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Correspondence may be addressed to the corresponding secretary-treasurer, Edna M. Buhrer, Division of Nematology, Plant Industry Station, Beltsville, Md., or to the editor, Jesse R. Christie, Division of Nematology, Plant Industry Station, Beltsville, Md.

PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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Effect of sulfamethazine on the coccidian parasite, *Eimeria tenella*, of chickens.

EVERETT E. WEHR and MARION M. FARR, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Maryland.

In the field of poultry therapeusis, the sulfonamid drugs have provided some of the most effective weapons for combatting coccidiosis in chickens. Although many of them have been tested experimentally, only a relatively small number have shown any real merit as prophylactic and/or curative measures for this disease. Of these latter, sulfamethazine ranks as one of the best.

Horton-Smith and Taylor (1942) presented data to show that sulfamethazine effected both a prophylactic and curative action on the course of infection. In 0.05-gram doses, this drug reduced to "almost nil" the mortality in chicks given sufficient numbers of *Eimeria tenella* oöcysts to cause acute cecal coccidiosis in untreated birds. Given in the mash in a 1 per cent concentration, sulfamethazine reduced considerably the severity of the infection in chickens infected 72 and 96 hours previously. These same authors (1943, 1945) reported good results from the use of a saturated solution of sulfamethazine as a substitute for the regular drinking water. Gordon (1944) wrote that 1 ounce of sulfamethazine powder to 8 gallons of tap water was an excellent prophylactic for cecal coccidiosis. Moreover, he stated that this medicated solution was of benefit to infected birds even when treatment was delayed until 3 days after infection. Swales (1944) conducted tests which showed that sulfamethazine inhibited the course of infection in chickens even after hemorrhage had started. Horton-Smith and Boyland (1946) found that the substitution of 0.2 per cent sodium sulfadimethylpyrimidine (sodium sulfamethazine) solution for the regular drinking water effectively controlled cecal coccidiosis in chickens.

The present paper presents data on the clinical and post-mortem findings in infected chickens to which had been fed sulfamethazine either in the mash or in capsules at various times during the course of the infection and, in case of the medicated mash, for varying lengths of time.

EXPERIMENTAL PROCEDURE

The chickens used in the experiments were obtained as day-old chicks from the Poultry Section of the Animal Husbandry Division, Bureau of Animal Industry, Agricultural Research Center, Beltsville, Md. As soon as the chicks were removed from the incubator, they were placed in clean electric brooders which had been previously placed in a large steam-heated room that had been used only for the brooding of parasite-free birds. When removed from the brooders for experimental use, the birds were transferred to clean all-metal cages which were equipped with wire floors, with metal pans 2 inches below the floors to catch the droppings, and with feeders and waterers attached to the outside of the cages. Newspapers were spread over the metal pans to facilitate the removal of the droppings and to keep the pans as dry as possible. The droppings were removed daily to prevent sporulation of oöcysts and subsequent ingestion of them by the experimental birds.

Infective oöcysts used to inoculate the birds were obtained in the usual manner. Scrapings of the cecal walls of the young birds which had been inoculated with

small doses of sporulated oöcysts 8 days previously were moistened with a 2.5 per cent solution of potassium dichromate. After 2 days were allowed for sporulation of the oöcysts, they were washed free of potassium dichromate solution by repeated decantations with tap or distilled water.

Prior to inoculation, the birds of each experiment were weighed and distributed into groups of equal number and of approximately equal weights. The birds were inoculated by introducing the sporulated oöcysts into the esophagus by means of a graduated pipette. The process of inoculation was carried out uninterruptedly and as quickly as possible so that the infection in the different groups would be as nearly comparable as possible. Immediately following inoculation, one uninoculated bird was placed in each cage of inoculated birds to serve as a check on the possibility of extraneous infection.

The number of bloody droppings and the relative amounts of blood in the droppings passed by each group of inoculated birds during the course of the experiment were noted.

Beginning with the seventh day and extending through the fifteenth and, in some cases, the seventeenth day following inoculation, records of the number of oöcysts per gram of total feces passed every 24 hours by each group were kept. Twenty-four hours prior to the collection of the first droppings, one-half of the surviving birds of each cage, including the uninoculated bird, were transferred to another cage. This procedure was followed in all the experiments, except No. 5. This was decided upon because (1) it was considered unnecessary to prepare for oöcyst calibration the droppings from all the surviving birds of each cage, since it was felt that the bulk of the material to be handled would be too great to assure a uniform mixture; and (2) it seemed best for the most accurate results to exclude the droppings of the uninoculated controls from those on which counts were being made. The procedure adopted by Farr and Allen (1942) for the determination of oöcyst counts was used by the writers. These data, together with mortality and hemorrhage records, were used as criteria in judging the effectiveness of the treatment.

Upon the termination of each experiment, all surviving birds were weighed and necropsied to determine the extent of lesions due to coccidiosis infection and to ascertain the possibility of any toxic effects of the drug.

In experiment 1, the drug was administered in the mash for 7 consecutive days, differing in this respect from any of the other experiments. Aside from the fact that the sulfamethazine powder was administered via capsules in $\frac{1}{4}$ and $\frac{1}{2}$ gram amounts to the infected birds of experiments 3 and 5, and in the mash in 1 per cent concentration to the infected birds of experiments 2 and 4, these 4 experiments were conducted similarly and with the same purpose in mind.

Groups 1, 2 and 3 were omitted from experiments 4 and 5 because the results of previous experiments showed that these groups had been decidedly less benefited from the sulfamethazine treatment than groups 4 and 5, and it was decided to concentrate attention on efforts to determine the reasons for this greater benefit.

RESULTS

Experiment 1 was conducted primarily as a preliminary test, the results of which were to furnish information for evaluating the prophylactic and/or curative properties of sulfamethazine. The data concerning this experiment are summarized briefly in tables 1 to 3.

Coccidiostatic Action of Sulfamethazine

The results of experiment 1 clearly demonstrated that sulfamethazine when administered in the mash in a 1 per cent concentration for 7 consecutive days to

infected chickens inhibited the development of *E. tenella*. This is shown by the mortality records (Table 1), the time of appearance of blood in the droppings (Table 2), and the oöcyst output of each of the groups in the experiment (Table 3).

Mortality.—As seen in table 1, the 1–T (started on treatment 1 day before inoculation), T–4 and T–5 groups (started on treatment 4 and 5 days respectively after inoculation) had 10, 25 and 80 per cent mortality, respectively, whereas the T–0 group (started on treatment at time of inoculation), T–1, T–2 and T–3 groups (started on treatment 1, 2 and 3 days after inoculation, respectively) had no mortality. The deaths among the IC group (inoculated controls), the T–4 and T–5 groups occurred on the fifth and sixth days after inoculation while deaths among the 1–T group occurred on the fifth and sixth days after withdrawal of the drug (11 and 12 days after inoculation).

Hemorrhage.—The data presented in table 2 (Experiment 1) show that there was a marked delay in the time of appearance of hemorrhage in the 1–T, T–0, T–1 and T–2 groups. Blood first appeared in the droppings of the 1–T and T–0 groups on the fourth day following withdrawal of the sulfamethazine and in the T–1 and T–2 groups on the fifth day after termination of therapy. The T–3, T–4 and T–5 groups passed from moderate to profuse amounts of blood on the same days as the inoculated controls. However, the T–3 group suffered a slight relapse and blood appeared again in the droppings on the fifth and sixth days after withdrawal of the drug, this being 15 and 16 days after inoculation.

Oöcyst output.—Just as there was a delay in the time of appearance of hemorrhage in the groups placed on treatment early in the infection, there was a comparable delay in the discharge of oöcysts. As with the time of appearance of hemorrhage, the first appearance of oöcysts in the droppings seemed to bear a close relationship to the time of withdrawal of medicated mash. In the 1–T, T–0, T–1 and T–2 groups, oöcysts appeared on the sixth day after termination of treatment and in the T–3 and T–4 groups they appeared on the seventh day after medication was stopped. In the T–3 and T–4 groups there was a considerable reduction in the numbers of oöcysts discharged. In the T–5 group, the number of oöcysts discharged on the seventh day after inoculation (two days after initiation of treatment) approximated the oöcyst output of the IC group on that day. However, on the eighth day after inoculation the output of the T–5 group dropped to almost one-half that of the previous day while that of the IC nearly doubled. Thereafter there was a sharp drop in the oöcyst output of the T–5 group as compared with that of the IC.

That the delay in time of appearance of hemorrhage and oöcysts in the above-mentioned groups of experiment 1 could not have been due to extraneous infections was confirmed by the results of the examination of the 14 uninoculated controls including the 7 kept in the cages with the inoculated treated birds. The ceca of all of them appeared normal while the ceca of all but one of the infected, treated birds that survived showed typical lesions of cecal coccidiosis. Smears taken from each of the control birds revealed the absence of any developmental stage of the coccidium, but the sugar flotation technique disclosed one oöcyst in each of the controls confined with the 1–T and the T–2 groups.

Toxicity.—The feeding to infected and noninfected chickens of a 1 per cent sulfamethazine mash for 7 consecutive days resulted in the development of necrotic lesions in the spleens of some of the birds; 30 of the 140 infected treated birds and 3 of the 20 uninfected treated birds had necrotic areas in the spleen. None of the 40 untreated birds had any spleen lesions at necropsy. The toxicity of the drug was further manifested by a retardation in the growth of the treated birds. The uninoculated, untreated controls at the end of the experiment had an average weight gain of 231 grams, while the uninoculated, treated controls had an average weight

TABLE 1.—*Effect of sulfamethazine on mortality from Eimeria tenella*

Age of birds in days at start of exp.	Method of administering drug	Group designation and treatments	No. birds per group	No. oöcysts given per bird	Mortality	
					Number	Per cent
		Experiment 1				
25	1 per cent in mash	1–T, 7-day treatment beginning 1 day before inoc.	20	89,000	2	10
		T–0, " " " " on " of "	"	"	0	0
		T–1, " " " " 1 " after "	"	"	0	0
		T–2, " " " " 2 days " "	"	"	0	0
		T–3, " " " " 3 " " "	"	"	0	0
		T–4, " " " " 4 " " "	"	"	5	25
		T–5, " " " " 5 " " "	"	"	16	80
		IC, Inoculated Controls	"	"	11	55
		UTC, Uninoculated-Treated Controls	"	0	0	0
		UUC, Uninoculated-Untreated Controls	"	0	0	0
		Experiment 2				
14	1 per cent in mash	1, treated for 24 hrs. before and 24 hrs. after inoc.	12	51,000	7	58
		2, treated for 24 hrs. beginning with inoculation	"	"	6	50
		3, " " 45 " " " "	"	"	4	33
		4, " from 45th to 72nd hrs. after inoc.	"	"	0	0
		5, " " 72nd " 96th " " "	"	"	0	0
		6, " " 96th " 144th " " "	"	"	4	33
		7, " " 144th " 192nd " " "	"	"	11	92
		8, Inoculated Controls	"	"	8	67
		9, Uninoculated Controls	"	0	0	0

TABLE 1.—(*Continued*)

Age of birds in days at start of exp.	Method of administering drug	Group designation and treatments	No. birds per group	No. oöcysts given per bird	Mortality	
					Number	Per cent
		Experiment 3				
14	In capsule	1, given ½ gm. 24 hrs. before inoculation	12	49,200	2	17
		2. " ¼ " at time of inoculation	"	"	0	0
		3, " ¼ " " " " " and ¼ " 24 hrs. after inoculation	"	"	0	0
		4, " ¼ " 45 " and ⅛ gm. 66 hrs. after inoc.	"	"	0	0
		5, " ¼ " 72 " after inoculation	"	"	0	0
		6, " ¼ " 96 " and ¼ gm. 120 hrs. after inoc.	"	"	0	0
		7, " ¼ " 144 " " ¼ " 168 " " "	"	"	6	50
		8, Inoculated Controls	"	"	10	83
		9, Uninoculated Controls	"	0	0	0
		Experiment 4				
38	1 per cent in mash	1–3, omitted				
		4, treated from 45th to 72nd hrs. after inoc.	15	100,000	0	0
		5, " " 72nd " 96th " " "	"	"	0	0
		6, " " 96th " 144th " " "	"	"	2	13
		7, " " 144th " 192nd " " "	"	"	2	13
		8, Inoculated Controls	"	"	5	33
		9, Uninoculated Controls	"	0	0	0
		Experiment 5				
19	In capsules	1, 3, used for tissue study	2	64,000		
		4, given ¼ gm. 45 hrs. and ⅛ gm. 66 hrs. after inoc.	12	64,000	1	8.3
		5, " ¼ " 72 " after inoculation	"	"	0	0
		6, " ¼ " 96 " and ¼ gm. 120 hrs. after inoc.	"	"	7	58
		7, " ¼ " 144 " " ¼ " 168 " " "	"	"	5	42
		8, Inoculated Controls	"	"	8	67
		9, Uninoculated Controls	"	0	0	0

TABLE 2.—*Time of appearance and extent of hemorrhage*

Experiment 1

No. days after inoculation	Group designation							
	IC	1–T	T–0	T–1	T–2	T–3	T–4	T–5
5	+++					++	+++	+++
6	+++					++	++	+++
7	+++					+	+	++
8								
9								
10		+						
11		+++	+					
12		++	++					
13		+	+	++				
14			+	++	+			
15				+	++	+		
16						+		

\+ trace of blood present.
++ several blood-stained droppings.
+++ severe hemorrhage, droppings largely blood.

