lateral groups in others; vitelline follicles are rarely found between the gonads although they somewhat invade the spaces laterally, particularly on the left side. The space between ovary and testes varies from almost none to approximately the length of a testis. Uterus preovarian, intercecal, often more to the left of median line; containing numerous, oval, thin-shelled eggs measuring 46 to 61 by 31 to 37 μ. The excretory vesicle extends anteriorly to the bifurcation of the ceca.

Comparisons.—The following species of *Lepidapedon* have been described: *L. rachion* (Cobbold, 1858) Stafford, 1904; *L. elongatum* (Lebour, 1908) Nicoll, 1915; *L. garrardi* (Leiper and Atkinson, 1915) Manter, 1926; *L. nicolli* Manter, 1934;[3] *L. lebouri* Manter, 1934; *L. gadi* (Yamaguti, 1934) Yamaguti,

[3] The "*L. nicolli*" listed by Manter (1947) as a part of the F. D. Barker collection was determined to be *L. trachinoti*.

1938;[4] *L. genga* Yamaguti, 1938; *L. luteum* Yamaguti, 1938; *L. hoplognathi* Yamaguti, 1938; *L. coelorhynchi* Yamaguti, 1938; *L. sebastisci* Yamaguti, 1938; *L. hancocki* Manter, 1940;[5] *L. clavatum* Linton, 1940; *L. levenseni* (Linton, 1907) Manter, 1947; *L. pugetensis* Acena, 1947; *L. calli* Acena, 1947.

L. trachinoti is distinguished from all other species in the genus by the possession of the lateral sucker-like organ. It is most closely related to *L. nicolli* and to *L. levenseni.* It differs from *L. nicolli* in sucker ratio (1: 1+ as compared with 1: 0.75), a generally more posterior genital pore, less anterior extent of the vitellaria, shorter external seminal vesicle, conspicuous and more lateral seminal receptacle, and relatively wider eggs as compared with length. From *L. levenseni* it differs in sucker ratio (1: 1+ as compared with 1: 0.75), a generally more posterior genital pore, lateral seminal receptacle, and shorter cirrus sac. The more posterior genital pore and the lengths of the prepharynx and esophagus combined with the extent of the vitellaria separate it from the other species.

Key to the species of Lepidapedon

1.	(8)	Excretory vesicle extending anterior to acetabulum	2
2.	(3)	Lateral sucker present	*L. trachinoti*
3.	(2)	Lateral sucker absent	4
4.	(5)	Vitellaria not reaching acetabulum	*L. levenseni*
5.	(4)	Vitellaria reaching acetabulum	6
6.	(7)	Genital pore distinctly anterior to acetabulum	*L. hancocki*
7.	(6)	Genital port not distinctly anterior to acetabulum	*L. nicolli*
8.	(1)	Excretory vesicle not reaching acetabulum	9
9.	(16)	Vitellaria at least reaching mid-acetabulum	10
10.	(11)	Eggs at least 100 μ long	*L. genge*
11.	(10)	Eggs less than 75 μ long	12
12.	(13)	Separate male and female pores	*L. luteum*
13.	(12)	Single genital pore	14
14.	(15)	Prepharynx very long, esophagus longer than pharynx	*L. lebouri*
15.	(14)	Prepharynx very long, esophagus shorter than pharynx	*L. rachion*
16.	(9)	Vitellaria not reaching mid-acetabulum	17
17.	(22)	Esophagus almost lacking	18
18.	(21)	Pharynx approximately twice as long as wide	19
19.	(20)	Prepharynx longer than pharynx, egg less than 75 μ	*L. hoplognathi*
20.	(19)	Prepharynx shorter than pharynx, egg 90 μ long	? *L. pugetensis*
21.	(18)	Pharynx approximately round	*L. clavatum*
22.	(17)	Esophagus distinctly present	*L. elongatum*

Yamaguti separated his *L. coelorhynchi* from *L. elongatum* on the basis of less anterior extent of the vitellaria. His specimens (from *Coelorhynchus*) agree in this respect with the *L. elongatum* of Manter (1934) from the same genus of host. Study of numerous specimens will be necessary to determine variation in this character. Until such study is made it seems best to consider *L. coelorhynchi* Yamaguti, 1938 a synonym of *L. elongatum* (Lebour, 1908) Nicoll, 1915.

L. sebastisci was not compared with any other species. It was characterized by "the esophagus and the beginning of the ceca being lined with a thick cuticle

[4] Acena (1947) reported the new combination *Lepidapedon gadi* (Yamaguti, 1934) Acena, 1947. Yamaguti (1938) in his discussion of *L. haplognathi* stated, "This species is easily distinguished from the related *Lepidapedon lebouri* Manter, *L. gadi* Yamaguti and *L. elongatum* (Lebour) . . .," so the combination should be *L. gadi* (Yamaguti, 1934) Yamaguti, 1938.

[5] Manter (1940, p. 354) stated that *L. hancocki* differed from all other *Lepidapedon* except *L. nicolli* "in its very short esophagus." Prepharynx should be substituted for "esophagus."

and not with epithelium, by the cirrus pouch extending only slightly beyond the acetabulum, etc.'' The short cirrus sac is not uncommon in *L. elongatum* since it is the external seminal vesicle which extends most posteriorly. Yamaguti further stated that the vitelline follicles begin ''at level of posterior end of vesicula seminalis externa or a little further behind.'' If this is true, the external vesicle extends more posteriorly than is figured or the vitellaria extend more anteriorly. The cuticular lining of a portion of the digestive system does not seem to be a good character, and *L. sebastisci* Yamaguti, 1938 is considered a synonym of *L. elongatum* (Lebour, 1908) Nicoll, 1915.

L. gadi was compared with *L. rachion* rather than with *L. elongatum* to which it seems more closely related. The latter comparison reveals little difference except that the egg of *L. gadi* has a ''knob-like projection at the antiopercular pole'' and is wider as compared with length. Other differences such as vitellaria interrupted opposite the gonads which are noted in the figure are not constant according to the description. *L. gadi* (Yamaguti, 1934) Yamaguti, 1938 is also considered a synonym of *L. elongatum* (Lebour, 1908) Nicoll, 1915.

The above synonyms are based on published descriptions and figures.

The validity of *L. pugetensis* is questionable. It is incompletely and in certain respects inaccurately described. Its position in the key is based upon Acena's figure. The acetabulum is described as being 1.97 mm. from the anterior end in a specimen 2.37 to 2.47 mm. long; this would place it in the testicular region, but it is figured between $\frac{1}{4}$ and $\frac{1}{5}$ of the body length from the anterior end. The prepharynx is described as twice as long as the pharynx (0.12 mm. for the former, 0.06 mm. for the latter) while the esophagus equaled the pharynx in length. According to the figure the prepharynx is less than half the length of the pharynx and the esophagus is almost lacking. The description indicates that the ovary is at least twice as long as the anterior testis and that the posterior testis is almost three times as long as the anterior one. The figure indicates that the ovary is only slightly smaller than the anterior testis while the posterior testis is not twice as large as the anterior one. An external seminal vesicle is not mentioned in the description and it is stated that ''both seminal vesicle and pars prostatica are enclosed within a large ovoidal cirrus sac.'' However, the enclosed external seminal vesicle characteristic of *Lepidapedon* seems to be figured. The type is not available for study. It is not on deposit at the U. S. National Museum as was stated in the paper. The specimen figured seems closely related to *L. hoplognathi* and would appear even closer if the prepharynx actually were twice as long as the pharynx. Further collection and verification should be undertaken before *L. pugetensis* is recognized as a valid species.

Acena's other species in the genus *Lepidapedon, L. calli* Acena, 1947, on the basis of the seminal vesicle cannot be classified as *Lepidapedon*. The seminal vesicle is described as small, surrounded by pars prostatica, and enclosed within the cirrus sac. Only the vas deferens is described or figured outside the cirrus sac.

One species, *L. garrardi,* is very incompletely described. Since the cirrus sac and seminal vesicle were neither described nor figured, its correct genus is uncertain. The body shape, ''delicate'' spines, wide ceca, large, rounded testes and large vitelline follicles suggest *Lepocreadium* rather than *Lepidapedon.*

10. *Pseudolepidapedon balistis* Manter, 1940. Host: Turbot, probably *Balistes capriscus* Gmelin. Location: Intestine.
11. *Pseudocreadium lamelliforme* (Linton, 1907) Manter, 1946. Host: Turbot, probably *Balistes capriscus* Gmelin. Location: Intestine.

Ten specimens were collected. They possess a straight, sac-like external seminal vesicle and eggs which tend to be nearer the upper limits of size established by Manter, 1946. The character of the excretory vesicle does not seem to have

been reported. It is observed in three specimens to be clearly saccular to the level of the testes where two slender tubules originate that extend to the level of the pharynx. A small branch on each side curves posteriorly around the testes.

Family OPECOELIDAE Ozaki, 1925

12. *Pseudopecoelus elongatus* (Yamaguti, 1938) Von Wicklen, 1946. Host: Squirrelfish, probably *Holocentrus ascensionis* (Osbeck).

This species has been previously reported from Japan only.

13. *Pseudopecoelus barkeri* n. sp. (Fig. 8) Host: Squirrelfish, probably *Holocentrus ascensionis* (Osbeck).

Description (based on 2 specimens).—Body unspined, elongated, 1.664 to 2.007 by 0.511 to 0.569 mm., wider and more bluntly rounded posteriorly. Oral sucker subterminal, 0.116 to 0.124 by 0.102 to 0.124 mm.; acetabulum nonpapillated, somewhat protrusible, 0.212 to 0.226 by 0.248 to 0.277 mm., $\frac{1}{4}$ to $\frac{1}{5}$ body length from anterior end, with transverse aperture; sucker ratio 1: 2.24 to 2.44. Prepharynx short, 0.012 to 0.026 mm. long; pharynx rounded, 0.105 to 0.124 by 0.092 to 0.102 mm.; esophagus 0.076 to 0.085 mm. long; ceca bifurcate anterior to acetabulum, terminate blindly near posterior end.

Testes large, ovoid, smooth, tandem; anterior testis 0.219 to 0.234 by 0.241 to 0.248 mm; posterior slightly larger, 0.241 to 0.263 by 0.247 mm.; posttesticular space approximately equal to forebody. Cirrus short, muscular; cirrus sac absent although longitudinal muscles located around cirrus; prostate gland lacking; seminal vesicle entirely external, extending posteriorly midway between ventral sucker and ovary, saccular posteriorly, tubular anteriorly. Ovary pretesticular, subglobular, smooth, 0.116 by 0.110 to 0.122 mm., adjacent to anterior testis, to right of median line; uterus loosely looped from mid-ovary to metraterm; metraterm muscular, approximately equal in length to cirrus. Genital pore to left opposite posterior half of pharynx. Vitelline follicles from posterior portion of acetabulum to very near posterior end; confluent posteriorly; dorsal, ventral and lateral to ceca. Eggs 44 to 51 by 31 to 34 μ (average, 48 by 31 μ). Excretory vesicle I-shaped, extending anteriorly almost to ovary.

Comparisons.—*P. barkeri* is distinguished from *P. priacanthi* (MacCallum, 1912) Manter, 1947 on the basis of the unnotched acetabulum, contiguous gonads, more median genital pore, smaller size, and much less numerous ova; from *P. elongatus* (Yamaguti, 1938) Von Wicklen, 1946 by its more pyriform shape, larger sucker ratio (1: 2.24 to 2.44 as compared with 1: 1.43 to 1.66), contiguous gonads and vitellaria, larger pharynx and shorter esophagus; from *P. japonicus* (Yamaguti, 1938) Von Wicklen, 1946 by its unindented testes and ovary, shorter esophagus, shorter cirrus, larger sucker ratio and smaller eggs; from *P. vulgaris* (Manter, 1934) Von Wicklen, 1946 by its unindented testes, globular ovary, and smaller eggs; and from *P. tortugae* Von Wicklen, 1946, which it resembles most closely, by its unindented testes, larger sucker ratio (1: 2.24 to 2.44 as compared with 1: 1.62), the presence of a metraterm, smaller but more robust size (1.66 to 2.01 by 0.51 to 0.57 mm. as compared with 2.94 by 0.67 mm.), and the fewer and smaller eggs (44 to 51 by 31 to 34 μ as compared with 57 to 66 by 39 to 44 μ).

14. *Genitocotyle atlantica* Manter, 1947. Host: Yellow grunt, probably *Haemulon sciurus* (Shaw). Location: Intestine.

15. *Plagioporus crassigulus* (Linton, 1910) Price, 1934. Host: Porgy, *Calamus* sp. Location: Intestine.

Family ACANTHOCOLPIDAE Lühe, 1909

16. *Tormopsolus orientalis* Yamaguti, 1934. Host: Bonito, possibly *Zonichthys fasciatus* (Bloch). Location: Intestine.

This species has hitherto been known only from Japan.

17. *Stephanostomum casum* (Linton, 1910) McFarlane, 1936. Host: Yellowtail, probably *Ocyurus chrysurus* (Bloch).
18. *Stephanostomum dentatum* (Linton, 1900) Manter, 1931. Host: Pot snapper, probably some species of Lutianidae. Location: Intestine.
19. *Stephanostomum elongatum* (Park, 1939) n. comb. Synonym: *Echinostephanus elongatus* Park, 1938. Host: Hogfish, probably *Lachnolaimus maximus* (Walbaum).

The type and only previously reported locality for this species is Korea.

Family ZOOGONIDAE Odhner, 1911

20. *Deretrema fusillum* Linton, 1910. Hosts: Yellowtail, probably *Ocyurus chrysurus* (Bloch); margate, probably *Haemulon album* Cuvier and Valenciennes. Location: Intestine.

A second species of *Deretrema* is represented by a single, twisted specimen collected from a bluefish. Although the specific determination is uncertain because it was flattened in a twisted position, the trematode appears closely related to *D. pacificum* Yamaguti, 1942.

Family FELLODISTOMATIDAE Odhner, 1911, *emend.* Nicoll, 1935

21. *Proctoeces subtenue* (Linton, 1907) n. comb. (Fig. 9) Synonyms: *Distomum subtenue* Linton, 1907; *Proctoeces erythraeus* Odhner, 1911. Host: Porgy, *Calamus* sp. Location: Intestine.

When Odhner (1911) described *Proctoeces erythraeus* from the Red Sea, he distinguished it from *P. maculatus* (Looss, 1901) Odhner, 1911 on the basis of smaller ventral sucker, less extensive vitellaria, and smaller eggs. Dawes (1946) considered *P. erythraeus* a synonym of *P. maculatus*, stating that of the above mentioned differences only the smaller egg size was of significance and that was "marred by the fact that only a solitary specimen was found." Manter (1947)[6] retained the species on the basis of six additional specimens collected from *Calamus bajonado* and *C. calamus* (porgies) at Tortugas. The two specimens from the porgy (*Calamus* sp.) at Bermuda also agree with the description of *P. erythraeus* in sucker ratio, vitelline extent, and egg size. Linton's (1907) description of *Distomum subtenue* also collected from *Calamus calamus* at Bermuda agrees with the description of *P. erythraeus* sufficiently to be considered the same species. The valid name of the species is, therefore, *Proctoeces subtenue* (Linton, 1907) n. comb.

Very conspicuous glands extending from the anterior to the posterior extremities of the body were first thought to be vitellaria. Sectioning proved the vitellaria to be located lateral to the anterior testis and obscured by the uterus. The glands were also noted by Looss (1901) in *P. maculatus* and by Yamaguti (1934) in his description of *P. major*.

Family HAPLOSPLANCHNIDAE Poche, 1926

22. *Haplosplanchnus acutus* (Linton, 1910) Manter, 1937. Host: "Houndfish," *Strongylura* sp. Location: Intestine.

[6] Manter (1947, p. 322) stated that ". . . the extent of the uterus varied some but never reached past the posterior testis as it does in *P. maculatus.*" The original manuscript contained the word "vitellaria" which should be substituted for "uterus" in the description. The statement should read: the extent of the vitellaria varied some but never reached past the posterior testis as it does in *P. maculatus.*

23. *Haplosplanchnus brachyurus* Manter, 1937. Host: Parrotfish, family Sparisomidae or Scaridae. Location: Intestine.

Family MONORCHIDAE Odhner, 1911

24. *Postmonorchis orthopristis* Hopkins, 1941. Host: Grunt, probably *Haemulon* sp. Location: Stomach.

Family GORGODERIDAE Looss, 1901

25. *Xystretrum solidum* Linton, 1910. Host: Turbot, probably *Balistes capriscus* Gmelin. Location: Urinary bladder.

Family CRYPTOGONIMIDAE Ciurea, 1933

26. *Metadena adglobosa* Manter, 1947. Host: Gray snapper, *Lutianus griseus* (L.). Location: Intestine and ceca.

Each of the 46 specimens is smaller than either *M. globosa* or *M. adglobosa*, but they are like *M. adglobosa* in all pertinent respects: proportion of oral sucker to body width; the small, weak acetabulum; greater sucker ratio than *M. globosa;* the relatively smaller pharynx; uterus not extending anterior to acetabulum. The differences are largely those coincident with smaller size.

It seems advisable to revise the lower limits of some of the measurements so that they include these specimens: length, 0.256 to 0.712 mm.; width, 0.175 to 0.502 mm.; width of oral sucker, 0.046 to 0.171 mm.; width of acetabulum, 0.022 to 0.034 mm.; width of pharynx, 0.017 to 0.042 mm.; eggs, 10 to 27 by 7 to 12 μ.

Metadena adglobosa differs from *M. globosa* in its more elongate body; the proportion of oral sucker to body width; the uterus which does not extend anterior to the acetabulum; the pharynx which is never more than half as wide and often only one-third as wide as the pharynx of *M. globosa.* Manter (1947) further differentiates the species on the basis of the number of lobes of the ovary and the egg size, but both of these measurements form a continuous series of questionable comparative value.

A study of type material led Tubangui and Masilugan (1944) to reduce the genus *Metadena* to synonymy with *Neochasmus* on the basis of overlooked taxonomic features, *i.e.* "the presence of small spines around the mouth opening (minute and easily detached), a voluminous receptaculum seminis, and a genital sinus that is intimately associated with the acetabulum; the submerged position of the acetabulum; and the ventral position of the ovary." A careful check of these 46 specimens revealed no spines. It seems best to retain the genus *Metadena* until further collection can validate one or the other condition.

27. *Siphodera vinaledwardsii* (Linton, 1899) Linton, 1910. Host: Amber, probably *Seriola dumerili* (Riso); pot snapper, probably some species of Lutianidae. Location: Intestine.

Family HEMIURIDAE Lühe, 1901

28. *Sterrhurus floridensis* Manter, 1934. Host: Squirrelfish, probably *Holocentrus ascensionis* (Osbeck).
29. *Sterrhurus fusiformis* (Lühe, 1901) Looss, 1907. Host: Moray, probably *Gymnothorax moringa* (Cuvier).
30. *Lecithaster acutus* (Linton, 1910) Manter, 1947. Host: Doctorfish, probably *Acanthurus hepatus* (L.).

These specimens from the doctorfish, averaging 0.7 by 0.3 mm., are about half the size of those recorded by Linton. The sucker ratio (1: 2.35 to 2.67) is smaller

than one to "three or four" (Manter, 1947) and "oral sucker . . . scarcely equal to one-third the ventral sucker" (Linton, 1910) previously recorded. The egg size (22 to 26 by 12 to 15 μ) includes some eggs that are slightly smaller than reported by Linton (24 by 14 to 15 μ) or Manter (27 by 12 to 15 μ), but it will be observed that the egg measurements overlap forming a continuous series. In all other respects, my specimens agree with the description of *L. acutus* as emended by Manter.

31. *Stomachicola rubea* (Linton, 1910) Manter, 1947. Host: Moray, probably *Gymnothorax moringa* (Cuvier).

