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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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Treatment of coccidial infections of lambs with sulfaguanidine. A. O. FOSTER, JOHN F. CHRISTENSEN, and ROBERT T. HABERMANN, U. S. Bureau of Animal Industry.

Of the many substances that have been tested for prophylactic and curative action against coccidial infections of the domesticated animals, only elemental sulphur and a few sulphur-containing compounds have shown sufficient promise to warrant their continued investigation. Herrick and Holmes (1936), studying cecal coccidiosis of poultry, first directed attention to the beneficial effects of sulphur, and the subsequent literature on this subject was summarized by Levine (1941a). Meanwhile, Spindler (1939) and Christensen (1940), in consequence of suggestions from earlier reports, showed that a combination of iron and copper sulphates, under properly guarded conditions of administration, caused a reduction of the oöcyst output of infected lambs and had a desirable effect upon the host animals. While these treatments were not generally accompanied by injurious effects when tested experimentally, both sulphur and copper are known to be more or less toxic under conditions of continued administration.

More recent studies with some of the sulfonamides have suggested that certain members of this series may hold more promise in the treatment of coccidiosis than any of the substances previously tested. With sulfanilamide, Levine (1939) demonstrated the first definitely inhibitory effect upon coccidia, although it was noted that the drug retarded the growth of the experimental chickens. Severin (1939) and Moore and Thompson (1941) indicated that this drug may be effective against coccidial infections of dogs. The latter workers showed also that it was of probable value in similar infections of cats and calves. Favorable results with sulfanilamide in coccidiosis of calves were also reported by McPeek and Armstrong (1941). In a study with sulfapyridine, Levine (1940) observed that this compound was about as efficacious as sulfanilamide and less toxic, although neither of these substances was significantly effective against the more pathogenic species of poultry coccidia, namely, *Eimeria tenella* and *E. necatrix*.

In September, 1940, Marshall, Bratton, White, and Litchfield published a pharmacological study of a newly synthesized compound of this series, sulfaguanidine (sulfanilylguanidine), that showed particular promise for the treatment of bacterial infections of the intestine. In spite of its present prohibitive cost, two properties of this substance seemed especially to recommend its trial against coccidiosis, viz.; its relatively high solubility in water and its low absorption from the alimentary tract. These properties permit the establishment and maintenance of an intraintestinal saturation with the drug and, at the same time, augur a low toxicity after oral administration. Accordingly, a preliminary experiment was initiated to test this compound for possible preventive and curative action against naturally acquired coccidial infections of lambs. When these studies were nearing completion, it was reported in a study by Levine (1941b) that this chemical "holds promise as a possible coccidiostatic agent for the control of all types of coccidiosis." His investigations dealt with the effect of this drug on experimentally induced infections in chickens with six different species of coccidia.

METHODS

The experiment described herein was begun March 24 and terminated June 14. 1941, and was conducted on experimental stock at the U. S. Department of Agriculture Beltsville Research Center, Beltsville, Md. Shortly after birth, 16 nursing lambs were divided into 4 groups and placed in separate pens indoors with their ewes. In order to accommodate both the ewes and their lambs, without undue crowding in any one pen, it was necessary to put 5 lambs in one group, 3 in another, and 4 in each of the others. Treatments with sulfaguanidine, obtained through the courtesy of a manufacturer, were commenced when the lambs were 11 to 14 days old, or about 2 or 3 weeks prior to the expected onset of oöcyst production resulting from naturally acquired coccidial infections. The drug was administered daily, except Sundays, in 2-gram and 1-gram doses, respectively, to each of the lambs of 2 groups. It was administered orally in gelatin capsules, using a balling gun. The lambs of the other groups were kept untreated until late in the experiment when 2-gram doses were given to the lambs of one group to test for curative action. Each day, after being treated, the lambs were confined for 2 to 4 hours in small pens with clean, concrete floors to facilitate the collection of fecal specimens. When the latter were collected, the lambs were returned to their ewes.

Management of the animals was designed to provide optimum conditions for the acquirement by the lambs of natural coccidial infections from oöcysts shed by the carrier ewes. Hay was fed upon the floor and grain in shallow open pans, and water given in open pails. Litter was allowed to accumulate for a week before pens were cleaned, permitting ample time for sporulation of oöcysts and appreciable fecal contamination of feeds. After weaning, which took place about midway in the experiment, the lambs of all 4 groups were kept together in a contaminated pen except for the intervals mentioned. This was done to insure a more or less uniform exposure of both the treated and the control lambs. Estimates of oöcyst output were made twice weekly, according to a method employed by Christensen (*loc. cit.*), on 3-day composite fecal specimens of each group. The general condition of the animals and the character and consistency of their feces were noted daily. The weight of each lamb was recorded weekly.

OBSERVATIONS

No oöcysts were detected in feces from group I lambs during nearly 6 weeks of treatment with 2-gram doses of sulfaguanidine, during another week with 1-gram doses, and for more than an additional week following cessation of treatment. Oöcysts were first found during the sampling interval of May 19 to 21, or 9 to 11 days after the last administration of the drug. For the remainder of the period without treatment, average levels of oöcyst production for lambs of this group were significantly high. (Table 1, Group I.)

Oöcyst discharge in the untreated group II lambs began during the April 14 to 16 sampling interval, or only 3 weeks after the beginning of drug administration in the treated lambs. Oöcysts were easily detected by dilution count in every sample throughout the remainder of the experiment, usually in considerable numbers. (Table 1, Group II.)

The lambs in group III, each of which received 1-gram doses of sulfaguanidine throughout the experiment, showed no oöcysts in the feces during the first 8 weeks of treatment. After infection was first detected in the composite sample of May 19 to 21, these lambs discharged oöcysts during the remainder of the test, but only in the sample of May 26 to 28 were appreciable numbers noted. It is significant that about 40 per cent of the oöcysts discharged during this interval were apparently unfertilized. The protoplasm of these abnormal oöcysts appeared densely

TABLE 1.—*Effect of sulfaguanidine on oöcyst production in natural coccidial infections of young lambs*

Sampling period (1941)	Group I (5 lambs)		Group II (3 lambs)		Group III (4 lambs)[a]		Group IV (4 lambs)	
	Sulfaguanidine dosage (grams per day per lamb)	Total average oöcyst output (per cc of pooled feces)	Sulfaguanidine dosage (grams per day per lamb)	Total average oöcyst output (per cc of pooled feces)	Sulfaguanidine dosage (grams per day per lamb)	Total average oöcyst output (per cc of pooled feces)	Sulfaguanidine dosage (grams per day per lamb)	Total average oöcyst output (per cc of pooled feces)
Mar. 24–26	2	...	0	0	1	0	0	...
Mar. 27–29	2	0	0	0	1	0	0	0
Mar. 31–Apr. 2	2	...	0	0	1	0	0	...
Apr. 3– 5	2	0	0	0	1	0	0	0
Apr. 7– 9	2	0	0	0	1	0	0	0
Apr. 10–12	2	0	0	0	1	0	0	0
Apr. 14–16	2	0	0	2,000	1	0	0	0
Apr. 17–19	2	0	0	48,000	1	0	0	0
Apr. 21–23	2	0	0	10,000	1	0	0	1,600
Apr. 24–26	2	0	0	1,800	1	0	0	2,200
Apr. 28–30	2	0	0	91,000	1	0	0	18,000
May 1– 3	2	0	0	84,000	1	0	0	50,000
May 5– 7	1	0	0	29,000	1	0	0	47,000
May 8–10	1	0	0	154,000	1	0	0	37,000
May 12–14	0	0	0	5,200	1	0	0	23,000
May 15–17	0	0	0	24,000	1	0	0	33,000
May 19–21	0	1,200	0	10,000	1	400	0	31,000
May 22–24	0	6,500	0	9,800	1	+	0	37,000
May 26–28	0	51,000	0	14,000	1	20,000	2	33,000
May 29–31	0	47,000	0	12,000	1	400	2	15,000
June 2– 4	0	6,800	0	1,200	1	+	2	+
June 5– 7	0	29,000	0	3,600	1	+	2	+
June 9–11	0	16,000	0	3,200	1	400	2	+
June 12–14	0	43,000	0	13,000	1	400	2	800

[a] This group was reduced to 2 lambs midway in the experiment.
+ Indicates detection by flotation only.

TABLE 2.—*Comparative growth gains in sulfaguanidine-fed and untreated young lambs*

Duration of period		Group I			Group II			Group III			Group IV		
Dates	Days	Total sulfaguanidine administered per lamb	Total average weight gain per lamb	Average daily gain per lamb	Total sulfaguanidine administered per lamb	Total average weight gain per lamb	Average daily gain per lamb	Total sulfaguanidine administered per lamb	Total average weight gain per lamb	Average daily gain per lamb	Total sulfaguanidine administered per lamb	Total average weight gain per lamb	Average daily gain per lamb
		grams	*pounds*	*pounds*	*grams*	*pounds*	*pounds*	*grams*	*pounds*	*pounds*	*grams*	*pounds*	*pounds*
March 24 to May 12	49	78	15.7	0.32	0	15.1	0.31	42	19	0.39	0	18.3	0.37
May 12 to June 14	33	0	5.8	0.18	0	8.2	0.25	30	8.5	0.26	36	8.4	0.25

granular throughout the oöcysts and showed no tendency to contract into pale, spherical sporonts characteristic of normal oöcysts. All other rates of discharge during the positive period were extremely low, infections being either barely detected by dilution count or by flotation only. (Table 1, Group III.)

Group IV lambs, which served as untreated controls until late in the test, initiated oöcyst production a week later than the group II controls and showed similar relatively high average levels of discharge for the next 5 weeks, or until about a week after 2-gram doses of sulfaguanidine were begun. When these administrations were started on May 26 to test the ability of the drug to reduce or terminate an existing natural coccidial infection, the average level of oöcyst production was relatively high and nearly all the oöcysts appeared normal. Although average discharge for the sampling period of May 29 to 31, or 3 to 5 days after the beginning of treatment, was 15,000 oöcysts per cc of feces, approximately two-thirds of these oöcysts were unfertilized. By the end of a week after the first treatment and for as long as the drug was given, oöcysts from group IV lambs were detected only once by dilution count, the remaining times by flotation. (Table 1, Group IV.)

In the amounts used and for the periods administered, the sulfaguanidine had no apparent deleterious effect upon the growth rate of the lambs. During 7 weeks of treatment with 2-gram doses of the drug, group I lambs showed nearly identical average daily weight gain per animal to that of the untreated control group II. Likewise, the lambs receiving 1-gram doses in group III showed average gain per lamb similar to that in untreated group IV lambs. During the last 5 weeks of the test, when group I lambs received no drug, average daily weight gain per lamb diminished in all groups, but were similar enough in 3 of the 4 treated and untreated groups to warrant the conclusion that in 1- and 2-gram daily doses per lamb the drug had no ill effect on growth rate of the lambs over the period of this experiment. (Table 2.)

The only instances of diarrhea during the entire experiment were noted in lambs untreated at the time with sulfaguanidine. One case of mild coccidiosis, with diarrhea and high oöcyst discharge, was observed during the height of the infection in the untreated group II lambs. Two or three of the lambs in group I scoured considerably after sulfaguanidine had been discontinued during the last 2 weeks of experiment, which probably accounted for the relatively low average daily gain per lamb recorded for this group during the last 5 weeks (Table 2). Two of the original 16 lambs were lost during the experiment, both from group III. One lamb died from malnutrition following death of the mother ewe, the other apparently from overeating of grain. That neither death could be linked to sulfaguanidine administrations is shown by the fact that all 5 of the group I lambs flourished during treatment with 2-gram doses of the drug.

CONCLUSIONS

The results of this experiment appear to justify the following conclusions: Administrations of sulfaguanidine in 2-gram amounts per lamb daily, except Sunday, prevented completely the acquirement of natural coccidial infections in 5 lambs, and rapidly reduced to insignificant proportions heavy existing subclinical natural infections in 4 lambs. Administrations of the drug in 1-gram daily doses per lamb postponed initial oöcyst discharge from natural infections for at least 4 weeks in 2 lambs and, once oöcyst production had started, kept it effectively subdued for as long as the treatments were continued. In the dosages specified, the drug had no apparent harmful influence on the growth and development of the lambs during the course of the experiment.

LITERATURE CITED

CHRISTENSEN, JOHN F. 1940. The effect of copper sulfate and ferric sulfate on coccidial oöcyst output in feeder lambs. Jour. Amer. Vet. Med. Assoc. **96**: 478–480.

HERRICK, C. A., and HOLMES, C. E. 1936. Effects of sulphur on coccidiosis in chickens. Vet. Med. **31**: 390–391.