Experiment 2

No. days after inoculation	Group number							
	1	2	3	4	5	6	7	8
5	++	+		+	+	+++	+++	+++
6	+++	+++	++		+	+++	+	++
7	+++	+++	+++	+	+		+++	+++
8	+	+	+++	+			+	
9				++	+			
10				+	+			

Experiment 3

5					+	+++	+++	+++
6	++				+	++	+++	+++
7	+++	+++	+				+++	+++
8	++	+++	+					+
9	+	++	++	+				
10			+	++				
11				++				
12				+				
13				+				

Experiment 4

No. days after inoculation	Group number				
	4	5	6	7	8
4		++	++	++	++
5		++	+++	++	+++
6		++	++	+++	+++
7	+		++	++	++
8	+				+

Experiment 5

4	+	+	+			
5		+	+++	+++		+++
6		+	+++	+++		+++
7				++		++
8						
9	++					
10	+++	+				
11	+++	+				

TABLE 3.—*Oöcyst output in thousands of oöcysts per gram of total feces*

Experiment 1

No. days after inoculation	Group designation							
	IC	1–T	T–0	T–1	T–2	T–3	T–4	T–5
7	876	0	0	0	0	0	0	853
8	1,463	0	0	0	0	0	0	462
9	235	0	0	0	0	0	0	10
10	40	0	0	0	0	0	0	3
11	20	0	0	0	0	0	0	0
12	13	225	0	0	0	0	0	0
13	3	870	186	0	0	0	0	3
14	10		660	40	0	0	0	6
15	10			1,073	50	0	0	3
16	3			446	535	0	0	0
17	6					100	0	0
18	0						10	0
19	0							0

Experiment 2

No. days after inoculation	Group number							
	1	2	3	4	5	6	7	8
7	0	32	0	566	213	0	433	1,540
8	2,633	503	876	340	140	0	1,266	273
9	1,100	1,090	2,496	980	550	0	100	910
10	286	170	806	966	176	0	30	300
11–12	123	83	76	100	33	3	0	16
13	3	43	23	23	47	160	0	16
14	3	53	26	36	50	175	0	3
15	0	16	32	43	53	166	0	3
16	3	6	13	43	10	100	13	20

Experiment 3

7	0	0	0	0	0	0	3,973	1,263
8	3	116	0	0	0	0	100	893
9	810	896	3	0	0	0	123	620
10	436	620	576	0	0	0	0	83
11	1,033	423	426	490	40	0	0	60
12	343	130	240	1,330	60	0	6	3
13	53	136	80	740	16	0	6	23
14	50	46	63	163	13	0	0	16
15	90	16	16	190	3	10	0	50

Experiment 4

7				210	0	0	830	1,870
8				173	0	0	113	350
9				573	3	0	13	236
10				220	0	0	6	46
11				60	0	0	13	76
12				90	3	10	16	13
13				66	6	23	10	13
14				50	10	70	0	20
15				20	33	16	0	10
16				33	3	6	3	13
17				16	13	0	0	6

TABLE 3.—(*Continued*)

Experiment 5								
No. days after inoculation	Group number							
	1	2	3	4	5	6	7	8
7				0	0	0	1,976	1,900
8				0	0	0	156	1,910
9				0	0	0	13	286
10				0	0	0	0	76
11				1,600	3	0	3	30
12				980	116	0	30	40
13				100	56	0	43	26
14				156	30	10	0	73
15				70	10	30	3	76
16				16	16	23	0	96
17				103	20	41	180	26

gain of 165 grams. The absence of spleen lesions in the untreated birds and the lower weight gains in the uninoculated, treated birds suggest that the drug might be toxic.

An excellent example of interference with the normal course of development of *E. tenella* by the administration of sulfamethazine was seen in experiment 6. Six 33-day-old chickens were placed together in a cage and given 1 per cent sulfamethazine. Twenty-four hours later 3 of the birds were inoculated with 108,000 oöcysts of *E. tenella*. The medication was continued for 5 more days after which the birds were returned permanently to plain mash. The first symptoms of coccidiosis appeared 9 days after inoculation (4 days after withdrawal of medicated mash) when 2 blood-stained droppings were seen. On the morning of the following day 8 bloody droppings were present; at this time the birds were killed and at necropsy the ceca of the 3 uninoculated controls were normal and no coccidial forms were found. In contrast to this, the ceca of the 3 inoculated birds were definitely affected; the walls were greatly thickened, the contents were either blood-stained or consisted of bloody cores. Smears from the cecal walls contained immature and mature second generation schizonts, second generation merozoites and immature gametocytes. No oöcysts were seen.

In this test there was evidence that the drug produced some toxic effects. Two of the inoculated and two of the uninoculated birds had small to large necrotic lesions in the spleen as well as eroded hemorrhagic areas in the duodenum.

Conclusions.—The marked delay in the time of appearance of blood in the droppings of the treated birds of experiments 1 and 6 and in discharge of oöcysts in treated birds of experiment 1 was strongly indicative of a coccidiostatic action of sulfamethazine. Moreover, the reduced oöcyst output in the T–4 group of experiment 1 suggested that the sulfamethazine mash apparently had exerted a coccidiocidal action on one or more stages in the life cycle of the parasite. Inasmuch as the time of appearance of blood, the amount passed and the period over which it was observed in this group of birds were somewhat similar to those of the inoculated controls, it appeared that the drug was affecting some stage in the life cycle of the parasite present in the tissues at or soon after bleeding began.

Coccidiocidal Action of Sulfamethazine

That the greatly delayed and much reduced oöcyst output in the T–4 group of experiment 1 was possibly the result of a coccidiocidal action of sulfamethazine has been suggested. In order to obtain more conclusive information on the proto-

zoacidal action of sulfamethazine, experiments 2, 3, 4 and 5 were undertaken for the purpose of making clinical and post-mortem studies and to determine any correlation between the two.

From the data presented (Tables 1 and 2) it appears that under the system of treatment followed in the above experiments, those groups started on treatment between the 45th hour and the 72nd hour after experimental inoculation were most benefited from the treatment. This benefit was manifested in (1) a distinctly lower mortality rate and (2) a reduced amount of hemorrhage.

Mortality.—Regardless of the method of administration of the drug used in these tests, the greatest mortality and morbidity occurred in those groups placed on treatment early and late in the course of the infection. The total absence of deaths in group 5 of all experiments and the one death in group 4 of experiment 5 definitely indicated that sulfamethazine was, in some way, affecting one or more stages of the parasite.

Hemorrhage.—As a rule, those groups which had the highest percentage of mortality (Table 1) and morbidity, namely, groups 1, 2, 6, 7, and 8 passed the largest amount of blood (Table 2). The birds of group 5 remained in a reasonably healthy condition during the entire test and, except for experiment 4, passed no more than a trace of blood on any single day. With the exception of experiment 4, the group 4 birds passed a moderate amount of blood on one or more days during the bleeding period and, in experiment 5, a considerable amount on the tenth and eleventh days after inoculation. There was a delay of several days in the time of appearance of blood in group 4 in experiment 3. In experiment 5 a trace of blood was observed in the droppings of this group on the fourth day after inoculation and no more was noted until the ninth day after inoculation.

Oöcyst output.—The data presented in table 3 for experiments 2, 3, 4 and 5 indicate no very great reduction in the oöcyst output of those groups treated before inoculation or within 72 hours following inoculation. But in those groups placed on treatment either at 72 or 96 hours after inoculation the oöcyst output was markedly reduced except for group 5 of experiment 2. On the other hand, the groups treated from the 144th to the 196th hours after inoculation discharged for a short time as many or more oöcysts than the inoculated controls, but the oöcyst output of this group (7) declined sharply thereafter. In general these observations also apply to the corresponding groups of experiment 1 in spite of the fact that the birds of this experiment were on medicated mash for 7 consecutive days. In experiments 3 and 5, in which the drug was administered in capsules, there was a delay in time of discharge of oöcysts in all the treated groups except group 7, the greatest delay being in group 6.

The regularity with which oöcysts were absent from the droppings of group 6 for several days after their appearance in the droppings of the other groups indicated that the drug was exerting a deleterious effect on some particular stage in the life cycle of the parasite. A study of the life cycle of *E. tenella* suggested that the second-generation schizont and/or second-stage merozoite were probably the stages that were being adversely affected by the administration of the drug from the 96th to the 144th hours after inoculation, the period over which group 6 was treated. At the termination of the treatment period in group 6, one or more of these birds were sacrificed for study. One cecum of each of the sacrificed birds was emptied of its contents and immediately preserved in Bouin's solution for sectioning and study at a later time. Fresh smears were taken from the other cecum and examined immediately under the microscope. In every case the smear revealed large numbers of destroyed second-generation schizonts and only a few motile second-generation merozoites. However, the presence of oöcysts in small numbers in the droppings of the birds of this group indicated that the drug was not destroying all the developing forms.

Toxicity.—One treated bird of experiment 2 showed a necrotic spleen lesion.

Conclusions.—In the foregoing experiments, data have been presented to show more precisely the stage or stages in the life cycle of *E. tenella* that were being affected by the test drug. Groups 4 and 5 of these experiments, just as in experiment 1, received the greatest benefit from sulfamethazine treatment. Several scrapings from the cecal walls of birds of each of these groups killed at the time of withdrawal of medicated mash were examined microscopically with the result that many first-generation schizonts with merozoites and hyaline bodies—the latter indicating degeneration—were seen in the scrapings from the birds of group 4. Cecal scrapings from birds of group 5 examined at 96 hours after inoculation contained a great many small, immature, second-generation schizonts which appeared to be normal. However, an examination of cecal scrapings of birds of group 5 killed at 140 hours after inoculation revealed many immature or degenerated second-generation schizonts. Scrapings from the cecal walls of birds of group 6 killed at 96 and 144 hours after inoculation, and examined similarly to those of groups 4 and 5, showed masses of degenerating and degenerated mature second-generation schizonts each containing a large hyaline body. There were also some motile and some non-motile second-generation merozoites. This evidence seems to demonstrate the coccidiocidal action of sulfamethazine.

DISCUSSION

The data obtained from the foregoing experiments indicated that sulfamethazine administered either as a 1 per cent concentration in the mash or in capsules exerted both a coccidiostatic and coccidiocidal effect on *E. tenella.* Evidence of its inhibitory action was shown by those groups started on treatment early in the course of the disease, the delay of several days in the time of appearance of blood and oöcysts in the droppings of the treated birds being manifestations of this phenomenon. In addition to the delay in the time of appearance of blood and oöcysts, there was, in some cases, a reduction in the amount of blood and the number of oöcysts passed in the droppings. This was particularly true of those groups started on treatment between the 45th and 72nd hours after experimental inoculation. In those cases where treatment was delayed until the 96th hour or later after inoculation, the drug did not appear to interfere with the course of the infection, that is, insofar as the time of appearance and amount of bleeding were concerned, blood appearing in the droppings at the usual time and in quantities similar to those observed in the inoculated, untreated controls. However, in those groups started on treatment on the 96th hour following inoculation, there was an exceptionally long delay in the time of appearance of oöcysts in the droppings. There were also only a few oöcysts recovered from the feces of the birds in these groups. It appeared that, in such cases, the drug destroyed the second-generation schizont rather than merely delaying the development of the parasite. Thus it has been shown that sulfamethazine exerts a coccidiocidal effect as well as a coccidiostatic effect on the protozoan parasite.

Horton-Smith (1945) remarked that the earlier in the course of the infection treatment was instituted the more beneficial the results. However, he noted that the most striking results were obtained when treatment was begun 24 to 48 hours after infection.

The present experiments indicate that those groups started on treatment from the 45th to the 72nd hours after inoculation and continued on treatment until the 72nd and 96th hours, respectively, after infection were the ones most benefited. Treatment instituted earlier in the infection reduced mortality and mitigated the severity of the infection. However, little or no amelioration in the condition of those birds placed on treatment 96 hours, or later, after inoculation was noticed.