Family PTYCHOGONIMIDAE Dollfus, 1936

32. *Ptychogonimus megastoma* (Rudolphi, 1819) Lühe, 1900. Host: Nurse (dogfish) shark, possibly *Cynias canis* (Mitchill).

These specimens were collected December 21, 1915, by W. J. Crogier and represent an American record. The species is hitherto known from European waters. It is one of the few European species found at Bermuda.

DISCUSSION

The number of species of digenetic trematodes collected from the marine fishes of Bermuda is now 43, 22 of which are reported for the first time in this paper. Of the remaining 21, 18 were found by Linton in 1907 (Table 1). Discussing the geographical distribution of the species occuring at Tortugas, Manter (1947) predicted that "the 12 species known from Bermuda are based on less knowledge than those known at Woods Hole and Beaufort and probably would be doubled or more if collections there corresponded to those made at Woods Hole." The collections at Bermuda are not yet as extensive as those at Woods Hole, but the number of species has doubled.

Although absent from the Barker Collection, *Atalostrophion epinepheli* MacCallum, 1917 from *Epinephelus striatus* from Bermuda is evidently the same as *Distomum tomex* Linton, 1907, also from *Epinephelus striatus* from Bermuda. Its correct name should be *Atalostrophion tomex* (Linton, 1907) n. comb. Nine additional species have been reported from Bermuda that did not appear in the Barker Collection. They are: *Accacladocoelium macrocotyle* (Diesing, 1858) Odhner, 1928; *Genolopa ampullacea* Linton, 1910; *Hirudinella beebei* Chandler, 1937; *Lepidapedon levenseni* (Linton, 1907) Manter, 1947; *Megapera gyrina* (Linton, 1907) Manter, 1934; *Multitestis chaetodoni* Manter, 1947; *Opecoeloides vitellosus* (Linton, 1900) Von Wicklen, 1946; *Steganoderma nitens* (Linton, 1898) Manter, 1947; *Sterrhurus monticellii* (Linton, 1898) Linton, 1910.

Twenty-eight (65.1 per cent) of the species found at Bermuda are also found at Tortugas, Florida. One other species, *Hirudinella beebei,* is known from the American Atlantic and probably occurs at Tortugas although it has not been collected there. Of the Bermuda species, seven have also been reported from Woods Hole, Mass.; six from Beaufort, N. C.; six from the tropical American Pacific; five from Japan; three from the European Atlantic; three from the Mediterranean Sea; one from the Red Sea; one from the Baltic Sea; one from the Canadian Pacific. Only the three new species reported in this paper and *Atalostrophion epinepheli* are endemic Bermuda species. It is evident that the trematode fauna of Bermuda is most closely related to that of Tortugas. This, too, was predicted by Manter (1947) when he stated that "considering the types of fishes found at Bermuda, it seems probable that the trematode fauna will be found to be very similar to that of Tortugas."

TABLE 1.—*The following table shows the currently accepted names of the 26 species of sexually mature Digenea reported from Bermuda by Linton* (1907).

Name Used by Linton (1907)	Name Now Accepted	Authority
1. *Aspidogaster ringens* Linton, 1905	*Lobatostoma ringens*	Eckmann, 1932
2. *Distomum monticellii* Linton, 1898	*Sterrhurus monticellii*	Linton, 1910
3. *Distomum vitellosum* Linton, 1900	*Opecoeloides vitellosus*	Von Wicklen, 1946
4. *Distomum subtenue* Linton, 1907	*Proctoeces subtenue*	this paper
5. *Distomum macrocotyle* Diesing, 1858	*Accacladocoelium macrocotyle*	Odhner, 1928
6. *Distomum nitens* Linton, 1898	*Steganoderma nitens*	Manter, 1947
7. *Distomum gyrinus* Linton, 1907	*Megapera gyrina*	Manter, 1934
8. *Distomum lamelliforme* Linton, 1907		
(Figs. 75 & 78)	*Dermadena lactophrysi*	Manter, 1946
(Figs. 76 & 77)	*Pseudocreadium lamelliforme*	Manter, 1946
9. *Distomum trulla* Linton, 1907	*Lepocreadium trulla*	Linton, 1910
10. *Distomum levenseni* Linton, 1907	*Lepidapedon levenseni*	Manter, 1947
11. *Distomum tomex* Linton, 1907	*Atalostrophion tomex*	this paper
12. *D.* sp. from *Tylosurus acus*	*Haplosplanchnus acutus*	Manter, 1937
13. *D.* sp. from *Chaetodon* sp. and from *Bodianus fulvus punctatus*	*Multitestis chaetodoni*	Manter, 1947
14. *D.* sp. from *Balistes carolinensis*	*Pseudolepidapedon balistis*	Manter, 1940
15. *Monostomum vinal-edwardsii* Linton, 1899	*Siphodera vinaledwardsii*	Linton, 1910
16. *Monostomum* sp. from *Bathystoma striatus* and *Haemulon flavolineatum*	*Genolopa ampullacea*	Linton, 1910
17. "Undetermined trematode" from *Balistes carolinensis*	*Xystretrum solidum*	Linton, 1910

18-26. Still unnamed are the species listed as *Distomum* species from the following hosts: *Seriola fasciatus, Angelichthys ciliaris, Seriola dumerili, Teuthis hepatus, Sphyraena sphyraena, Paranthias furcifer, Salariichthys textilis, Teuthis coeruleus,* and *Gasterostomum* sp. from *Mycteroperca apua. Distomum fenestratum* is an immature form.

It will be noted that modern names can be given to 18 species collected by Linton. Nine species still have uncertain status. To the 18 named species, 22 additional species are reresented in the present Barker Collection; three other species are now known from Bermuda so that the total list is now 43.

SUMMARY

The Barker Collection contained 32 species of digenetic trematodes collected from marine fishes of Bermuda. Three new species, and three new combinations are named. Emended descriptions are given for two.

The new species are *Dollfustrema macracanthum*, *Lepidapedon trachinoti*, and *Pseudopecoelus barkeri*.

The new combinations are:

Proctoeces subtenue; formerly *Distomum subtenue* Linton, 1907; *Proctoeces erythraeus* Odhner, 1911.

Atalostrophion tomex; formerly *Distomum tomex* Linton, 1907; *Atalostrophion epinepheli* MacCallum, 1917.

Stephanostomum elongatum; formerly *Echinostephanus elongatus* Park, 1939.

Synonyms not involved in the new combination are:

Prosorhynchus atlanticus Manter, 1940; synonym of *P. pacificus* Manter, 1940.

Lepidapedon coelorhynchi Yamaguti, 1938; synonym of *L. elongatum* (Lebour, 1908) Nicoll, 1915.

Lepidapedon sebastisci Yamaguti, 1938; synonym of *L. elongatum* (Lebour, 1908) Nicoll, 1915.

Lepidapedon gadi (Yamaguti, 1934) Yamaguti, 1938; synonym of *L. elongatum* (Lebour, 1908) Nicoll, 1915.

Lepidapedon garrardi (Leiper and Atkinson, 1915) Manter, 1926 and *Lepidapedon calli* Acena, 1947 are considered *species inquerenda* on the basis of insufficient description. The description of *Lepidapedon pugetensis* Acena, 1947 is not sufficient to permit determination of the actual genus involved.

Forty-three species of digenetic trematodes have now been reported from marine fishes of Bermuda. These 43 are: *Accacladocoelium macrocotyle* (Diesing, 1858) Odhner, 1928; *Atalostrophion tomex* (Linton, 1907) n. comb.; *Barisomum erubescens* Linton, 1910; *Deretrema fusillum* Linton, 1910; *Dermadena lactophrysi* Manter, 1946; *Dollfustrema gravidum* Manter, 1940; *Dollfustrema macracanthum* n. sp.; *Genitocotyle atlantica* Manter, 1947; *Genolopa ampullacea* Linton, 1910; *Hapladena ovalis* (Linton, 1910) Manter, 1947; *Haplosplanchnus acutus* (Linton, 1910) Manter, 1937; *Haplosplanchnus brachyurus* Manter, 1937; *Hirudinella beebei* Chandler, 1937; *Lecithaster acutus* (Linton, 1910) Manter, 1947; *Lepidapedon levenseni* (Linton, 1907) Manter, 1947; *Lepidapedon trachinoti* n. sp.; *Lepocreadium trulla* (Linton, 1907) Linton, 1910; *Lobatostoma ringens* (Linton, 1905) Eckmann, 1932; *Megapera gyrina* (Linton, 1907) Manter, 1934; *Metadena adglobosa* Manter, 1947; *Multitestis chaetodoni* Manter, 1947; *Opecoeloides vitellosus* (Linton, 1900) Von Wicklen, 1946; *Plagioporus crassigulus* (Linton, 1910) Price, 1934; *Postmonorchis orthopristis* Hopkins, 1941; *Postporus mycteropercae* (Manter, 1947) Manter, 1949; *Proctoeces subtenue* (Linton, 1907) n. comb.; *Prosorhynchus pacificus* Manter, 1940; *Pseudocreadium lamelliforme* (Linton, 1907) Manter, 1946; *Pseudolepidapedon balistis* Manter, 1940; *Pseudopecoelus barkeri* n. sp.; *Pseudopecoelus elongatus* (Yamaguti, 1938) Von Wicklen, 1946; *Ptychogonimus megastoma* (Rudolphi, 1819) Lühe, 1900; *Siphodera vinaledwardsii* (Linton, 1899) Linton, 1910; *Steganoderma nitens* (Linton, 1898) Manter, 1947; *Stephanostomum casum* (Linton, 1910) McFarlane, 1936; *Stephanostomum dentatum* (Linton, 1900) Manter, 1931; *Stephanostomum elongatum* (Park, 1939) n. comb.; *Sterrhurus floridensis* Manter, 1934; *Sterrhurus fusiformis* (Lühe, 1901) Looss, 1907; *Sterrhurus monticellii* (Linton, 1898) Linton, 1910; *Stomachicola rubea* (Linton, 1910) Manter, 1947; *Tormopsolus orientalis* Yamaguti, 1934; *Xystretrum solidum* Linton, 1910.

Twenty-eight (65.1 per cent) of the 43 species are also found at Tortugas, Florida; seven at Woods Hole, Massachusetts; six at Beaufort, North Carolina; and six in the tropical American Pacific. The trematode fauna of Bermuda is clearly most closely related to that of Tortugas.

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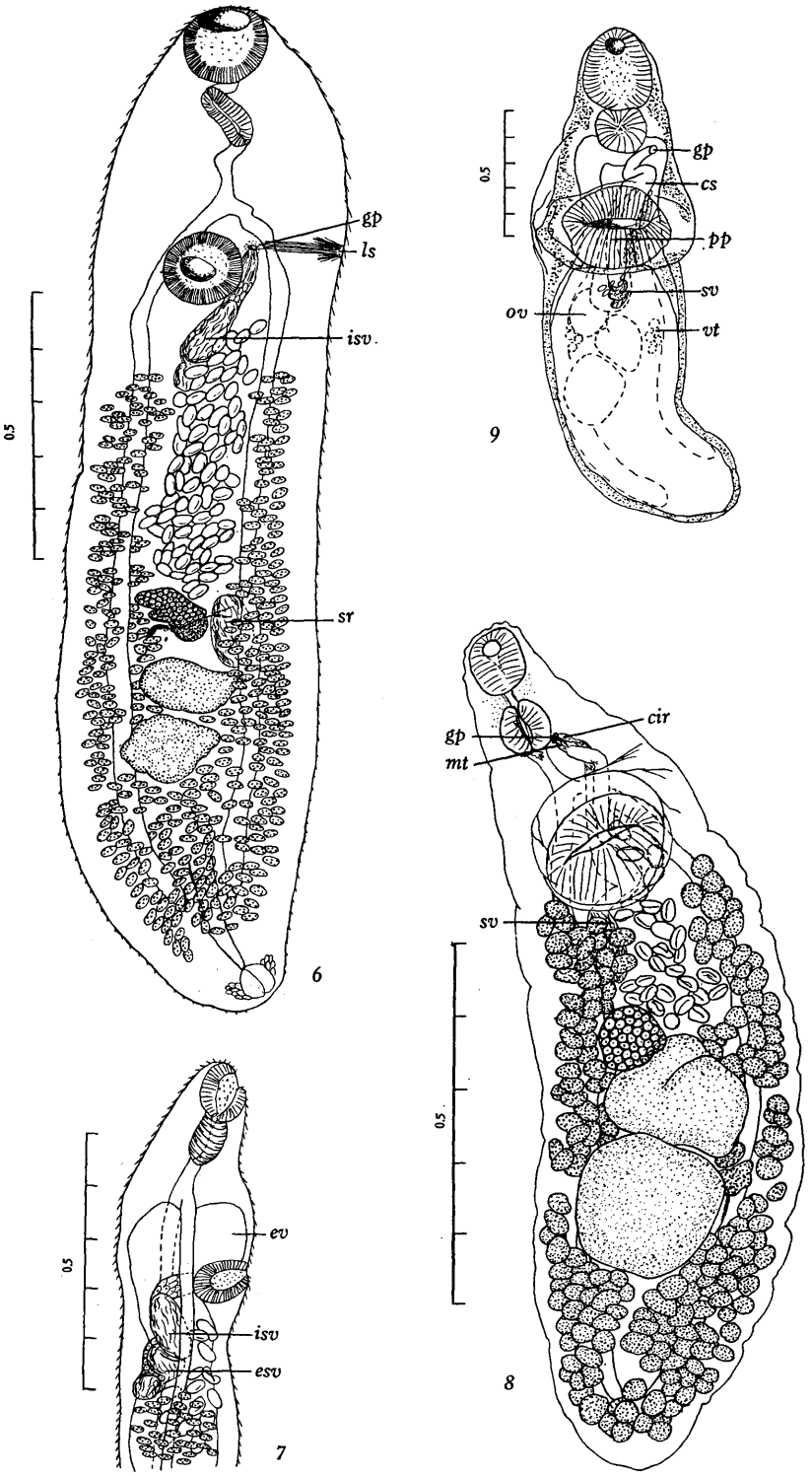

FIGS. 6–9. Trematodes of marine fishes of Bermuda, cont. 6—*Lepidapedon trachinoti* from permit (ventral view). 7—*L. trachinoti* (side view of seminal vesicle). 8—*Pseudopecoelus barkeri* from squirrelfish (ventral view; terminal reproductive ducts from a sectioned specimen). 9—*Proctoeces subtenue* from *Calamus* sp. (ventral view). The projected scale has its value indicated in mm. Abbreviations: *cir*, cirrus; *cs*, cirrus sac; *esv*, external seminal vesicle; *ev*, excretory vesicle; *mt*, metaterm; *vt*, vitellaria. Other abbreviations as before.

Freedom from Viable Trichinae of Pork Products Prepared to be Eaten without Cooking under Federal Meat Inspection

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Meat products such as frankfurters, salami, bologna sausage, liverwurst, Canadian style bacon, cooked ham, Westphalia style ham, coppa, cappocollo, farmer style sausage, thuringer and others which are composed wholly or in part of pork muscle tissue, constitute important articles of diet of the American public. The large amount of these products consumed yearly in the United States is attested by the fact that in 1937, according to the National Provisioner (vol. 100, no. 12, p. 17, 1939) more than 460,000,000 pounds of frankfurters alone were made by the American sausage industry. Under Federal meat inspection, products of the type named are regarded as products that are customarily eaten by the consumer without cooking. Consequently, in meat packing establishments operating under Federal inspection, these products are processed by the manufacturer in such a manner as to destroy the vitality of trichinae that may be contained in the pork muscle tissue incorporated therein.

Methods of processing pork and pork products to destroy trichinae as prescribed in the Federal meat inspection regulations include heating until the internal temperature of the meat reaches 137° F., or higher; refrigeration at temperatures of 5° F., −10° F., or −20° F. for specified periods; and special curing methods. The last-named procedures involve the addition to the meat of salt in specified amounts prior to curing. (Regulations Governing the Meat Inspection of the United States Department of Agriculture, Ed. 1947.)

EXAMINATION OF FEDERALLY INSPECTED PORK PRODUCTS FOR TRICHINAE

During the periods March 1934, to August 1939, and July 1948, to December 1949, examinations for trichinae were made of a series of half-pound samples of pork and other products containing muscle tissue of pork, the products in question being of the kinds customarily eaten without cooking, and, therefore, processed at the time of manufacture to destroy the vitality of contained trichinae. The purpose of the examinations was to ascertain (1) the number of samples that contained trichinae, (2) the number of trichinae in the individual samples, and (3) whether any of the contained trichinae were alive. The examinations were carried out at the laboratories of the Zoological Division, Bureau of Animal Industry, located at Beltsville, Maryland, and Chicago, Illinois. Samples were collected at weekly intervals in meat packing establishments operating under Federal inspection and sent to one or both of the aforementioned laboratories. On arrival, the samples were ground finely through food choppers, digested in artificial digestive fluid, and the sediment from the digestions examined for trichinae; the technique employed was similar to that described by Schwartz, (1939, Proc. Helm. Soc. 6 (2): 35–37). The findings are summarized briefly in this paper.

During the period from March 1934, to August 1939, 13,013 samples collected from meat packing establishments located in 38 cities of 27 states were examined. Of this number, 433 (3.32 per cent) contained trichinae. Of the 433 positive samples, 429 contained only dead trichinae; the number of dead organisms per sample ranged from 1 to 2,400. From each of the remaining 4 samples, (0.03 per cent), there were recovered from 1 to 2 trichinae which exhibited some signs of life; in all cases the larvae in question responded feebly to stimulation by heat, were somewhat pale in color and partially relaxed, these conditions indicating that

their vitality had been impaired, if not largely destroyed. Experience has shown that such devitalized larvae rarely, if ever, are capable of establishing infections in laboratory rats, host animals that are highly susceptible to infection with trichinae.

In the second series of examinations carried out during the period July 1948, to December 1949, there were examined a total of 3,171 samples collected from meat packing establishments located in 62 cities of 26 States. Of the samples examined, only 26 (0.82 per cent) contained trichinae. Of the 26 positive samples, 24 were found to contain only dead trichinae, the number of larvae per individual sample ranging from 1 to 32. Larvae showing signs of life were found in 2 (0.063 per cent) of the samples examined. One of these samples contained 2 living larvae and, in addition, 7 dead larvae. All the larvae recovered from this sample were administered by duodenal tube to a susceptible laboratory rat; 30 days later the rat was killed, the carcass ground through a food chopper and digested in artificial digestive fluid. No trichinae were found, a fact which shows conclusively that the vitality of the larvae fed had been completely destroyed. In the case of the other sample, 120 larvae showing various degrees of devitalization were recovered. The sample had been digested imperfectly which resulted in leaving the majority of the larvae still within their cysts, a fact which made an accurate determination of their vitality extremely difficult. Although for purposes of this survey all the encysted larvae referred to are considered as being potentially alive, it appears that their vitality had been destroyed. This conclusion is supported by the fact that a rat-feeding test with the larvae in question failed to produce a trichina infection in the host animal so fed.

CONCLUSIONS

In light of the data presented in this paper, it is evident that meat products containing pork muscle tissue, which are customarily eaten without cooking, were free from infective trichinae after having been processed for the destruction of these organisms in accordance with methods prescribed in the Federal meat inspection regulations. The data indicate, moreover, that there has been a decline in the extent of trichina infections in hogs during the past decade. This conclusion is attested by the fact that in the first series of examinations, conducted from March 1934, to August 1939, 3.32 per cent of the samples examined contained trichinae with numbers ranging as high as 2,400 per half pound sample of product. In contrast to this, in the series of examinations made from July 1948, to December 1949, only 0.82 per cent of the samples contained trichinae, the maximum number per sample being 120.

The extremely small number of trichinae showing signs of life recovered from samples in the two series of examinations, and the failure of the feebly viable larvae to infect rats which are highly susceptible host animals, show conclusively that processing of pork for the destruction of trichinae as prescribed in the Federal meat inspection regulations is an effective safeguard against human trichinosis.