LEVINE, P. P. 1939. The effect of sulfanilamide on the course of experimental avian coccidiosis. Cornell Vet. **29**: 309–320.

————. 1940. The effect of sulfapyridine on experimental avian coccidiosis. Jour. Parasitol. **26**: 233–235.

————. 1941a. Sulphur therapy in experimental avian coccidial infection with *Eimeria necatrix*. Cornell Vet. **31**: 120–126.

————. 1941b. The coccidiostatic effect of sulfaguanidine (sulfanilylguanidine). *Ibid.* **31**: 107–112.

MARSHALL, E. K., JR., BRATTON, A. CALVIN, WHITE, H. J., and LITCHFIELD, J. T., JR. 1940. Sulfanilylguanidine: a chemotherapeutic agent for intestinal infections. Bull. Johns Hopkins Hosp. **67**: 163–188.

MOORE, G. R., and THOMPSON, W. W. 1941. Sulfanilamide in coccidiosis. Jen-Sal Journal, Jan.–Feb., pp. 8, 9, 29.

MCPEEK, RAYMOND, and ARMSTRONG, FERNANDO E. 1941. Bovine coccidiosis treated with sulfanilamide. Vet. Med. **36**: 199–201.

SEVERIN, J. E. 1939. Sulfanilamide in canine coccidiosis. Jour. Amer. Vet. Med. Assoc. **94**: 240.

SPINDLER, L. A. 1939. Effect of ferric sulphate and copper sulphate on coccidiosis in lambs. North Amer. Vet. **20** (5): 33–38.

Further tests with unconditioned phenothiazine as an anthelmintic in cattle. DALE A. PORTER, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama.

The efficacy of unconditioned phenothiazine as an anthelmintic in cattle, indicated in critical tests conducted by Swanson, Porter, and Connelly (1940, Jour. Amer. Vet. Med. Assoc. **96**: 704–707) has been substantiated by numerous clinical observations (Taylor and Sanderson, 1940, Vet. Rec. 52: 635–647; LaPage, 1940, Vet. Rec. **52**: 648–657; Foggie, 1940, Vet. Rec. 52: 783–785; Porter, Simms, and Canthen, 1941, Jour. Amer. Vet. Med. Assoc., in press). In view of the fact that the doses of phenothiazine administered in the first tests were rather large, further experiments were conducted to determine the efficacy of smaller doses for the removal of nematodes most commonly encountered in bovines.

Of the 6 animals used in the critical tests, 4 (Nos. 57, 62, 63, and 64) were naturally parasitized yearlings purchased near Auburn, Alabama, and 2 (Nos. 75 and 89) were calves obtained the day after birth and raised free of parasites until experimentally infected. The yearlings were steers of mixed breeding. Yearling 57 had suffered a dislocated shoulder joint shortly after birth and had remained in a stunted condition. Nos. 62, 63, and 64 were from a herd having a history of parasitism and particularly poor grazing with little or no supplemental feed. Calf 75, a Hereford-Jersey cross bull, and calf 89, a grade Jersey bull, were in good condition and 3 months old at the time of treatment. Calf 75 was fed infective larvae of *Haemonchus contortus* 48 and 42 days before treatment and calf 89 was fed infective larvae of *Ostertagia ostertagi, Cooperia* spp., and *Oesophagostomum radiatum* 41 days before treatment. In addition to those used in the critical tests, 2 yearlings were treated in a field test.

The unconditioned phenothiazine used was obtained from commercial sources and was administered either in hard gelatin capsules or mixed with the grain ration and placed before the individual animal. The experimental animals were confined in concrete-floored pens and fed alfalfa hay and grain. Calves 75 and 89 were also receiving about 3 quarts of whole milk daily at the time of treatment. Some of the animals were treated without fasting, while others were fasted 16 hours before

TABLE 1.—*The efficacy of unconditioned phenothiazine for the removal of gastrointestinal nematodes from cattle*

Host[a] No.	Treatment				Worms eliminated following treatment					
	Weight	Period of fasting	Dose	Date 1940	*H. contortus*	*O. ostertagi*	T. *axei*	*B. phlebotomum*	*Cooperia* spp.	*O. radiatum*
	pounds	*hours*	*grams*[b]							
57	120	16	20F	3–20	11	28	28	0	0	3
62	200	0	20F	4–18	39	160	3636	0	0	172
63	160	16	8C	5–18	100	0	1553	0	0	123
64	170	16	17C	5– 4	48	0	2142	9	0	71
75	110	0	11C	9–23	3	0	0	0	0	0
89	110	0	22C	12– 9	0	158	0	0	929	54

Host No.	Date 1940	Worms recovered at necropsy					
		H. contortus	*O. ostertagi*	*T. axei*	*B. phlebotomum*	*Cooperia* sp.	*O. radiatum*
57	3–29	0	60	0	0	240	0
62	4–25	0	120	60	0	940	0
63	5–27	0	1880	180	65	9,760	70
64	5–13	0	60	40	49	5,120	0
75	10– 4	0	0	0	0	0	0
89	12–16	0	0	0	0	19,550	0
		Percentage of efficacy					
57	3–29	100	31.8	100.0	.....	0.0	100.0
62	4–25	100	57.1	98.4	.....	0.0	100.0
63	5–27	100	0.0	89.6	0.0	0.0	63.7
64	5–13	100	0.0	98.2	15.5	0.0	100.0
75	10– 4	100	.....	.....	.....	.....	.....
89	12–16	.....	100.0	.....	.....	4.5	100.0

[a] Calves 75 and 89 were 3 months old, all others were yearlings.
[b] Phenothiazine given in feed (F); in capsules (C).

the drug was given. The number of parasites passed after treatment and the number of worms remaining at necropsy, 7 to 11 days after the administration of the drug, were determined by the method outlined in another paper (Swanson, Porter, and Connelly, *loc. cit.*). Details of the tests and the results obtained are set forth in table 1.

Doses of unconditioned phenothiazine ranging from 8 to 22 grams (0.05 to 0.2 gram per pound body weight) removed 100 per cent of *Haemonchus*, and 89.6 to 100 per cent of *Trichostrongylus axei*. The drug was 100 per cent effective against *Oesophagostomum* except in yearling 63 to which 8 grams were given (0.05 gram per pound body weight). It will also be noted that the lowest efficacy against *Trichostrongylus* also occurred in this case. All of the *Ostertagia* were removed from calf 89, which was given 22 grams. This was the largest dose employed; smaller doses were ineffective against this parasite. Yearling 63, given 8 grams, and yearling 64, given 17 grams, of phenothiazine, eliminated 0 and 15.5 per cent of their hookworms, respectively. Phenothiazine failed to remove any of 4 *Trichuris* from yearling 57, 260 *Capillaria* from yearlings 63 and 64, 940 *Nematodirus* from yearling 63, and 60 *Strongyloides* from calf 89. The drug was also ineffective against the cooperids in that only one of the animals so infected passed any of these worms and then only 4.5 per cent of those present.

No toxic effects or pathology that could be associated with the drug was observed in any of the animals regardless of the method of administration. Apparently the phenothiazine was equally effective when given in the feed or by capsule, with or without a preliminary period of fasting.

Two experiments with phenothiazine not reported in table 1 are also of interest. A yearling Jersey heifer, 10 months old and weighing 200 pounds, was given, on February 7, 1941, 20 grams of the drug by capsule without preliminary fasting. At the time of treatment the animal was passing 4,200 nematode eggs per gram of feces and the volume of packed red blood cells (hematocrit) was 17 cc per 100 cc of blood. For the most part the eggs were those of *Haemonchus* and *Oesophagostomum*, although a few eggs of *Cooperia* spp. were present. Seven days after treatment the egg count was 86 cooperid eggs per gram and the packed cell volume was 25 cc per 100 cc of blood. Twenty-four days after treatment the egg count was 20 per gram and the packed cell volume 32 cc. Another Jersey yearling, 15 months old and weighing 310 pounds, similarly parasitized, was given 31 grams of phenothiazine in the same manner and examined at the same intervals as in the case cited above. The number of eggs per gram of feces decreased from 374 to 26 to 12, and the packed cell volume increased from 23.5 to 26 to 28 cc per 100 cc. Considerable improvement was also evident in the appearance of these two animals. The first gained 24 pounds and the second 50 pounds during this period, although remaining under the same pasture conditions as before treatment.

The tests summarized here indicate that phenothiazine is very effective against *Haemonchus, Oesophagostomum,* and *Trichostrongylus,* when given in doses as small as 0.1 gram per pound of body weight and against *Haemonchus* in doses even as small as 0.05 gram per pound of body weight. The drug was not so effective against *Bunostomum phlebotomum* and *Ostertagia* at these dose rates, as it was in doses ranging from 0.2 to 0.5 gram per pound of body weight (Swanson, Porter, and Connelly, *loc. cit.*). This suggests that administration of phenothiazine in larger doses may be necessary only in those cases where these parasites are known to be of greater importance than the common stomach worm and the nodular worm.

SUMMARY

Critical tests on 4 yearlings and 2 calves and field tests on 2 yearlings gave results that indicate that unconditioned phenothiazine was effective for the removal

of *Haemonchus, Oesophagostomum,* and *Trichostrongylus* in doses as low as 0.1 gram per pound of body weight. Apparently the drug was equally effective whether given in feed or by capsule and whether given with or without a preliminary period of fasting.

Adenomatous tumors in the large intestine of cats caused by *Strongyloides tumefaciens*, n. sp. EMMETT W. PRICE and G. DIKMANS, U. S. Bureau of Animal Industry.

In 1929, the writers reported in abstract form the occurrence of multiple adenomata of the large intestine of a cat caused by an apparently new species of *Strongyloides.* This appears to have been the only report of the occurrence of tumors of the epithelial type associated with worms of this genus, the usual pathological changes resulting from parasitism with *Strongyloides* being inflammatory in nature.

Darling (1911) found that sections of the duodenum and jejunum of man heavily parasitized with *Strongyloides stercoralis* showed infiltrations of round and plasma cells in certain areas. Similar infiltrations were seen in the supporting reticulum of Brunner's glands, together with an increased number of polymorphonuclear leucocytes, both neutrophilic and eosinophilic, associated with the round and plasma cells. In one section this author found a break in the muscularis mucosae and an attenuated downgrowth of epithelial cells from a crypt, at which point a female worm had entered Brunner's gland. This downgrowth of epithelial cells may represent a duct as he suggests, or it may indicate the beginning of a pathological process such as described in this paper. Darling also found inflammatory changes, similar to those occurring in man, in the ileum of monkeys (*Cebus hypoleucus*) infested with *Strongyloides cebus* and in the ant bear (*Nasua nasica panamensis*) infested with *Strongyloides nasua.*

Blacklock and Adler (1922) reported a lymphoid tumor caused by *Strongyloides* occurring in the jejunum of a chimpanzee. This tumor consisted of a core of muscle tissue surrounded by a thick layer of lymphoid cells and was situated beneath the muscularis mucosae. Adult worms were found in the tumor as well as in the adjacent connective tissue and in the mucous membrane. The intestinal wall in this case was three times its normal thickness owing to the large increase in lymphoid tissue in the mucosa and submucosa.

Ware and Ware (1923) report thickening of the intestine of a dog dying from a naturally acquired infestation with *Strongyloides stercoralis,* and Sandground (1926) found similar lesions in experimentally infested dogs; in none of these cases was any evidence of epithelial proliferation or tumor formation observed.

The case of adenomatous tumors in cats associated with *Strongyloides* referred to above (Price and Dikmans, 1929) was found by one of us (G.D.) on April 28, 1927, at Jeanerette, La. The cat was brought to the laboratory for necropsy to determine the cause of death. Upon examination of the large intestine a number of tumor-like lesions were observed, some of them being somewhat hemorrhagic. The entire section of gut was removed, preserved in formalin, and forwarded to the Bureau of Animal Industry for study.

A second case was found in 1930 by Dr. John Wells, West Palm Beach, Fla., in a cat which had been sent to his hospital for treatment. The cat had been suffering from intestinal disturbances for some time and finally died. Upon necropsy the mucosa of the large intestine was found to be studded with small tumorlike masses. The entire section of gut was sent to the Bureau of Animal Industry for diagnosis.

An examination of the nodules in both of these cases showed them to be similar in nature and caused by small nematodes which were found only in the nodules.

These worms belong to the genus *Strongyloides* and appear to be a new species for which the name *Strongyloides tumefaciens* is proposed.