In experiment 1, there were evidences of retarded growth resulting from the administration of the drug. Post-mortem examination of the treated birds revealed the presence of white necrotic areas in the spleens of some of the birds. Spleen lesions were not observed in any of the untreated controls.

The toxicity of sulfamethazine and sodium sulfamethazine has been reported briefly by Hawkins and Kline (1945) and Asplin, Boyland and Horton-Smith (1946). In concentrations of 0.4 to 1.0 per cent in the mash, the former authors stated that sulfamethazine powder interfered with the normal weight gains of the birds if continued to be fed longer than 3 consecutive days. The latter investigators reported that the blood clotting time in chicks which had received an aqueous solution of sodium sulfamethazine was lengthened, possibly owing to the decreased synthesis of vitamin K in the gut. They also claimed that an aqueous solution of the sodium salt caused hyperplasia of the seminiferous tubules of the testes in cockerels, which was accompanied by precocious development of the comb and wattles.

SUMMARY AND CONCLUSIONS

1. Experiments were conducted with 588 chickens to determine the effect of sulfamethazine on experimental *Eimeria tenella* infections.

2. One per cent sulfamethazine fed in the mash for 7 consecutive days beginning at the time of inoculation or within 3 days after inoculation materially benefited the birds.

3. The chickens derived little or no benefit when treatment was initiated on the 4th, 5th or 6th day after inoculation whether the drug was administered for 7 days in 1 per cent medicated mash or for 2 days in ¼-gram capsules.

4. One per cent mash fed for 6 or 7 days beginning 1 day before inoculation delayed the appearance of clinical symptoms until 4 days after the drug was withdrawn and the discharge of oöcysts until 6 days after completion of therapy.

5. The administration of the drug (1) in the mash at a level of 1 per cent for 24 hours before and after inoculation or (2) in a ½-gram capsule 24 hours before inoculation was of little if any benefit.

6. When treatment was initiated with 1 per cent medicated mash 45 hours after inoculation and discontinued 72 hours after inoculation or when ¼-gram capsule was administered on the 45th and ⅛-gram on the 66th hour after inoculation the chickens were considerably benefited. Smears from the ceca of one of these birds contained first-stage schizonts packed with merozoites but there were hyaline bodies among the merozoites.

7. When sulfamethazine was fed in the mash from the 72nd to the 96th hour after inoculation or in a single ¼-gram dose at 72 hours after inoculation all the chickens survived, suffered moderate to slight hemorrhage, and passed reduced numbers of oöcysts.

8. Direct smears from the ceca of chickens placed on treatment at 96 hours after inoculation revealed that many second-generation schizonts were destroyed.

9. The effect of sulfamethazine on *E. tenella* appeared to be both coccidiostatic and coccidiocidal depending upon what time in the course of the infection the treatment was initiated.

10. Necrotic lesions were present in the spleens of some of the birds which received the 1 per cent medicated mash for 6 and 7 days.

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Developmental stages in the life cycle of *Eimeria tenella* affected by sulfamethazine treatment. MARION M. FARR and EVERETT E. WEHR, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Maryland.

Since the discovery that some of the sulfa drugs are effective in controlling cecal coccidiosis of poultry, the question has often arisen as to just how these and other drugs which have been generally recommended for this purpose affect the causative protozoan parasite—whether they destroy the organism or merely prevent its development. Various investigators have expressed their views as to the possible action of drugs on *Eimeria tenella,* but few have presented experimental evidence to show just how the parasite actually was affected.

Goff (1941) and Goff and Upp (1943) reported that the sporozoite of *E. tenella* was the stage affected by sulfur. Herrick *et al.* (1944) stated that sulfadiazine stopped the production of oöcysts. Ripsom and Herrick (1945) noted that the sporozoites "have a higher threshold of tolerance for sulfadiazine than the merozoites" and concluded that the drug "exerts its influence on the second generation merozoites and possibly on the developing gametocytes as well." Swales (1944) believed that the motile merozoites were weakened or destroyed by sulfamethazine. With specific reference to the effect of sulfamethazine, Horton-Smith (1945) stated that the "asexual stages developed normally to the second generation" but development in the later stages was completely stopped. Moreover, he observed that "gamete formation was inhibited if the drug had acted long enough." Horton-Smith and Taylor (1945) stated that in the ceca of infected chickens treated with sulfamethazine, the second generation schizonts showed little or no merozoite formation, many of them having a dead appearance.

The present studies were undertaken to determine by histological studies the effect of sulfamethazine on the different stages in the life cycle of *E. tenella.*

MATERIALS AND METHODS

The material on which this study is based was obtained from the ceca of 16 treated and 14 untreated birds in experiments 4 and 5, and 12 treated birds in experiment 2, of the study reported in an accompanying paper by Wehr and Farr (1947). The methods of handling and infecting the birds, method of administration, and the amount of sulfamethazine given, are discussed fully in the paper mentioned above and need not be repeated here.

Whenever a treated bird of a particular group was removed from the experiment and killed for further study, a control bird which had been inoculated at the same time was likewise removed and handled similarly for comparison of findings. This procedure was followed in all instances, except in the case of birds

of experiment 2; no cecal tissues of the control birds of this experiment were fixed and stained, since it was felt that these tissues from birds of the same groups involved in other experiments would serve the purpose.

A portion of one cecum of each of the sacrificed birds was immediately fixed in Bouin's picro-formol fluid for subsequent histopathological study. Smears were prepared from the other cecum and examined microscopically, in a fresh condition, to determine as accurately as possible the developmental stages present. The preserved tissue was embedded in paraffin, sectioned, and stained either in Heidenhain's or Harris' hematoxylin, using standard methods.

Corresponding groups of both the capsule-fed birds and those receiving the drug in the mash were removed from the experiment and killed at the same time following initiation of treatment.

RESULTS OF EXAMINATIONS

Birds killed 24 hours after inoculation.—No coccidia were found in direct smears or in stained slides of cecal tissues of birds treated for 24 hours before and for 24 hours after inoculation with oöcysts of *E. tenella.* A few apparently normal sporozoites were observed within the apparently unaltered epithelial cells of the cecal glands of the birds placed on medicated mash at the time of inoculation.

Birds killed 45 hours after inoculation.—In a bird treated with medicated mash for 45 hours beginning with inoculation, the invading sporozoite was located below the very much enlarged nucleus of the host cell. In other cecal glands there were a few early generation 1 schizonts. No developmental stages were found in the birds which had received the drug in capsules at the time of inoculation, and again 24 hours later.

Birds killed 72 hours after inoculation.—Fresh smears from birds treated with medicated mash from 45 to 72 hours after inoculation contained generation 1 schizonts filled with normal-appearing merozoites among which were small and large, rounded refractile bodies. In stained sections from these birds there were seen a few apparently normal growing forms derived from the sporozoites, early generation 1 schizonts (Plate I, Fig. 1) still showing the darkly staining rounded bodies that were formerly seen in the sporozoites, and multinucleated and mature generation 1 schizonts. The growing forms derived from the sporozoites and the early stages of generation 1 schizonts could not be distinguished from those found in the inoculated control, but the contents of some of the nearly mature generation 1 schizonts had degenerated into a mass of granules while the contents of other schizonts (Plate I, Figs. 3 and 4) consisted of a central darkly staining clump of material surrounded by either normal-appearing merozoites or by small, poorly stained ragged bodies. The central clump of material seemed to have somewhat the same character as the multinucleated generation 1 schizont in that there were scattered through it darkly staining bodies which might have been remnants of nuclei.

The feeding of the drug from the 45th to the 72nd hours after inoculation did not destroy the majority of generation 1 merozoites, since a large number of them were seen to have invaded the cecal glands. In some parts of the tissue the parasitized epithelial cells were still *in situ,* but in other parts these host cells had moved into the interglandular spaces and had begun to enlarge. Growing forms derived from generation 1 merozoites (Plate I, Fig. 6) and 2-nucleated generation 2 schizonts were present in these epithelial cells.

In a bird given sulfamethazine in capsules 45 hours and again at 66 hours after inoculation, normal-appearing and partially broken down generation 1 schizonts as well as normal-appearing generation 2 schizonts were observed.

In fresh cecal smears from the inoculated control birds a few generation 1

schizonts filled with generation 1 merozoites were found. In stained sections an occasional growing form derived from the sporozoite, many multinucleated generation 1 schizonts, and a number of mature generation 1 schizonts filled with small merozoites (Plate I, Fig. 2) were found within the glandular epithelium. In the fundi of some of the glands numbers of generation 1 merozoites had penetrated into the epithelial cells and had rounded up. In other sections these rounded forms had grown and developed into 2 and 4-nucleated generation 2 schizonts and the parasitized host cells had moved into the interglandular spaces (Plate I, Fig. 5); the latter had increased in size.

Birds killed 96 hours after inoculation.—Fresh cecal smears from birds treated with medicated mash from the 72nd to the 96th hours after inoculation revealed masses of normal second-generation schizonts and a few free generation 2 merozoites. Stained sections from these same birds showed normal second-generation schizonts in all stages of development, a large number of multinucleated generation 2 schizonts being shown in plate II, figure 8.

The cecum of a bird which had received a 0.25-gram capsule of sulfamethazine at the 72nd hour after inoculation contained masses of small- to medium-sized multinucleated generation 2 schizonts which appeared to be normal.

In the inoculated control birds masses of small and large immature generation 2 schizonts, an occasional generation 2 schizont containing merozoites, and a few free motile generation 2 merozoites were found in fresh smears. The stained sections revealed the presence of an occasional generation 1 schizont with merozoites, some very early generation 2 schizonts, and a great number of the larger multinucleated generation 2 schizonts (Plate II, Fig. 7).

Thus, it is apparent that the condition of the parasites in the birds treated with sulfamethazine from the 72nd to the 96th hours after inoculation, and killed at the latter hour, was not markedly different from that of the parasites in the inoculated controls. However, stained sections from a bird given 0.25 gram sulfamethazine at 72 hours after inoculation, and killed at 140 hours after inoculation, showed a somewhat different picture. A few small normal-appearing multinucleated generation 2 schizonts and great numbers of generation 2 schizonts that had undergone degeneration were present. A few of these affected schizonts were large and contained a dark central clump, but most of them were small and the contents consisted of very faintly stained strands and fragments. The surface epithelium was apparently normal and no gametocytes were seen.

Stained sections prepared from a bird which had received medicated mash from the 72nd to the 96th hours after inoculation, and killed at 146 hours after inoculation, revealed very much the same conditions as were found in the bird

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PLATE I

Photomicrographs from cecal tissues of chickens experimentally infected with *Eimeria tenella* and killed at 72 hours after inoculation. × 800.

FIG. 1. Multinucleate generation 1 schizonts in gland of chicken treated with 1 per cent sulfamethazine from the 45th to the 72nd hour after inoculation. The greatly enlarged nuclei of the host cells have been pushed toward the lumen of the gland.

FIG. 2. Generation 1 schizont containing generation 1 merozoites in gland of an inoculated control.

FIG. 3. Partially degenerated generation 1 schizont in cecal gland of chicken treated with medicated mash from 45 to 72 hours after inoculation. Some normal merozoites are distributed around the central clump of material.

FIG. 4. Degenerated generation 1 schizont from chicken treated with medicated mash from the 45th to the 72nd hour after inoculation.

FIG. 5. Early generation 2 schizonts in interglandular space just outside invaded cecal gland of inoculated control.

FIG. 6. Growing forms derived from generation 1 merozoites in cecal gland of a chicken treated with medicated mash from 45 to 72 hours after inoculation.