The Status of the Cestode Genus *Meggittiella* Lopez-Neyra, 1942

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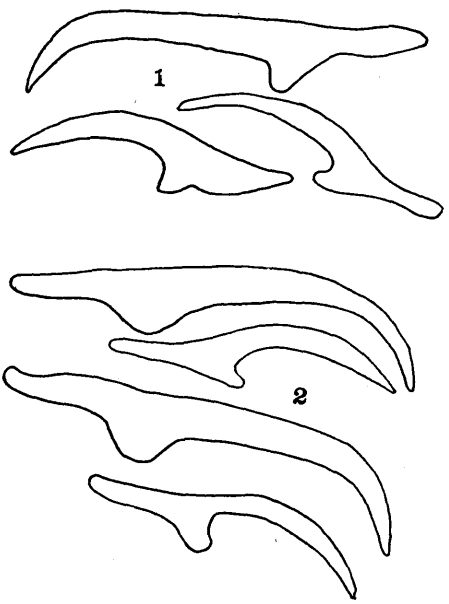
Lopez-Neyra (1942) has recently attempted to split up the classical and somewhat unwieldy genus *Hymenolepis* Weinland, 1858 into smaller and well defined genera. While such an attempt might be useful in itself, it has unfortunately been based almost entirely on descriptions of the species and not on the original speci-

mens. Moreover, this revision has been published with the most complete disregard for correct spelling of the names both of the species of tapeworms and of their hosts. New genera such as *Diplomonorchis* Lopez-Neyra, 1944 and *Meggittiera* Lopez-Neyra, 1942 are still-born since no type species has been designated and no description published. It is obvious, however, that *Meggittiera* is a lapsus for *Meggittiella* Lopez-Neyra, 1942.

The genus *Meggittiella* was erected to accommodate cyclophyllidean tapeworms possessing a double crown of rostellar hooks and three testes per segment. The type species by subsequent designation is *Hymenolepis multihamata* Meggitt, 1927 from the kite, *Milvus migrans aegyptius* (Gm.). The specimens studied by Meggitt came from a collection made by A. Looss in Cairo and later deposited in the British Museum after the first World War. Previously to the latter, however, Looss had divided his collection of tapeworms into two lots, one of which was presented to the late Professor Fuhrmann in Neuchâtel and the other being the col-



FIGS. 1–2. Rostellar hooks of *Paradilepis urceus*. 1—Specimen from *Milvus migrans aegyptius* (Gm.).

lection mentioned above. As Professor Meggitt presented one of the writers (J.G.B.) with an extensive collection of paratypes of the genus *Hymenolepis*, it has thus become possible to compare the specimens from the entire Looss collection.

The specimens determined by Meggitt as *H. multihamata* Meggitt had been labeled by Fuhrmann *Dilepis urceus* (Wedl), a species that normally occurs in ibises, *Platalea leucorodia* L. Since the above species also occurs in Looss' collection from this host we have been able to compare the three series of slides and fail to find any difference between them. The size and shape of the rostellar hooks are identical as shown in Figs. 1 and 2; the internal anatomy is also the same. These specimens are also identical with tapeworms from *Plegadibis falcinellus* (L.) that occur in the Neuchâtel collections. Consequently, *Hymenolepis multihamata* Meggitt, 1927 becomes a synonym of *Dilepis urceus* (Wedl, 1855).

The presence of tapeworms normally occurring in ibises, in a kite, might of course be attributed to a mistake made in labeling the vials. Yet Looss was a

very careful collector and such errors are hardly likely. Moreover, Johri (1934) has reported this same species, under the name of *Hymenolepis multihamata,* from an Indian kite, *Milvus migrans govinda* Sykes. From the same host, Johri also described a tapeworm under the name *Oligorchis hieraticos* Johri. This species does not belong to the genus *Oligorchis* as it possesses a double crown of rostellar hooks. The latter are both in size and shape identical with those of *H. multihamata,* although there appear to be four testes instead of three in each proglottis. It is no doubt by mistake that Johri states the length of the large hooks as varying from 113 to 190 μ.

Kites are scavengers that mostly feed on dead fishes and certainly cannot attack and devour an ibis. Ibises build their nests in the vicinity of water and feed on aquatic organisms even on fishes. It seems therefore possible that the larval forms of *D. urceus,* probably plerocercoids similar to those of *Gryporhynchus,* also occur in fishes.

Dilepis urceus is usually considered as having only three testes per segment although we have found specimens that occasionally have four. The presence of three testes per segment and a double crown of rostellar hooks caused Lopez-Neyra (1942) to transfer this species to the genus *Meggittiella.* On closer examination of *D. urceus* it is found that an external seminal vesicle is lacking and that both the double crown of rostellar hooks, their general shape as also the internal anatomy of the proglottids agree with the definition of the genus *Paradilepis* Hsü, 1935 that has so far been recorded with a single exception from cormorants only.

The species of the genus *Paradilepis* apparently all possess four testes but we do not consider that this character alone is sufficiently important to exclude species with five or three testes. Consequently, we propose to transfer *Dilepis urceus* (Wedl) to the genus *Paradilepis* Hsü with the following synonymy: PARADILEPIS URCEUS (Wedl 1855) syn. *Dilepis urceus* (Wedl) Fuhrmann, 1908; *Hymenolepis urceus* (Wedl) Megitt, 1927; *Hymenolepis multihamata* Meggitt, 1927; *Oligorchis hieraticos* Johri, 1934; *Meggittiella multihamata* (Meggitt) Lopez-Neyra, 1942.

The consequence of this transfer is to leave the genus *Meggittiella* without its type species but since the latter is tranferred to the genus *Paradilepis,* the former genus also becomes a synonym of the latter.

In his original conception of the genus *Meggittiella,* Lopez-Neyra also included two other species namely *Dilepis kempi* Southwell, 1921 and *Hymenolepis ficticia* Meggitt, 1927. *D. kempi* is closely related to the other species of *Paradilepis* from which it had been separated so far on account of its possessing only three testes per segment. We no longer consider this character as being sufficiently important taxonomically to justify this view all the more so that *Paradilepis delachauxi* (Fuhrmann) has five testes per proglottid.

On re-examining the cotypes of *Hymenolepis magniuncinata* Meggitt, 1927 from a pelican we have found this species to be identical to *H. ficticia* Meggitt, 1927 from the same host. Both possess a double crown of rostellar hooks yet their internal anatomy is of the typical *Hymenolepis*-type there being both an internal and an external seminal vesicle. These species will be discussed in a further note.[1]

On the basis of both original specimens and descriptions we include in the genus *Paradilepis* Hsü the following six species together with their synonyms.

[1] From descriptions of the recently published species of the genus *Hymenolepis* from pelecaniform-birds, we find that *H. childi* Burt, 1940 from *Haliëtor niger* (Vieill.) is a synonym of *H. cormoranti* Ortlepp, 1938 from *Hal. africanus* (Gm.) and that *H. gyogonka* Johri, 1941 from *Hal. niger* (Vieill.) is also a synonym of the latter species. Moreover, *H. furcouterina* Davis, 1945 from *Anhinga melanogaster* Pennant belongs to the genus *Echinorhynchotaenia* Fuhrmann, 1909.

1. PARADILEPIS DELACHAUXI (Fuhrmann, 1909) syn. *Dilepis scolecina* Joyeux & Baer, 1928 *nec* Rudolphi, 1819; *Paradilepis lepidocolpos* Burt, 1936. Hosts: *Haliaëtor africanus* (Gm.), *Hal. niger* (Vieill.).
2. PARADILEPIS KEMPI (Southwell, 1921) syn. *Dilepis kempi* Southwell, 1921; *Hymenolepis kempi* (Southwell) Mayhew, 1925; *Oligorchis burmanensis* Johri, 1941:*Meggittiella kempi* (Southwell) Lopez-Neyra, 1942. Hosts: *Haliaëtor pygmaeus* (Pall.), *Hal. niger* (Vieill.).
3. PARADILEPIS MACRACANTHA Joyeux & Baer, 1936 syn. *Dilepis delachauxi* Joyeux & Baer, 1928 *nec* Fuhrmann, 1909. Host: *Haliëtor africanus* (Gm.).
4. PARADILEPIS SCOLECINA (Rudolphi, 1819) syn. *Paradilepis duboisi* Hsü, 1935; *Paradilepis brevis* Burt, 1940. Hosts: *Phalacrocorax carbo* L., *Ph. capillatus* (Temm. & Schleg.), *Ph. fusciocollis* Stephens, *Haliaëtor africanus* (Gm.).
5. PARADILEPIS SIMONI Rausch, 1949. Host: *Pandion haliaetus carolinensis* (Gm.).[2]
6. PARADILEPIS URCEUS (Wedl, 1855) syn. *Dilepis urceus* (Wedl) Fuhrmann, 1908; *Hymenolepis urceus* (Wedl) Meggitt, 1927; *Hymenolepis multihamata* Meggitt, 1927; *Oligorchis hieraticos* Johri, 1934; *Meggittiella multihamata* (Meggitt) Lopez-Neyra, 1942. Hosts: *Platalea leucorodia* L., *Plegadibis falcinellus* (L.), *Milvus migrans aegyptius* (Gm.), *M. m. govinda* Sykes.

Key to the species of Paradilepis

1	Length of strobila not exceeding 10 mm	2
	Length of strobila exceeds 10 mm	3
2	Number of testes usually 3, exceptionally 4	*P. urceus*
	Number of testes usually 4	*P. scolecina*
3	Large hooks more than 300 μ in length	*P. macracantha*
	Large hooks less than 300 μ in length	4
4	Number of rostellar hooks 20–22	5
	Number of rostellar hooks 36	*P. simoni*
5	Small hooks 135 μ in length; 3 testes	*P. kempi*
	Small hooks 80–87 μ in length; 5 testes	*P. delachauxi*

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Laboratory Evaluation of Two Dinitro-phenols as Molluscacides[1]

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INTRODUCTION

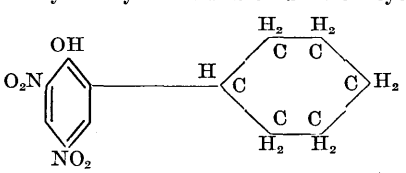
The medical and economic significance of snail control became evident with the discovery that molluscs play an obligatory role in the life cycle of trematode parasites. As a matter of fact, Thomas (1883), who is credited with the first description of a trematode life cycle, that of *Fasciola hepatica*, advocated control of the snail host by the liberal use of salt and lime on infested pastures.

Other suggestions for mollusc control have included: drying of irrigation canals in conjunction with rotation of the water supply (Leiper, 1915); clearance of canals (Barlow, 1937); trapping of snails (Abdel Azim and Barlow, 1947); introduction of snail predators [e.g., crayfish (Alicata, 1941); carnivorous firefly larvae as used by the Japanese (*fide* Alicata, 1941); fish (Cawston, 1937a; Oliver-González, 1946); birds (Cawston, 1937b); etc.]; concoctions derived from crushed plants and their fruits (Archibald, 1933; Wager, 1936; Mozley, 1944); and the use of chemicals (Leiper, 1915; Chandler, 1920; Mozley, 1944; Halawani, 1946; McMullen and Graham, 1947; and many others).

In spite of all this, methods of control of schistosomiasis in current practice are admittedly inadequate. Eradication of the snail intermediate hosts of the schistosomes is, as yet, impractical except in small areas, and has been accomplished then only with repeated applications of high concentrations of toxicants after clearing the areas of aquatic plants and debris.

In the face of these facts, the development of a practical, effective molluscacide assumes great importance. Ideally, such a preparation must be toxic to snails in low concentrations, yet nonpoisonous to human beings and domestic animals which use the water, and to plant crops which are grown in irrigated fields; it must be sufficiently water soluble to reach the snail tissues, but not so soluble that it is lost quickly; it must not be greatly affected by the organic content of the water; and it should be available in quantity at a reasonable price. This study was undertaken to search for a molluscacide which would qualify in as many of these particulars as possible.

Investigators in this laboratory have had opportunity to evaluate, under laboratory conditions, two compounds, dinitro-*o*-cyclohexylphenol (hereafter referred to as DCPH), and the dicyclohexylamine salt of dinitro-*o*-cyclohexylphenol (K604,



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Dow). The dinitrophenols from which these compounds were derived have a recognized high insecticidal value, and much phytotoxic activity (Roark, 1940; Howard and Weigel, 1946). The latter was greatly reduced by the addition of the cyclohexyl radical in the ortho position. Their molluscacidal activity was indicated in early reports from the Philippine Islands by the Army Commission on Schistosomiasis (Abbott, 1945; McMullen and Graham, 1947). Since these preliminary reports gave only scant data, it seemed desirable to make more detailed studies of the chemicals in the laboratory. Samples were provided by the Dow Chemical Company.

MATERIALS AND METHODS

The molluscs tested were *Australorbis glabratus,* the intermediate host of *Schistosoma mansoni* in Puerto Rico, and *Bulinus contortus,* a vector of *S. haematobium* in Egypt and Africa. Snails of uniform medium size were chosen for study.

Clutches of eggs used to test the effectiveness of chemicals on developing snails were deposited on microslides (77 × 54 mm.) placed vertically in the culture dishes of *A. glabratus* and handled according to procedures described earlier (Stirewalt and Kuntz, 1946). In many instances the embryos were allowed to develop to late gastrula or beyond before slides on which they had been collected were transferred to the test dishes.

Cercariae of *S. mansoni* were obtained from infected *A. glabratus* and subjected to toxicants according to procedures followed in testing the cercaricidal efficiency of DDT (Kuntz and Stirewalt, 1946).

Goldfish were used to determine the tolerance of small fish to the test preparations. *Carrasius auratus* was chosen because of its availability and the ease with which it could be handled in testing. Individuals were selected for excellence of condition and conformity to selected size range.

Plants employed in the preparation of the test aquaria were *Elodea* collected from a local fish pond, duckweed and green algae taken from stocks in laboratory aquaria.

The two compounds applied as toxicants, DCHP and K604, are stable compounds, apparently not affected by ordinary conditions of moisture and temperature. Though they are only moderately soluble in cold water, stock solutions of one part to ten thousand were prepared without difficulty. Further dilutions with aged tap water for testing at scheduled concentrations were made as needed after the stock solutions had been allowed to stand for 24 hours.

In the majority of tests run in this laboratory an arbitrary contact period of 24 hours has been employed. Preliminary tests were made in 150 ml. of conditioned tap water in finger bowls which allowed for easy observation and which stacked well. After the 24-hour exposure, the snails were removed from the test solution, washed, and transferred to fresh water. At this time the percentage of snails killed during the test was recorded. A second recording of dead molluscs was made 48 hours later, i.e., 72 hours after beginning of the test period.

Later in the course of investigation it became desirable to test the molluscacidal efficiency of the compounds under conditions more nearly resembling those of natural waters, and to determine also the susceptibility of fish and aquatic plants to concentrations lethal to snails. For this purpose wide-mouthed mouse jars were filled with three liters of conditioned tap water. Three handfuls of soil (sand and humus) were placed in each jar. After the water had cleared, *Elodea,* small amounts of duckweed, and green algae were added to the jars. Three to six days later one fish and five snails were placed in some aquaria; in others only snails

were added. Half of the jars allocated to each concentration of each test compound were used as described above, and half were aerated by bubbling a stream of air through the water. All aquaria were allowed to stand undisturbed for three or more days to permit thorough settling of the soil and to bring the water to room temperature at which all of the tests were run. This temperature varied during the two-year period over which the experiment extended, from 16° to 27° C.

Desired dilutions of the toxicants were prepared by adding proper amounts of stock solutions, or in several cases, the dry powder itself, to the water of the aquaria. In some instances one aquarium-setup was used for two tests. After the original test was completed and the animals were removed, the aquaria were allowed to stand unmolested at room temperature of 18° to 23° C. for three weeks. After that interval they were used again, five snails being added to each.

One test vessel was maintained as a control throughout each experiment; no toxicant was added. For the sake of comparison, also, snails both in finger bowls and in aquaria were treated with different concentrations of copper sulfate.

Observations on the viability of snails, fish, and water plants were made hourly for the first five hours and at the end of each 24 hours thereafter for a week. In determining minimal lethal concentrations the criterion used throughout was the death point of all the organisms tested. While the exact time of death of such organisms as snails is difficult to establish, it has been found that reliable proof of death was a gradual loss of motility followed by the absence of response to tactile stimulation when the bodies of the snails were relaxed and protruding from the shell, by cessation of heartbeat, and by the presentation of an opaque appearance with the body withdrawn far into the outer whorl of the shell. Fish were pronounced dead when they had suffered progressive loss of equilibrium, of ability to swim, and finally of irritability. In plants, the loss of turgidity and green color, and the absence of oxygen bubbles which, in sunlight, are evidence of active photosynthesis indicated loss of vitality.

RESULTS OF EXPERIMENTS

Dinitro-*o*-cyclohexylphenol applied in concentrations of 2 ppm., either in powder form or in aqueous solution was a completely effective killing agent for *A. glabratus* and its eggs in laboratory tests (Tables 1 and 3). All of these snails which were so exposed for 24 hours died within the 72-hour observation period, only one of 194 surviving more than 24 hours. This was true, regardless of experimental conditions, such as aeration, presence of soil and water plants, mineral or organic content of the water, etc. Dow K604, the dicyclohexyl amine salt of DCHP, was slightly less toxic since applications of 3 ppm. were necessary to kill all the *A. glabratus* within 72 hours. Applied at a concentration of 1 ppm., both compounds gave over 73 per cent kill within 72 hours.

B. contortus showed a variable resistance to both molluscacides. During some tests they succumbed to the toxicants as quickly as did *A. glabratus;* in others, often in the same aquaria, they withstood concentrations fatal to the latter.

In a series of ten tests exposing 20 *Oncomelania fausti* to DCHP, these snails exhibited a response similar to that of *A. glabratus.* Since *O. fausti* were available in such small numbers the results were not included in Table 1. It is not believed that so few tests provide a reliable basis for judgment.

Also, *Physa* and *Ancylus,* two local snails added to some aquaria for comparative purposes, sometimes withstood concentrations fatal to all *Australorbis.* Usually they were inactivated for part of the exposure period, but many recovered after being replaced in fresh water.

Experiments run at different seasons of the year supplied the data included in

Table 1. The first, conducted during the months of July, August, and September (1945), when the temperature was consistently high, gave constant and uniform kill of all the snails within 24 hours at 2 ppm. with either compound. The second, during the winter and early spring months (1946), gave less uniform kill and it was necessary to increase the concentration of the molluscacides slightly to insure complete kill within 24 hours. Such variations may be the result of light and

TABLE 1.—*Resistance of* AUSTRALORBIS GLABRATUS *and* BULINUS CONTORTUS *to* DCHP *and* K604

Conc. in PPM[a]	Tests/ No. Snails	Finger bowls: Percentage of snails dead 24 hrs	Finger bowls: Percentage of snails dead 72 hrs	Aquaria with soil, plants and water with high organic content[b]: Aerated: Tests/ No. Snails	Aerated: Percentage of snails dead 24 hrs	Aerated: Percentage of snails dead 72 hrs	Nonaerated: Tests/ No. Snails	Nonaerated: Percentage of snails dead 24 hrs	Nonaerated: Percentage of snails dead 72 hrs
Australorbis glabratus exposed to dinitro-*o*-cyclohexylphenol (Dow)									
2	9/55	100	.....	11/65	98	100	16/74	100	.....
1	33/160	84	86	12/78	95	95	24/160	90	92*
½	21/105	59	68	10/50	50	68			
Bulinus contortus exposed to dinitro-*o*-cyclohexylphenol (Dow)									
3	6/18	89	94	8/44	94	98	4/27	94	100
2	6/26	54	69*	9/46	70	77*	5/29	70	74*
1	12/37	32	38*						
½	11/29	21	38				1/5	40	60*
Australorbis glabratus exposed to K-604[c] (Dow)									
3	9/46	100	.....	11/86	98	100	12/98	100	.....
2	18/76	67	73	12/74	89	92	20/118	88	94
1	25/111	56	59	3/15	50	90	6/30	73	88
½	5/25	25	25				6/30	27	40
Bulinus contortus exposed to K-604 (Dow)									
4	4/18	100	.....	6/40	100	.....	6/38	100	.....
2	8/24	42	46*	5/24	46	54*	5/35	96	100
1	10/30	33	36*						
½	5/15	26	34						

[a] Parts per million (weight/volume).
[b] Aquaria set up 1 to 3 weeks prior to test.
[c] Dicyclohexyl amine salt of dinitro-*o*-cyclohexylphenol.
* Percentage of snails dead 3 to 7 days after test much greater.

temperature differences during the two periods; of variations in the two lots of chemicals used; or of differences in normal snail activity in summer and winter.