DESCRIPTION OF THE LESIONS

The specimens from both cases present a similar appearance. The mucous membrane shows numerous tumor-like elevations which are irregularly distributed over the entire length of the large intestine (Fig. 1, A). These tumors are from 2 to 10 mm in diameter and each shows a small pit or depression at its summit. No evidence of hyperemia or hemorrhage can be detected, but this may be due to the bleaching effect of the formalin in which the tissues have been preserved for a considerable length of time. The nodules and adjacent mucous membrane are

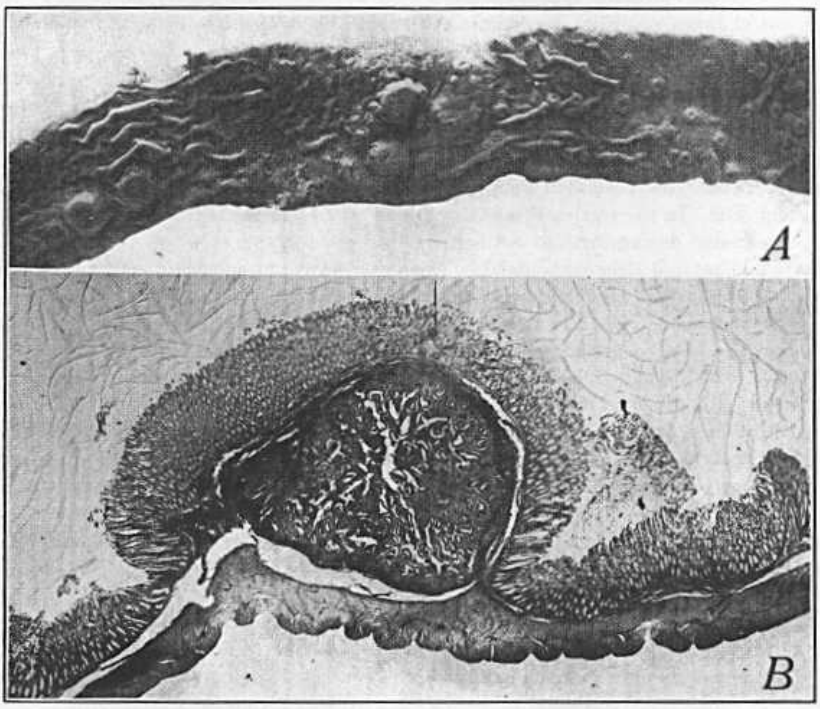

FIG. 1. Lesions caused by *Strongyloides tumefaciens*, n. sp. A—Gross appearance of a portion of large intestine of cat (Jeanerette, La., case). B—Section through one of the tumors.

covered with a moderate amount of mucus. The wall of the gut appears normal in thickness and the serosa shows no evidence of inflammation. On section the nodules are found to be well encapsulated, more or less spherical in shape, and situated between the muscularis mucosae and muscular coats.

Sections prepared for histological study show considerable desquamation of the superficial epithelium of the mucous membrane and the membrane is covered with mucus. The cells lining the crypts are normal in appearance except for an apparent increase in the number of goblet cells. The submucous connective tissue shows a marked infiltration with leucocytes, both round cells and polymorphonuclears, but only a few eosinophiles are present. The submucosa appears normal except for an occasional circumscribed accumulation of round cells in the region of the nodules,

these masses being similar to those described and figured by Hung and Höppli (1923) in *Strongyloides* infestation in monkeys. The muscular coat shows a slight round cell infiltration; the serosa appears to be normal.

The tumors are regular in outline due to a well developed connective tissue capsule (Fig. 1, B). They are adenomatous in nature and consist of irregular acini of columnar cells, which are more or less distended with exudate. The parenchymatous portion of the nodule is supported by a delicate stroma of connective tissue which shows a marked infiltration with lymphoid cells. Scattered throughout the stroma and in the lumena of the acini are the adult worms, usually seen in cross section, and in some areas numerous larvae are present. In none of the sections examined were adult worms found outside the nodules.

DISCUSSION

The lesions described above appear to be new growths of tissue which have resulted from the presence of the worms. Whether these legions should be regarded as true adenomata or cases of acquired heterotopia, or the abnormal snaring or displacement of cells with "subsequent growth out of place," is a matter which is difficult to determine. It is possible that cells of the intestinal mucosa might have become displaced by the penetration of the tissue by the infective larvae and that these cells proliferated in a form resembling an adenoma. Some writers have described heterotopia following trauma of the gut and in these cases the growth was limited to a mass of epithelial cells arranged as a simple, glandular loop. The present writers are of the opinion, however, that to make a distinction between heterotopia of glandular epithelium and adenoma is largely a matter of opinion and of little consequence since the two conditions are so similar and, under certain conditions, practically identical.

DESCRIPTION OF THE PARASITE

This description is based upon specimens dissected from the nodules. Unfortunately, it was impossible to obtain complete specimens owing to the brittleness of the formalin-fixed tissue. The total length of the worm as given below is based upon a specimen complete except for a small portion of the anterior end, the probable length of the missing portion being obtained from another specimen.

Strongyloides tumefaciens, new species

Description.—Parasitic *female* about 5 mm long by 109 μ wide. Cuticle finely striated transversely, the striae being indistinct except under very high magnification. Head 23 μ wide; oral opening oval and surrounded by the usual number of small papillae; buccal capsule very shallow and rudimentary. Esophagus slender, 0.75 to 1 mm long by 63 μ in diameter at the widest portion. Nerve ring situated about 200 μ from the anterior end of body. Vulva inconspicuous, situated about 1.6 mm from posterior extremity. Uterus containing an immature egg in each branch; ovaries simple and recurved, forming U-shaped bands. Tail short and pointed; anus situated 106 to 114 μ from the tip of the tail. Egg, as found in material dissected from the nodules, 114 to 124 μ long by 62 to 68 μ wide, containing a fully developed larva. Rhabditiform larvae found in the tissue are about 200 μ long by 10 μ wide.

Specimens.—U. S. N. M. Helm. Coll. No. 28190 (type); 28191 (paratypes), and 43857.

Strongyloides tumefaciens falls more nearly within the range of measurements given for *S. papillosus* of ruminants than with those given for the other species of the genus. It differs from this form, however, in that it is more robust, the tail is

pointed instead of bluntly rounded, and the characteristic twisting of the ovaries is absent. The only other species which approaches *S. tumefaciens* in size is *S. westeri* of the horse, this worm being from 8 to 9 mm long by 80 to 95 μ wide and in other respects resembling *S. papillosus*.

In proposing *S. tumefaciens* as a new species, the writers realize that the morphological characters are not outstanding. However, in view of the fact that the genus is composed of species which are more or less homogeneous and lack such well defined differential characters as are present in many other genera, the writers feel that the large size of this form, its location, and the type of lesion produced are characters that justify its recognition as a distinct species.

REFERENCES

BLACKLOCK, B., and ADLER, S. 1922. The pathological effects produced by *Strongyloides* in a chimpanzee. Ann. Trop. Med. & Parasitol. **16**: 283–290.

DARLING, S. T. 1911. *Strongyloides* infections in man and animals in the Isthmian Canal Zone. Jour. Exp. Med. **14**: 1–24.

HUNG-SEE-LÜ and HÖPPLI, R. 1923. Morphologische und histologische Beiträge zur *Strongyloides*-Infektion der Tiere. Arch. f. Schiffs- u. Tropen-Hyg., Leipz., 27: 118–129, pl. 4, figs. 1–5.

PRICE, EMMETT W., and DIKMANS, G. 1929. Multiple adenomata of the large intestine of a cat caused by a species of *Strongyloides*. Jour. Parasitol. **16**(2): 104.

SANDGROUND, J. H. 1926. The role of *Strongyloides stercoralis* in the causation of diarrhea. Some observations on the condition of dogs and cats experimentally infected with this parasite. Am. Jour. Trop. Med. **6**: 421–432.

WARE, FRANK, and WARE, MARTHA. 1923. *Strongyloides stercoralis* in a dog. Jour. Comp. Path. & Therap. **36**: 104–108.

Hot-water-formalin treatment (at 110° to 111° F.) of field-grown and of forced narcissus bulbs infected with the bulb or stem nematode, *Ditylenchus dipsaci*. B. G. CHITWOOD, Bureau of Plant Industry, U. S. Department of Agriculture; F. A. HAASIS, Department of Plant Pathology, Cornell University; and F. S. BLANTON, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.[1]

INTRODUCTION

Hot-water treatment of narcissus bulbs for the control of the bulb or stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, was introduced by Ramsbottom (1918a, b) and Van Slogteren (1919). In order to protect narcissus planting in the United States, imported and domestic bulbs have been subjected to this hot-water treatment or modifications of it. Originally treatment consisted of the exposure of bulbs to water at 110° F. for 3 to 4 hours. Failure of such treatments (Chitwood and Blanton, 1941) to control the disease adequately was responsible for continued investigations to improve this treatment, by the authors and other members of the Bureau of Plant Industry and the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. These investigations have led to the hot-water-formalin treatment as here discussed. In this treatment water containing 0.5 per cent formalin (1 part commercial formalin to 199 parts water) is used. Chitwood and Blanton (1941) reported that a 4-hour hot-water-formalin treatment of such concentration, at 110° to 111° F. is highly effective for the control of *D. dipsaci* in narcissus bulbs.

The present paper contains additional and more conclusive data on hot-water-formalin treatments of 3, 3½, and 4 hours' duration at 110° to 111° F. Data con-

[1] The writers gratefully acknowledge the assistance of L. B. Reed, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, who verified the statistical analyses.

cerning the efficacy of such treatments against *D. dipsaci* in field-grown and in forced narcissus bulbs are presented. Data on the tolerance of forced bulbs to a 4-hour treatment in hot water-formalin are also given. Some studies have already been published in part (Blanton and Chitwood, 1940) on the tolerance of field-grown bulbs to a 4-hour hot-water-formalin treatment (with a 2-hour aqueous presoak at 70° to 80° F.). Another manuscript on this subject is in preparation.[2]

EFFICACY OF TREATMENTS OF INFECTED FIELD-GROWN NARCISSUS BULBS

Problem.—In a previous paper Chitwood and Blanton (1941) have shown that a therapeutic treatment of narcissus bulbs should have a predictable efficacy of 90 per cent, preferably 95 per cent, against *D. dipsaci,* in order that it be considered recommendable for the control of that organism. The per cent efficacy which may be predicted on the basis of experimental data is obtained from binomial distribution formulae. The question therefore arose if a hot-water-formalin treatment would result in the required efficacy of control of the nematode in field-grown narcissus bulbs.

Methods.—In the present experiment field-grown bulbs of the varieties King Alfred and Emperor, supposedly infected with *D. dipsaci,* were treated for 3, 3½, and 4 hours at 110° to 111° F. in hot water-formalin. The treatments were conducted on August 27 and 28, 1940, in a commercial treating tank provided with an agitator. The ratio of solution weight to bulb weight was approximately 9: 1. Timing of the treatments was begun when the treating tank reached the desired temperature, the approach period being about 10 to 15 minutes. The individual samples of bulbs were segregated in jute onion bags and these were placed loosely in rigid cages made of hardware cloth. The infected narcissus had been grown in the vicinity of Babylon, N. Y.; bulbs of the Emperor variety were 9 to 10 cm in circumference, those of lots 1 and 2 of the King Alfred variety were 10 to 12 cm and 18 to 20 cm in circumference, respectively.

Experimental results.—The bulbs were examined individually 1 to 2 months after treatment, the various lots for examination being taken at random. Controls were examined later than the treated bulbs in order to be certain that the nematodes were not adversely affected by storage.

The data from examinations of all treated bulbs are given in table 1. There were 3 replicates of each treatment for each variety-lot of bulbs. In each block of figures 3 numbers are given, the first indicating the number of bulbs containing living nematodes, the second indicating the number of bulbs containing nematodes either living or dead and the third indicating the total number of bulbs in the sample.

Examination of the controls gave the following results: King Alfred: Lot 1, total 44 bulbs, of these 30 contained living *D. dipsaci* and 1 contained dead specimens only; Lot 2, total 29 bulbs, of these 18 contained living nematodes. Emperor: total 9 bulbs, of these 8 contained living nematodes. Therefore, less than 2 per cent of the bulbs containing dead nematodes in a treatment might be considered as "cured" without treatment. Actually the death of the nemas in such a case is probably due to complete decomposition of the bulb by secondary organisms. The controls also showed that 57 out of 82 bulbs or 69 per cent were infected with *D. dipsaci,* while the treatments showed only 371 out of 795 or 46 per cent infected. Chitwood and Blanton (1941) have already noted that when bulbs are taken at random from a given lot and then treated those subjected to the most severe treatment will show the lowest percentage of infected bulbs on subsequent examination.