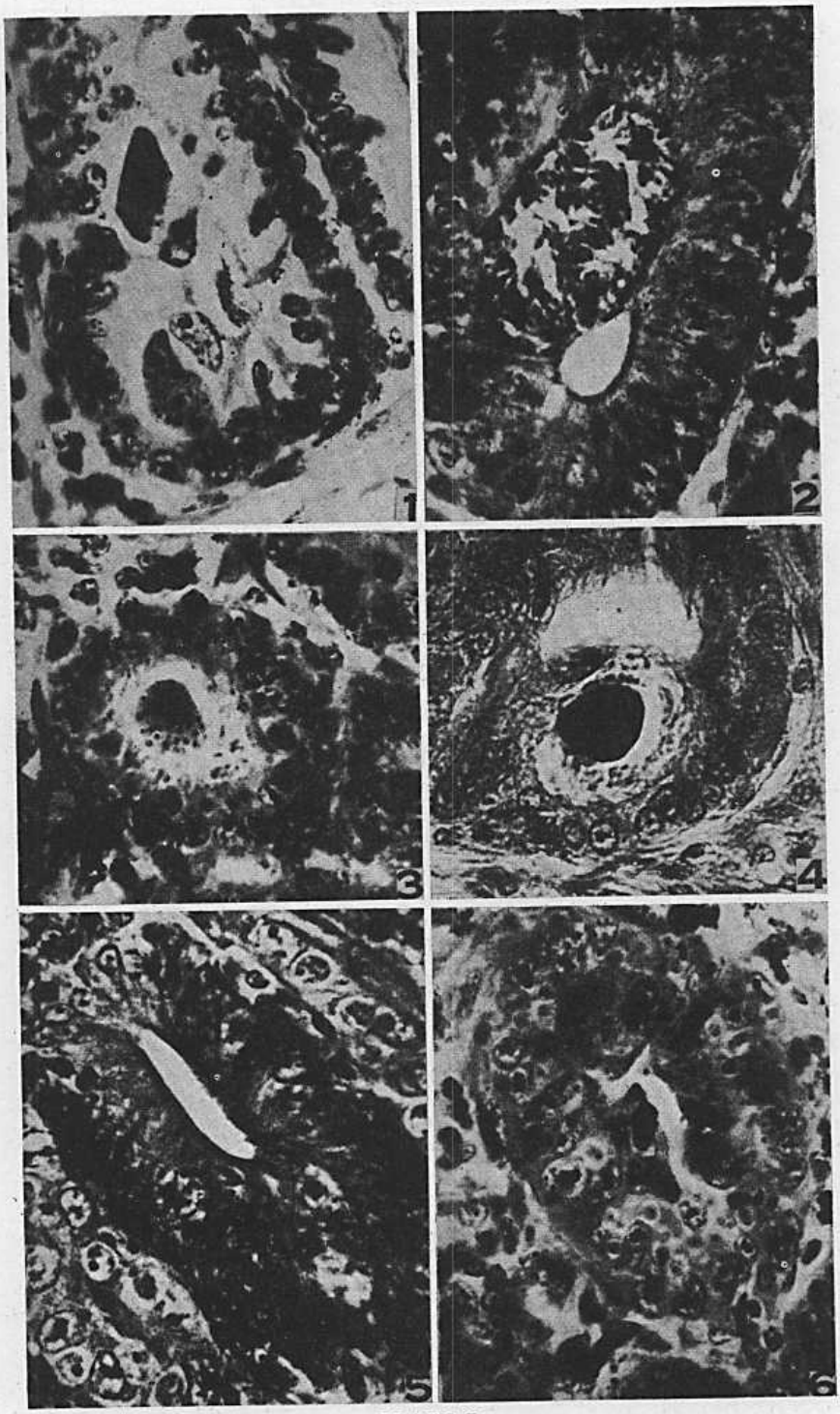

PLATE I

killed at 140 hours after inoculation, that is, masses of degenerated second generation schizonts with faintly stained fragmented contents were seen. However, in this bird a few multinucleated normal-appearing generation 2 schizonts, and an occasional fully developed generation 2 schizont containing normal merozoites, were observed.

Birds killed 144 hours after inoculation.—The deleterious effect of sulfamethazine was clearly demonstrated in the birds which had received medicated mash, or the drug in capsules, from the 96th to the 144th hours after inoculation. In fresh cecal smears of some of these birds masses of what appeared to be immature generation 2 schizonts, and only a few free generation 2 merozoites, were seen. In stained sections of cecal tissues of these same birds the generation 2 schizonts contained pale fragments surrounding one or two clumps of darkly staining material (Plate III, Fig. 13). Scattered throughout many of these clumps were small, dark, rounded objects which may have been remnants of nuclei. In some cases, the pale outlines of degenerating generation 2 merozoites could be discerned in the schizonts. Deep in the fundi of the glands, beneath the nuclei of the epithelial cells, were vacuolated, shrunken remnants of young gametocytes and possibly of generation 3 schizonts.

Other birds of this group showed somewhat different conditions. In fresh cecal smears there were masses of mature generation 2 schizonts, numbers of motile generation 2 merozoites, and many small gametocytes. In stained sections the entire mucosa showed marked destruction, however, in the remnants were seen small and large multinucleated generation 2 schizonts, some normal-appearing mature generation 2 schizonts filled with merozoites, many generation 2 schizonts containing merozoites among which were scattered darkly staining clumps of material, a very few normal-appearing generation 3 schizonts with merozoites, and a few normal gametocytes and an occasional vacuolated gametocyte.

A bird treated from the 96th to the 144th hours after inoculation and examined at 146½ hours after inoculation showed a much different condition than that discussed above. Examination of fresh cecal smears revealed that the generation 2 schizonts contained clumps of hyaline material among the merozoites and many of the latter were shrunken; only a few free and motile merozoites were seen and gametocytes were present but not abundant. In stained sections there were some immature generation 2 schizonts with dividing nuclei, but most of the large generation 2 schizonts contained a central dark body surrounded by fragments. In some of the cecal glands vacuolated remains of the young gametocytes were present, and an occasional normal-appearing young gametocyte was seen in the surface epithelium.

In fresh smears from the inoculated controls, there were found great numbers of mature generation 2 schizonts, many motile generation 2 merozoites, and in the epithelium of the cecal glands masses of young gametocytes. In the stained sections the cytoplasm of the gametocytes was stained dark blue and the nuclei

PLATE II

FIG. 7. Multinucleated generation 2 schizonts in interglandular spaces of inoculated control killed at 96 hours after inoculation. ×400.

FIG. 8. Multinucleated generation 2 schizonts in chicken treated with 1 per cent sulfamethazine mash from 72 to 96 hours after inoculation and killed at 96 hours after inoculation. ×400.

FIG. 9. Developing gametocytes in cecal gland of inoculated control killed at 192 hours after inoculation. A pocket of collapsed oöcysts can be seen to the right of the cecal gland. ×400.

FIG. 10. Section through cecum of chicken treated with sulfamethazine mash from 144 to 192 hours after inoculation and killed at the end of treatment. Developing gametocytes are present in a cecal gland and a pocket of collapsed oöcysts is at the left of the gland. ×400.

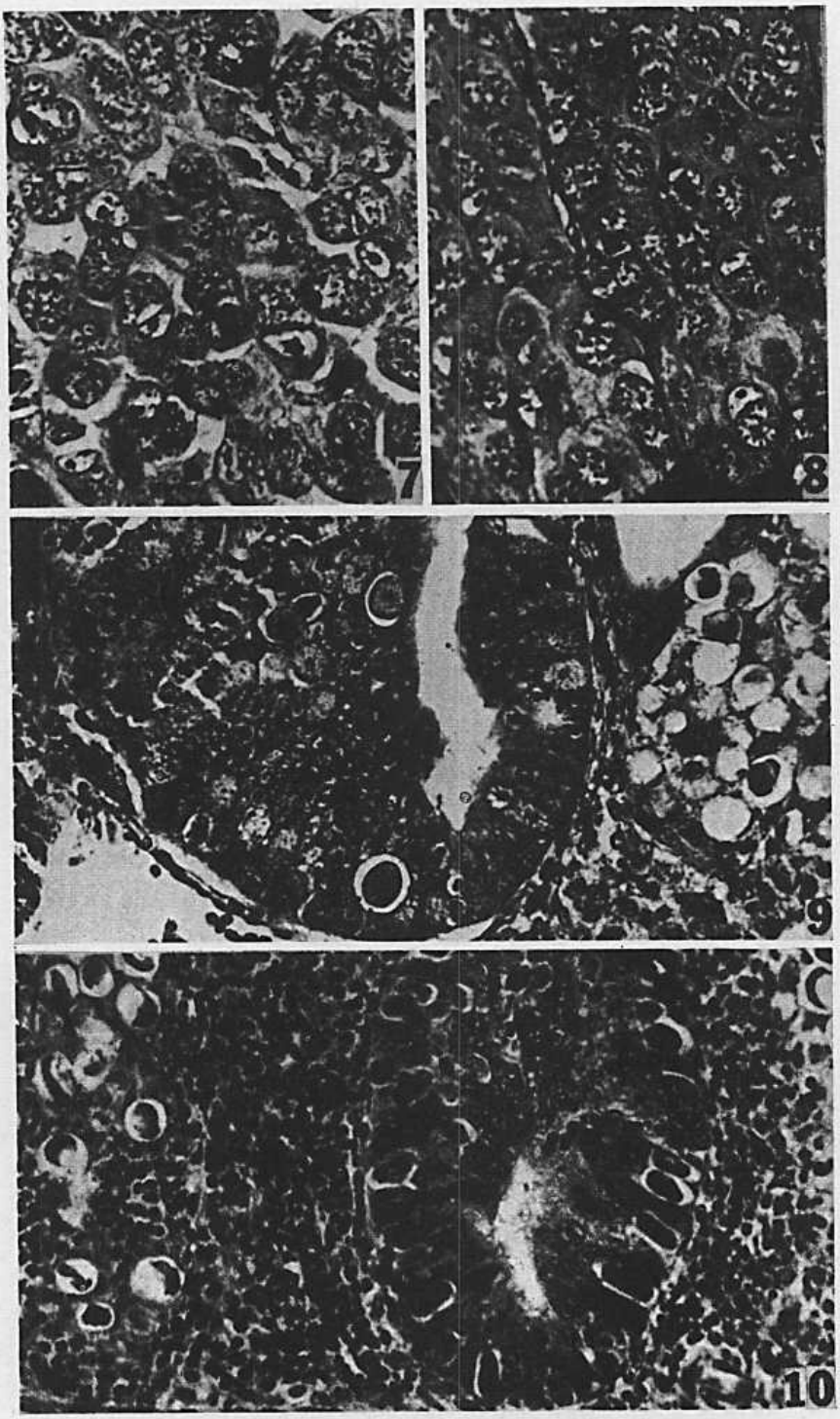

PLATE II

stood out sharply. In the interglandular spaces great numbers of small to large multinucleated generation 2 schizonts as well as mature generation 2 schizonts filled with merozoites (Plate III, Fig. 11) were present. Deep in the tissues were some large generation 2 schizonts which contained a number of irregularly shaped bodies along with normal-appearing merozoites.

In fresh smears from the bird treated with 0.25 gram of sulfamethazine in capsule at 96 and again at 120 hours after inoculation and killed at 144 hours after inoculation there were great numbers of generation 2 schizonts, each containing a large hyaline body and granules. There were also a very few motile merozoites and a number of non-motile merozoites which were either misshapen or filled with granules; no gametocytes were observed. In stained sections there were masses of destroyed generation 2 schizonts (Plate III, Fig. 12) most of which contained only pale fragments. The epithelium in the fundi of some of the glands contained vacuolated, weakly stained young gametocytes and a few generation 3 schizonts containing a dark central clump and fragments.

Birds killed 192 hours after inoculation.—Masses of oöcysts were present in fresh smears from the ceca of birds treated with medicated mash from the 144th to the 192nd hours after inoculation. Stained sections revealed gametocytes in various stages of development (Plate II, Fig. 10), degenerated generation 2 schizonts containing large, dark, central bodies surrounded by pale fragments, a few normal multinucleated generation 2 schizonts, and a few mature generation 2 schizonts with normal merozoites. As in the inoculated control, there were pockets of collapsed oöcysts deep in the tissues (Plate II, Fig. 10). Fresh cecal smears and stained sections from the chicken which received the drug in capsules showed quite similar conditions to those just described.

In fresh smears of the inoculated control bird there were numbers of motile merozoites, an occasional generation 2 schizont filled with merozoites, great numbers of gametocytes in all stages of development, and many mature oöcysts. Stained sections revealed deep in the mucosa groups of darkly stained collapsed oöcysts (Plate II, Fig. 9) which probably had been trapped in the tissue. There were a few large generation 2 schizonts which contained some faintly stained, irregularly shaped bodies along with normal merozoites.

DISCUSSION AND SUMMARY

The foregoing studies have shown that in the presence of sulfamethazine (1) sporozoites were not prevented from penetrating into the epithelium and continuing their development; (2) the generation 1 schizonts were somewhat affected but not completely destroyed; (3) generation 1 merozoites were resistant to the effects of the drug; (4) the greater part of the generation 2 schizonts and their contained merozoites were destroyed; (5) the young gametocytes were either destroyed or damaged but the larger gametocytes were not affected.