Combined results of all the tests indicated little effect of soil and water plants, organic content, or aeration on the toxicity of the compounds to snails. The slight differences shown in the table were not constant and are probably the result of the evasion of long exposure to the toxicants by the more active snails which crawled above the surface of the water.

In all tests which resulted in incomplete kill at the end of 24 hours, snail deaths continued until a week after the beginning of the experiment. Thus the total percentages of snails killed by the molluscacides under different conditions were more uniform than appears in Table 1. Since deaths occurred after the regular three-day period of observation, it may be that field control of molluscs will be possible with lower concentrations than are indicated by laboratory results. The number of deaths in untreated controls was negligible.

A comparison of either compound with copper sulfate (Table 2) is favorable to the dinitros irrespective of the experimental conditions (Table 1). Especially is it worthy of note that while dosages of copper sulfate must be increased with greater organic content of water, this is not necessary with the dinitro compounds. In fact, in some tests they appeared to be more active toxicants in the aquaria set up to simulate natural waters than in the finger bowls without soil, aquatic plants, etc. This may be an attribute of great value in field work where the waters requiring treatment will undoubtedly contain a great deal of organic matter plus aquatic vegetation.

TABLE 2.—AUSTRALORBIS GLABRATUS *exposed to Copper Sulfate*

Conc. in PPM[a]	Finger bowls			Aquaria with soil, plants and water with high organic content[b]							
				Aerated				Nonaerated			
	Tests/ No. Snails	Percentage of snails dead		Tests/ No. Snails	Percentage of snails dead			Tests/ No. Snails	Percentage of snails dead		
		24 hrs	72 hrs		24 hrs	72 hrs	5 days		24 hrs	72 hrs	5 days
1	16/80	30	58	4/60	2	38	40				
2	9/53	45	71	7/108	54	82	86	6/97	15	49	68
5	8/38	76	83	7/101	34	70	81	7/105	34	76	85
10	7/35	100	.....	10/132	56	76	84	6/71	55	96	98
15				2/80	90	97	97	6/90	95	97	99

[a] Parts per million (weight/volume).
[b] Aquaria set up 1 to 3 weeks prior to test.

If one is to regard the effect of the molluscacide upon other members of the biotic community, it is unfortunate that DCHP and K604 are toxic to goldfish as well as to snails. All goldfish died within 6 to 15 hours when exposed to dosages of the test compounds which were lethal to snails. At concentrations slightly less than this, however, some fish survived. Aeration increased the resistance of the fish. It is quite possible that fish could avoid lethal exposure in natural waters undergoing treatment by swimming to toxicant-free areas.

No injurious effects of treatment to the plants in the experimental aquaria were evident during a period of two weeks. Since long exposure is not necessary to kill snails, it seems unlikely that these compounds will be lethal to vegetation when applied as molluscacides.

It is worthy of note that the solutions, at concentrations which killed snails, had little or no effect on the many protozoa, rotifers, crustaceans, etc., present in the test aquaria; however, all cercariae of *S. mansoni* were killed within a few hours after exposure to DCHP and K604 at 1 to 2 ppm. It appears probable, then, that no drastic imbalance will follow the treatment of limited water areas with these compounds.

Developing snail embryos were all killed at concentrations of DCHP and K604 lethal to average-sized snails (Table 3). The majority of the embryos did not hatch from the gelatinous covering of the egg clutch if exposed to either of the test toxicants at concentrations as low as ½ ppm. Though early observation showed that a few of these embryos had survived (Table 3), examination of the clutches of eggs two or three weeks after the 24-hour exposure period revealed that most of those still surviving had not hatched. Living embryos in the test dishes were retarded markedly in their activity and growth, remaining within their capsules 10 to 14 days after embryos in the untreated control dishes had hatched. Some of the snails that were so retarded and had remained in the egg membranes for longer than the normal developmental period showed indications of anomalies. The majority of these died, if they did finally emerge.

TABLE 3.—*Eggs (developing embryos) of* AUSTRALORBIS GLABRATUS *exposed to* DCHP *and* K604

Dinitro-*o*-cyclohexylphenol						K 604				
Conc. in PPM	Tests/ No. Eggs	Percentage of eggs (developing embryos) dead				Tests/ No. Eggs	Percentage of eggs (developing embryos) dead			
		24 hrs	72 hrs	5 days	after 5 days		24 hrs	72 hrs	5 days	after 5 days
2	6/83	100	.....	.....	.....	8/117	100	.....	.....	.....
1	19/220	84	91	99	100	8/182	88	92	96	100
½	59/1164	78	86	91	99	23/398	74	76	82	98

A high residual toxicity was indicated in the aquaria in which retests were made two to four weeks after the first test period. Those aquaria in which DCHP had given complete kill of *A. glabratus* at 2 ppm. within 24 hours after application of the molluscacide possessed lethal concentrations for snails placed in the aquaria three weeks later. Aquaria treated with K604, however, showed slightly less toxicity upon second trial. In aquaria killing 80 per cent of the snails in the original tests, only 60 per cent died within 72 hours following the usual 24-hour exposure in the same aquaria on second test. During the standing period the test aquaria supported apparently normal populations of protozoa, rotifers and unicellular algae.

DISCUSSION

Laboratory experiments indicate that both DCHP and K604 show considerable promise as molluscacides. DCHP seems to be the better of the two since its killing efficiency is more consistent than that of K604, it is lethal to snails at lower concentrations, and is considerably cheaper. These compounds, more especially DCHP, fill in part the requisites for a suitable molluscacide: (1) DCHP is toxic to snails in low concentrations, i.e., 1 to 2 ppm.; (2) DCHP, although moderately toxic to higher animals in concentrated mixtures (Spencer, Rowe, Adams and Irish, 1948) would be of little danger to livestock or man at concentrations employed in mollusc eradication; (3) these compounds are only slightly toxic to aquatic plants and are not sufficiently toxic to most invertebrates to cause a drastic biological imbalance in nature; (4) although slowly soluble, the dinitro compounds are sufficiently soluble to kill snails within reasonable contact periods and retain their toxicity to snails for several days; and (5) these compounds are not adversely affected by water with unusually high organic content. It is regrettable,

however, that the dinitro compounds at present are not obtainable at a price that would permit their use on a large scale if proven acceptable in the field.

DCHP and K604 ranked high in the list of 19 chemicals which were tested for their molluscacidal activity to *O. quadrasi* by McMullen and Graham (1947). In their investigation the ease of handling, quantity of compound required to kill the amphibious snail, *O. quadrasi*, and cost per unit area treated were used as criteria in evaluating their comparative effectiveness. These investigators, as did we, noted the persistency of the dinitro compounds. This property we believe is one of the most promising attributes of the dinitro compounds and one which should bear considerable weight if such chemicals are to be given field trials against the non-operculate schistosome-carrying snails.

The findings with reference to the kill of developing embryos agree with those of Abbott (1945) who demonstrated that DCHP and K604 at 10 ppm. kill the ova of *O. quadrasi* within 24 hours, and with the earlier observations of Stirewalt and Kuntz (1946). Our work contradicts the statement of McMullen and Graham (1947) that "in the control of non-operculated snails it is known that the eggs are more resistant than the snails."

The actual mechanism responsible for the death of snails following exposure to lethal agents has not been explained. Obviously a knowledge of the physiological effects of promising toxicants upon snails would give investigators a more fundamental approach to the molluscacide problem. In our experiments marked hemorrhage was observed in many of the snails killed during tests and it occurred more frequently in snails exposed to the dinitro compounds than in those tested with copper sulfate. Hemorrhage was detectable microscopically in 40 to 45 per cent of *Australorbis* exposed to DCHP at 2 ppm., and occasionally there were blood-filled swellings on the snails' tentacles. It was noted also in *Bulinus*, though less frequently.

The initial knockdown of snails was greater with copper sulfate than with the dinitro compounds. Many of the snails exposed to the latter gradually became less active and for a period of several hours appeared to be anesthetized. This may well be another factor favorable to the dinitros since snails subjected to chemicals which evoke immediate escape reactions may seek protection by burrowing into the mud. A compound acting as a mild anesthesia would probably be effective on a greater part of the mollusc population. It must be noted that, in the tests described here, snails on occasion did crawl above the water line in test containers to escape introduced toxicants.

Since the results of testing of DCHP and K604 in the laboratory have been so favorable, a project has been set up to evaluate the worthiness of these compounds as molluscacides against snails in their natural habitats in Egypt. A report on the field testing of these compounds will follow.

SUMMARY

1. Two dinitrophenols, dinitro-*o*-cyclohexylphenol (DCHP) and its dicyclohexylamine salt (K604), have been tested under laboratory conditions to evaluate their promise as molluscacides.

2. Both DCHP and K604 show considerable promise as snail killing agents.

3. DCHP is the better of the two compounds since it fulfills in greater part the requisites for a practical molluscacide. DCHP is lethal to the non-operculate snails, *Australorbis glabratus* and *Bulinus contortus*, within a 24-hour contact period under the conditions of these experiments at 2 and 3 ppm., respectively; it is only slightly toxic to mammals and to aquatic plants, and is not sufficiently harmful to most aquatic invertebrates to cause a drastic biological imbalance in

natural waters; it is very persistent; and it is not adversely affected by water with high organic content.

4. Both DCHP and K604 are lethal to goldfish at concentrations required to kill snails.

5. Laboratory tests indicate that further study of these compounds under field conditions is warranted.

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The Efficacy of Nitrofurazone Fed Continuously for the Control of Avian Coccidiosis under Conditions of Natural Infection

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INTRODUCTION

For many decades, drugs or chemicals have been used profitably for the treatment of poultry or livestock which suffered severely from the depredations of animal parasites. Although the treatment of infected animals has proved economically sound, the general practice has been criticized because the host suffers much injury before the medicaments can be applied. Therefore, livestock and poultry raisers have long demanded some method of preventing the tremendous losses suffered by the host before remedial measures can be applied. Several authorities have advocated sanitation which has not proved satisfactory for control of some parasitic infections. Until recently the attempts to use medicaments continuously for the prevention of parasitic diseases have failed because of the toxicity of the known medicaments. However, the discovery of the anthelmintic efficacy of phenothiazine prompted a reinvestigation of the problem.

Within the last decade, two investigators from the U. S. Bureau of Animal Industry (Shorb and Habermann, 1940; Habermann and Shorb, 1942) developed a method of self-medication for sheep by mixing 1 part of phenothiazine in from 9 to 14 parts of salt. Recently this principle of continuous medication was applied to the control of avian coccidiosis with various effective drugs, and it is newly advocated for the prevention of blackhead in turkeys by means of Enheptin-T (Waletzky, Brandt, Bliznick, and Hughes, 1949).

HISTORICAL

Delaplane, Batchelder and Higgins (1947) first described the efficacy of sulfaquinoxaline against avian coccidiosis when administered for 4 days in the feed at levels of 0.05 to 0.1 per cent. Grumbles and Delaplane (1947) foreshadowed the use of sulfaquinoxaline continuously in the feed when they reported that 0.0125 per cent of the drug in an all-mash feed prevented losses among chickens placed on litter heavily contaminated with *E. necatrix*. Subsequent investigations by Grumbles, Delaplane and Higgins (1948a and b); Hart, Wiley, Delaplane, Grumbles and Higgins (1949); Grumbles, Tower, Oglesby and Upp (1949); Jungherr and Winn (1949); Peterson and Munro (1949) have established the efficacy of sulfaquinoxaline fed continuously for the prevention of avian coccidiosis. Subsequently, Johnson, Mussell and Dietzler (1949) advocated the continuous feeding of 4,4′ Isopropylidene-bis (2-Isopropylphenyl) for preventing coccidiosis. Also Waletzky, Hughes, and Brandt, as well as Brackett and Bliznick (1949) have described the usefulness of nitrophenide for this purpose.

Harwood and Stunz (1949a and b) determined that nitrofurazone was effective for preventing death losses among birds infected experimentally with oocysts of *Eimeria tenella*. The investigations to be described herein were undertaken to ascertain whether that drug could be used to prevent losses from coccidiosis if fed continuously.

MATERIALS AND METHODS

The birds were purchased as day-old chicks from various commercial hatcheries. We purposely used several breeds in the several tests to determine if there was any

difference in response of the various breeds. Indeed, the conditions under which the experiments were conducted, were varied slightly with each test. Consequently, only general arrangements will be described under this heading.

Recently Henderson and Stafseth (1950) have indicated that coccidiosis research can not be conducted successfully unless the genetic history of each individual chick is known because resistance to coccidiosis may be inherited. This suggestion is partially justified by the poor experimental design employed in some recent investigations of the treatment of coccidiosis, but it may not prove to be the *sine qua non* of all coccidial therapeutics. Chicks with known histories regarding coccidiosis susceptibility or resistance were not available to us, but we attempted to control this factor, as well as other variables, through randomization or experimental design which is described below.

In the experiments reported herein, the day-old chicks were received in the usual boxes which are divided into 4 sections or compartments. There are 25 to

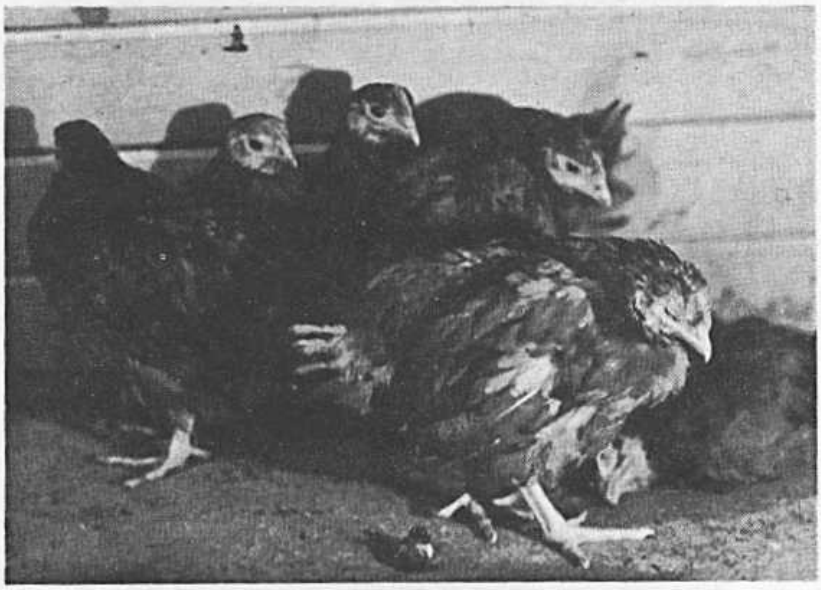

FIG. 1. At 8 weeks of age 6 runts from pen 1 (nitrophenide) weighed 500 grams or less.

27 chicks in each compartment, but the birds in one compartment may not be genetically comparable with those in another compartment or box. Therefore, whenever the chicks were placed directly in the experimental pens from the shipping cases, the operator removed 5 birds from each compartment of each box for each pen. Thus 5 chicks of each one-fourth box as received were placed in each experimental pen.

But unconsciously human beings exercise selection. Consequently, the last 5 chicks removed from each compartment will contain a preponderance of the poorer chicks. At least we have demonstrated that fact with 3 of our workers repeatedly at our laboratory. To distribute the poor chicks evenly, the first box is taken to pen 1, and the first 5 chicks from each quarter section removed, then the box is taken to pen 2 and 5 more from each section removed. Therefore, pen 5 receives the last 5 chicks from each section of box 1, but box 2 is taken to pen 2 first, and the last 5 chicks from each section of this box are removed in pen 1.

Thus by rotating the pens systematically the chicks of low vitality are distributed at random. The one or two per cent of chicks remaining after 25 are removed from each section are evenly distributed among the 5 pens after all the remaining birds have been placed.

Apparently Henderson and Stafseth (1950) did not exercise any such care in the distribution of their experimental birds since they acknowledge that some segregation of the coccidiosis susceptibles may have been associated with the use of pedigree baskets and bands. Of course, they are quite correct in declaring that statistical analysis is impotent for the correction of such poor design. Indeed randomization or careful distribution of the experimental subjects among the various groups is necessary to equalize a number of uncontrolled, and to a large extent uncontrollable, factors. In experiments of this type, where decisions are based upon deaths and upon weight gains, the general vitality and ability to

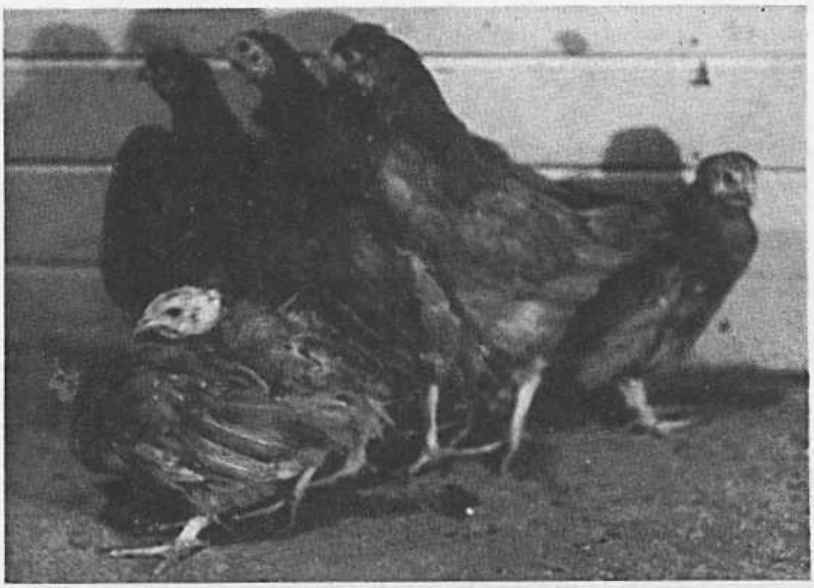

FIG. 2. At 8 weeks of age 6 runts from pen 3 (controls) weighed 500 grams or less.

gain weight are very important. These variables must be controlled as fully as practicable by experimental design. Any adequate design may also control the variations referable to degrees of susceptibility to coccidial infection. Such randomization is particularly necessary if the data are to be subjected to the usual statistical tests for significance. On the other hand, it may yield such consistent and striking results that the usual statistical computations are unnecessary to demonstrate significance.

The buildings used to house the experimental birds are of somewhat different construction. The brooder house is of wooden frame upon a concrete base. The five pens have concrete floors, and are separated by wooden partitions for two feet above the floor and woven-wire fence to the ceiling. A service alley 4 feet wide runs along the front of these pens. The long house is of wooden construction throughout. The wood floors are raised two feet above the ground: There are 6 pens in this house, each of which opens on a service alley. Both electric and gas hovers were used, but hovers of only one type were used in any one test. The

individual pens were 10 feet wide and 15 feet long in each building, therefore, 240 birds were needed to stock each pen at the rate of 2 birds for each 3 square feet.