[2] Blanton, F. S. and Chitwood, B. G. The tolerance of 40 varieties of narcissus to a hot-water-formalin treatment based on 1939–1940 and 1940–1941 experiments. (In preparation.)

TABLE 1.—*Results from hot-water-formalin treatments at 110° to 111° F. of Ditylenchus dipsaci-infected, field-grown narcissus bulbs examined 1 to 2 months after treatment. Babylon, N. Y., 1940.*

Lots and varieties	Duration of treatments in hours	Replicate number				Efficacy in per cent	
		1	2	3	All replicates	Observed	Predictable[b]
Emperor	3	1– 6– 9[a]	0– 5– 8	0– 5– 9	1– 16– 26	93	76
	3½	0– 3– 9	1– 6– 9	1– 6– 8	2– 15– 26	86	63
	4	0– 4– 9	0– 4– 9	0– 4– 8	0– 12– 26	100	77
King Alfred Lot 1	3	0–16–49	0–16–50	0–20–49	0– 52–148	100	94
	3½	0–21–50	0–29–47	0–33–49	0– 83–146	100	96
	4	0–21–46	0–21–47	0–28–48	0– 70–141	100	95
King Alfred Lot 2	3	0–11–31	1–12–44	0–14–29	1– 37–104	97	87
	3½	0–17–31	0–10–30	0–16–29	0– 43– 90	100	93
	4	0–14–29	0–15–29	0–14–30	0– 43– 88	100	93
Combined lots	3				2–105–278	98	94
	3½				2–141–262	98	95
	4				0–125–255	100	97

[a] In each block the first number represents the number of bulbs containing living *D. dipsaci*, the second indicates the number of bulbs containing living or dead *D. dipsaci*, and the third is the number of bulbs examined.

[b] On the basis of the binomial distribution the odds are at least 19 to 1 that the true efficacy is above the values given.

This fact is explained on the basis that, after the bulb is macerated, living nematodes tend to crawl out of the resulting bulb fragments while dead nematodes are observed only if they happen to rest on the surface of a fragment.

One of the 57 infected bulbs in the control series contained no living nematodes, therefore it would appear proper to discount 2 per cent from the number of bulbs containing nematodes in a given treatment, since according to the records from the control samples, unaccountable nema death amounted to approximately this figure. It would also appear proper to make correction for the number of bulbs in which dead nematodes were probably present but escaped observation; such a correction would be obtained by multiplying the number of bulbs containing nematodes by the factor 69/46. But, since uncorrected data place the treatments at a disadvantage while corrected data might give the treatments more advantage than is warranted without resorting to extensive statistical methods, we have made no correction and, therefore, the predicted efficacies are based on the actual data.

From these data (Table 1) disregarding varieties and lots, the odds are 19: 1 that the efficacies of 3-, 3½-, and 4-hour treatments in hot water-formalin at 110° to 111° F. are better than 94, 95, and 97 per cent, respectively. Therefore any of these treatments would be recommendable but the 3½- and 4-hour treatments give the preferable efficacy of better than 95 per cent.

Considering the 3 variety-lots separately, the Emperor stock, though consisting of the smallest bulbs, gave the lowest predictable efficacies. The observed efficacies of the 3- and 3½-hour treatments, 93 and 86 per cent, are also low and there is no obvious explanation. The observed efficacy of the 4-hour treatment, 100 per cent, shows that the low predictable efficacy, 77 per cent, of this treatment may be due entirely to the limited number of bulbs available.

The differences between the King Alfred bulbs of lots 1 and 2 may easily be due to chance. The fact that the only living nematodes occurred in the 3-hour treatment of lot 2 might cause some suspicion that this treatment might not be saisfactory. However, the observed efficacy of this treatment, 97 per cent, is better than the predictable efficacy of either the 3½- or 4-hour treatments as based on the same or other variety-lots.

A 4-hour treatment in hot water-formalin at 110° to 111° F. is, therefore, an excellent means of controlling *Ditylenchus dipsaci* in field-grown narcissus bulbs. In the present experiment it gave an observed efficacy of 100 per cent as based on the examination of 125 field-grown bulbs containing this organism. The predictable efficacy with odds of 19: 1, as based on binomial distribution, is better than 97 per cent. This figure compares favorably with the observed efficacy of 96 per cent obtained by Chitwood and Blanton (1941) in which records were included of treatments where the technic was known to be faulty.

A 3½-hour treatment of field-grown narcissus bulbs in 0.5 per cent formalin at 110° to 111° F. is probably very good since the efficacy with odds of 19: 1, as based on binomial distribution, is better than 95 per cent. However it might not be satisfactory for other varieties or lots of bulbs since it showed an efficacy of only 86 per cent with the lot of Emperor bulbs studied.

A 3-hour treatment of field-grown narcissus bulbs in 0.5 per cent formalin at 110° to 111° F. is also successful since it has an efficacy of better than 94 per cent. Like the 3½-hour treatment, however, it may not prove satisfactory for other varieties or lots of bulbs.

EFFICACY OF TREATMENTS OF INFECTED FORCED BULBS

Problem.—Ordinarily narcissus bulbs which have been used in winter forcing are discarded after flower production. Exceptional varieties, however, are often retained for field growing in order to restore such bulbs to normal vigor. Since

forced bulbs are "softer" than field-grown bulbs, it is commonly supposed that treatments for those infected with the bulb or stem nematode need not be as long as for field-grown bulbs. It is also commonly supposed that the treating date has a bearing on the efficacy of treatments. The problem, therefore, was to determine the significance of these factors on the efficacy of the hot-water-formalin treatment of infected forced bulbs.

Methods.—An experiment was conducted using forced bulbs supposedly infected with *D. dipsaci.* In this experiment treatments of 3, 3½, and 4 hours at 110° to 111° F. in hot water-formalin were given on June 7, 1940, June 21, 1940, and July 7, 1940. The timing of the treatments was begun when the treating bath reached the desired temperature. The bulbs used were of the variety King Alfred measuring 10 to 13 cm in circumference. Treatments were made in a standard small commercial hot-water treating tank equipped with an agitator.

As a result of the work by Chitwood and Blanton (1941) the number of supposedly infected bulbs for each treatment was set at 43 in order to be assured that sufficient records would be available for analysis on the basis of binomial distribution. Thirty bulbs were taken at random from the same stock of supposedly diseased bulbs and examined untreated. Twenty-seven contained living *D. dipsaci,* 1 contained dead *D. dipsaci* and 2 contained no *D. dipsaci.* Hence for practical purposes it is permissible to assume that if a treated bulb contained specimens of dead *D. dipsaci,* their death was due to the treatment. If one is to use binomial distribution formulae for obtaining statistical odds on efficacy, supposedly infected bulbs in which *D. dipsaci* is not found either dead or alive had best not be included in the data used.

Experimental results.—The bulbs were examined individually 2 to 3 weeks after treatment. The results of these examinations are given in table 2.

TABLE 2.—*Number of forced bulbs out of lots of 43 found infected with D. dipsaci 2 to 3 weeks after treatment with hot water-formalin 110° to 111° F. Babylon, N. Y., 1940*

Date of treatment	Duration of treatment in hours	Number of bulbs containing nematodes	
		Living	Living and dead
June 7	3	1	40
Do.	3½	1	39
Do.	4	0	39
June 21	3	2	36
Do.	3½	3	36
Do.	4	1	34
July 7	3	0	35
Do.	3½	0	36
Do.	4	0	33
All dates	3	3	111[b]
Do.	3½	4	111[a]
Do.	4	1	106[c]

[a] On the basis of binomial distribution, efficacy better than 91 per cent with odds of 19:1.

[b] On the basis of binomial distribution, efficacy better than 92 per cent with odds of 19:1.

[c] On the basis of binomial distribution, efficacy better than 95 per cent with odds of 19:1.

On the basis of these data, satisfactory treatments of forced narcissus bulbs may be made on or before July 7. Up to that date, a 4-hour treatment in hot

water-formalin (0.5 per cent formalin at 110° to 111° F.) had an observed efficacy of as much as 99 per cent and a predictable efficacy of not less than 95 per cent. A 3½-hour treatment under the same conditions appeared to be successful also, since an observed efficacy of 96 per cent was obtained and the treatment was shown to have a predictable efficacy of better than 91 per cent. The 3-hour treatment had an observed efficacy of 97 per cent and a predictable efficacy of 92 per cent. The apparent difference of 1 per cent in favor of the 3-hour treatment over the 3½-hour treatment is probably due to chance.

TOLERANCE OF FORCED NARCISSUS BULBS

Problem.—Since forced bulbs differ from field-grown bulbs in their physiological condition, the reactions of the two types of bulbs must be determined on the basis of separate experiments. Lots of forced bulbs usually contain a considerable amount of *D. dipsaci,* the amount varying according to the original infection. Grown in crowded conditions the percentage of infection is greatly increased. The same may also be true for basal rot. Determination of the tolerance of bulbs to therapeutic treatment is based on, (1) the increase in weight of the bulbs after one season's planting following treatment, and (2) the decrease in amount of basal rot shown in the bulbs of such planting. One of the main disadvantages of the original hot-water treatment is its tendency to increase and spread basal rot (Weiss, 1929). The purpose of the present experiment is to determine the comparative tolerance of forced bulbs to hot-water versus hot-water-formalin treatments.

Methods.—Reasonably healthy forced King Alfred narcissus bulbs were treated for 4 hours in hot water or hot water-formalin at 110° to 111° F. Each treatment consisted of 8 lots of 200 bulbs each, selected at random. The bulbs were treated June 30, 1939, and stored at random in screen-bottom trays until Sept. 16, 1939, when they were planted; after growth they were reharvested July 31, 1940, and restored until Sept. 17, 1940. Rot counts were made at the end of each storage period as well as at harvest in 1940. The analysis is based on total rot encountered in bulb samples beginning immediately after June 30, 1939, and terminating Sept. 17, 1940.

Experimental results.—The average per cent increase in weight of the hot-water-treated bulbs was 21 per cent and that of the hot-water-formalin-treated bulbs was 28 per cent. This difference, in favor of the hot-water-formalin treatment, was not significant and could have been due to chance. However, in some cases heat treatments at temperatures not injurious to bulbs stimulate growth.

In the hot-water-treated bulbs an average of 14.4 bulbs per lot were lost due to basal rot while in the hot-water-formalin-treated bulbs an average of only 6.6 bulbs were lost due to basal rot. The actual rot counts of the treatment replicates were as follows: (1) hot-water treatment 13, 14, 5, 9, 23, 15, 17, 19; (2) hot water-formalin 6, 7, 7, 5, 3, 9, 0, 16. Analysis showed that with a standard error of the difference between means of 2.59 the minimum difference for significance with 99: 1 odds is 7.7. Since the difference is actually 7.8, it is highly significant. Fewer bulbs are lost due to basal rot after hot-water-formalin than after hot-water treatment.

SUMMARY

With field-grown narcissus bulbs a 4-hour hot-water-formalin treatment at 110° to 111° F. is highly successful for the control of *D. dipsaci* since an efficacy of 100 per cent was obtained in 125 bulbs. An efficacy of better than 97 per cent can be predicted. Treatments of 3 or 3½ hours may also be successful since the efficacies observed were 94 and 95 per cent, respectively. However, it is possible that these treatments might be effective for one variety or lot and not for another variety

or lot since all of the nematodes were killed in only 86 per cent of the stock of diseased Emperor bulbs by a 3½-hour treatment. All of the nematodes were killed in 100 per cent of the bulbs of 2 stocks of King Alfred bulbs by a 3½-hour treatment and in 98 per cent of the bulbs of one stock and 100 per cent of the bulbs of the other stock of King Alfred bulbs by a 3-hour treatment.

Treatment of forced King Alfred variety narcissus in hot water-formalin at 110° to 111° F. between June 7 and July 7, 1940, showed that a 4-hour treatment of such bulbs at this season is also highly successful. An efficacy of 99 per cent against *D. dipsaci* was obtained and an efficacy of better than 95 per cent can be predicted. Similar treatments of 3 and 3½ hours duration demonstrated efficacies of 97 and 96 per cent with predictable efficacies of better than 92 and 91 per cent, respectively. No difference in efficacy between treatments of forced and field grown bulbs was demonstrated.

Forced narcissus bulbs appear to tolerate a 4-hour treatment in hot water-formalin at 110° to 111° F. as well as, or better, than they tolerated a 4-hour treatment in water at the same temperature. Basal rot is controlled better by hot water-formalin than by hot water.

LITERATURE CITED

BLANTON, F. S., and CHITWOOD, B. G. 1940. The tolerance of 40 varieties of narcissus to a combined hot-water and formalin treatment based on the 1938–1939 experiments. Proc. Helminth. Soc. Wash. **7**(2): 91–94.