Before the present studies were undertaken and completed, it was not clear why certain groups of birds represented in the experiments reported by Wehr and

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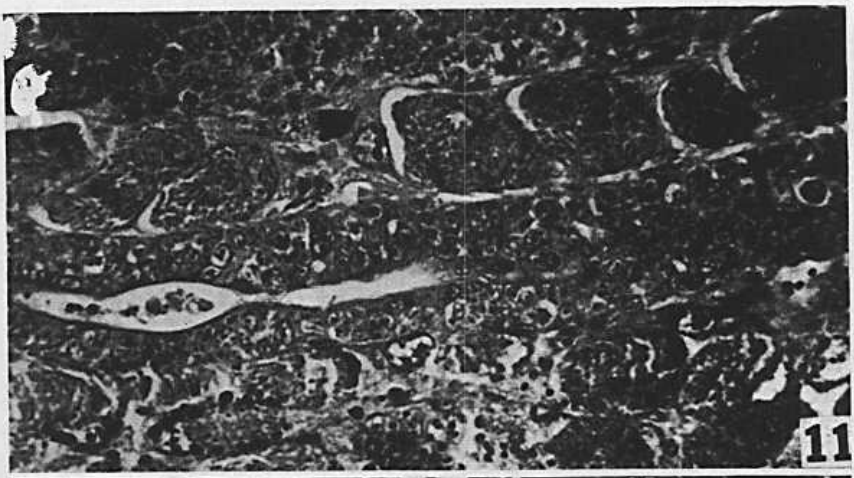
PLATE III

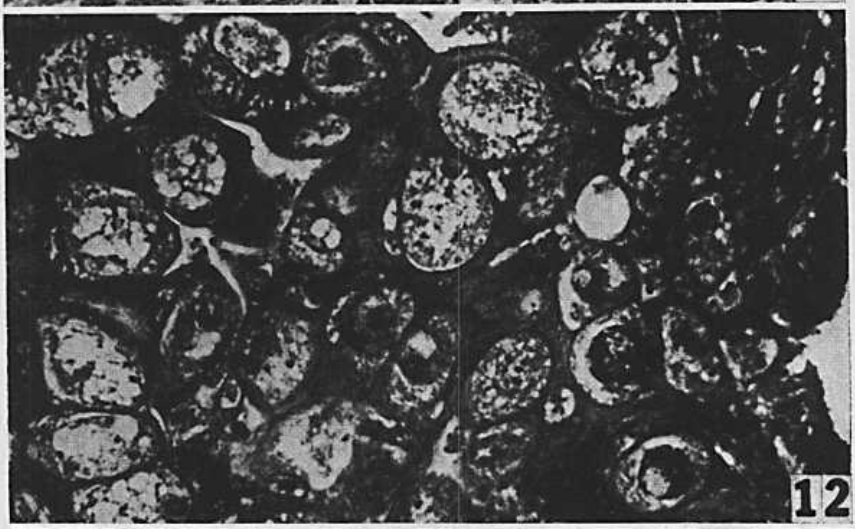
Photomicrographs from cecal tissue of chickens killed at 144 hours after inoculation. × 400.

FIG. 11. Mature generation 2 schizonts in interglandular spaces and developing gametocytes in epithelial cells of cecal gland from inoculated control.

FIG. 12. Degenerated generation 2 schizonts in interglandular spaces of chicken treated with 0.25 gm. sulfamethazine at 96 and at 120 hours after inoculation.

FIG. 13. Degenerated generation 2 schizonts in interglandular spaces and vacuolated young gametocytes in epithelial cells of cecal gland from chicken treated with 1 per cent medicated mash from 96 to 144 hours after inoculation.





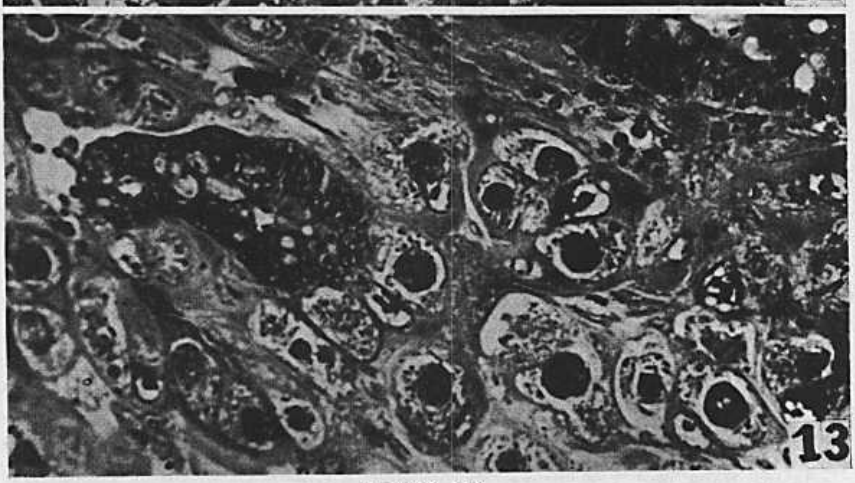


PLATE III

Farr (1947) were benefited clinically more than others. The reason for this is now clear. The survival of all birds and the reduced amount of blood in the droppings of the group treated from 72 to 96 hours after inoculation can now be attributed in a large measure to the high degree of susceptibility of the second generation schizont to the toxic effects of the drug and to the early destruction of these forms. Although the administration of the drug as late as the 96th hour after inoculation destroyed many of the existing second generation schizonts and their contained merozoites, the destruction of these forms did not take place soon enough to prevent hemorrhage and death. The reduction in the number of oöcysts in those groups placed on treatment at the 96th hour after inoculation may also have been partly due to the destruction of some of the very early gametocytes. The drug, however, had little or no effect on the partly or fully grown gametocytes as was evidenced by the large numbers of oöcysts discharged by members of those groups placed on treatment at 144 hours after inoculation.

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The relative susceptibility of 41 sweetpotato varieties, introductions, and seedlings to the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. L. J. KUSHMAN and J. H. MACHMER.[1]

INTRODUCTION

Root-knot nematode injury to sweetpotatoes has been reported on several occasions (Tyler, 1941), but in only a few cases have definite comparisons been made between varieties of sweetpotatoes (Poole and Schmidt, 1929; Weimer and Harter, 1925; Whittle and Drain, 1935) and these comparisons were made in field plot experiments. The results presented here were obtained in the greenhouse at Beltsville, Maryland, under fairly well controlled conditions.

METHODS

Two tests were conducted with the population of root-knot nematode found to occur naturally on sweetpotato plants in the greenhouse benches employed. In the first test 4 replications of 16 varieties were planted, one randomized replication in each of 4 different benches. Each replication of each variety consisted of 6 rows of 12 plants each planted across a 4-foot-wide bench. For examination, one row of each variety in each replication was taken up at monthly intervals. The

[1] Assistant Physiologist, Division of Fruit and Vegetable Crops and Diseases, and Junior Nematologist, Division of Nematology, respectively, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

soil was removed from the roots by dipping in a beaker of water. Then the roots were examined for egg masses in a beaker of clear water. As occasion demanded, roots were examined under a low-power binocular microscope.

The plants were divided into 4 classes depending on the number of egg masses observed—no infection, slightly infected, moderately infected, and heavily infected. Nancy Hall and Red Brazil were used as the standard of heavy infection each month, because they ultimately became thoroughly infected, and all other varieties were scored accordingly. This largely eliminated the factor of absolute amount of infection and limited the classification to the relative degree shown by the several varieties.

Since some plants died of causes other than nematode injury and since rows 5 and 6 were taken up together, varying numbers of plants made up the samples. The degree of infection of each plant was scored as follows:

No infection	0 points per plant
Slight infection	1 " " "
Moderate infection	3 " " "
Heavy infection	5 " " "

The sum of the plant scores for each sample was then calculated as a percentage of a "possible" score with all plants heavily infected, by the formula:

$$\frac{\text{Sum of scores}}{\text{Number of plants} \times 5} \times 100 = \text{Infection Index}$$

The infection indices were analyzed by the variance method (Snedecor, 1934). Results are shown in table 1. Highly significant varietal differences in degree of infection exist and the varieties seem to fall into 3 categories with all but 2 varieties, Big Stem Jersey and 021745, falling into moderately or heavily infected groups.

TABLE 1.—*Relative nematode infection of 16 varieties of sweetpotatoes in greenhouse test 1, conducted August to December, 1942*

Variety	Number of plants showing various degrees of infection				Total number of plants	Average infection index[a]
	None	Slight	Medium	Heavy		
Big Stem Jersey	18	212	23	.....	253	22.7
021745	3	89	124	18	234	43.6
Porto Rico (Old)	.....	52	172	22	246	55.1
Porto Rico (Unit I)	.....	28	145	62	235	62.3
Mameyita	.....	25	152	66	243	63.6
Triumph	.....	20	186	49	255	64.5
Florida (White)	.....	17	76	150	243	82.2
029878	.....	2	38	210	250	91.9
B–33	.....	1	38	206	245	92.8
Director	.....	1	27	208	236	94.8
47442	.....	.....	20	234	254	95.6
Myer's Early	.....	.....	14	206	220	97.6
Red Bermuda	.....	.....	6	240	246	98.8
Red Brazil	.....	.....	5	228	233	98.9
85985	.....	.....	5	228	233	98.9
Nancy Hall	.....	.....	2	229	231	99.7

[a] Difference required for significance at 1 per cent level, 17.2; at 5 per cent level, 13.2.

In the second test only a few plants and 2 replications were used for the 28 varieties tested. An attempt was made to include several varieties related to Big

Stem Jersey in order to see whether they also would show little infection. Examinations were made the second and third months from planting. The average infection index and number of plants examined are given in table 2.

TABLE 2.—*Relative nematode infection of 28 varieties of sweetpotatoes in greenhouse test 2, conducted May to July, 1943*

Variety	Number of plants examined	Average infection index
Yellow Jersey	60	11.3
Southern Queen	30	12.0
85986	13	13.8
Vineland Bush	76	13.9
Big Stem Jersey	44	14.1
Orange Little Stem	59	14.2
Wenholz II	30	15.3
Wenholz I	15	16.0
Red Jersey	40	16.5
B–204	51	16.9
22437	23	17.4
Yellow Strasburg	23	19.1
Norton	33	30.9
Kansas 40	64	34.1
47443	47	37.4
Porto Blanco	33	38.8
B–219	26	44.6
029881	30	52.7
Purple Stem Triumph	30	59.3
Yellow Spanish	33	85.5
Nancy Gold	21	96.2
129651	17	97.6
Pierson	53	98.5
Red Brazil	49	99.2
L–5	14	100.0
Nancy Hall	14	100.0
Vivrovsky	22	100.0
Wennop	38	100.0

The Jersey types as a group were the most consistently resistant varieties studied, while Porto Rico and its selections and mutants (Unit I, Mameyita, Porto Blanco, Kansas 40) were intermediate between the Jerseys and the highly susceptible Nancy Hall–Red Brazil group.

At no time were mature roots available, but observations made on some small potatoes that developed showed damage comparable to the degree of infection of the root system as a whole. It is interesting also that seedlings and introductions gave readings ranging from highly susceptible to slightly susceptible.

Apparently reliable comparisons of nematode susceptibility can be made under greenhouse conditions. The readings given here for standard varieties agree for the most part with those already reported, except in the case of Southern Queen which has been variously reported as both slightly and heavily infected. In additional several varieties have been tested for the first time. Such greenhouse tests may be of special importance in determining suitability of new varieties for release to commercial channels in view of the desirability of keeping the root-knot-nematode population at a minimum in rotations employing sweetpotato in alternation with other crops easily damaged by this nematode, as well as because of occasional severe damage incurred by susceptible sweetpotato varieties.

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Preliminary tests to determine the nematocidal and fungicidal properties of certain chemical compounds when used as soil fumigants. JESSE R. CHRISTIE, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Md.

In a previous paper (Christie, 1945, Proc. Helminth. Soc. Wash. 12(1): 14–19), the writer reported the results of some preliminary tests to determine the efficacy of certain chemicals when used as soil fumigants for control of the root-knot nematode, *Heterodera marioni* (Cornu). These tests were conducted in the open field and the distance from the point of injection to which a given amount of the chemical proved effective in killing the root-knot nematode was used as a basis for evaluating efficacy.