Three diets were employed as follows: Standard diet: 320 lbs. yellow corn; 150 lbs. wheat middlings; 100 lbs. wheat bran; 100 lbs. ground oats; 50 lbs. meat scraps; 50 lbs. fish meal; 150 lbs. soybean meal; 50 lbs. 17 per cent alfalfa meal; 2.5 lbs. cod liver oil (400 D); and 30 lbs. of a commercial mineral mixture. High energy diet with meat scraps: 500 lbs. yellow corn; 150 lbs. wheat middlings; 65 lbs. meat scraps; 200 lbs. soybean meal; 30 lbs. 17 per cent alfalfa meal; 25 lbs. Sol-O-Meeno; 2.5 lbs. cod liver oil (400 D); and 30 lbs. of a commercial mineral mixture. High energy diet with A.P.F.: 500 lbs. yellow corn; 100 lbs. wheat middlings; 40 lbs. meat scraps; 290 lbs. soybean meal; 30 lbs. 17 per cent alfalfa meal; 12 lbs. limestone; 4 lbs. salt; 0.8 oz. Dry D (2000 D per gram); 1 lb. cod liver oil (400 D; 2000A); and 24 lbs. of a commercial mineral-vitamin concentrate containing the so-called animal protein factors.

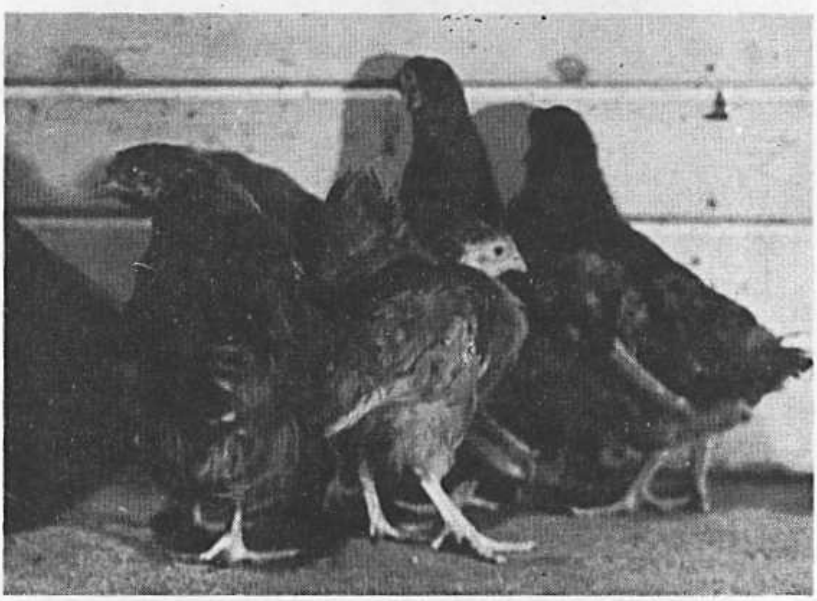

FIG. 3. At 8 weeks of age 5 runts from pen 4 (sulfaquinoxaline) weighed 500 grams or less.

The birds in each pen were weighed every two weeks throughout the course of the tests. All birds which died were necropsied, and the cause of death ascertained in so far as possible.

All infections with coccidia were acquired naturally. At necropsy, we attempted to identify the species of Eimeria which were chiefly responsible for the death of the individual bird. *Eimeria tenella* was usually the species which initiated the coccidial epidemics, but before the *E. tenella* epidemic had run its course, one or more of the intestinal species appeared in large numbers among some of the birds. Among the intestinal species *E. maxima* and two races of *E. acervulina* were most frequent and apparently most significant pathologically.

In some cases we encountered in nearly pure culture an *Eimeria* sp. having a very small oocyst. Since the maximum diameter of this oocyst corresponded

closely to measurements given for *E. mitis* this parasite was at first confused with that species. However, its biological characteristics such as sporulation time and the production of large clumps of oocysts which show as white opacities in the intestinal wall indicate that this species is closely related to *E. acervulina* with which it is identified. The race has pathogenic potentialities since very large doses (1 million sporulated oocysts per chick on each of 3 successive days) proved fatal to 20 per cent of the White Leghorn cockerels which were infected experimentally when 7 weeks old.

In addition we observed occasional fatalities in which *Eimeria necatrix* and *E. hagani* predominated. Individual oocysts of *E. praecox* and *E. mitis* were discovered but were not thought to be of significance in disease production. Usually the infections with intestinal forms were by two or more species. Therefore, it

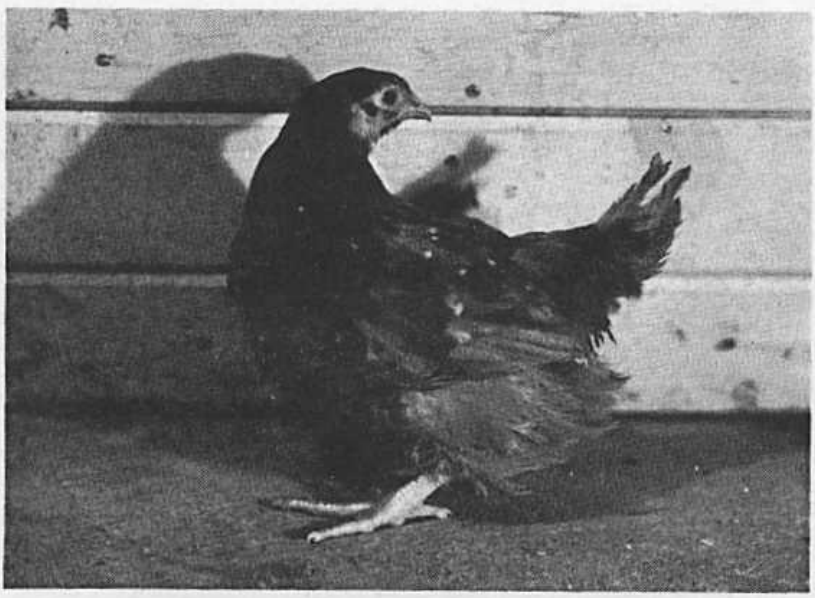

FIG. 4. At 8 weeks of age only 1 runt from pen 5 (nitrofurazone) weighed as little as 500 grams.

was not feasible to assign each death to a single species as cause. On the contrary many deaths seemed to be due to *E. tenella* alone. Therefore, the deaths from coccidiosis are reported under the following headings: "Cecal," showing *E. tenella* alone; "Mixed," showing *E. tenella* as well as one or more intestinal species and "Intestinal," showing intestinal species only.

EXPERIMENTAL RESULTS

The details of experiment 1 are set forth in Table 1. The birds employed in this test were intended as replacements for our laying flock. The brooder house was thoroughly cleaned and disinfected before the birds were placed in the pens. Medication was started at two weeks of age because the disease does not appear earlier in our experience.

Coccidiosis appeared late in this test. Indeed the epidemic was still in progress when the test was terminated and the birds were placed on range on June 15, 1949. The next morning, June 16, 3 birds which had died of cecal coccidiosis

were found in the range shelter that housed the birds from pen 3 (controls). These deaths are not included in the totals given in Table 1.

Criteria for judging the efficacy of the medication are weights, and deaths. By both tests the birds receiving nitrofurazone 0.0067 per cent (pen 5) are superior to those from all other pens.

The details of experiment 2 are presented in Table 2. This test closely paralleled experiment 1. More birds per pen were used, and they were of mixed sexes. Also the high energy ration with scraps was used in pen 3; the standard diet in the other 5 pens. The pens in the long house were swept out before the chicks were placed in them, but they were not washed or disinfected. Shortly before these tests were started, a flock of birds which had suffered severely from coccidiosis of mixed species were removed from pens 5 and 6. The other four pens had been used to house livestock and were not seeded with the avian species of *Eimeria*. Consequently, coccidiosis appeared earlier in pens 5 and 6. This is shown by the depressed weights obtained in those pens on May 18 and June 1. However, as the epidemic spread to other pens, the value of the medication in pen 6 became apparent.

By June 15, when it became necessary to terminate the test, the birds receiving 0.0067 per cent nitrofurazone outweighed those from any of the other pens by at least an ounce. Likewise there were no more deaths with coccidiosis in pen 6 than in any other pen. As in the brooder house, the epidemic of coccidiosis was by no means complete in the long house when the test was terminated. However, it was relatively more advanced particularly in pens 5 and 6, presumably because of the greater quantity of infective material present in those pens.

Details of experiment 3 are presented in Table 3. The weights of the birds receiving 0.0125 per cent sulfaquinoxaline and of those receiving 0.0067 per cent nitrofurazone closely parallel each other throughout the test. The sulfaquinoxaline birds averaged 7 grams more at the final weighing. However, pen 5 is slightly ahead in feed efficiency since the birds receiving nitrofurazone (0.0067 per cent) consumed 2.45 grams of feed per gram of gain and the birds receiving sulfaquinoxaline consumed 2.79. Likewise there were 4 more deaths with coccidiosis among the birds receiving sulfaquinoxaline. All the remaining pens made poorer weight gains and exhibited more deaths with coccidiosis. The pens receiving nitrofurazone intermittently (pens 1 and 2) are superior to the controls as regards deaths with coccidiosis, but they show no advantage in weight gains.

The results obtained in experiment 4 are detailed in Table 4. Although this test closely parallels experiment 3, there are differences in detail which seem to be significant. During the first 3 weeks of life the birds employed in experiment 3 were raised on the floor, those used in experiment 4 on woven wire. Consequently, in experiment 3 the birds carried subclinical infections with coccidia when placed on test and resistance to the infection was increasing daily. On the other hand the birds used in experiment 4 were uninfected and fully susceptible. Consequently, death losses with coccidiosis among the controls of experiment 4 were much higher than among similar birds of experiment 3. Also the birds of experiment 4 were overcrowded during the first 3 weeks. This circumstance may have increased the spread of pullorum infection which was present in a small percentage of the chicks when received. Possibly the higher losses from conditions other than coccidiosis may be explained on the basis of the spread of pullorum infection.

The birds in pen 6, which received 0.0067 per cent nitrofurazone outgained those in all other pens, and showed fewer deaths with coccidiosis. Pen 1 which received 0.0125 per cent sulfaquinoxaline is superior to all remaining pens, but when this pen is compared to pen 6 one observes that it consumed more feed, suf-

TABLE 1.— *The efficacy of nitrofurazone for the control of coccidiosis under conditions of natural infection.*

Note: The birds employed were female Rhode Island Reds which were hatched April 20, 1949. They were brooded under gas hovers in the brooder house. Clean wood shavings were used for litter. The birds were first weighed May 4, 1949, at two weeks of age. Medication which was confined to the standard diet began at this time and continued to the end of the test.

Pen No.	Treatment		No. of Birds	Mean Weight in Grams				Deaths		
	Drug	Amount (Per cent)		5/4	5/18	6/1	6/15	Coccidiosis		Other Causes
								Cecal	Mixed	
1	Nitrofurazone*	0.011	161	125.4	284.6	449.3	652.9	1	0	2**
2	Nitrofurazone*	0.011	165	119.6	270.8	450.8	661.5	0	1	2
3	Control		166	116.1	270.5	402.1	596.3	11	1	3**
4	Nitrofurazone*	0.011	166	118.9	277.3	448.1	642.3	2	0	2
5	Nitrofurazone	0.0067	163	123.8	272.7	443.7	722.4	0	0	2

* The medicated feed was given in pen 1 on Tuesday and Friday of each week; in pen 2, on Monday, Tuesday and Wednesday; in pen 4, on Monday, Wednesday and Friday. The standard diet alone was fed on the remaining days of each week.

** One chick in each of these pens developed a crossed beak. Because of the malformation these birds were killed.

TABLE 2.—*The efficacy of nitrofurazone for the control of coccidoisis under conditions of natural infection.*

Note: The birds employed were Rhode Island Reds of mixed sexes which were hatched April 20, 1949. They were brooded under electric hovers in the long house. Shavings were used for litter. The birds were first weighed May 4, 1949, at two weeks of age. Medication began at this time, and was confined to the diet.

Pen No.	Treatment		No. of Birds	Mean Weight in Grams				Deaths		
	Drug	Amount (Per cent)		5/4	5/18	6/1	6/15	Coccidiosis		Other Causes
								Cecal	Mixed	
1	Nitrofurazone**	0.011	242	123.8	288.6	429.0	645.6	4	2	1
2	Nitrofurazone**	0.011	245	122.0	251.8	389.7	622.5	2	2	2
3	Control*		246	122.5	285.6	390.4	658.4	9	0	0
4	Control*		240	117.9	289.7	371.7	648.4	16	9	2
5	Nitrofurazone**	0.011	243	125.2	268.4	413.0	664.1	8	13	2
6	Nitrofurazone	0.0067	235	122.0	268.2	385.9	693.4	1	3	4

* The controls in pen 3 received the high energy diet with meat scraps: those in pen 4 the standard diet which was fed to all remaining pens.

** The medicated feed was given in pen 1 on Tuesday and Friday; in pen 2, on Monday, Tuesday, and Wednesday; in pen 5, on Monday, Wednesday, and Friday. The unmedicated standard diet was fed on the remaining days.

TABLE 3.—*The efficacy of nitrofurazone for the control of naturally occurring epidemics of avian coccidiosis among White Rock chicks.*

Note: The chicks were obtained from a commercial hatchery on May 29, 1949. They were brooded until June 16 under electric hovers in a building which never housed chickens before. When three weeks old they were divided evenly into 5 pens and transferred to the brooder house where they were placed on test. When received these chicks were lightly infected with pullorum which accounted for many of the extraneous deaths.

Pen No.	Treatment		Pounds of Feed eaten**	No. Birds per pen	Mean Weight in Grams						Deaths			
	Drug	Amount (Per cent)			6/16	6/29	7/13	7/27	8/10	8/24	Coccidiosis			Other Causes
											Cecal	Mixed	Intest.	
1	Nitrofurazone*	0.011	847 P 410 M	240	154.0	228.9	393.6	617.0	877.0	1039.9	12	3	3	22
2	Nitrofurazone*	0.011	746 P 580 M	240	154.0	216.5	407.4	626.4	866.6	1080.4	6	2	1	32
3	Control		1256 P	240	153.1	236.1	397.4	627.8	886.0	1060.3	20	4	0	19
4	Sulfaquinoxaline	0.0125	1363 M	240	153.1	266.9	450.6	674.5	949.0	1142.0	6	1	0	5
5	Nitrofurazone	0.0067	1324 M	240	153.1	255.9	427.6	676.4	952.1	1135.0	2	1	0	14

* The medicated feed was given in pen 1 on Tuesday and Friday of each week; in pen 2 on Monday, Tuesday and Wednesday. The standard diet alone was fed on the remaining days of each week.

** In this column P indicates standard diet without medication; M indicates the same diet to which a medicament has been added.

TABLE 4.—*The efficacy of nitrofurazone for the control of naturally occurring epidemics of avian coccidiosis among White Rock chicks.*

Note: The chicks were obtained from a commercial hatchery on May 29, 1949. They were brooded for the first three weeks in wire-floored, battery-brooders. They were overcrowded which possibly resulted in increased spread of pullorum among these birds. They were placed on test in the long house on June 16, 1949.

Pen No.	Treatment		Pounds of Feed eaten***	No. Birds per pen	Mean Weight in Grams						Deaths			
	Drug	Amount (Per cent)			6/16	6/29	7/13	7/27	8/10	8/24	Coccidiosis			Other Causes
											Cecal	Mixed	Intest.	
1	Sulfaquinoxaline	0.0125	1452 M	211	163.9	265.3	425.8	684.1	918.5	1038.3	16	0	0	26
2	Nitrofurazone*	0.011	685 P 476 M	212	156.8	243.2	357.7	628.9	838.7	965.1	24	19	8	24
3	Control**		850	211	152.6	250.9	346.5	588.1	830.9	956.8	36	38	7	25
4	Control		800 P	212	150.2	247.3	334.2	567.0	823.4	1007.1	42	38	4	20
5	Nitrofurazone*	0.011	700 P 687 M	212	156.2	253.3	372.9	604.3	856.9	1043.8	5	17	5	29
6	Nitrofurazone	0.0067	1313 M	212	151.8	249.4	414.8	649.0	924.6	1082.8	0	1	5	32

* The medicated feed was given in pen 1 on Monday, Tuesday and Wednesday; in pen 5 on Monday, Wednesday, and Friday. The standard diet was used on the remaining days of the week.

** The high energy diet with meat scraps was used in pen 3.

*** In this column P indicates basal diet without medication; M indicates the same diet to which a medicament has been added.

fered significantly higher loss (chi-square test) with coccidiosis and attained lower average weights. Again 0.0067 per cent nitrofurazone appears at least as effective as sulfaquinoxaline.

The results of the first 4 experiments clearly demonstrate that intermittent administration of nitrofurazone at 0.011 per cent is not nearly so effective for the control of coccidiosis as 1 to 15,000 fed continuously. Therefore, in further tests the intermittent method of administration was abandoned.

Experiment 5 has been presented in Table 5. In this test the birds were placed on the litter which was used in the previous experiment. This was done because various agencies are recommending use of old litter. Electric hovers were employed, but the birds did not use them readily. The weather was exceptionally cool and, consequently, a number of birds died early in the tests from smothering or chilling. This factor accounts for most of the deaths listed under "Other causes" between August 31 and October 6. Shortly after medication was terminated, symptoms of a respiratory disease were noted, first in pen 1. This proved to be the earliest sign of an epidemic of Newcastle disease which soon involved all pens. The deaths under "Other causes" between October 6 and November 9, were largely due to this disease.

An error of a decimal point was made in pen 1. The birds received exactly ten times the indicated dose of nitrophenide for one week, when the error was detected and corrected. By this time the chickens were showing postural abnormalities and other symptoms of intoxication. They recovered promptly after the error was corrected, but the growth of these birds was retarded. Consequently, no comparison of weights between pen 1 and the other pens is of any possible value.

In experiment 5 we may best evaluate the various treatments by comparing the pens shortly after treatments were terminated. On October 12, pens 2 and 5 which received nitrofurazone at 0.0067 per cent were heavier on the average than the birds in any other pen. At present no reason is available to explain the superior gains of pen 5 as compared with pen 2. While on medication one bird from pens 2 and 5 died from coccidiosis. During the same period 12 birds from pen 4, which received sulfaquinoxaline, and 20 from pen 1, which received nitrophenide, died with coccidiosis. Again nitrofurazone appears slightly, but in the case of deaths significantly, superior to sulfaquinoxaline.

The medication was removed as soon as we considered the epidemic in pen 3, the controls, had run its course. Although some deaths showing coccidia occurred in this pen subsequent to October 6, they were fewer than those which occurred in the treated pens subsequent to medication. This indicates that medication once instituted should be continued until danger of recurring epidemics are past, but interpretation is complex owing to the superimposed Newcastle infection. Probably immunity to coccidiosis was developing in the treated pens, but more slowly than in the controls. At 12 weeks of age several of these birds were necropsied. Approximately 40 per cent of them were infected with *Ascaridia*. Apparently this hazard accompanying the use of old litter has not received much attention.

Experiment 6 was undertaken as the final test in this series. The birds employed in this experiment were studied more closely than previously, because some of the variations in the preceding tests can not be explained adequately. Details of the experiment are presented in Tables 6 and 7 which present most of the essential data.

As may be seen in Table 6, the birds receiving nitrofurazone were superior to both the controls and those receiving other medicaments. In this test the coccidiosis epidemic appeared just before the weights were taken on February 8 (pen 2

TABLE 5.—*Comparative efficacy of nitrofurazone, sulfaquinoxaline and nitrophenide against naturally acquired coccidiosis of chickens.*

Note: The chicks employed were a Rhode Island Red–Plymouth Rock cross which were hatched August 19, 1949. They were kept in wire-floored battery-brooders until August 31, when they were divided into five lots of 243 birds each and placed in the brooder house. The litter employed had been used in experiment 3. Medication was started August 31 and terminated October 6.