CHITWOOD, B. G., and BLANTON, F. S. 1941. An evaluation of the results of treatments given narcissus bulbs for the control of the nematode *Ditylenchus dipsaci* (Kühn) Filipjev. Jour. Wash. Acad. Sci., **31**(7): 296–308.

RAMSBOTTOM, J. K. 1918a. Investigations on the narcissus disease. Jour. Roy. Hort. Soc. **43**: 51–64.

————. 1918b. Experiments on the control of eelworm disease of narcissus. *Ibid.* **43**: 65–78.

SLOGTEREN, E. VAN. 1919. De Toepassing van warmte als Bestrijdingsmiddel van eenige Bloembollensiekten. Weekblad voor Bloembollcult **30**: 63–66, 69–71.

WEISS, F. 1929. The relation of the hot-water treatment of narcissus bulbs to basal rot. Phytopath. **19**: 100.

The use of methyl bromide to control the root-knot nematode, *Heterodera marioni*.[1] CHARLES E. GINGRICH and C. M. HAENSELER.

INTRODUCTION

In a recent paper A. L. Taylor and C. W. McBeth (1940. Proc. Helminth. Soc. Wash. **7** (2): 94–96) presented the results of their studies on the destruction of the root-knot nematode, *Heterodera marioni*, in soils by means of fumigation of the soil in a closed chamber containing a low concentration of methyl bromide. Perfect control of the nematode was obtained by exposure of nematode-infested soil for 72 hours to an atmosphere containing 80 ml methyl bromide per cubic meter.

Similar studies were conducted at the New Jersey Agricultural Experiment Station during 1939 and 1940 as part of a detailed study of the relative value of several chemicals for the control of the root-knot nematode. Only that phase of the studies which deals with methyl bromide will be presented here.

EXPERIMENTAL WORK

Three methods of applying methyl bromide to soil infested with the root-knot nematode, *Heterodera marioni*, were used in these experiments, and all the tests

[1] Extract from a thesis by Charles E. Gingrich presented to Rutgers University in partial fulfillment of the requirements for the degree of Master of Science, June, 1940. Journal Series paper of the N. J. Agricultural Experiment Station, Rutgers University, Department of Plant Pathology.

were conducted under greenhouse conditions. In the first method the soil was placed in containers and exposed to a methyl bromide atmosphere in a relatively tight fumigating chamber into which measured amounts of methyl bromide were released; in the second method a mixture of methyl bromide and ethyl alcohol was injected into the soil; and in the third method pure methyl bromide was injected into the soil. A sandy loam soil with a moisture content of approximately 15 per cent was used throughout. Before treatment, the soil was thoroughly mixed to ensure uniform distribution of the nematode population within each series. After treatment, the various lots of treated soil as well as appropriate untreated checks were planted with cucumber seedlings, which were used as indicator plants to determine the degree of root-knot nematode control. When the plants in the untreated check soils showed abundant root knot, all plants were harvested and graded into groups showing different degree of infection.

The chamber fumigations were made with methyl bromide used in amounts ranging from ¼ ml to 4 ml per cubic foot of space in a chamber of 2 cubic yards capacity and for exposure periods ranging from ¼ to 24 hours. The extent of control of the root-knot nematode obtained in this experiment, as determined by the degree of root-knot infection on the cucumber indicator plants, is shown in graphic form in figure 1. Complete nematode control was obtained by this chamber method of fumigation with the following time-dosage combinations: ¼ ml of methyl bromide per cubic foot of chamber volume, required 12 hours; ½ ml, 6 hours; 1 ml, 3 hours; 2 ml, 1 hour; 3 ml, ½ hour; and 4ml, ¾ hour. (A mere trace of root knot occurred in the 4 ml-½ hr. treatment.)

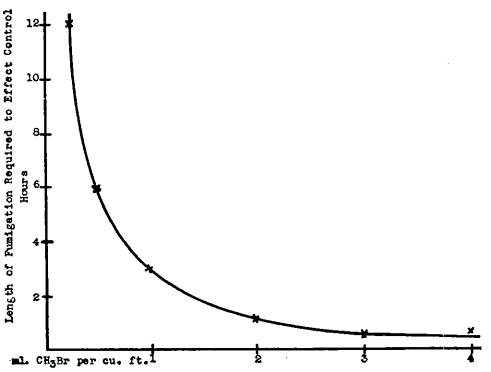

FIG. 1. The relation of the fumigation period and amount of fumigant to effect complete nematode control in methyl bromide chamber fumigations.

Another test in the fumigation chamber was conducted in which the soil to be treated varied in depth from 2 to 8 inches. In this test it was found that perfect root-knot control was obtained in soil to a depth of 2, 4, 6, or 8 inches, respectively, in containers open only at the top and exposed for 3 hours in an atmosphere containing 1 ml methyl bromide per cubic foot of chamber space. It was evident that the methyl bromide gas readily penetrated the soil to a depth of 8 inches.

The above results were obtained by using a relatively small amount of soil (approximately ½ cu. ft.) in relation to the volume of the fumigating chamber (54 cu. ft.). They show that satisfactory control of the root-knot nematode can be obtained by exposing such small volumes of soil in layers ranging from 2 to 8 inches in depth and for a specified time in a relatively large volume of atmosphere containing a specified concentration of methyl bromide. They give no information, however, on the very practical question of whether a satisfactory nematode control could be obtained by treating soil in chambers which are almost or completely filled with soil. To obtain information on this point soil in metal chambers of one cubic foot capacity and containing ¼, ½, ¾, and 1 cubic foot of soil respectively were treated with liquid methyl bromide in amounts ranging from ½ to 4 ml per container. Immediately after the required amount of slightly chilled methyl bromide was delivered on the surface of the soil, each chamber was covered with a glass

plate and tightly sealed with vaseline. After 3 hours the various soil lots were aerated, placed in pots, and planted with cucumber seedlings which were allowed to grow until root knot developed abundantly on plants set in untreated soil from the same source. The results showed that complete control of nematodes was obtained in all of the treated series, even those which received only ½ ml methyl bromide per cu. ft. container, indicating that this method of applying methyl bromide was more efficient than the former. In the large chamber fumigation, for example, where a relatively small amount of soil was used, the 3-hour exposure required a methyl bromide dosage of 1 ml per cubic foot of chamber space in order to effect complete control, whereas in the small chambers with a relatively large amount of soil, ½ ml per cu. ft., the lowest dosage used, gave perfect control with a 3-hour exposure.

The greater efficiency of methyl bromide where the fumigation chamber contained a large amount of soil in proportion to the chamber volume is probably due to the fact that the air space was materially reduced by the relatively large volume of soil, thus allowing a smaller dosage of methyl bromide to give a toxic concentration in the soil atmosphere. It would also indicate that the soil does not adsorb or otherwise affect the methyl bromide with sufficient rapidity to quickly destroy its nematocidal properties. The results would also suggest that, for the most effective use of methyl bromide in soil fumigation for nematode control, the vapors should be confined as far as possible to the soil atmosphere, allowing as little free air above the soil as possible.

In the second soil treatment method used, soil injections in open containers were made with a mixture containing 1 volume of methyl bromide and 5 volumes of ethyl alcohol. The mixture, which does not boil at room temperature, was used in dosages to give methyl bromide in increasing increments from ½ to 10 ml per 1-gallon pot of soil. A corresponding series received equivalent amounts of alcohol without the methyl bromide, and a third series received no treatment. Glazed pots of ¼ sq. ft. soil surface area and with a capacity of 1 gallon were used throughout.

The results obtained by this method showed that a methyl bromide-ethyl alcohol mixture in amounts equivalent to 5 ml of methyl bromide per pot was necessary for partial control of nematodes. This is in sharp contrast to the results obtained in the third method discussed below in which dosages as low as ½ ml methyl bromide per gallon pot gave a corresponding degree of control. Furthermore the alcohol series showed that with dosages of the alcohol-methyl bromide mixture large enough to effect control of nematodes the alcohol carrier alone caused injury to the roots of seedlings set into the soil 7 days after treatment.

More efficient nematode control than was obtained by the methyl bromide-ethyl alcohol mixture resulted from the use of the third method, in which pure methyl bromide was injected into the soil and a "water seal," similar to that which has been successfully used in soil fumigation with chloropicrin, was made by wetting the soil surface immediately after treatment to delay the rapid escape of the gas. Injections of pure methyl bromide were made into soils in glazed earthenware pots in amounts ranging from ¼ ml to 15 ml per pot of 1 gallon capacity and ¼ sq. ft. soil surface area. Immediately after injection of the methyl bromide each pot was "water sealed" by adding 125 ml water to the surface of the soil. The efficiency of the treatment for the control of the root-knot nematode was determined as in the earlier tests, by growing cucumber indicator plants in the treated as well as in untreated soil. In this test partial control of root knot was obtained in soils treated with ½ ml methyl bromide per pot and complete control in soils treated with 1½ ml or more per pot. The results of this experiment seem to indicate that the methyl bromide injection of soils in open containers, although less efficient than the chamber fumigation method, may be of practical value in nematode control if precautions are taken to prevent the rapid escape of the gas.

The response of the cucumber indicator plants to both the direct injection method and the chamber fumigation method of methyl bromide application showed that no seedling injury occurred even where 15 ml of methyl bromide per gallon pot, or 10 times the dosage required for complete control, was used and where the cucumber seedlings were set in the soil only 4 to 5 days after treatment. On the contrary, there was evidence of decided plant growth "stimulation" in many cases which seemed to be greater than that attributable to nematode control alone (Fig. 2).

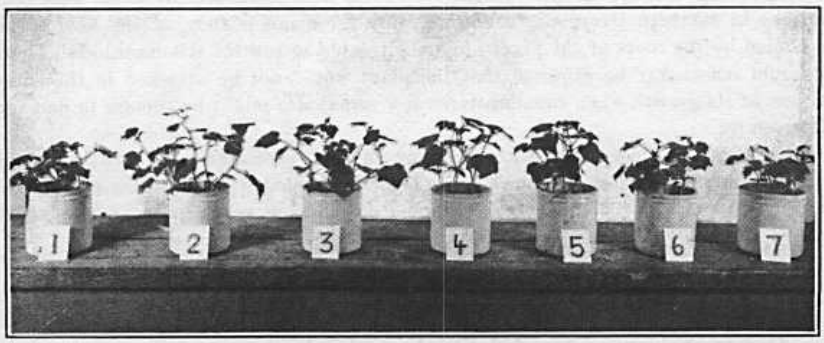

FIG. 2. Response of cucumber plants in a root-knot nematode infested soil fumigated for 3 hours in various concentrations of methyl bromide in a fumigation chamber. Pot Nos. 1 to 7 treated in chamber with 0, 4, 2, 1, ½, ¼, and ⅛ ml methyl bromide per cu. ft. chamber space, respectively. Nos. 1, 6, and 7, no root-knot control; No. 5, partial control; Nos. 2, 3, and 4, perfect control.

SUMMARY

1. Methyl bromide proved to be a very good nematocide for the control of the root-knot nematode, *Heterodera marioni*, when infested soils were treated in a closed fumigation chamber.

2. The amount of methyl bromide per cubic foot of fumigation chamber required to give complete control of the root-knot nematode in a sample of soil varied with the length of the fumigation period as follows: ¼ ml, 12 hours; ½ ml, 6 hours; 1 ml, 3 hours; 2 ml, 1 hour; 3 ml, ½ hour.

3. A given amount of methyl bromide per unit volume of chamber space seems to be more efficient in nematode control when the chamber is filled with soil than when a small amount of soil is treated in a large chamber.

4. Injection of 1½ ml methyl bromide into soil in 1-gallon glazed pots gave complete nematode control.

5. A given amount of methyl bromide used in a methyl bromide-ethyl alcohol mixture was far less efficient in nematode control than was methyl bromide alone.

6. Cucumber seedlings made better growth in methyl bromide treated soils than in untreated lots even at dosages which failed to give nematode control.

Spot treatments with chlorpicrin and ethylene dichloride for control of root knot.

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The use of chlorpicrin for control of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, in the soil has become a well known practice. The regular procedure in the use of this chemical is to inject 1 to 4 cc into the soil at 10- to 14-inch intervals over the whole area where the pest is to be controlled. Since 150 to 400 lbs. of chlorpicrin are required for 1 acre, the method is much too expensive

for general use at the present price of 80¢ to $1.00 per pound. The use of ethylene dichloride for the same purpose was investigated in some preliminary experiments. It was found to have some value as a nematocide when 5 to 10 cc were injected into the soil at 7-inch intervals. This requires more than 2000 lbs. per acre for the smaller amount, at a cost of about $140 for the chemical alone.