The tests reported in this paper were conducted in closed containers (fumigating boxes) filled with sand; otherwise the procedure was essentially the same as that used in the earlier experiments. However, the present tests have been enlarged to indicate fungicidal as well as nematocidal properties, the aster-wilt fungus, *Fusarium oxysporum* f. *callistephi* (Beach) Synder and Hansen, being used as a test organism. Lists of the materials tested are given in tables 2 and 3. Most of the chemicals now in use as soil fumigants are included together with several that hitherto have not been tested for this purpose.[1]

Calcium phosphide is a solid that, on contact with water, gives off a gas (phosphine) and it was tested by placing a weighed amount in the moist sand at the point of "injection." Cyanogen bromide is a solid and was tested as a 20 per cent solution, benzene serving as the solvent. All the other materials are liquids. Soil fumigants known by trade names are listed in table 1. D–D is a mixture said to contain about equal parts 1,3-dichloropropene[2] and 1,2-dichloropropane with small quantities of other chlorinated compounds and impurities. Presumably Dowfume N is of similar composition. Dowfume G is a mixture of which the principal active ingredient constitutes about 10 per cent by volume while Iscobrome No. 1 and Dowfume W-15 are mixtures of which the principal active ingredient constitutes about 15 per cent by volume. The other liquids were chemical compounds in a more or less pure form and were tested without diluting.

PROCEDURE

Inoculum of the root-knot nematode was prepared by growing susceptible plants in the greenhouse in pots containing infested soil. The roots of these plants were passed through a food chopper and then mixed with a small quantity of soil taken from the pots in which the plants had been grown. Inoculum of the aster-

[1] The writer thanks the following companies who have cooperated by furnishing materials for testing: Dow Chemical Company; Innis, Speiden and Company; Koppers Company, Inc.; Monsanto Chemical Company; Pennsylvania Salt Company; Shell Chemical Corporation; and Westvaco Chlorine Products Corporation.

[2] Propene and propylene are synonyms, propene being the name approved by the International Union of Chemistry.

wilt *Fusarium* was prepared by growing the fungus in flasks on autoclaved oats. Immediately before using, the infested oats were thoroughly mixed with a small quantity of sand. Portions of these two kinds of inoculum, each portion being about 35 to 40 cc. in volume, were enclosed in cheesecloth. For convenience these portions of inoculum will be referred to as inoculum units.

The fumigating boxes were of the type shown in figure 1, A, each 4″ × 4″ × 48″, inside measurements. The boxes were painted on the inside with several coats of asphalt varnish and on the outside with ordinary paint and were provided with tight-fitting covers attached with screws. They were filled with moderately fine bank sand. The point of injection was equidistant from the ends of the box. One unit of the nematode inoculum and one of the fungus were placed, side by side in the sand, to the right of, and 3 inches from the point of injection, a second pair 6 inches from the point of injection, etc., at 3-inch intervals, the eighth and last pair being 24 inches from the point of injection and against the end of the box. An equal number of inoculum units was placed in corresponding positions to the left of the point of injection. Intervals from the point of injection indicate dis-

TABLE 1.—*Soil fumigants discussed in this paper that are known by trade names*

Trade name	Principal active ingredient	Diluent(s)	Manufacturer
Larvacide	Chloropicrin	None	Innis, Speiden & Co.
D–D	Dichloropropene	See text	Shell Chemical Corp.
Dowfume N	do	See text	Dow Chemical Co.
Dowfume G	Methyl bromide	Carbon tetrachloride & ethylene dichloride	do
Iscobrome No. 1	do	Xylene	Innis, Speiden & Co.
Dowfume W–15	Ethylene dibromide	Naphtha	Dow Chemical Co.
Iscobrome C	Ethylene chlorobromide	None	Innis, Speiden & Co.

tances, not to the center of each unit, but to the side farthest away. The box was then closed and the fumigant injected into the sand through a hole in the cover. This hole was then closed with a cork through which extended a thermometer.

After one week the box was opened and the inoculum units removed for testing to determine the effect of the fumigant on the organisms in question. Each unit of nematode inoculum was removed from the enclosing cheesecloth and placed near the center of a 5-inch pot filled with autoclaved soil. Squash seeds were placed in each pot and the plants allowed to grow for at least 4 weeks, after which they were removed and their roots examined. Each unit of fungus inoculum was removed from the cheesecloth, placed in a beaker, and a small quantity of water added. After this mixture had been thoroughly stirred the roots of small aster plants (a wilt-susceptible variety) were dipped in the resulting suspension and potted in 4-inch pots filled with autoclaved soil. Five aster plants were exposed in this manner to each inoculum unit.

The procedure is intended primarily as an elimination or "screening" test that can be conducted in the laboratory and greenhouse at any time of the year and by which chemicals having little promise as soil fumigants can be separated from those that appear to merit further trial. Confinement of the fumes is complete or nearly so and, by using sand, adsorption and other factors that impede diffusion should be less than with most soils, hence, results reflect diffusibility and toxicity when the chemicals are acting under favorable conditions. A material may look promising in a test of this kind yet fail in the open field, but the reverse is

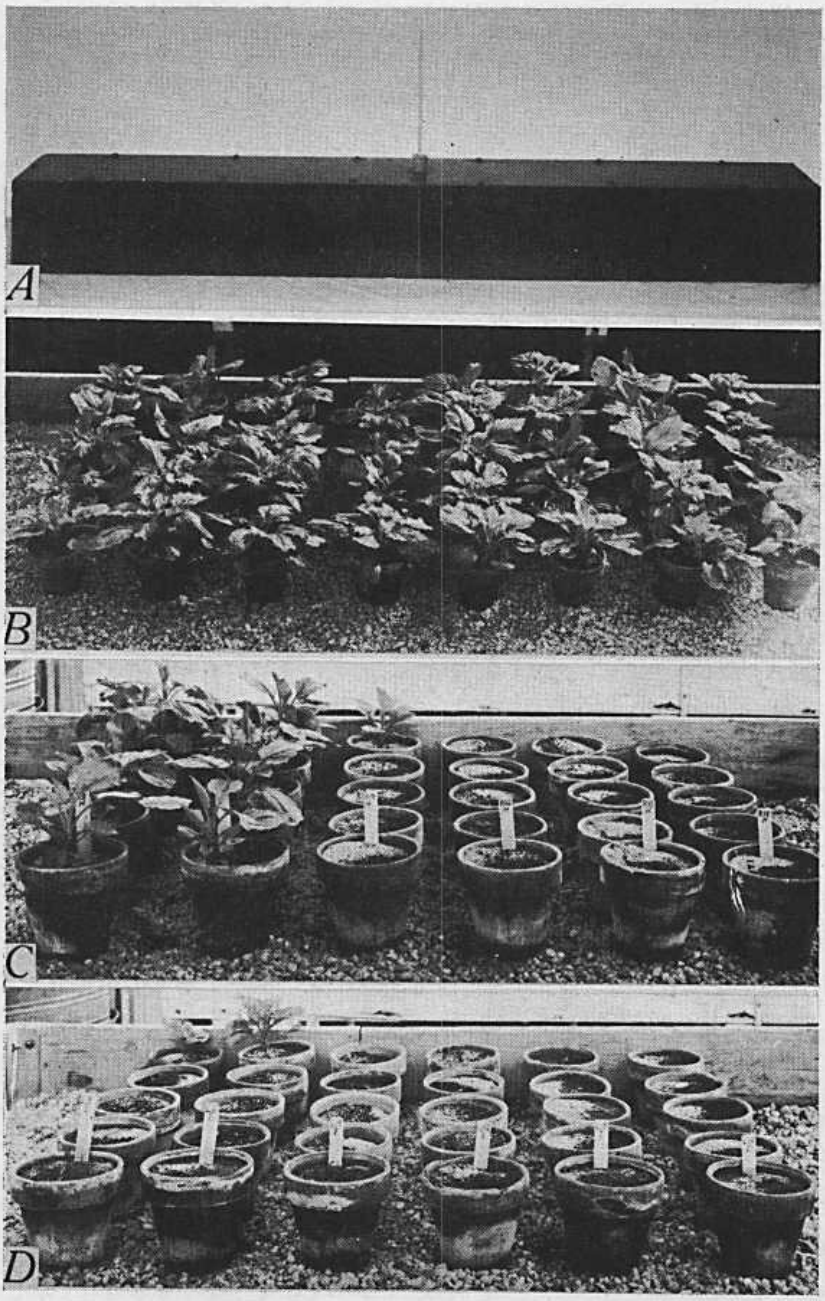

FIG. 1. A—One of the fumigating boxes. B–D—Effect on aster plants produced by inoculum units of *Fusarium oxysporum* f. *callistephi* that previously had been placed in a fumigating box at regular intervals from the point where 2 cc. of the fumigant were injected. The five plants comprising the row at the left were exposed to an inoculum unit that had been placed 3 inches from the point of injection, the five plants comprising the second row from the left were exposed to an inoculum unit that had been placed 6 inches from the point of injection, etc., the interval in each case being increased by 3 inches. B—Results from test with chloropierin. C—Results from test with Dowfume N. D—Results from test with Dowfume W–15.

TABLE 2.—*Comparative nematocidal properties of certain substances when used as soil fumigants, comparisons based on the distance from the point of injection to which a given amount will kill the root-knot nematode in a fumigating box filled with sand. The amount was 2 cc. in all cases unless otherwise stated*

Material	Sand		Degree of galling on squash plants (see text for explanation of rating)							
	Water by weight	Temp.	3″	6″	9″	12″	15″	18″	21″	24″
	%	°F.								
Allyl bromide	6.5	72–74	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
do (1 cc.)	7.4	73–74	0	0	0	0	0	0	2	3
			0	0	0	0	0	1	2	3
Calcium phosphide	4.7	72–75	3	3	3	3	4	3	3	3
			3	3	3	3	3	3	3	3
Carbon disulfide (6 cc.)	7	74–77	0	0	0	0	0	1	0	0
			0	0	0	0	0	1	1	0
Chloropicrin	5	72–76	0	0	0	1	3	4	4	4
			0	0	0	0	3	3	4	4
do (1 cc.)	7.4	73–74	0	0	1	2	4	4	4	4
			0	0	1	1	2	4	4	4
Cyanogen bromide (20% solution in benzene)	4.7	72–76	0	0	3	3	3	3	3	3
			0	0	4	3	3	3	3	2
"D–D"	7.5	72–75	0	0	0	0	0	0	0	2
			0	0	0	0	0	0	0	1
Dichloroisopropyl ether	6.2	74–76	4	4	4	4	4	.....	.....	.....
			4	4	4	4	4	.....	.....	.....
Dichloroethyl ether	6.2	74–76	2	4	4	4	4	.....	.....	.....
			2	4	4	4	4	.....	.....	.....
"Dowfume G"	8.7	70–72	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	1
"Dowfume N"	7.5	72–76	0	0	0	0	0	0	1	2
			0	0	0	0	0	0	1	3
"Dowfume W–15"	7.5	72–76	0	0	1	1	1	0	1	3
			0	0	1	1	0	2	1	2
Ethylene chlorobromide	5.7	72–75	0	0	0	1	0	0	0	1
			0	0	0	0	0	0	0	0
"Iscobrome No. 1"	8.7	70–72	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	1
Monochlorothiophene	5.7	72–75	0	4	4	4	4	4	4	3
			1	4	4	4	4	4	4	3
Pentachloroethane	6	78–80	4	4	4	4	3	4	3	4
			4	4	4	4	4	3	3	4
Polychlorobenzene	5.7	72–75	2	4	4	4	4	4	4	4
			2	4	4	4	4	4	4	4
Tetrachloroethane	7	74–77	4	4	4	4	4	4	.....	.....
			4	4	4	4	4	4	.....	.....