Pen No.	Medication	Mean Weight in Grams						Dates for Duration of Treatment and after Treatment	Feed consumed (Pounds)	Deaths			
		8/31	9/14	9/28	10/12	10/26	11/9			Coccidiosis			Other Causes
										Cecal	Mixed	Intest.	
1	Nitrophenide 0.0125 per cent	93.8	170.1	301.7	452.3	718.9	1038.4	8/31 to 10/6	653.0	7	8	5	29
								10/6 to 11/9	1074.0	4	1	5	25
2	Nitrofurazone 0.0067 per cent	93.8	180.7	343.1	516.9	788.2	1039.3	8/31 to 10/6	712.5	0	0	0	11
								10/6 to 11/9	1277.5	4	6	7	33
3	Control	93.8	184.0	327.2	469.0	767.0	1072.3	8/31 to 10/6	643.5	25	11	6	21
								10/6 to 11/9	1099.5	1	1	5	15
4	Sulfaquinoxaline 0.0125 per cent	93.8	191.4	322.0	507.5	769.8	1068.6	8/31 to 10/6	635.0	12	0	0	21
								10/6 to 11/9	1190.0	4	1	8	20
5	Nitrofurazone 0.0067 per cent	93.8	195.1	364.1	552.8	832.9	1162.0	8/31 to 10/6	712.5	0	0	1	20
								10/6 to 11/9	1356.0	7	3	6	20

TABLE 6.—*Comparative efficacy of nitrophenide, sulfaquinoxaline and nitrofurazone for the prevention of deaths and weight losses caused by a naturally induced outbreak of coccidiosis.*

Note: 1,226 straight-run New Hampshire chicks were received from Christie Poultry Farms on January 13, 1950. They were divided among the 5 pens of the brooder house as soon as received. Fresh wood shavings were used for litter. Medication was started at once.

Pen No.	No. Birds	Medication	Mean Weight in Grams				Mean Weight in Grams			Deaths			
			1/25	2/8	2/22	3/8		3/22	4/5	Coccidiosis			Other Causes
										Cecal	Mixed	Intest.	
							(Sex)						
1	240	Nitrophenide 0.0125 per cent	123.0	310.0	472.8	747.7	Mixed	1109.4	1372.9	0	0	0	8
							Male	1203.7	1511.7				
							Female	994.1	1210.8				
2	245	Nitrofurazone 0.004 per cent	128.7	316.4	514.5	788.3	Mixed	1154.3	1459.0	0	0	0	4
							Male	1259.5	1613.2				
							Female	1031.0	1261.0				
3	246	Control	125.9	296.4	430.1	702.1	Mixed	1046.1	1368.9	3	2	5	7
							Male	1135.0	1521.0				
							Female	967.2	1213.3				
4	245	Sulfaquinoxaline 0.0125 per cent	131.2	312.1	485.2	786.5	Mixed	1155.5	1451.9	2	0	2	6
							Male	1279.6	1647.8				
							Female	1060.2	1305.9				
5	250	Nitrofurazone 0.0067 per cent	130.8	322.3	547.6	838.5	Mixed	1194.7	1511.0	0	0	1	5
							Male	1317.5	1698.7				
							Female	1071.0	1323.3				

is already significantly heavier than pen 3 : t = 3.1 : P at the 5 per cent level = 2.8), and had nearly run its course by February 22 when the 6-weeks' weights were taken. On the latter date the nitrofurazone pens are clearly superior to all others.

On February 27 an accident occurred which marred the further course of this experiment. A farmer brought to our laboratory a chicken which showed a typical picture of coryza. Not finding anyone in the main laboratory, he entered the brooder house and wandered along the feeding alley as far as pen 2 before he was detected. He was carrying the sick bird from which *Hemophilus* was subsequently isolated. On March 2 the birds in pens 1 and 2 began to sniffle. By March 4 the birds in all 5 pens were showing marked symptoms of colds and seemed about equally affected. The epidemic seemed to be declining in severity by March 8, although at this time symptoms were still apparent in all pens. Observations suggested that pens 4 and 5 suffered less from coryza than any of the other pens. Whether this was due to later exposure or to the medicaments used in these two pens cannot be ascertained.

However, on March 8, when the birds were first weighed individually, pen 2, which received 0.004 per cent nitrofurazone in the mash, had lost its weight advantage over pen 4 which received sulfaquinoxaline. At this time pen 2 averaged 40 grams more per bird than pen 1 (nitrophenide) and the difference was highly significant statistically. (t = 4.26: At the 1 per cent level P = 2.59). On the other hand the birds in pen 5 which received 0.0067 per cent nitrofurazone maintained their weight advantage over the birds from pen 4 (sulfaquinoxaline) until the last weighing on April 5. At this time the value of t for the difference between the means of the males was 4.25 (P at the 1 per cent level = 2.6) ; t for the difference between the means of the females equalled 2.9.

Likewise deaths from coccidiosis are significantly fewer in the pens receiving nitrofurazone than in the control pen by the chi-square test. If pen 5 is tested against pen 3, chi-square equals 8: At the 1 per cent level P equals 6.6. Therefore, all pens except pen 4 exhibit significantly fewer deaths from coccidiosis than pen 3 (controls). The deaths from causes other than coccidiosis were in large measure accidental. Thus 2 birds from each of pens 1 and 5, as well as one bird from each of pens 2 and 4, were killed accidentally while weighing the group. Thus the deaths from unascertained causes are fewer in the nitrofurazone pens than in any other pen, but the difference is not significant and no emphasis is intended.

When the birds were examined individually for any abnormalities, the presence of curled toe paralysis was determined. The feed given to these birds was estimated to contain 1.7 mg. of riboflavin per pound, which should be adequate although marginal. However, the prevalence of curled toe paralysis, which is considered pathognomonic for riboflavin deficiency, in 13 per cent of the controls (pen 3: table 7) indicates that the estimate was optimistic. This abnormality was present at approximately the same degree of severity in all pens except pen 5 where it was both more severe in individual birds and present in a significantly greater number of birds. If pen 5 is tested against pen 3 for this character, chi-square equals 16.5, which is highly significant. Therefore, nitrofurazone at 0.0067 per cent possibly caused a slight exacerbation of a dietary deficiency in this test. The full importance of this observation can not be estimated until additional data are available. However, the findings of nutritionists (Stokstad and Manning, 1938) suggest that slight riboflavin deficiency retards growth slightly, but that curled toe paralysis does not appear until the deficiency and growth retardation are more marked. Therefore, growth retardation due to riboflavin deficiency was probably present in all pens, but it was most serious in pen 5 where the deficiency was greatest. The effect of 0.0067 per cent nitrofurazone for control of weight losses

due to coccidiosis may have been sufficient to overcome this disadvantage, since the birds receiving this medication outgrew all others. Furthermore, Becker (1942) showed that an excess of riboflavin in the diet of rats "definitely curbed the multiplication of *Eimeria nieschulzi* in its host. . . ." If the *Eimeria* spp. of the chicken are likewise affected adversely by riboflavin, correction of this deficiency may result in a marked increase of efficacy for nitrofurazone medication.

Poultrymen, who have used sulfaquinoxaline under field conditions for the control of coccidiosis, have complained about the large number of culls or runts in their treated flocks. Therefore, we carefully studied each group of birds for this characteristic, and on March 8, when the coccidial epidemic was complete, we sorted out from each pen every bird weighing less than 500 grams. Our observations lend no support to the contention that sulfaquinoxaline increases the number of culls or runts. On the other hand the photographs (Figures 1 to 4) show that runts developed in pen 4 (sulfaquinoxaline) during the coccidial epidemic almost as readily as in the pen 3 (controls). On the other hand such culls were nearly eliminated from the pens treated with nitrofurazone, since only one bird from these 2 pens weighed as little as 500 grams.

TABLE 7.—*Additional data and statistical computations of data obtained from the test detailed in Table 6.*

Pen No.	Medication	Birds with Curled Toes	March 8	Standard Deviation of Weights				Feed efficiency
				March 22		April 5		
				Males	Females	Males	Females	
1	Nitrophenide	29	123	156	122	191	126	3.99
2	Nitrofurazone	40	106	131	101	155	113	3.75
3	Controls	32	129	171	139	224	147	3.86
4	Sulfaquinoxaline	34	122	180	119	205	136	3.85
5	Nitrofurazone	72	118	133	106	188	137	3.61

The degree of variation present in each pen may be a more exact measure of the amount of injury produced by coccidia. Consequently, the standard deviations were calculated and these are recorded in table 7. On March 8, this statistic suggests that pens 2 and 5 were less variable than the other three. Since much of the variation present in the pens, was due to the differential growth of the two sexes, the birds were sexed in subsequent weighings. On March 22 the males receiving nitrofurazone proved significantly less variable than those receiving other medicaments ($t = 3.3$: At the 5 per cent level $P = 3.2$). The value of t for the standard deviations of the females falls short of significance, but combining the two groups, and weighing the standard deviations of the females to make them comparable with those statistics for the males, the results of analysis of the combined statistics is highly significant ($t = 4$: At the 1 per cent level $P = 3.4$). The increasing variation present in all pens makes the difference between the standard deviations non-significant by March 22.

Because deaths were few in this experiment, we have calculated the feed efficiencies for each pen. Again the nitrofurazone pens are superior to all other pens, but this superiority is not statistically significant. Furthermore, the experiment was not designed to test feed efficiencies and a number of factors, namely: frequent and intensive handling of birds, the presence of a riboflavin deficiency, the epidemic of coryza, and excessive wastage because the feeders were kept filled to the rims, combined to make the feed-utilization by these birds inefficient. How-

ever, as all birds were exposed to these factors equally, the figures may be of some comparative value.

SUMMARY AND CONCLUSION

1. The value of nitrofurazone fed continuously or intermittently was explored in 6 experiments involving a total of 7,183 chickens. When the drug was fed intermittently for three or four days each week, the effects upon the natural outbreaks of coccidiosis were slight. However, when fed continuously at 0.0067 per cent of various all-mash feeds, nitrofurazone proved very effective. Thus, 15 birds (1 per cent) out of 1,586 died in the pens receiving 0.0067 per cent nitrofurazone. On the other hand 287 (15.9 per cent) of 1,804 similar, but unmedicated controls died of the infection. In one experiment the concentration of nitrofurazone was reduced to 0.004 per cent and at this level it was very effective for controlling both death losses and weight losses from coccidiosis. However, 0.004 per cent nitrofurazone was significantly less effective for the control of weight losses than 0.0067 per cent nitrofurazone in the feed.

2. In four experiments sulfaquinoxaline was employed as a reference standard. This drug proved effective, since 39 (4.2 per cent) of 939 chicks died with coccidiosis, and in the same tests 241 (20.9 per cent) of 1,152 control birds died of the disease. In these four tests only 11 (0.9 per cent) of 1,188 chicks receiving 0.0067 per cent of nitrofurazone in the mash died of the disease. In every test the deaths with coccidiosis were fewer in the pens receiving nitrofurazone than in the pens receiving sulfaquinoxaline and the difference is highly significant by the chi-square test. The pens receiving nitrofurazone outgained the pens receiving sulfaquinoxaline by more than an ounce on the average. However, the variation was so extreme that this mean difference falls a little short of significance, according to the usual t test. In the one experiment where individual weights were taken, the birds receiving nitrofurazone at 0.0067 per cent were significantly heavier than those receiving 0.0125 per cent of sulfaquinoxaline. An economically more important feature of nitrofurazone medication was the almost complete elimination of the culls or runts which resulted from the coccidial attacks in control pens and pens treated with other medicaments.

3. In one test where the ration was unexpectedly submarginal for riboflavin, 0.0067 per cent nitrofurazone apparently increased the severity and frequency of curled toe paralysis. Also the plumage was a little rougher in this pen than in the controls. Since 0.004 per cent nitrofurazone had no such adverse effects upon the birds, we may consider in further tests reducing the level of nitrofurazone below the 0.0067 per cent level and adding an excess of riboflavin to the diet. An excess of riboflavin depresses reproduction in other species of coccidia (*E. nieschulzi* of the rat), and a deficiency of the vitamin depresses growth rate of the chick. When the proper balance of drug and vitamin are established, it is possible that nitrofurazone will exhibit a more consistent and more powerful coccidiostatic action than demonstrated heretofore.

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The Occurrence of a Gubernaculum in *Thelazia californiensis* Price, 1930 (Nematoda: Thelaziidae)

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Some eyeworms collected from a dog at Stockton, California by Dr. W. L. Peterson were received recently for identification. They were identified as *Thelazia californiensis*, since the worms of both sexes presented all the characteristics which are described for this species. However, in the first male closely studied, the presence of an inconspicuous, apparently discrete, sclerotized body, situated in close proximity to the dorsal margin of the distal portion of the right spicule, was noted. It appeared to be a small weakly-developed gubernaculum.

A gubernaculum was not mentioned by Price [1930. J. Parasitol., 17(2): 112–113; 1931. North. Am. Vet., 2(11): 49–58.] in his descriptions of *T. californiensis*, nor by Kofoid and Williams (1935. Arch. Ophthal., 13: 176–180), who described specimens of Price's species from man. As far as the writer is aware,

a gubernaculum, or gubernaculum-like structure, is unreported for all species of *Thelazia*, except *T. chui* Hsü, 1935. In that species, which differs from *T. californiensis* in the number and distribution of its caudal papillae, and which is based on only a single male from a falcon from "French Indo-China," Hsü [1935. Ztschr. Parasitenk., 7(5): 579–600] observed a "small gubernaculum-like chitinous structure . . . near the region of the cloaca."

In view of these circumstances, the writer first attempted to determine whether a minute body, accessory to the right spicule, like the one first noted in the aforementioned specimen, could be found in all available males of Price's species and, secondly, the exact nature of this body.

In preparing the full description of *T. californiensis*, Price (1931. *loc. cit.*) evidently examined three lots of specimens: (1) 25 (number of each sex unspeci-

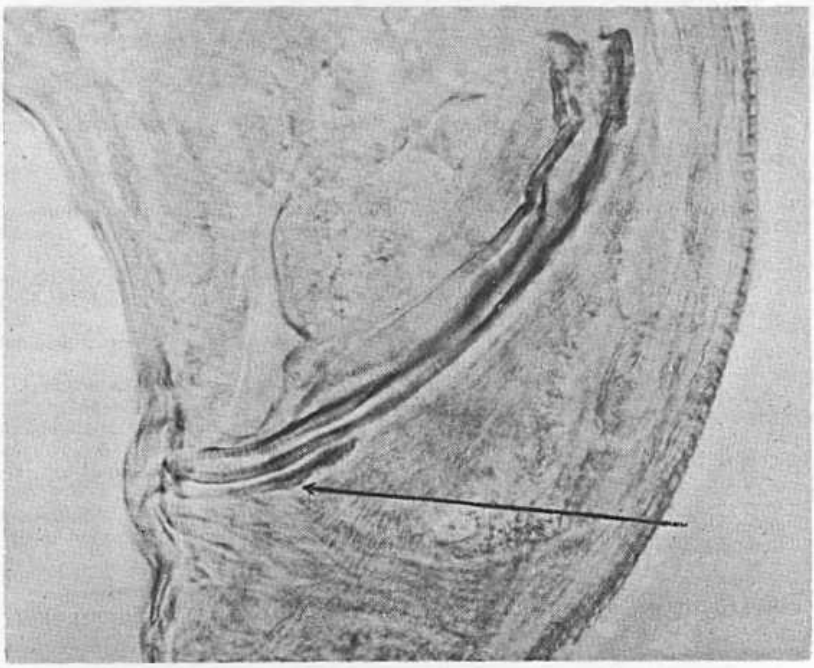


FIG. 1. *Thelazia californiensis* (male). Caudal region; showing gubernaculum (indicated by the arrow) and right spicule (Photomicrograph; magnification, approx. ×500).

fied) collected by Dr. E. C. Jones at Hollywood, California; (2) two, a male and a female, received from Dr. Charles E. Crowe, but collected at Redding, California, and (3) 28 (number of each sex unspecified) received from Dr. Agnew of Pasadena, California.

Unfortunately, only two males from this original material, the type male, which was collected by Dr. Jones, and the male (U. S. N. M. No. 30860) received from Dr. Crowe, were found in the U. S. National Museum Helminthological Collection. In addition, in this Collection were found six males, which Price had identified after, or apparently after, his species description had been completed, and five identified by the writer in 1942.

These 13 males and the five (U. S. N. M. No. 46879) received on November

17, 1949 from Dr. Peterson were studied comparatively as carefully as their condition permitted.

suspect gubernaculum was observed in four of the five specimens of the last-mentioned lot; it typically appeared like the body indicated by the arrow in Figure 1. The degree of its development was approximately equal in the four specimens; efforts to orient the fifth specimen satisfactorily for study failed.

The type specimen appeared to possess a smaller accessory piece, sclerotized

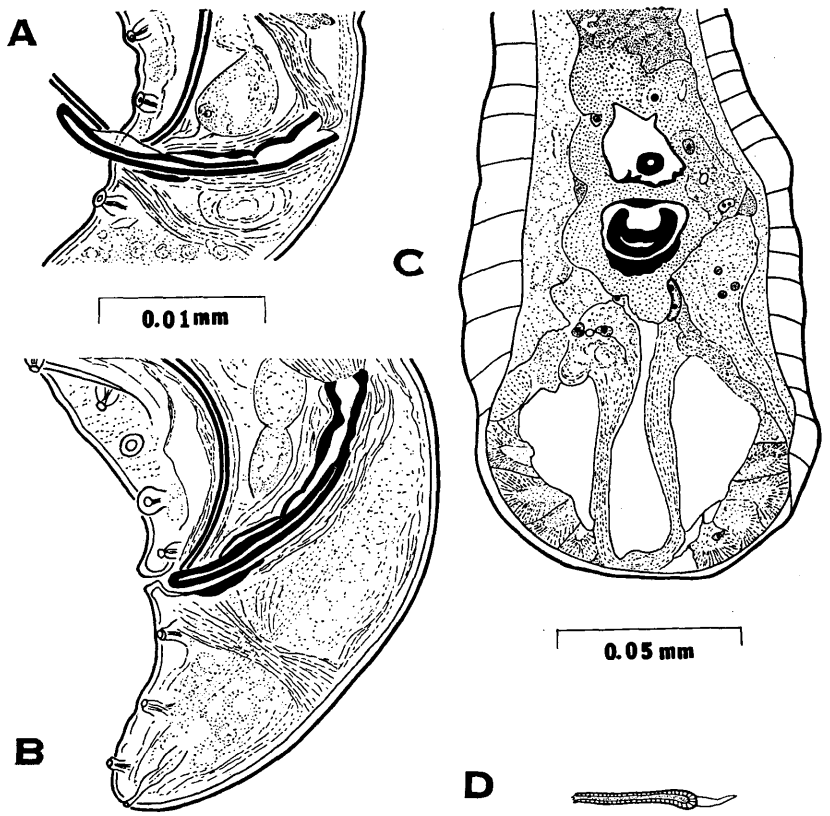


FIG. 2. *Thelazia californiensis* (males). A—Cloacal region; note position of gubernaculum relative to distal tip of partially extruded right spicule. B—Caudal region; note position of gubernaculum relative to distal tip of right spicule. C—Oblique, longitudinal frontal section through caudal region, showing left and right spicules, spicular pouches and gubernaculum in cross section. D—Distal tip of left spicule. (Figs. A, B and D drawn to the same scale.)

to a lesser extent, but the discoloration of the specimen interfered with satisfactory observation. The remaining male specifically mentioned by Price (1931. *loc. cit.*) can be considered a paratype, though he did not designate it so; the dorsal wall of its right spicular pouch, though comparatively thin, was highly refractive, had a wavy appearance, probably due to contraction, and seemed to be slightly thickened.