In devising a cheaper method of using these chemicals in the field, consideration was given to two facts: (1) Many crops are planted in widely spaced rows or hills and the roots occupy only a fraction of the field. (2) The root-knot nematode moves through the soil at an average rate of less than 1 cm per day under field conditions in southern Georgia. Therefore, only the small portion of the field to be occupied by the roots of the plant might be treated to control this nematode. Thus it could reasonably be expected that the plant would not be attacked in the early stages of its growth when comparatively few nematodes might be enough to damage it severely.

To test the theory, 2 Latin squares of 16 plots each were arranged in a field of Norfolk sandy loam soil known to be heavily infested with root-knot nematodes. Each plot was 30 by 30 feet and contained 25 planting spots spaced 6 feet apart each way.

One series of plots was treated with chlorpicrin as follows:

A. No chlorpicrin.
B. 2 cc of chlorpicrin applied directly on the planting spot.
C. 3 cc of chlorpicrin applied directly on the planting spot.
D. Three 1-cc applications of chlorpicrin spaced symmetrically around the planting spot and 6 inches from it.

The second series of plots was treated with ethylene dichloride as follows:

A. No ethylene dichloride.
B. 5 cc of ethylene dichloride applied directly on the planting spot.
C. 10 cc of ethylene dichloride applied directly on the planting spot.
D. Three 5-cc applications of ethylene dichloride spaced symmetrically around the planting spot and 6 inches from it.

The method of applying both chemicals was: Soil plowed, harrowed and leveled. Planting spots marked. Chemicals placed 6 inches beneath the soil surface by means of an accurately calibrated applicator. Hole made by the applicator spike filled with soil. About 1 square yard of soil surface around the planting spot sprinkled with enough water to wet the top inch or two of soil. Soil temperature at time of treatment was 20° C. Soil moisture 6.5 per cent.

Five days after treating, 5 seeds of the Thomas Watson variety of watermelon were planted at each planting spot. Ten days later approximately ½ lb. of 4–8–4 fertilizer was applied to each hill.

Six weeks after planting all but 2 plants were removed from each hill and examined for root knot. Average per cent of infested plants in the 4 plots in each treatment of the chlorpicrin series was:

A. Control—no chlorpicrin ..... 71.0% infested plants.
B. 2 cc of chlorpicrin ..... 1.0% infested plants.
C. 3 cc of chlorpicrin ..... 1.0% infested plants.
D. Three 1-cc applications of chlorpicrin ..... 2.5% infested plants.

In the ethylene dichloride series the results were:

A. Control—no ethylene dichloride ..... 73.0% infested plants.
B. 5 cc of ethylene dichloride ..... 72.0% infested plants.
C. 10 cc of ethylene dichloride ..... 76.0% infested plants.
D. Three 5-cc applications of ethylene dichloride ..... 83.0% infested plants.

In the chlorpicrin series, differences between the treated plots and the control were all highly significant when analyzed by standard statistical methods.

In the ethylene dichloride series, there were no significant differences.

Ninety-nine days after planting the melons were harvested and the roots of the remaining plants in the chlorpicrin series were again examined for root knot. Results were as follows:

A. Control	86.8% infested plants.
B. 2 cc of chlorpicrin	12.0% infested plants.
C. 3 cc of chlorpicrin	4.2% infested plants.
D. Three 1-cc applications of chlorpicrin	7.1% infested plants.

Differences between control and all treatments highly significant. No significant differences between treatments.

It was noted that the roots of plants from the control plots were generally very heavily infested and many had started to decay. Infested roots from the treated plots had evidently been attacked quite late in the season and were only slightly knotted.

DISCUSSION AND CONCLUSIONS

The method of using chemicals outlined above might be called "spot" treating or fumigation to distinguish it from the ordinary soil fumigation methods. It is evident that the chlorpicrin treatment reduced root knot to a very low point in the early stages of growth and that most of the plants were protected all through the growing season. The failure of ethylene dichloride to produce results cannot be explained at present.

With the hills spaced at 8-foot intervals, about 5 lbs. of chlorpicrin would be required to treat an acre of soil for watermelons if 2 cc were injected at each planting spot. Labor of applying the chemical is not great, perhaps about 4 hours per acre if a commercial applicator is used. If running water is not available, 1-yard squares of gas impervious paper might be substituted for the sprinkling. Other experiments have shown that one or the other is absolutely necessary if good results are to be obtained. Soil to be treated should be loose, well broken up and just moist enough to retain its shape when moulded in the hand. Soil temperature (measured at a depth of 1 foot) should be at least 15° C. at time of treatment.

While watermelons were used in the above experiment, there appears to be no reason why similar technique could not be used on any other crop planted in hills or in rows.

SUMMARY

Spot treatments with chlorpicrin, i.e., fumigation of only that portion of the soil which is to be occupied by the roots of the plants, was demonstrated to be a practical method of controlling the root-knot nematode disease of watermelons. Two or three cc of the chemical were used to each hill in sandy loam soil with no significant advantage for the larger amount. Root knot was almost completely controlled in the first 6 weeks of the growing season and less than 12 per cent of the plants in the treated areas were infested at the end of the 99-day growing period. Cost of the treatment is approximately $5.00 per acre for the chemical alone.

A similar experiment, using 5, 10, and 15 cc of ethylene dichloride to each planting spot, failed to produce any appreciable reduction of root knot.

The number of cercariae of *Fasciola hepatica* developing in snails infected with a single miracidium. WENDELL H. KRULL, U. S. Bureau of Animal Industry.

INTRODUCTION

Experimental infections of snails with *Fasciola hepatica* have been carried out to some extent and reports of such work may be found in the voluminous literature concerning this economically important parasite. However, so far as the writer

is aware, no work has been carried out to determine the number of cercariae that may develop in snails infected with a single miracidium. Experience with various intermediate hosts of *F. hepatica* during the last several years indicates that the snails possess inherent characteristics, as well as differences in environmental requirements, that may influence the infection with *F. hepatica*. For these reasons the snail, *Pseudosuccinea columella*, previously reported as an intermediate host, was selected for this investigation. This snail also appeared to be especially suitable for this work because of the ease with which it could be infected regardless of age, of the low mortality rate of the infected snails, and comparatively rapid development of the infection.

MATERIALS AND METHODS

The snails used in the experiment were raised under controlled conditions by Mr. Lawrence Avery, Jr., at the U. S. Department of Agriculture Beltsville Research Center, Beltsville, Maryland. The snails were received in January by air mail and subjected to infection 3 days after their arrival. An attempt was made to repeat the experiment a year later without satisfactory results as it was impossible to maintain any of the snails for the duration of the experiment, although some of those exposed to a single miracidium became infected. Although the snails in both shipments were distributed in many aquaria and discharged numerous eggs, only a few of the eggs hatched and none of these newly hatched snails remained alive any length of time under laboratory conditions at the Utah Agricultural Experiment Station, Logan, Utah, where the experiments were conducted.

In subjecting the snails to infection small Bureau of Plant Industry stender dishes were used as containers. The miracidia were caught individually and removed with a finely drawn out pipette, one miracidium being placed in each container; this procedure was carried out under a wide-field binocular microscope. Each miracidium was removed in the smallest possible amount of water to permit rapid and accurate determination of the presence of the organism. The stender dishes were then half filled with water, without forming a contact between the dropper and the water in the receptacle. After a second check to see that the miracidia were in good condition and that only one was present in each container, a snail was placed in each dish with the aid of forceps, without contacting the water. The containers with the snails were then left undisturbed for one hour, after which each stender dish was examined; a dead miracidium observed in only one dish and the snail that the miracidium failed to penetrate was, therefore, discarded.

The 21 snails, which were infected in the manner outlined, were kept in separate containers for 24 hours, after which they were placed together in a single aquarium. At the time the snails were subjected to infection on January 26, they varied in height from 4 to 9 mm. At intervals, the snails were examined for infections that can be seen through the transparent shell. In this species it is very easy to determine the presence of infection in this way because of the transparency of the shell.

RESULTS

Infections were observed in 7 snails 57 days after exposure to miracidia; rediae could be identified in 6, and rediae and cercariae were present in the remaining snail. The infected snails were placed in separate fingerbowls at this time, and the remaining one was isolated subsequently. The snails ranged in height from 6.0 to 11.5 mm. Infection data concerning the 8 snails which became infected have been summarized in table 1.

Cercariae were observed in snail A 57 days after infection; this snail lived for 39 more days without shedding any cercariae. The largest number of cercariae

produced by any of the snails in this experiment was 629, of which 613 were discharged. The shedding period was terminated by death of the snail in all except snail E, which continued to live for 24 days after the last cercariae had escaped. Although all of the snails were infected on the same day, there was a difference of 21 days in the time before the first cercariae were shed.

TABLE 1.—*Summary of data concerning eight snails infected with single miracidia of Fasciola hepatica*

	Designation of snail							
	A	B	C	D	E	F	G	H
Days between infection and initiation of shedding of cercariae	—	68	68	68	68	71	78	89
Duration of shedding (days)	—	22	57	65	49	77	45	10
Number of cercariae shed during first half of period	—	30	110	128	44	139	78	6
Number of cercariae shed during last half of period	—	26	352	380	39	474	130	8
Total number of cercariae shed	—	56	462	508	83	613	208	14
Cercariae shed per day (average)	—	2.5	8.0	7.8	1.7	8.0	4.6	1.4
Number cercariae shed on first day	—	14	5	2	8	2	12	3
Largest number of cercariae shed during single day	—	14	55	68	10	204	20	4
Number of days no cercariae were shed	—	9	13	19	27	23	14	4
Number of cercariae recovered on dissection	13	2	63	19	0	16	5	0
Total number of cercariae produced	—	58	525	527	83	629	213	14
Cercariae encysted on container	—	53	340	435	74	536	186	9
Cercariae encysted on lettuce	—	3	89	36	5	57	12	2
Cercariae encysted on dead leaves	—	0	21	17	0	10	2	2
Cercariae encysted on surface of water	—	0	9	16	3	9	4	1
Cercariae encysted on shell of snail	—	0	1	4	0	0	0	0
Cysts recovered in excrement	—	0	2	0	1	1	4	0

Since the approximate time of death of the infected snails could be determined a day or so in advance by the behavior of the mollusks, it was possible to dissect the snails while the contained parasite larvae were still alive. In this way the number of rediae could be determined and peculiarities of infection ascertained. In snail A, besides the 13 live cercariae, 16 rediae containing cercariae and 71 rediae without cercariae were recovered. In addition to these larvae, there was a number of dead rediae and cercariae that appeared as shrunken, opaque, hard and dense granular masses. In snail B, the visceral mass was infiltrated with rediae and the digestive gland was literally replaced by them. In addition to the 10 rediae containing cercariae, all from the region of the digestive gland, there were 81 rediae which contained no cercariae. Although 415 rediae were recovered from snail C, the digestive gland was almost intact, except for the extreme tip which had been replaced by rediae. Most of the other rediae were in the viscera, and had invaded extensively the edge of the mantle. Of the 63 cercariae recovered from snail C, 3 were found in the terminal part of the digestive tract. The condition of the digestive gland in snail D was the same as in snail C, and 174 rediae were recovered. The digestive gland was little disturbed in snail E, and the rediae all appeared to be emaciated; 71 rediae were recovered but none contained cercariae. In snail F, about two-thirds of the gland had been destroyed; the rediae were

dispersed throughout the viscera and 320 were recovered, 6 of which contained cercariae. As regards the disposition of the rediae in this snail, it was noted that the edge of the mantle was packed with them. In snail G a large portion of the digestive gland was intact and 331 rediae distributed throughout the viscera were recovered. The digestive gland in snail H was practically intact and 17 of the 48 rediae recovered contained cercariae.

The rediae in all of the snails ranged from some exceedingly small to large well-developed ones containing not more than 2 well-developed cercariae.

The activity of the rediae was studied through the shells of these experimental snails as well as in other snails and, although these larvae were very active and, apparently, consumed considerable food, no evidence of cannibalism was observed.

In considering the postmortem findings in snails A, B, D, E, and H, there is evidence to indicate that death of the snail was, apparently, not always due directly to the infection, or to the same cause, and certainly was not always due to the insufficiency of digestive gland material which is the source of food for the larvae. It is not entirely clear why some snails should harbor so few infective elements and others so many, and why there should exist such extensive variations in the proportion of rediae and cercariae; however, these differences may be due, at least in part, to individual host resistance.