TABLE 3.—*Comparative fungicidal properties of certain substances when used as soil fumigants, comparisons based on the distance from the point of injection to which a given amount will kill the aster-wilt Fusarium in a fumigating box filled with sand. The amount was 2 cc. in all cases unless otherwise stated*

Material	Sand		Number of aster plants that died out of a total of 5 exposed to each inoculum unit							
	Water by weight	Temp.	3″	6″	9″	12″	15″	18″	21″	24″
	%	°F.								
Allyl bromide	6.5	72–74	0	0	0	0	0	0	.....	.....
			0	0	0	0	0	0	.....	.....
do (1 cc.)	7.4	73–74	0	0	0	0	0	0	0	0
			0	1[a]	0	0	0	0	0	0
Calcium phosphide (2 g.)	4.7	72–76	5	5	5	5	5	5	.....	.....
			5	5	5	5	5	5	.....	.....
Carbon disulfide (6 cc.)	7	74–77	0	2	1	4	3	4	5	5
			0	1	4	2	3	5	4	5
Chloropicrin	6	75–76	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
do (1 cc.)	7.4	73–74	0	0	0	0	0	0	0	0
			0	0	0	0	0	1[a]	0	0
Cyanogen bromide (20% solution in benzene)	4.7	72–76	5	5	5	5	5	5	.....	.....
			5	5	5	5	5	5	.....	.....
"D–D"	7.5	72–75	0	1	4	5	5	5	5	5
			0	2	4	5	5	5	5	5
Dichloroisopropyl ether	6.2	74–76	5	5	5	5	5	5	.....	.....
			5	5	5	5	5	5	.....	.....
Dichloroethyl ether	6.2	74–76	5	5	5	5	5	5	.....	.....
			5	5	5	5	5	5	.....	.....
"Dowfume G"	8.7	70–72	5	5	5	5	5	5	.....	.....
			5	5	5	5	5	5	.....	.....
"Dowfume N"	7.5	72–76	0	0	4	5	5	5	.....	.....
			1	2	4	5	5	5	.....	.....
"Dowfume W–15"	7.5	72–76	5	5	5	5	5	5	.....	.....
			4	4	5	5	5	5	.....	.....
Ethylene chloro-bromide	5.7	72–75	4	5	4	5	5	5	5	5
			4	5	4	5	5	5	5	5
"Iscobrome No. 1"	8.7	70–72	5	5	5	5	5	5	.....	.....
			5	5	5	5	5	5	.....	.....
Monochlorothiophene	5.7	72–75	5	5	5	5	5	5	5	5
			5	5	5	5	5	5	5	5
Pentachloroethane	6	78–80	3	5	5	5	5	5	5	5
			1	5	5	5	5	5	5	4
Polychlorobenzene	5.7	72–75	5	5	5	5	5	5	5	5
			5	5	5	5	5	5	5	5
Tetrachloroethane	7	74–77	2	5	5	5	5	5	5	5
			1	5	5	5	5	5	5	5

[a] Death of plant probably due to some cause other than action of fungus.

not likely to happen. Fumigants now in general use, the efficacies of which are already fairly well known, are included for comparison.

RESULTS

A list of the materials tested and the results with each are given in tables 2 and 3. In table 2 the effects of the fumigants on root-knot-nematode inoculum placed at regular intervals from the point of injection is expressed in terms of the amount of galling each inoculum unit subsequently produced on the roots of squash plants as follows: 0, none; 1, trace; 2, slight; 3, moderate; 4, severe. In table 3 the effects of the fumigants on inoculum of the aster-wilt *Fusarium* is expressed in terms of the number of aster plants that died out of a total of 5 that was exposed to each inoculum unit.

Typical results from these tests to determine fungicidal action are shown in figure 1.

Based primarily on killing range as a criterion, the most pronounced nematocidal action was exhibited by mixtures containing dichloropropene (D–D and Dowfume N), by mixtures containing methyl bromide (Dowfume G and Iscobrome No. 1), by carbon disulfide, by ethylene chlorobromide, and by allyl bromide. Other investigators have already noted that methyl bromide and carbon disulfide are very effective in controlling root knot when soil is treated in containers but that usually they do not maintain quite the same relative efficacy, as compared with some other fumigants, when used in the open field. The mixture containing ethylene dibromide (Dowfume W–15) showed a killing range comparable to mixtures containing dichloropropene but it failed to effect a complete kill throughout this range. The killing range of chloropicrin was less than that of the most effective materials and the killing range of cyanogen bromide was less than that of chloropicrin. None of the other materials appear to have much promise as a soil fumigant for the control of root knot.

With regard to fungicidal properties, chloropicrin and allyl bromide are outstandingly the best of the materials tested while mixtures containing dichloropropene (D–D and Dowfume N), and carbon disulfide are slightly to moderately fungicidal. The tests do not indicate that any of the other materials would be of much value in controlling fungi that are as difficult to kill as the one employed unless the rate of application was far in excess of that at which soil fumigants are usually applied.

A note on the fungoid nature of certain internal structures of Miescher's sacs (*Sarcocystis*) from a naturally infected sheep and a naturally infected duck. L. A. SPINDLER, U. S. Bureau of Animal Industry.

Miescher's sacs, the muscle stage of *Sarcocystis*, have been determined by some investigators to be individual parasites, each containing a series of "septa" which divide the bulk of the sac into "chambers." The "chambers" nearest the periphery of the sac have been considered to be the most recently formed, because they generally are smaller than those nearest the center, and often contain variable numbers of spherical cells which are thought to be an early stage in the development of the spores, called Rainey's corpuscles. The latter are the crescent-shaped bodies, commonly referred to as spores, that fill the larger "chambers" or compartments of Miescher's sacs.

Different opinions have been expressed regarding the origin of the "septa." Some investigators have considered that these structures arise from the host tissues and are probably of connective tissue origin. Other investigators have considered that the "septa" are derived from the parasite. In the past, little

attention has been paid, however, to the morphology and staining characteristics of these structures.

In a study of the internal structures of Miescher's sacs, histologic sections were prepared from sacs lodged in the muscle of a naturally infected sheep and a naturally infected wild duck. The sections were stained with Gram's stain. The technique utilized was MacCallum's modification of Goodpasture's method as described by Conant, Martin, Smith, Baker and Callaway (Manual of Clinical Mycology, 1945, W. B. Saunders Company, Philadelphia).

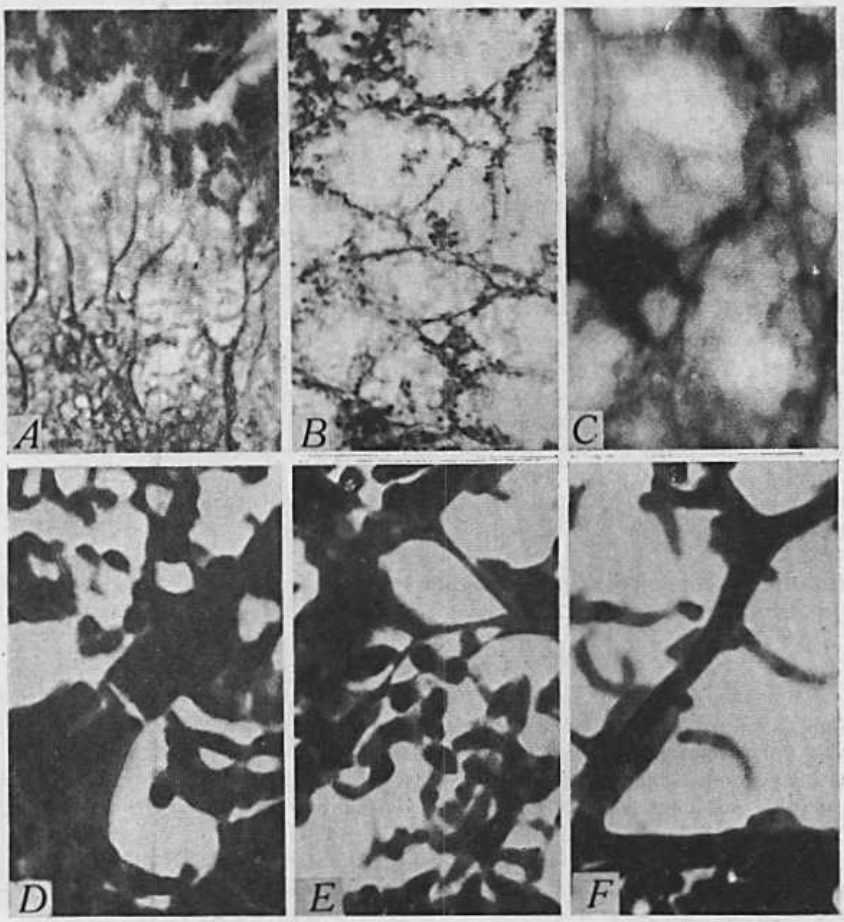

FIG. 1. Enlarged photomicrographs of small segments of histologic sections from Miescher's sacs showing: A, B, and C, arrangement of "septa"; C, D, and F, their jointed structure; and E and F, attachment of the Rainey's corpuscles (spores) to the "septa." Sections stained with Gram's stain. A, B, and C, Miescher's sacs from the flesh of a naturally infected wild duck; D, E, and F, Miescher's sacs from the flesh of a naturally infected sheep.

Studies of the sections revealed that the sacs contain a network of jointed, hypha-like structures (Fig. 1, A, B, C, and D). The structures appear to divide the sac into compartments (Fig. 1, B and C). The widths of the hypha-like structures were seen to vary with (1) the location in the sac, those nearest the center generally being wider than those nearest the periphery, and (2) the size of the sac,

the strands being much more delicate in small sacs than in large ones from the same host. The structures exhibit staining reactions characteristic of fungi. This finding is confirmed by the fact that on numerous occasions a delicate septate mycelium has been found by heating, in 30 per cent potassium hydroxide solution, sacs from the flesh of naturally infected sheep, cattle, and birds, and staining the residue with lacto-phenol-cotton blue solution. This stain is widely used for the demonstration of fungi in tissues.

In the histologic sections studied, the Rainey's corpuscles appeared to be exogenous growths on the jointed hypha-like structures, particularly the more delicate portions of the strands (Fig. 1, E and F). Each Rainey's corpuscle is apparently capable of giving rise from its free end to another corpuscle somewhat in the manner in which conidia are formed in the saprophytic growth of certain fungi. In microscopic examinations of the contents of unpreserved Miescher's sacs from the hosts named, Rainey's corpuscles have from time to time been observed attached to the hypha-like structures, and short chains of corpuscles, arranged like conidia, have occasionally been observed. The attachment of the corpuscles to the succeeding ones and to the hypha-like parent structures is apparently by means of a short pedicle. This pedicle is very weak and generally breaks during the sectioning and during manipulation of the contents of sacs for examination as fresh preparations. For that reason the arrangement of the Rainey's corpuscles was observed in only the most carefully prepared slides.

Invasion of sacs by strands of the host's connective tissues has also been observed. These strands are distinct from the hypha-like structures, however, and Rainey's corpuscles have not been observed attached to them.

The observations herein summarized indicate that Miescher's sacs are not individual parasites but are small mycetoma. This supports the conclusion of Spindler and Zimmerman (1945, Jour. Parasitol. **31** (Dec. Suppl.): 13) that *Sarcocystis*, in reality, belongs to the fungi instead of to the protozoa as hitherto supposed.

Growth rate of pigs fed skim milk to control intestinal parasites. DOYS A. SHORB and L. A. SPINDLER, U. S. Bureau of Animal Industry.

BACKGROUND OF THE INVESTIGATION

Spindler, Zimmerman and Hill (1944, Proc. Helm. Soc. **11**(1): 9–12) reported that pigs deprived of all solid feed and water for periods of 3 to 5 days, and fed only fluid skim milk, expelled during that time all, or nearly all, whipworms and nodular worms, and varying numbers of ascarids. Pigs so fed remained in good condition and continued to gain weight during the time they were fed milk. Spindler and Zimmerman (1944, Proc. Helm. Soc. **11**(2): 49–54) also reported that pigs maintained on soil grossly contaminated with infective stages of the parasites named acquired no, or only small numbers of, helminths when fluid skim milk was fed once daily in place of grain and water, or in place of grain and water for periods of 3 days at intervals of 2 weeks. Control pigs, not fed milk but fed a balanced grain ration, became heavily parasitized when kept with the milk-treated pigs.

During the experiment, the pigs fed milk daily made an average daily gain of 1.17 pounds; those fed milk at intervals of 2 weeks gained 0.87 pound daily and the controls made an average daily gain of 0.32 pound. Consequently, the question arose as to whether the superior weight gains of the pigs fed milk were due to the milk consumed or to freedom from parasitism. An investigation was begun, therefore, in which the weight gains of pigs kept on clean ground and fed fluid skim milk in place of grain, once daily, and for 3 consecutive days at intervals of

2 weeks, were compared with those of pigs fed grain only. The diets utilized, the method of feeding and handling the pigs, and other conditions except exposure to parasites, were entirely comparable with those of the investigation described by Spindler and Zimmerman (*loc. cit.*). The investigation was carried out during the period from May to October, 1945, at the field station of the Zoological Division, Agricultural Research Center, Beltsville, Maryland.

EXPERIMENTAL PROCEDURE

The number of animals involved in the investigation, conditions under which they were maintained, method of feeding, and other pertinent information are briefly summarized below.

Experimental animals.—Six littermate pigs of mixed breeds, weaned at 8 weeks of age and divided at that time into 3 groups on the basis of weight and sex, were involved. The pigs were farrowed and maintained under conditions that precluded acquisition of large numbers of ascarids, nodular worms, and whipworms. All pigs acquired small numbers of *Strongyloides* before weaning. As determined by fecal examinations, these worms disappeared during the first week of the experiment.

Method of feeding.—Each evening the pigs comprising group 1 (Nos. 1491 and 1497) were fed as much fluid skim milk as they would consume before the next morning; grain and water were withheld during that time. Each morning the pigs were given fresh water and fed as much of a well-balanced grain ration as they would consume during the day.