Four of the males [U. S. N. M. Nos. 31719, 43801, and 43802 (2 specimens)] subsequently identified by Price and four of the five (U. S. N. M. No. 45361) iden-

tified by the writer in 1942 showed a suspect gubernaculum similar to the refractive body indicated in Figure 1. In the fifth male of the lot last mentioned and, as far as could be determined, in one of the "additional specimens" (U. S. N. M. No. 41051) identified by Price, the wall of the spicular pouch appeared as in the "paratype" male. Practically no evidence of thickening dorsal to the right spicule was seen in the remaining male (U. S. N. M. No. 31766) identified by Price.

The possibility that the suspect accessory piece definitely observed to be present in 12 of the 18 males examined might actually be a special and unusual alate region of the right spicule seemed satisfactorily excluded by the fact that its position was identical in specimens in which the right spicule was extruded (Fig. 2, A) and in specimens in which this spicule was not extruded (Figs. 1 and 2, B).

Nevertheless, it seemed desirable to study sections through the cloacal region. Since in *T. californiensis* usually the right spicule is directed nearly at a right angle to the longitudinal axis of the worm's body and the tail region is somewhat coiled in helical fashion, serial oblique, longitudinal sections through the precloacal and caudal portions of a specimen were examined. Study of them showed beyond reasonable doubt the gubernacular nature of the body (Fig. 2, C) which had been observed in cleared whole specimens. The gubernaculum did not, however, appear to be sclerotized to the same degree as the spicules, since it took the stain to some extent, whereas the spicules were unstained. In those sections showing the greatest thickening of the dorsal and dorso-lateral walls of the right spicular pouch, the thickening had a spongy appearance.

As previously noted, some of the specimens examined either lacked a gubernaculum or exhibited only very little thickening, and apparently no sclerotization, of the wall of the spicular pouch. Possibly its development is related to the age of the individual specimen. As many observers no doubt have noted, the spicules of agamic parasitic nematodes generally are feebly sclerotized, or "chitinized," as compared with the spicules of mature males of the same species.

Incidental observations indicated that males of *T. californiensis* have, in addition to three pairs of postcloacal papillae, a pair of subterminal nervous organelles which appear to be identifiable as phasmids. Eight pairs of precloacal papillae were counted in one male. For the left spicule, measurements up to 2.2 mm were obtained; this spicule terminates distally in a sharply pointed hyaline process (Fig. 2, D).

A New Genus of Amphistome (Trematoda) from a Tasmanian Marine Fish[1]

HAROLD W. MANTER[2] and PETER W. CROWCROFT[3]

This paper describes a new genus of trematode collected by the junior author from the intestine of *Dactylosargus arctidens* Gill (kelp fish) from the southern coast of Tasmania. The trematode is an "amphistome" of unusual interest because of the peculiar character of the acetabulum which possesses a posterior pore as well as a ventral opening.

Choanomyzus tasmaniae n. gen., n. sp.

(Figures 1–5)

Description.—Amphistomes measuring 1.442 to 1.806 by 0.868 to 1.064 mm. Body rounded at each end, somewhat more tapering anteriorly, widest posterior

[1] Studies from the Department of Zoology, University of Nebraska, No. 247.
[2] University of Nebraska.
[3] Bureau of Animal Population, Oxford, England, until 1951.

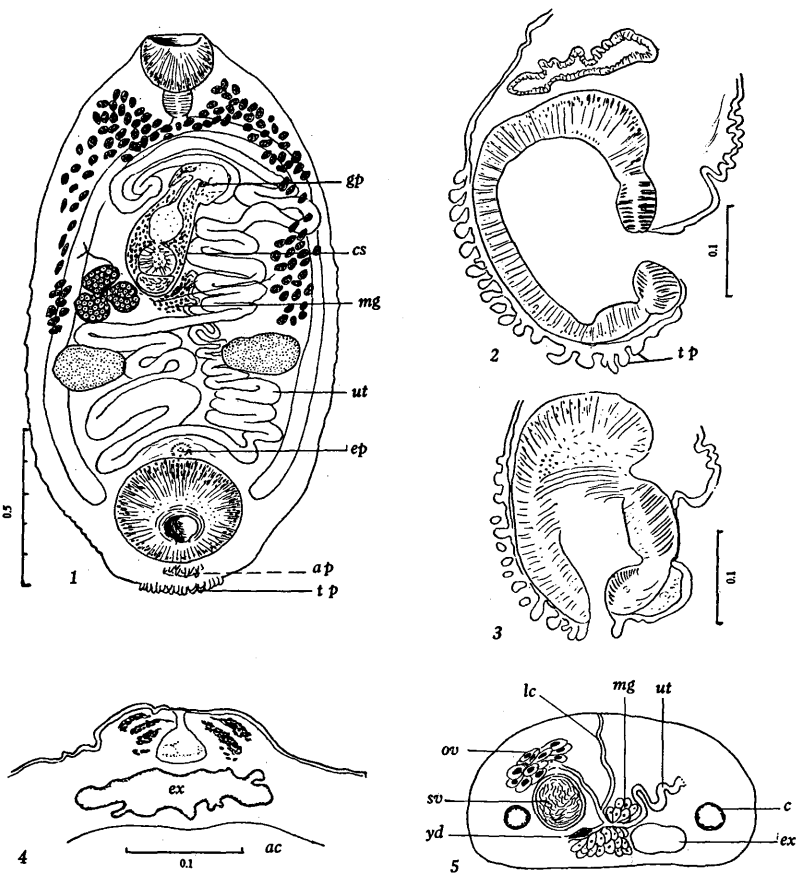
to midbody, somewhat flattened but rather thick-bodied and muscular, without spines or scales but somewhat rugose with low cuticular elevations particularly posterior to midbody. Eye spots or pigment spots lacking. At or near the posterior end are numerous papilla-like or finger-like processes bunched closely together. These vary somewhat in size but are usually about twice as long as wide and are bluntly rounded. They occupy an area approximately opposite the middle 2/4 of the acetabulum (transversely). They may be chiefly terminal but usually extend a short distance dorsally. Oral sucker somewhat wider than long; 0.223 to 0.277 mm. wide by 0.154 to 0.239 mm. long; acetabulum ventral; subterminal; wider than long; 0.400 to 0.470 mm. wide by 0.292 to 0.392 mm. long; ratios of sucker widths (oral sucker-acetabulum) usually about 1: 1.7 but varying from 1: 1.38 to 1.79. The acetabulum is unique in possessing in addition to the usual ventral opening a dorsal or terminal pore (Figs. 1 and 3). The ventral aperture of the sucker is subcircular and relatively small. It shows a small posterior depression or notch due to an interruption in the circular muscles which almost completely surround it. The entire circular muscle region of the acetabulum is separated from the remainder of the sucker by a slight constriction evident only in sections. The circular muscles consist of an external and a slightly smaller internal ring (Fig. 2). The acetabular pore is terminal or subterminal and dorsal, rarely slightly ventral, without special musculature. It opens among the papillae.

Digestive system.—Postoral ring lacking; prepharynx lacking; pharynx somewhat wider than long, 0.063 to 0.087 mm. long by 0.078 to 0.136 mm. wide; esophagus very short or lacking; ceca divergent, extending to near posterior end of body, ending blindly at the sides of the acetabulum.

Excretory system.—The excretory pore is dorsal, on a small elevation, immediately anterior to the acetabulum. It leads to a small spherical chamber surrounded by conspicuous, deeply staining cells (Fig. 4). The posterior portion of the excretory vesicle has a convoluted lining, at least when it is not inflated, containing deeply staining cells. It resembles the intestinal lining. At the level of the testes this portion of the tube leads to a more ventral, more inflated tube lined with a thin membrane. This portion extends close to the ventral body wall, to the posterior edge of the ovary where it divides into two crura which continue forward ventral to the ceca to the level of the pharynx. One crus may be more inflated than the other. The smooth or convoluted inner surface of the vesicle or its crura may be due to amount of fluid within the tube. Since no difference can be noted in the structure of the median vesicle and the crura, the entire organ is considered to be Y-shaped.

Lymphatic vessels are lacking.

Male reproductive system.—Testes two, ovoid, symmetrical, unlobed, wider than long, a little posterior to midbody, well separated by the uterus. Cirrus sac pyriform, 0.319 to 0.385 by 0.123 to 0.208 mm., extending diagonally from ovary to the inconspicuous genital pore located slightly to left of midbody at a level about halfway between ovary and intestinal bifurcation. It contains a sac-like seminal vesicle, a large subspherical prostatic vesicle, followed by a subspherical sac of about the same size which leads to the short cirrus. Deeply staining prostatic cells surround all these parts except the seminal vesicle. The character of the cirrus is not evident from study of toto-mounts. Sections show that near the genital pore the cirrus spreads and folds back around itself so that a portion of it seems to project into a narrow enveloping cavity. This cavity is not the genital atrium, as at first believed, because its lining is identical with that of the cirrus and it is enclosed in the cirrus sac. There is some variation in the two specimens sectioned in the folding of this portion of the cirrus, and the projecting portion



FIGS. 1–5. *Choanomyzus tasmaniae* n. gen., n. sp. 1—Ventral view of *C. tasmaniae*. This specimen is unusual in that the acetabular pore is ventral rather than terminal. 2—Sagittal section through posterior end, showing ventral aperture of the acetabulum and terminal papillae. 3—Sagittal section through posterior end, showing acetabular pore. This section is from the same specimen and is a few sections beyond the one shown in Fig. 2. 4—A portion of a cross section showing excretory pore, the flask-shaped sac into which it leads, and the excretory vesicle. 5—Diagram of the female ducts of *C. tasmaniae*. The diagram is composed of several cross sections near the anterior end of the ovary. All figures except Fig. 5 were made with the aid of a camera lucida. The projected scale has its value indicated in mm. Abbreviations: *ac*, acetabulum; *ap*, acetabular pore; *c*, intestinal cecum; *cs*, cirrus sac; *ep*, excretory pore; *ex*, excretory vesicle; *gp*, genital pore; *lc*, Laurer's canal; *ov*, ovary; *mg*, Mehlis' gland; *sv*, seminal vesicle; *tp*, terminal papillae; *ut*, uterus; *yd*, yolk duct.

may not be oriented toward the genital pore. The uterus enters the posterior side of a small genital atrium. A portion of the cirrus and cirrus sac lie anterior to the genital pore. In contracted specimens the cirrus sac may overlap the pharynx.

Female reproductive system.—The ovary is deeply 4-lobed. It lies to the right approximately at midbody between the right cecum and the base of the cirrus sac, a short distance anterior to the right testis from which it is separated by a coil of the uterus. The oviduct leads from the anterior end of the ovary. Cells of Mehlis' gland are conspicuous, without membrane, preovarian, ventral to

base of cirrus sac. Laurer's canal is present; a seminal receptacle lacking (Fig. 5). The uterus extends posterior to the ovary and testis to near the base of the elevation of the excretory pore. The early coils of the uterus extend anterior to the ovary a short distance, dorsal to the cirrus sac, then turn dorsally and posteriorly. Descending coils of the uterus are in the left half of the body separating the left testis and the acetabulum; ascending coils are on the right, one of them separating the right testis and ovary. At near the level of the cirrus the uterus extends far to the left overlapping the left cecum, then stretches in an arc across the body anterior to the genital pore as far as the right cecum which it may overlap, then it turns back to the left arching along the anterior border of the cirrus sac and enters the posterior edge of the genital atrium. The anterior loop of the uterus suggests the condition in *Opistholebes adcotylophorus* Manter, 1947. Vitellaria extend from oral sucker to the anterior edges of the testes; they are confluent ventrally at level of bifurcation of the ceca. Cells in posterior part of body resemble vitelline cells but lack yolk granules. The yolk ducts meet at the anterior-median edge of ovary. Eggs are yellowish, fairly thin-shelled, 23 to 28 by 11 to 13 μ.

The name *Choanomyzus* is from *choan* = funnel, and *myzus* = sucker. It refers to the acetabulum which is like a funnel, not in general shape, but in possessing a large and a small aperture.

Host.—*Dactylosargus arctidens* Gill, kelp fish. This fish is a member of the Cirrhidae, a family limited to the Pacific.

Habitat.—Intestine.

Locality.—Nubeena, Tasmania.

Type specimens.—To be deposited in the United States National Museum.

Discussion.—This trematode differs considerably from other genera with which it can be compared and its family allocation is not clear. Certainly it is not related to true amphistomes (Paramphistomidae). It is placed tentatively in the family Opistholebetidae because of its posterior acetabulum and glandular papillated region posterior to the acetabulum. The rugose appearance of the body cuticula occurs also in *Opistholebes*. The transverse uterine loop anterior to the genital pore is remarkably similar to the condition in *Opistholebes adcotylophorus* Manter, 1947. The peculiar folded character of the cirrus is similar to a condition described by Ozaki (1937b) for *Glyiauchen* and *Flagellotrema* in the related family Gyliauchenidae. However, *Choanomyzus* differs from the Opistholebetidae in a number of important characters such as lack of seminal receptacle, lack of pigment flecks, lack of postoral ring, more posterior extent of uterus, 4-lobed ovary, and a Y-shaped excretory vesicle.

Choanomyzus shows some interesting resemblances to the Fellodistomatidae including the folded cirrus, lack of seminal receptacle, and posterior extent of the uterus. Furthermore, fellodistomid genera usually have a lobed ovary, a Y-shaped excretory vesicle, and symmetrical testes. Perhaps the Opistholebetidae have connections with the Fellodistomatidae rather than, or in addition, to the Lepocreadiiae as has been suggested (Manter, 1947). Some genera of Fellodistomatidae such as *Discogaster* Yamaguti, 1934, *Paradiscogaster* Yamaguti, 1934, and *Piriforma* Yamaguti, 1938 have highly modified acetabula in the posterior half of the body. *Piriforma* has an acetabulum with transverse slit and conspicuous extra-acetabular muscles as occur in *Opistholebes*, and a deeply lobed ovary as in *Choanomyzus*.

The dorsal or terminal pore of the acetabulum is unique, so far as we can learn. Its significance is not evident. Its size is probably too small to interfere with the suction efficiency of the acetabulum. Other peculiar characters are the

swollen region anterior to the prostatic vesicle in the cirrus sac, and the interrupted circular muscles of the acetabular aperture.

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Two Species of *Lernaeodiscus* (Crustacea: Rhizocephala) from North Carolina and Florida[1]

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The genus *Lernaeodiscus*, as Brinkmann (1936) has shown, can validly contain only species that have a symmetrical internal structure. This clarification has resulted in the transfer to the genus *Triangulus* of five or six species of Rhizocephala that had previously, through error, been included in *Lernaeodiscus* (see van Baal, 1937). The genus, as now understood, includes only three well-established species, viz. *L. porcellanae* Müller 1862, *L. ingolfi* Boschma 1928, *partim*, Brinkmann 1936 and *L. okadi* Boschma 1935. The status of two others, *L. strigosae* Smith and *L. squamiferae* Pérez, is uncertain since these were not carefully studied anatomically at the time they were described. Boschma (1946), however, was able to give further particulars about *L. squamiferae* but could not decide with certainty whether or not this parasite is specifically distinct from *L. ingolfi*.

In the present paper a new species of *Lernaeodiscus* is described, based on a specimen collected by Dr. Waldo L. Schmitt at Tortugas, Florida. This parasite was found infesting *Munida iris* A. Milne-Edwards. The paper also includes notes on *Lernaeodiscus porcellanae* Müller, a species that is especially interesting because it was one of the first Rhizocephala to be described and is the type species of the genus. With the exception of a single specimen collected by Dr. Th. Mortensen's Pacific Expedition at Tobago, B.W.I. and studied by Boschma (1931) this species has not been noted since the original discovery by Müller (1862) on the coast of Brazil.

The specimens of *L. porcellanae* reported on here were collected by Dr. A. S. Pearse during the summer of 1949 at Beaufort, N. C. The host at Beaufort is *Petrolisthes galathinus* (Bosc.). That this crab is parasitized by a rhizocephalid at Beaufort was mentioned more than 30 years ago by Hay and Shore (1918). These authors stated that one or two individuals of *Petrolisthes galathinus* (Bosc.) taken by the *Fish Hawk* at depths of 6 or 7 fathoms off New River Inlet and off the mouth of Cape Fear River bore rhizocephalan parasites. Having studied Dr. Pearse's material, which was also collected off New River, the writer can assert that the unnamed parasite noted by Hay and Shore was undoubtedly also *L. porcellanae*.

[1] Supported in part by a grant from The Catholic University of America Research Fund.

The only other rhizocephalid that has thus far been found at Beaufort is a parasite of the crab *Pilumnus dasypodus* Kingsley, two examples of which were collected by Dr. A. S. Pearse during June and July, 1949, at Black Rocks off New River, N. C. These specimens were sent to the writer for identification and they proved to be *Sacculina hirsuta* Boschma. Previous records of this parasite are from the type locality, Caracas Bay, Curaçao (Boschma, 1925) and from St. Thomas, West Indies (Boschma, 1931). In both of these cases the host was also *Pilumnus dasypodus* Kingsley.

Lernaeodiscus porcellanae Müller 1862

(Fig. 1)

Boschma 1931, pp. 374–378, figs. 54–56.

Material examined.—Black Rocks off New River, N. C., 4 specimens on 4 *Petrolisthes galathinus* (Bosc.), June–July, 1949. A. S. Pearse coll. (Sectioned two.)

The smallest specimen measured 3 mm. in length (mantle opening to stalk), 5 mm. in breadth, and 2 mm. in thickness (dorso-ventral diameter). The largest measured 6 mm. in length, 11 mm. in breadth and 4 mm. in thickness.

The external appearance of the smallest specimen in which the lappets are particularly numerous is shown in Fig. 1, A, B. This specimen did not have eggs in the mantle cavity and was probably immature. The larger specimens had fewer lappets and resembled Boschma's figure (Fig. 54a, b) in general appearance except that the mantle opening was not a narrow slit but more like the aperture shown in Müller's figure.

Where the short, narrow stalk joins the mantle it flares out to form an arcuate shield made up of distinct concentric rings. This feature was also characteristic of Müller's specimens, for, in his original description he speaks of "ein gewölbtes Chitinschild mit concentrischen Streifen." In sections it can be seen that the chitinous flange at the base of the stalk is composed of a substance different from that of the external cuticle. It stains heavily with haematoxylin and has a granular appearance. The layers of which it is composed are arranged perpendicularly to the body surface.

The external cuticle of the mantle varies in thickness from 7 to 14 μ. There are no excrescences on the surface. The internal cuticle lacks retinacula.

The ventral mesentery is short and narrow, occurring only in the posterior half of the body, while the very broad dorsal mesentery extends from the posterior margin of the mantle opening to the stalk.

Boschma (1931) has given a good account of the internal anatomy of this parasite. However, a few points have hitherto escaped attention. One of these is the presence of a spacious connective tissue sac that encloses the testes and continues on as a sheath closely surrounding the vas deferens. There is a considerable space between the wall of the testis and the enveloping sac which in life is probably filled with a fluid. Müller's Fig. 2 clearly shows the presence of such a sac and it is also apparent in Boschma's Fig. 54a, although neither of these authors alluded to it in their text.

The ganglion of this species has also not been previously described. It consists of two masses, one on each side of the midline, the space between them bridged by a heavy strand of nervous tissue. A ganglion divided into two widely separated lateral portions with a commissure between was described and figured in the case of *Lernaeodiscus ingolfi* by Brinkmann (1936, pp. 43–44 and Pl. I, Fig. 11) and it appears likely that this type of ganglion is characteristic of the genus.