***Crenosoma microbursa*, n. sp. from the skunk.** F. G. WALLACE, Lake Itasca Biological Station, University of Minnesota.

In a skunk, *Mephitis minnesotae*, collected near Itasca State Park, Minnesota, 25 female and 11 male nematodes of an undescribed species were found. The ring-like folds around the body, most conspicuous anteriorly, as well as the character of the bursa and the location in the bronchi of the host, marked them as belonging to the genus *Crenosoma* Molin, 1861, of the family *Metastrongylidae*.

Crenosoma microbursa n. sp.

Description.—Female (Fig. 1, A) 18 mm to 22 mm long and .40 mm to .47 mm wide. Ring-like folds, 140 to 170 in number, encircle body at intervals throughout length of worm; the first 25 or 30 folds relatively conspicuous and apparently fringed because of delicate longitudinal striation of cuticula. Folds become progressively less conspicuous and farther apart posteriorly until those at posterior end are barely visible. En face view of mouth (Fig. 1, B) shows inconspicuous dorsal and ventral lips, each bilobed and possessing 2 papillae. Amphids present, between lateral extremities of lips. Oesophagus .30 to .35 mm (about 1/60 of body length) long. A pair of excretory glands open through excretory pore just posterior to nerve ring. Vulva about 2/5 of body length from anterior end. Ova in uterus 56 μ to 61 μ × 29 μ to 33 μ. Tail with 2 very inconspicuous papilla-like structures near tip.

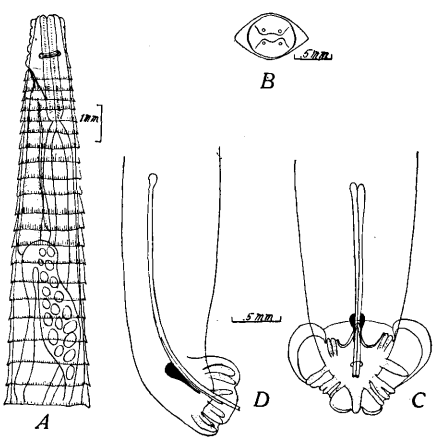

FIG. 1. *Crenosoma microbursa*. A — Anterior end of female. B—En face view. C—Lateral view of tail of male. D—Ventral view of tail of male.

Male 9 mm to 11 mm long and .21 mm to .23 mm wide. Body encircled by 70 to 80 ring-like folds of which about the first 20 are conspicuous and fringed while those farther back become so inconspicuous as to be visible only under the 4 mm lens. Oesophagus .30 mm to .33 mm (about 1/30 of body length) long. Bursa (Fig 1, C) .15 mm to .18 mm in greatest transverse diameter and consisting of 2 lateral lobes, each of which is divided into 3 sub-lobes by 2 slight indentations. Ventro-ventral and latero-ventral bursal rays close together and parallel; externo-lateral separate; medio-lateral and postero-lateral arise from common stem; externo-dorsal separate but close to postero-lateral. Dorsal unbranched. Spicules .29 mm to .31 mm and provided with a dorsal branch which is closely applied to main shaft. Gubernaculum as seen in lateral view (Fig. 1, D) thickened dorsally at anterior end and tapering to a slender shaft posteriorly; 61 μ to 81 μ long.

Host.—Mephitis minnesotae.

Location.—Bronchi.

Locality.—Itasca State Park, Minnesota.

Type Specimens.—U.S.N.M. Helm. Coll. No. 44830.

In the genus *Crenosoma* Molin, 1861, there are now recognized 5 species[1]: *C. striatum* Zeder, 1800, *C. vulpis* Dujardin, 1845, *C. taiga* Skrjabin and Petrow, 1928, *C. potos* Buckley, 1930, and *C. skrjabini* Pologentsev, 1935. *C. mustelae* Galli-Valerio, 1930, was described from a fragment of a worm and eggs and larvae found in smears from the lungs of *Mustela putorius.* The only basis for distinguishing the species was the size of the eggs which were said to be 82.5 μ × 6 μ [sic]. The inadequacy of the description forbids acceptance of this species as valid.

Crenosoma potos was found in the kinkajou, *Potos flavus,* presumably from South or Central America. The only other species of the genus known from the western hemisphere is *C. vulpis* which is listed (as *C. decoratum*) from foxes in Canada by Law and Kennedy (1932). The life cycle of *C. vulpis* has been studied by Wetzel and Müller (1935) who found that various land snails serve as intermediate hosts.

The following key will serve to differentiate *Crenosoma microbursa* from other members of the genus.

Key to Species of Crenosoma

A. Gubernaculum slender in lateral view.
  B. Dorsal ray of bursa with lateral projections.
    C. Externo-lateral arising with medio-lateral and postero-lateral. Externo-dorsals arising with dorsal from common stem. Spicules less than .1 mm long and with short dorsal process which is entirely within proximal half or spicule's length ..... *C. skrjabini*
    CC. Externo-lateral arising apart from dorsal. Spicules more than .2 mm long. Dorsal process of spicule arising near middle and extending to within 1/6 of spicule's length from distal end.
      D. Longitudinal striations of cuticula present. Spicules .24 mm long ..... *C. striatum*

[1] Since this paper was written, the description of another species, *C. mephitidis* from the skunk, has appeared (M. Hobmaier, 1941, Jour. Parasitol. 27(3): 229–232). Specimens of this form, kindly provided by Dr. Hobmaier, have been compared with *C. microbursa* from which they differ in a number of respects. In *C. mephitidis* the bursa is larger, the externo-lateral bursal ray arises from a common stem with the medio-lateral and postero-lateral, and the cuticular rings are confined to the anterior part of the body. In the key presented here *C. mephitides* would fall with *C. potos* except for the length of the spicules which are only .38 mm long in the former.

DD. Longitudinal striations of cuticula absent. Spicules .37 mm long ..... *C. vulpis*

BB. Dorsal ray of bursa without lateral projections ..... *C. taiga*

AA. Gubernaculum thick in front and tapered behind in lateral view.

B. Spicules .97 mm long and about 1/15 length of body. Bursa large, rays being more than half as long as spicules. Externo-lateral arising with medio-lateral and postero-lateral from a common stem. *C. potos*

BB. Spicules .29 mm to .31 mm long; about 1/30 length of body. Bursa small, rays being less than 1/4 the length of spicules. Externo-lateral ray separate ..... *C. microbursa*

REFERENCES

BAYLIS, H. A. 1926. *Strongylus annulatus* v. Siebold and the genus *Crenosoma*, Molin. Ann. and Mag. Nat. Hist. **17**: 519–521.

BUCKLEY, J. J. C. 1930. On a lungworm, *Crenosoma potos*, n. sp. from the kinkajou, *Potos flavus* (Schreber). Jour. Helminth. **8**: 229–238.

DUJARDIN, F. 1845. Histoire naturelle des helminthes ou vers intestinaux. xvi + 654 + 15 pp. Paris.

GALLI-VALERIO, B. 1930. Notes de Parasitologie. Centbl. f. Bakt. [etc.] Abt. 1, Orig. **115**: 212–219.

LAW, R. G., and KENNEDY, A. H. 1932. Parasites of fur-bearing animals. 25th Ann. Rept. Game and Fisheries Dept., Ontario. pp. 20–43.

MOLIN, R. 1861. Il sottordine degli acrofalli ordinato scientificamente secondo i resultamenti delle indagini anatomiche ed embriogeniche. Mem. R. Ist. Veneto di Sci. Let. ed Arti, Venezia. **9**: 427–633.

POLOGENTSEV, P. A. 1935. On the nematode fauna of the shrew-mouse, *Sorex araneus* L. Jour. Parasitol. **21**: 95–98.

SKRJABIN, K. I., and PETROW, A. M. 1928. A description of the genus *Crenosoma* Molin 1861 (Metastrongylidae, Nematoda). Parasitol. **20**: 329–335.

WETZEL, R., and MÜLLER, F. R. 1935. Die Lebensgeschichte des Schachtelhalmförmigen Fuchslungenwurmes, *Crenosoma vulpis*, und seine Bekämpfung. Deut. Pelztierzüchter. Heft 19, pp. 361–365.

A new dilepidid cestode, *Catenotaenia linsdalei*, from a pocket gopher in California. ALLEN MCINTOSH, U. S. Bureau of Animal Industry.

In this paper is described a new species of the genus *Catenotaenia* Janicki, 1904. The new species is the ninth assigned to the genus and the first described from America. The material on which the species is based, two fragmented specimens, was collected by J. M. Linsdale, Monterey, California, in February, 1941, from *Thomomys bottae bottae*.

Catenotaenia linsdalei, n. sp.

Strobila comparatively long and slender, about 135 mm long with a maximum breadth of 1 mm. Mature proglottid (Fig. 1, A) longer than broad, 1.45 mm by 0.93 mm; gravid proglottid (Fig. 1, B) usually more than 3 times as long as broad, typical segment measuring 3.37 mm by 1 mm. Excretory system consisting of a conspicuous longitudinal vessel on each side lateral to testes and ventral to the reproductive ducts; a transverse vessel in the posterior part of the segment connects the longitudinal vessels. The genital pores are irregularly alternate and near the basal level of the anterior fourth of the proglottid. No heads of the species were available for study.

Male reproductive system.—Testes average about 130 per segment, about 50 μ by 70 μ in diameter, arranged, for the most part, in 2 layers and in 2 longitudinal fields, forming a commissure in posterior portion of segment. Cirrus sac pyriform, 70 μ by 140 μ; vas deferens forming a few loops before entering cirrus sac; cirrus

not observed protruding but in at least one instance it was inserted in the vagina (Fig. 1, A) indicating intraproglottic fertilization.

Female reproductive system.—Ovary fan-shaped, mutilobed, about 350 μ long by 500 μ wide, situated medially in anterior third of proglottid, occupying in breadth the space between the longitudinal excretory vessels. Vitellarium posterior to ovary, multilobed, the lobes in shape and size resembling the lobes of the ovary; shell gland about 50 μ in diameter, on poral side between ovary and vitellarium; seminal receptacle lateral to shell gland, about 85 μ in diameter. Vagina about 200 μ long, extending diagonally to lateral margin of segment. Outline of developing uterus in mature proglottid observable, especially in area anterior to ovary. Uterus (Fig. 1, B) in gravid or partly gravid segments consists of a median stem with from 40 to 50 sacculations on either side. Eggs numerous, small, 6 to 7 μ.

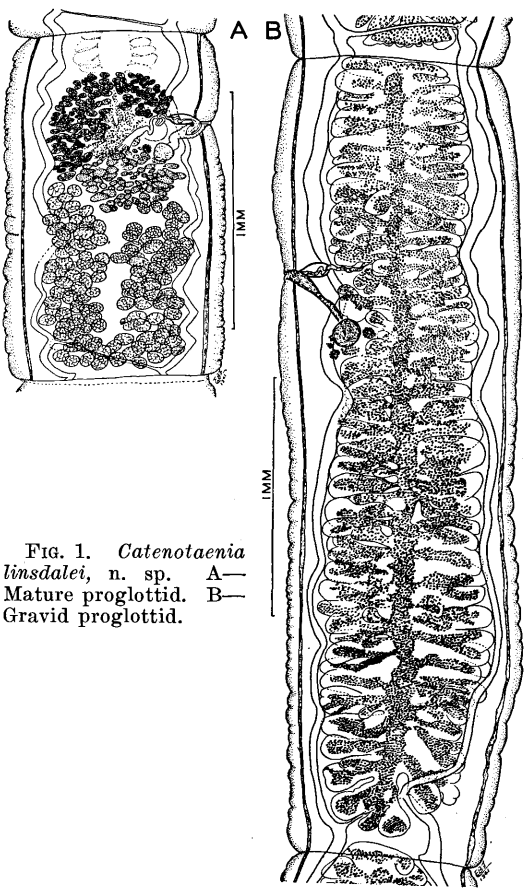

FIG. 1. *Catenotaenia linsdalei*, n. sp. A—Mature proglottid. B—Gravid proglottid.

Habitat.—Small intestine of a pocket gopher, *Thomomys bottae bottae* (Eydoux and Gervais, 1836).

Distribution.—Monterey, Calif. (Hastings Natural History Reservation).

Specimens.—U. S. N. M. Helm. Coll. No. 44806 (type, longest fragment on slide mount).