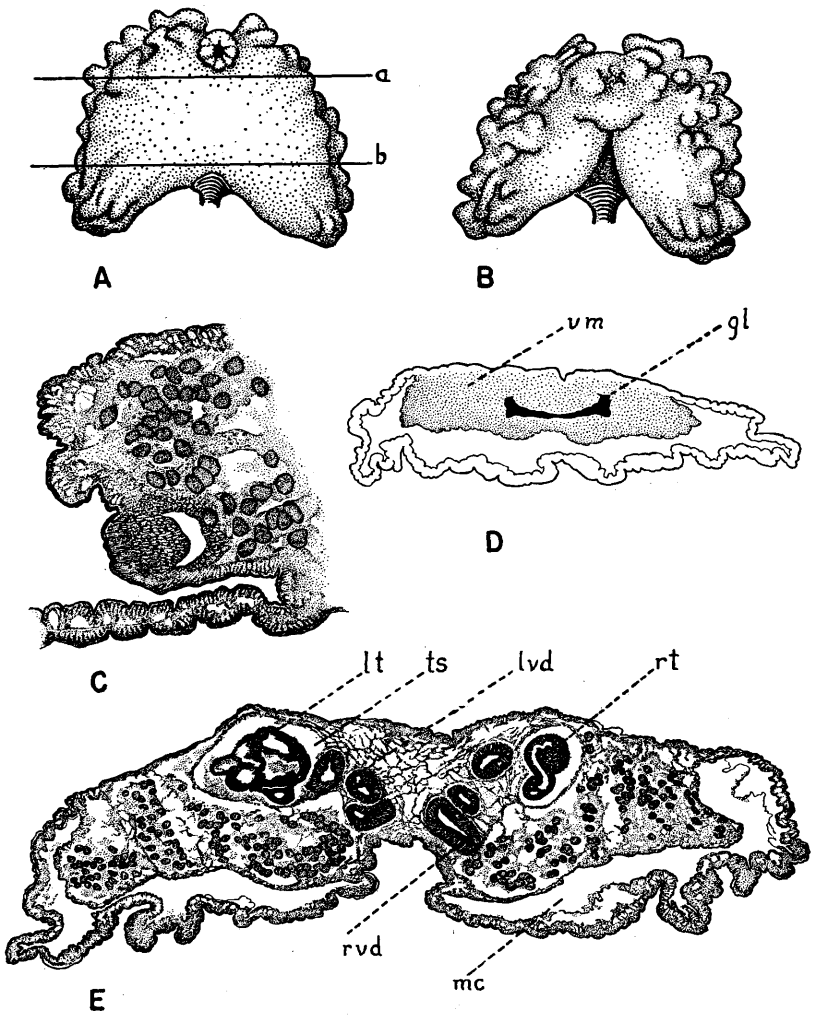

FIG. 1. *Lernaeodiscus porcellanae* Müller. A—Dorsal surface. B—Ventral surface. C—Portion of transverse section showing colleteric gland. D—Schematic transverse section at level of line "a" to show ganglion. E—Transverse section at level of line "b." *Gl.*, ganglion; *lt.*, left testes; *lvd.*, left vas deferens; *mc.*, mantle cavity; *rt.*, right testis; *rvd.*, right vas deferens; *ts.*, testicular sac; *vm.*, visceral mass. (C, D, E drawn by Florence Lambeth.)

In *L. porcellanae* it is found towards the anterior end of the visceral mass, close behind the beginning of the dorsal mesentery (Fig. 1, D).

The testes proper are wide and contorted. They lie in the dorsal posterior part of the visceral mass. The vasa deferentia are divisible into a short proximal portion that runs from the testes mesially and ventrad, and a much longer distal portion that runs along the margin of the ventral mesentery in an anterior direction. These thick-walled tubes are tortuous during the first part of their course but become straight externally, although retaining a strongly coiled lumen, during their course along the ventral mesentery. The vasa deferentia terminate on genital

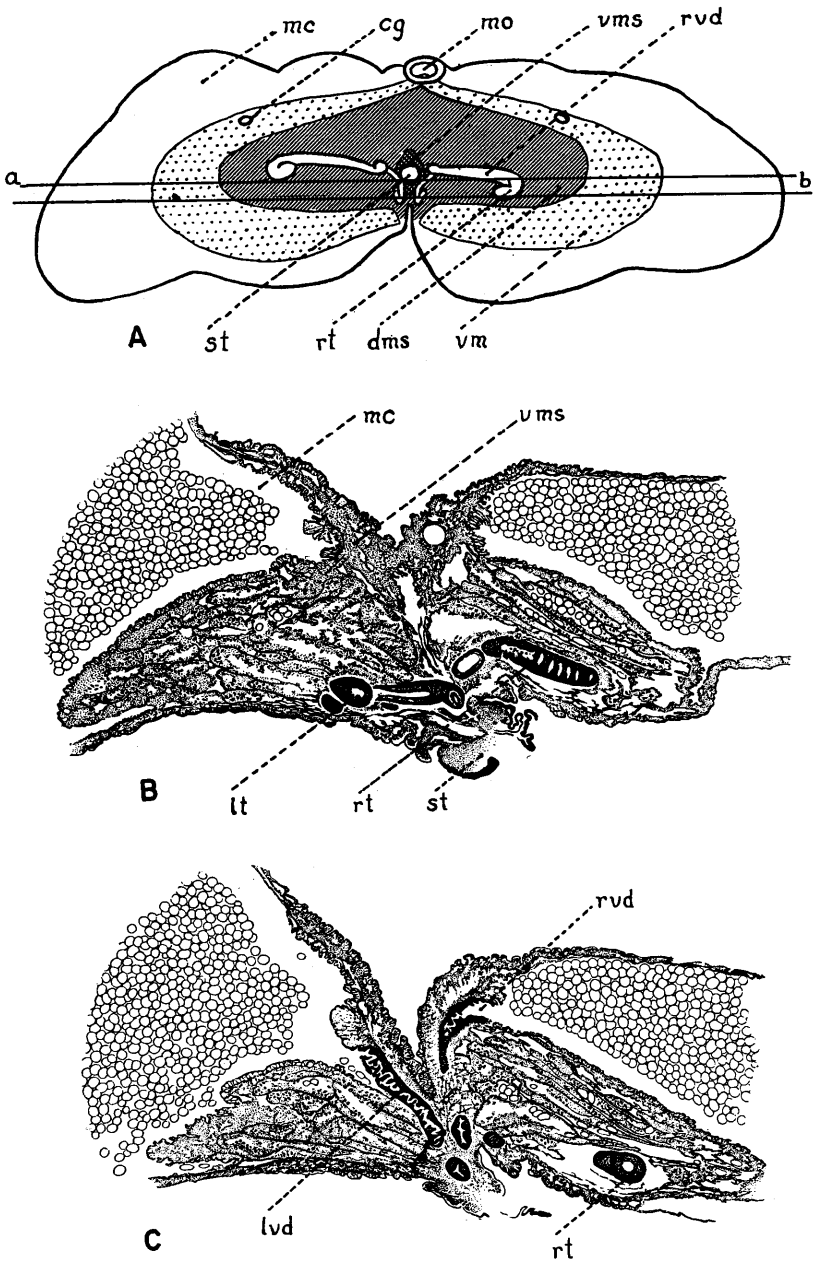

FIG. 2. *Lernaeodiscus schmitti* n. sp. A—Diagram illustrating the position of the principal organs, seen from the dorsal surface. B—Median portion of a transverse section at level of line "a" showing the testes and vasa deferentia. C—Median portion of a transverse section at level of line "b" showing the terminations of the vasa deferentia. *Cg.*, colleteric gland; *dms.*, dorsal mesentery; *lt.*, left testis; *lvd.*, left vas deferens; *mc.*, mantle cavity; *mo.*, mantle opening; *rt.*, right testis; *rvd.*, right vas deferens; *st.*, stalk; *vm.*, visceral mass; *vms.*, ventral mesentery. (B, C drawn by Florence Lambeth.)

papillae that lie close to each other and project ventrally into the mantle cavity at the anterior end of the ventral mesentery.

In the same sections that contain the genital papillae, or immediately in advance of these, one finds the small and inconspicuous colleteric glands. They occur on the dorso-lateral surfaces of the visceral mass about midway between the stalk and mantle opening.

The mantle cavity of one of the specimens contained fully developed nauplii measuring about 170 μ in length and about 100 μ in width. Each nauplius was enclosed in a clear shield, which was likewise true of the specimens described and figured by Müller.

Lernaeodiscus schmitti n. sp.

(Fig. 2)

Type.—Off Tortugas, Florida, 135–156 fathoms, July 2, 1932; one specimen on *Munida iris* A. Milne-Edwards, W. L. Schmitt coll. Transverse sections were made of this parasite and the set of slides will be deposited in the U. S. National Museum.

Diagnosis.—External form bilaterally symmetrical, flattened dorso-ventrally, with large wing-like lateral lobes but without lappets. Antero-posterior axis short. Mantle opening and stalk in the median plane, the former at the anterior extremity, the latter at some distance from the posterior margin.

Dorsal mesentery very broad and as long as the antero-posterior axis; ventral mesentery narrow and extremely short, confined to the region of the stalk. Male genital organs at the level of the stalk, extending to the right and to the left, with their closed ends recurved dorsad. Vasa deferentia made up of three divisions: the proximal running in a lateral direction towards the midline, the medial passing posteriorly along the dorsal mesentery, and the distal proceeding ventrad to terminate near the posterior end of the ventral mesentery. Colleteric glands small, ovate, with undivided lumen, in anterior half of body, on ventro-lateral surface of visceral mass. Stalk without internal chitinous projections.

Description.—The parasite, which was attached to the second abdominal segment of the host, has the form of two broad lobes that project laterally from the constricted midline on which the stalk and mantle opening are located. Each lobe appears to be subdivided into a smaller anterior and a larger posterior portion. The right lobe is somewhat larger than the left, but this is probably not a character of the species, since the animal had been injured on the right side. The parasite measures 4 mm. in length (antero-posterior axis) and 17 mm. in breadth. Its thickness (dorso-ventral diameter) varies from 3 mm. at the midline to 5 mm. at the expanded portion of the lateral lobes. The extreme shortness of the antero-posterior axis in proportion to the breadth of the animal is a noteworthy feature that may assist in differentiating this species from the other known species of Lernaeodiscus.

The round mantle opening at the anterior extremity of the animal is surrounded by a rather thick elevated ring which is thrown into folds by the contraction of the sphincter muscle. The stalk, which is subterminal on the dorsal surface and somewhat prolonged, arises from a swollen pad-like base covered with mal is smooth and the external cuticle thin, measuring 3.5 to 10 μ in thickness much thicker chitin than that of the rest of the mantle. The surface of the ani- except in the immediate vicinity of the stalk where it gradually attains a thickness up to about 40 μ.

Apart from the well-developed sphincter muscles surrounding the mantle open-

ing, the mantle musculature is not prominent. The mantle proper is thicker in the mid-ventral region than elsewhere.

The visceral mass, which reaches from the mantle aperture to the posterior margin of the animal, is closely applied to the mantle along the dorsal surface. This area of attachment constitutes the dorsal mesentery. The ventral mesentery is found only in the immediate vicinity of the stalk. It is short and narrow and is connected with the dorsal mesentery by a mass of muscle fibers.

A transverse section through the mid-region of the animal shows the visceral mass as a low and broad elevation, highest in the center and sloping gradually towards the sides. Laterally and ventrally (except at the ventral mesentery) the mantle cavity is greatly distended with eggs undergoing development.

The left colleteric gland is a simple, papilla-like aperture situated on the ventro-lateral margin of the visceral mass in the anterior third of the animal. Its greatest width, including epithelium, is only about 390 μ; its depth 250 μ. The right colleteric gland could not be found.

The testes lie in the dorsal portion of the visceral mass and extend as narrow tubes to the right and to the left of the midline at the level of the stalk. The closed ends of the testes are recurved towards the dorsal surface of the parasite. Before the testes reach the midline they pass into the vasa deferentia, tubes of approximately the same thickness as the testes. The vasa converge as they proceed posteriorly, run parallel with each other along the margin of the dorsal mesentery for a short distance, then pass abruptly from the dorsal to the ventral surface where each empties into the mantle cavity at the summit of a genital papilla immediately to the right and to the left of the main axis of the body. The male genital openings occupy a position posterior to the testes, at the posterior end of the ventral mesentery.

Each vas deferens has a narrow, sinuous lumen, but the organ proper is, for the most part, a straight tube, bent sharply in two places where it changes its course. A sheath of muscle and connective tissue surrounds the vas deferens. In cross section its lumen appears as an irregular slit.

Remarks.—This new species is named for the collector, Dr. Waldo L. Schmitt, Head Curator of Zoology, U. S. National Museum.

L. schmitti is morphologically nearest to *L. ingolfi*. These two species both have relatively narrow testes and are similar with respect to the position of the male genital openings. The stalk of *L. schmitti*, however, does not have internal chitinous projections and the external proportions of the body are significantly different. Moreover, the present species is readily distinguishable from *L. ingolfi* and the other known species of the genus by the fact that the vasa deferentia run along the margin of the dorsal mesentery and the colleteric glands are on the ventral surface of the visceral mass, whereas in the other species the vasa deferentia run along the ventral mesentery and the colleteric glands are lateral in position.

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Notes on Toto-mount Technique

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Frequently, it is necessary for taxonomists to restudy collections of animals which have been stored in cork-stoppered vials of alcohol for periods of years. If stained toto-mounts are required, such material is usually of little value, since it often is stained with extracts from the cork. However, these extraneous substances can be removed by treating with fat solvents and oxidizing agents. The following method has proved to be most successful.

The specimens are placed in dishes of distilled water for 5–10 minutes, and then are transferred to pyridine. Microcrustacea, small trematodes and small cestodes are allowed to remain in the pyridine for 30 minutes. Large cestodes require about two hours; i.e., twice the time necessary for them to become clear. The pyridine is washed out with many changes of distilled water, and when the odor of the reagent is no longer discernible, the specimens are covered with three times their volume of 0.25% potassium permanganate. Here, too, the length of time for the treatment is dependent upon the size of the organisms and may require from 20 minutes to one hour. Generally, the rate of penetration is quite slow, but can be hastened by turning the material over. Following the permanganate bath, the specimens should be thoroughly washed in distilled water, and then transferred to a decolorizer consisting of equal parts of 1.0% oxalic acid and 1.0% sodium bisulfite. This phase of the procedure requires from 20–40 minutes, and when completed leaves the treated objects a bone-white or a slightly yellowish color. Once the reaction between the decolorizer and the permanganate ceases, the specimens begin to shrink, and thus, the process must be frequently observed. A prolonged washing in distilled water is necessary following the bisulfite treatment. A further bath in pyridine may be required, but usually is not necessary. Satisfactory staining results can be obtained if the specimens are allowed to remain in 70% alcohol for 24 hours after treatment.

The staining of specimens *in toto* differs markedly from the methods used in histology and cytology. The greatest difficulty which one encounters is that of "destaining" or differentiating. The familiar solid red masses labelled "Taenia" which are found as slides in the zoology departments of our universities have their counterparts in the type specimens which have served as the bases for the descriptions of many species of worms and microcrustaceans. A staining solution suggested by Dr. F. Proescher, of San Jose, Calif. avoids the usual difficulties encountered in staining whole mounts. It is a more stable modification of the Coelestin blue B-lake described by Proescher, Zapata, and McNaught (1946) and is prepared as follows:

Dissolve 2 grams of ferric ammonium sulfate (violet crystals) in 100 cc of cold distilled water. Add 2 cc of concentrated sulfuric acid. Bring to a boil and add 1 gram of Coelestin blue B. Boil for a few minutes. Cool and add 10 cc of absolute methyl alcohol and 10 cc of glycerin.

Although this is an instantaneous stain for sections, *toto* preparations must remain in the solution for from 15 minutes to 1 hour depending upon thickness. Much of the excess dye washes out in distilled water, and the remainder is extracted by 70% alcohol. There is no necessity for using acid in the process of differentiation. The excess dye frequently is completely removed after the second change of 70% alcohol. The alcohol should be changed as often as it becomes discolored, and the specimens are completely differentiated when the dye is no longer given up to the alcohol.

This staining procedure follows most fixatives. Material killed in Carnoy or any of the modifications of AFA will not differentiate if stained soon after it is fixed. It must be washed in water for 24 hours before staining, or it should be stored in 70% alcohol for two or more weeks. Bouin's fixative is not recommended for the preparation of whole mounts of flatworms at any time, and the Coelestin blue lake has no particular advantage over any other stain following this agent except when the specimens are treated with pyridine following the alcoholic removal of the picric acid.

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MINUTES

Two Hundred Eighty-fifth to Two Hundred Ninety-second Meetings

The 285th meeting was held October 12, 1949, at The Catholic University of America, Washington, D. C. The following were elected to membership: Harold J. Jenson, E. G. Vogelsang, H. M. Martin, Donald J. Ameel, David R. Lincicome, E. Francis Ervin, and Robert E. Kuntz. Papers were presented by Spaeth, Lynn, Steiner, Reinhard, and Chitwood.

The 286th meeting was held November 16, 1949 at the Station of the Zoological Division, U. S. Bureau of Animal Industry, Beltsville, Maryland. The following were elected to membership: Harriet K. Howard, Judith Humphrey, Charles G. Durbin, Bakir A. Oteifa, and Luke Sinclair. Papers were presented by Kates, Dikmans, Enzie, and Tarjan.

The 287th meeting was held at The Catholic University of America, Washington, D. C. on December 14, 1949. Officers elected to serve in the year 1950 were: L. J. Olivier, President; M. A. Doss, Vice-president; W. G. Jahnes, Recording Secretary; E. M. Buhrer, Corresponding Secretary-Treasurer. Rev. Richard Timm and Dr. Wilford Olsen were elected to membership. Papers were presented by Dr. Shortt, English Protozoologist, Alicata, Steiner, Wehr, Baker, Timm, Tarjan and Reinhard.

The 288th meeting was held January 18, 1950 at The Catholic University of America, Washington, D. C. Elected to membership were: Bruce P. Phillips, Paul P. Weinstein, Nathan W. Riser, and Berwin A. Cole. Dr. Otto was reappointed to the Editorial Board of the *Proceedings;* Dr. Kates was reappointed to the Executive Committee; Dr. Price was reappointed as the Society's representative to the Washington Academy of Science. Society dues were reduced from $5.00 to $4.00 and subscription rates increased from $1.75 to $2.50, other rates to be adjusted proportionately. Papers were presented by Olivier and by Young, U. of Montana.

The 289th meeting was held on February 15, 1950 at Wilson Hall, National

Institutes of Health, Bethesda, Maryland. The following were elected to membership: John R. Elsea, Joseph C. Hwang, Lyman P. Frick, Doris Newman, Jack D. Tiner. Papers were presented by Phillips, Greenberg, and Cram.

The 290th meeting was held at Sternberg Auditorium, AMDR&GS, Army Medical Center, Washington 12, D. C. on March 22, 1950. The following were elected to membership: Harold W. Manter, Mary L. Hanson, William B. DeWitt, Ethyl L. Ballard and Donald L. Price. Dr. Shorb was appointed Chairman of the committee to formulate plans for the 40th anniversary celebration of the Society. Papers were presented by Alicata and Tarjan.

The 291st meeting was held April 14, 1950 at the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland. Papers were presented by Baily, Chernin, Eyles, Foote, and Kartman.

The 292nd meeting was held June 3, 1950 in the form of an annual picnic meeting at the Plant Industry Station, Beltsville, Maryland. Elected to membership were: L. DeConick, M. J. Fielding, C. M. Herman, J. G. Baer.

WILLIAM G. JAHNES,
Recording Secretary

Report of the Brayton H. Ransom Memorial Trust Fund

FUNDS ON HAND, Jan. 1, 1949	$1668.41
RECEIPTS: Interest rec'd in 1949	58.76
DISBURSEMENTS: Expenses and grant to Helminthological Society of Washington	31.00
BALANCE ON HAND, Dec. 31, 1949	$1696.17

ELOISE B. CRAM,
Secretary-Treasurer

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The following membership list, arranged geographically, includes life, resident, and nonresident members, as defined in Art. 3 of the Constitution (Vol. 13, No. 1 of the Proceedings).

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Doss, M. A.
Durbin, C. G.
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Farr, M. M.
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Habermann, R. T.
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Huff, C. G.
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