Remarks.—The 8 species previously assigned to the genus *Catenotaenia* are as follows: *C. capensis* Ortlepp, 1940, *C. dendritica* (Goeze, 1782), *C. geosciuri* Ortlepp,

1938, *C. lobata* Baer, 1925, *C. oranensis* Joyeux and Foley, 1930, *C. pusilla* (Goeze, 1782), *C. rhombomidis* Shul'ts and Landa, 1935, and *C. symmetrica* Baylis, 1927. The last-named species was regarded by Meggitt (1934, Jour. Parasitol. **20**: 181–189) as a member of the genus *Oochoristica;* Ortlepp (1938, Onderstepoort Jour. Vet. Sci. Anim. Indus. **11**: 23–50; 1940, *Ibid.* **14**: 97–110), however, appears not to have accepted the action of Meggitt since he reviewed the species in question as a member of the genus *Catenotaenia.* In view of the large eggs and the apparent lack of a uterus with definite walls, the action taken by Meggitt in transferring *C. symmetrica* to the genus *Oochoristica* may be further justified when more material is available for study.

The new species, *Catenotaenia linsdalei,* shows closer affinity to *C. dendritica* and *C. goesciuri* than to any of the other species. In these 3 species the excretory system consists of 2 longitudinal vessels instead of a complicated network as described for some of the other species; the testes are posterior to the ovary and for the most part grouped in 2 lateral fields. In the number of uterine branches the new species appears to be more closely related to *C. geosciuri* than to any other member of the genus; the new species, however, can be differentiated from the last named species on the length of the vagina which is exceedingly long in *C. geosciuri* and of average length in *C. linsdalei,* n. sp.

A note on the parasite fauna of Georgia. LEONARD E. SWANSON, U. S. Bureau of Animal Industry.

During the years 1938 to 1941, the writer had an opportunity to conduct post-mortem examinations on various domestic animals at Moultrie, Georgia. The following list gives the species encountered, as well as the location in their respective hosts. Only those parasites found on necropsies are listed, but this does not signify that the hosts mentioned may not harbor other species of parasites. Only one dog was necropsied.

	CESTODA	
Moniezia benedeni	Sheep and cattle	Small intestine
Moniezia expansa	Cattle	do
Cysticercus bovis	do	Muscles
Cysticercus cellulosae	Swine	do
	NEMATODA	
Habronema muscae	Horse and mule	Stomach
Habronema microstoma	do	do
Oxyuris equi	do	Large intestine
Setaria equina	do	Body cavity; once in scrotal sac of horse
Strongylus equinus	do	Large intestine
Strongylus vulgaris	do	do
Strongylus edentatus	do	Large intestine; once in scrotal sac of horse
Cylicostomes	Horse and mule	Large intestine
Oesophagostomum radiatum	Cattle	do
Bunostomum phlebotomum	do	Small intestine
Strongyloides papillosus	do	do
Cooperia punctata	do	do
Cooperia pectinata	do	do
Ostertagia ostertagi	do	Stomach

Haemonchus contortus	Cattle and sheep	do
Haemonchus similis	Cattle	do
Setaria labiato-papillosa	do	Body cavity
Trichostrongylus axei	do	Caecum
Oesophagostomum columbianum	Sheep	Large intestine
Ostertagia circumcincta	do	Stomach
Bunostomum trigonocephalum	do	Small intestine
Trichuris ovis	do	Caecum
Cooperia spp.	do	Small intestine
Trichostrongylus spp.	do	Stomach
Ascaris suis	Swine	Small intestine
Stephanurus dentatus	do	Liver and kidney regions, occasionally lungs
Stephanurus dentatus	Cattle	Liver
Oesophagostomum dentatum	Swine	Large intestine
Oesophagostomum longicaudum	do	do
Oesophagostomum brevicaudum	do	do
Oesophagostomum georgianum	do	do
Globocephalus urosubulatus	do	Small intestine
Hyostrongylus rubidus	Swine	Stomach
Ascarops strongylina	do	do
Physocephalus sexalatus	do	do
Metastrongylus elongatus	do	Lungs
Metastrongylus salmi	do	do
Choerostrongylus pudendotectus	do	do
Gongylonema pulchrum	Swine and cattle	Oesophagus
Trichuris suis	Swine	Caecum
Strongyloides ransomi	do	Small intestine
Ancylostoma caninum	Dog	do
ACANTHOCEPHALA		
Macracanthorhynchus hirudinaceus	Swine	Small intestine
ARTHROPODA		
Gastrophilus nasalis	Horse and mule	Stomach
Oestrus ovis	Sheep	Nasal sinuses
Hypoderma spp.	Cattle	Oesophagus and under hide and back
Demodex folliculorum suis	Swine	Skin
Sarcoptes scabiei var. *suis*	do	do

Additional notes on North American Physalopterinae (Nematoda).[1] BANNER BILL MORGAN, Department of Zoology, University of Wisconsin.

In a previous paper the writer (1941, Proc. Helminth. Soc. Wash. **8**: 28–30) presented a host list of all known Physalopterinae occurring in North America. Specimens on which the doubtful determinations or unverified species mentioned in the above report were based have since been examined by the writer and the necessary revisions are herein recorded. A list of additional hosts of members of the subfamily also is included.

Specimens of *Physaloptera papillotruncata* Molin, 1860, of Canavan, 1931 from *Taxidea taxus* (Badger) have been identified by the writer as *P. torquata* Leidy,

[1] This investigation was aided by a grant from the Wisconsin Alumni Research Foundation.

1886. Specimens of *P. turgida* Rudolphi, 1819, of Leigh, 1940, from *Procyon lotor* (Eastern Raccoon) appear to be *P. rara* Hall and Wigdor, 1918 and those from *Mephitis mephitis* (Skunk) were identified by the writer as *P. maxillaris* Molin, 1860. The writer wishes to express his appreciation to Dr. Canavan and to Dr. Leigh for the opportunity to examine their material.

Through the kindness of J. A. McLeod, specimens of *P. spinicauda* McLeod, 1933 from *Citellus franklini* (Franklin Ground Squirrel) were forwarded to the writer. This material agreed in most respects with *P. massino* Schulz, 1926, originally described from *Mus muscularis wagneri* (Wagner Rat) from Russia. With this finding, *P. spinicauda* becomes a synonym of *P. massino*.

In addition to the hosts recorded in the writer's previous paper, the following North American animals have been found to harbor Physalopterinae not identified as to species because of the lack of mature male or female specimens.

All of the parasites were from the stomach unless otherwise noted.

Skrjabinoptera sp.

Anolis carolinensis (American Chamaeleon)

Abbreviata sp.

Sceloporus consobrinus (Yellow-banded Swift Lizard)

Physaloptera sp.

Heloderma horridum (Gila Monster Lizard)
Colinus virginiana (Bob-white Quail; from breast muscles)
Bonasa umbellus (Ruffed Grouse; from breast muscles)
Falco mexicanus (Prairie Falcon)
F. peregrinus anatum (Duck Hawk)
Elanus leucrus (White-tailed Kite)
Astur a. atricapillus (Eastern Gosbawk)
Sorex f. fumeus (Smokey Shrew)
Sciurus n. nigra (Squirrel)
Lutra c. canadensis (Otter)
Mustela c. cicognani (Short-tailed Weasel)
M. frenata (Long-tailed Weasel)

Immature Physalopterinae:

Ambystoma opacum (Marbled Salamander)
Desmognathus f. fuscus (Salamander)
Plethodon glutinosus (Salamander)
Pseudotriton m. montanus (Salamander)
Rana pipiens (Leopard Frog)
Bufo fowleri (Fowler Toad)
B. compactilis (Toad)
B. c. cognatus (Toad)
Hyla crucifer (Tree Frog)
Pseudacris brimleyi (Swamp-tree Frog)
Acris crepitans (Cricket Frog)
Scaphiopus holbrooki (Spade-foot Frog)

MINUTES

Two Hundred Thirteenth to Two Hundred Twentieth Meetings

The 213th meeting was held October 16, 1940. The following officers were elected for the year: C. W. Rees, President; J. T. Lucker, Vice-president; Edna M. Buhrer, Corresponding Secretary-Treasurer; A. O. Foster, Recording Secretary. The following individuals were elected to membership: Dr. Nicholas Gelormini, Dr. Theodor von Brand, Mr. Merlin Allen, Dr. Mervin C. Myer, Dr. J. C. Lotze, and

Mr. J. H. Machmer. Dr. Christie was reelected to the editorship of the Proceedings. Dr. R. A. Cooley, U.S.P.H.S., a visitor to the Society, spoke on the problems encountered in controlling ticks. Other papers were presented by Hoffman, von Brand, Harwood, Price, and Steiner.

The 214th meeting was held November 16, 1940, at the Johns Hopkins University School of Hygiene and Public Health. Preceding the business session a dinner was enjoyed as guests of the Baltimore members and friends of the Society. Dr. Hegner was elected chairman of the scientific meeting. Contributions were made toward the purchase of flowers for Dr. Stiles who was a patient at the Johns Hopkins Hospital. Dr. Cort took charge of procuring the flowers and of presenting them to Dr. Stiles. Among the visitors who presented papers were Drs. Mauss, Rozeboom, Brook, Herber, and Laird. Members who gave papers were Sarles, Hoffman, Kates, and Steiner. Dr. Cort summarzied the papers given at the last meeting of the American Society of Tropical Medicine.

The 215th meeting was held December 18, 1940. Dr. Christie was elected the Society's representative in the Washington Academy of Sciences. Dr. Price, who had served in this capacity for several years, had indicated earlier his desire to relinquish the post at the time of this election. Mr. Banner Bill Morgan and Dr. Harold O. Peterson were elected to membership. Papers were given by Morgan, Spindler, Christie, and Steiner.

The 216th meeting was held January 15, 1941. Dr. Charles G. Dobrovolny was elected to membership. Mr. Daniel M. Jobbins of the Gorgas Memorial Laboratory, Panama, gave an illustrated discussion of the activities of the Laboratory. Foster, Habermann, and Peterson discussed the effect of phenothiazine on horses.

The 217th meeting was held February 19, 1941. Dr. Virginius E. Brown was elected to membership. On learning of the death of Dr. Stiles, the Society drew up the following resolution:

> "The Helminthological Society of Washington wishes to record its deep sense of the great loss which the scientific and medical world has suffered in the death of its esteemed and distinguished member, Doctor Charles Wardell Stiles, on January 24, 1941. Dr. Stiles was a founder of the Society and one of its most active members for many years. His versatility and painstaking research are evidenced by more than 800 contributions of lasting value to the literature of medical and veterinary zoology. Among these are several volumes of the unique and invaluable Index Catalogue which was jointly prepared by him and Dr. Albert Hassall. This will be for all time an indispensable reference work for medical and veterinary zoologists the world over. Everyone will remember him for having done so much toward rescuing thousands of shiftless and helpless victims from a disease hitherto regarded as inherent in their temperaments and their soil. Those who knew him will remember him as a profound scholar and stimulating friend.
>
> "The Society extends its sincerest sympathy to the members of his family.
>
> "Be it resolved, that a copy of these minutes be communicated to Dr. Stiles' family and be recorded in the Proceedings of the Society."

Drs. Schwartz, Wright, and Bartsch reviewed the work of Dr. Stiles, recalling especially its relation to the Bureau of Animal Industry and to the National Institute of Health. The second half of the meeting was given to a consideration of the influence of bacterial flora on the cultivation of *Endamoeba histolytica* by Dr. Ben D. Chinn, a consultant in protozoology at the National Institute of Health.

The 218th meeting was held March 19, 1941. The Treasurer's report for 1940 was read and approved with a vote of appreciation to Miss Buhrer for her loyal services to the Society. Dr. Luis Mazzotti, of the Instituto de Salubridad y Enfermedades Tropicales of Mexico City, and Mr. Rex. W. Allen were elected to membership. Papers were given by Scott (J. A.), Mazzotti, Jacobs, and Lotze.

The 219th meeting was held April 16, 1941. President Rees announced the Society's receipt of Vol. 1 of the Arquivos de Zoologia do Estado de Sao Paulo. It was voted to present this volume to the Library of the Zoological Division. Dr. Ewing stressed the importance of reviewing the status of the Proceedings, with particular reference to the preservation of back volumes and to the printing of a sufficient number of current numbers to avoid future embarrassment. It was voted to accept Miss Buhrer's invitation to hold the annual picnic at the recreational center of the Horticultural Station at Beltsville on Saturday, May 17. Dr. Kates was appointed chairman of a committee on arrangements. Dr. Bartsch invited the Society to meet at his home either in May or July. Dr. Cornelius Phillip, U.S.P.H.S., a visitor to the Society, spoke on the work at the Rocky Mountain Spotted Fever Laboratory at Hamilton, Montana. Dr. Sarles discussed experimental studies on nodular worms of sheep.

The 220th meeting was held May 21, 1941. Papers were presented by Christensen, Sarles, Schwartz, and von Brand.

A. O. FOSTER,
Recording Secretary

CONTENTS