At intervals of 2 weeks the group 2 pigs (Nos. 1494 and 1496) were fed fluid skim milk exclusively for a period of 3 days. The milk was fed night and morning, as much being given as the pigs would consume before the next feeding. All grain feed and water were withheld during the time milk was being fed. At all other times the animals were given water twice daily and fed as much of the grain ration as they would consume.

The group 3 pigs (Nos. 1492 and 1493) were fed twice daily as much of the grain ration as they would consume and were not fed milk at any time; these animals, therefore, served as controls. An adequate supply of fresh water was given the pigs twice daily. In the case of all groups the grain ration was fed on the ground.

Experimental pens.—Each group was maintained in a separate pen. The pens were constructed on an area that had not been used for pigs prior to initiation of this investigation. In order that the pigs of all groups would be exposed equally to conditions existing in the 3 pens, they were moved each week to the adjoining pen; rotation was always in the same direction, so that each group made the circuit of the pens every 3 weeks.

The pigs were weighed twice weekly until they reached a size and weight that made them difficult to handle. Weighings were then discontinued until the animals were removed from experiment, at which time each was given a final weighing.

Rectal samples of feces were collected from each animal at weekly intervals and examined for parasite eggs by the salt-flotation technique.

SUMMARY OF FINDINGS

The investigation was begun May 10, 1945. During the first 11 days of the experiment the pigs were maintained indoors to enable them to become accustomed to their respective diets and to await the beginning of warmer weather before they could be placed outdoors.

As stated previously, examinations for parasite eggs were made on fecal samples taken from each pig at weekly intervals. At no time were parasite eggs,

other than *Strongyloides,* found in the feces of the pigs fed milk. In the case of the pigs fed grain only, small numbers of ascarid eggs were found in the feces of each animal beginning 3 weeks after the pigs had been placed on the experimental plots, or approximately 4 weeks after the beginning of the experiment. The fact that approximately 7 weeks is required for ascarids to reach fertile maturity indicates that the infections in question had been acquired by the pigs prior to initiation of the experiment.

On September 26, 139 days after the beginning of the experiment, one of the group 2 pigs, No. 1494, died suddenly; the cause of death was not definitely determined. Lesions due to ascarids and kidney worms were seen in the liver, but the damage is not considered sufficient to have been responsible for death of the animal. Up to the time of its death this pig appeared to be in good health. During the time on experiment the average of the daily weight gain was 1.11 pounds, or a total gain of 154.75 pounds.

On September 28, 141 days after inception of the experiment and 2 days after death of the group 2 pig, one pig of each group, a male, was removed for slaughter and post-mortem examination. The remaining pigs, which were females, were kept 6 days longer and were then weighed and incorporated in the breeding herd.

On post-mortem examination, liver lesions due to ascarids and kidney worms were found in all the pigs. The control pig (1493) which had been fed grain harbored only 3 mature ascarids and one immature nodular worm. The 3 pigs which had been fed milk during the experiment were free of these worms.

The data pertaining to weight gains of the three groups of pigs are summarized in table 1. As can be seen from the data, daily weight gains of 1.14 and 1.48 pounds, respectively, were made by the group 1 pigs which had been fed milk each day. The group 3 pigs, those fed grain only, made daily weight gains of 1.03 and 1.28 pounds while on experiment. Weight gains made by the group 2 pigs, those fed milk at intervals of 2 weeks, were somewhat less than those of the other two groups, being 1.11 and 1.15 pounds per day, respectively. In each group the greater weight gain was made by the male pig. Differences in weight gains of the 3 male animals were reflected in the quality of the carcasses at slaughter. The pig which had been fed milk daily (No. 1497) and the pig which had been fed grain only (No. 1493) were adjudged to be "choice fat" with a slight advantage being given to the animal which had been fed milk daily. The carcass of the pig which had been fed milk at intervals of 2 weeks (No. 1496) and which had made the smallest weight gains was graded as "low choice" since it was somewhat less well-finished than the other two. The grading was conducted by workers in the Meats Laboratory of the Animal Husbandry Division, Bureau of Animal Industry, Agricultural Research Center, Beltsville, Maryland.

DISCUSSION AND CONCLUSIONS

In the investigation herein reported, feeding fluid skim milk in place of grain either once daily (Group 1 pigs) or for 3 consecutive days at intervals of 2 weeks (Group 2 pigs) was associated with weight gains in the pigs so fed that at best were only slightly greater than those of littermate pigs fed entirely on a balanced grain ration (Group 3). For example, the average daily weight gain of the pigs fed milk daily in place of one grain feeding was 1.31 pounds whereas pigs fed grain only made an average gain of 1.15 pounds per day. The difference, which is 0.16 pound per day in favor of the pigs fed milk, may be attributed to the milk consumed. In the case of pigs fed milk in place of grain for periods of 3 days every 2 weeks, the average daily gain was somewhat less than that of pigs fed grain only, being 1.13 pounds. Weight gains made by the group 3 pigs, those fed entirely on grain, show that the grain ration employed was in itself adequate to produce satisfactory weight gains.

TABLE 1.—*Comparison of the growth rate of three groups of pigs, each group fed a different ration and kept on clean ground, with the growth rate, as reported by Spindler and Zimmerman, of three groups of pigs similarly fed and constantly exposed to natural infections of worm parasites. See text for explanation of rations fed and methods of feeding*

Group and pig designation numbers	Days on test	Pigs kept on clean ground, not exposed to infections of worm parasites								Exposed to infections[a]
		Weight at beginning of test		Weight at end of test		Total gains		Daily gains		Daily gains
		Individual weights	Av. for group	Individual weights	Av. for group	Individual	Av. for group	Individual	Av. for group	Av. for group
	number	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Group 1, fed milk daily	.....	.....	21.50	.....	209.00	.....	187.50	.....	1.31	1.17
Pig 1491, female	147	18.00	.....	185.00	.....	167.00	.....	1.14	.....	.....
Pig 1497, male	141	25.00	.....	233.00	.....	208.00	.....	1.48	.....	.....
Group 2, fed milk every 2 weeks	.....	.....	20.12	.....	178.50	.....	158.37	.....	1.13	0.85
Pig 1494, female	139	20.25	.....	175.00	.....	154.75	.....	1.11	.....	.....
Pig 1496, male	141	20.00	.....	182.00	.....	162.00	.....	1.15	.....	.....
Group 3 (controls), fed grain only	.....	.....	26.12	.....	191.75	.....	165.62	.....	1.15	0.32
Pig 1492, female	147	26.75	.....	177.50	.....	150.75	.....	1.03	.....	.....
Pig 1493, male	141	25.50	.....	206.00	.....	180.50	.....	1.28	.....	.....

[a] Pigs constantly exposed to natural infections of worm parasites. Weights in this column from Spindler and Zimmerman (1944, *loc. cit.*).

In the experiment reported by Spindler and Zimmerman (1944, *loc. cit.*) the grain ration employed was the same as that utilized in the investigation herein reported. In view of this fact, and of the weight gains of pigs involved in the investigation herein reported, it may be concluded that the wide differences reported by Spindler and Zimmerman (see Table 1, this paper) as occurring between weight gains of pigs fed milk and those fed grain only were primarily due to freedom of the milk-fed pigs from parasites and only secondarily to the milk consumed.

A note on the caudal papillae of the male of *Wehrdikmansia cervipedis* (Wehr and Dikmans, 1935) Caballero, 1945. G. DIKMANS, U. S. Bureau of Animal Industry.

The Zoological Division of the U. S. Bureau of Animal Industry recently received from Dr. G. W. Stiles, Denver, Colorado, a number of nematodes recovered from the subcutaneous tissue of a deer killed near Cimarron Reservoir, Gunnison County, Colorado. They were identified as *Wehrdikmansia cervipedis* (Wehr and Dikmans, 1935) Caballero, 1945. This nematode was originally described by Wehr and Dikmans (1935, Zool. Anz. **110**: 202–208) as *Onchocerca cervipedis* and it was reported as having been found under the skin of the hindquarters and feet. In the present instance the worms were reported as occurring both free and in nodules under the skin of the belly, brisket and forelegs. Caballero (1945, Rev. Brasil. Biol. **5**: 557–562) after examining the specimens of *O. cervipedis* Wehr and Dikmans, 1935, decided that these authors had been in error in placing the species in question in the genus *Onchocerca* and created for it a new genus, *Wehrdikmansia*.

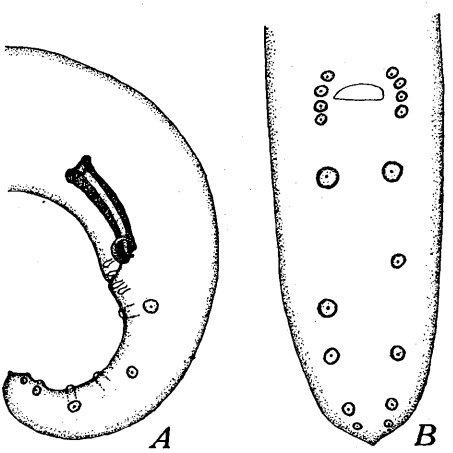

FIG. 1. *Wehrdikmansia cervipedis*, posterior portion of male showing number and arrangement of caudal papillae. A—Lateral view. B—Ventral view (diagrammatic).

In the original description and also in that of Caballero, it is stated that the tail end of the male is provided with 6 pairs of papillae, 4 adanal and 2 near the tip of the tail. The material recently received from Dr. Stiles contained 2 males and an examination of the terminal portions of these males shows that, so far as the number of papillae on the tail end is concerned, both previous descriptions are in error. Instead of 6 papillae on each side of the tail there are 9, 4 adanal and 2 near the tip of the tail as previously reported, and 3 between the adanal papillae and those near the tip of the tail. On the right side these papillae are placed about equidistantly from each other, but on the left side the sixth papilla is closer to the seventh than to the fifth (Fig. 1, B).

Another interesting feature noted on examination of one of the males mentioned above was that it contained only one spicule, namely the right (Fig. 1, A). No trace could be found of the left spicule.

The anthelmintic action of toluenes in dogs. F. D. ENZIE, U. S. Bureau of Animal Industry.

In pursuing anthelmintic investigations with comparatively simple benzene derivatives (Enzie, 1944, this journal **11**: 55–58; 1945, *ibid.* **12**: 19–24), a number of tests were conducted with toluene (methyl benzene) and certain halogen substitution products. Specifically, the compounds studied were toluene (sulfur-free), 12 mono-substituted halogen derivatives, namely, the ortho-, meta-, and para-isomers of chloro-, bromo-, iodo-, and fluoro-toluene, and 2 di-substituted products, namely, 2,6-dichloro- and 2,5-dibromo-toluene.

In so far as could be determined, the only reference pertaining to the anthelmintic action of toluene and immediate derivatives thereof is that of Hall and Wigdor (1926, Jour. Amer. Vet. Med. Assoc. **69**: 195–217). According to these writers, toluene in dosages ranging from 0.1 to 0.4 cc. per kilogram of body weight removed only 6 per cent (9) of 132 ascarids from 3 dogs and none of 42 whipworms from 1 dog. One dog died within 24 hours after treatment.

The tests reported herein were conducted at the station of the Zoological Division, Agricultural Research Center, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland. From 1 to 2 days before treatment, the dogs were confined in individual cages and the feces screened daily in order to detect spontaneous elimination of parasites. The kinds of parasites in each animal were determined by fecal examination prior to treatment. The drugs were given in hard gelatin capsules after a fast of 18 to 24 hours; and 3 to 4 hours after treatment the dogs were returned to regular feed. The feces of each dog were collected individually every 24 hours and examined for parasites. After the elimination of parasites ceased, usually within 3 or 4 days, the dogs were autopsied and the entire gastrointestinal tract examined for parasites and lesions. Representative tissue sections were preserved in the event studies of histopathology become necessary.

RESULTS

The results obtained are shown in tables 1 to 4. In preliminary tests with the compounds (Table 1), the drugs were given at a dose rate of 0.2 cc. per pound of body weight. Six of the compounds, namely, toluene, o-chlorotoluene, p-bromotoluene, and the o-, m-, and p-fluorotoluenes, were completely effective against both ascarids and hookworms. Moreover, two additional compounds, o-bromotoluene and m-iodotoluene, were wholly effective against ascarids, and 2,6-dichlorotoluene exhibited comparable action against hookworms. In the latter instance, the test animal did not harbor large roundworms. The efficacy of several of the compounds against whipworms was also rather marked, but this parasite is not suitable for comparative anthelmintic studies because trichuricidal action is contingent upon the fortuitous entrance of the drug into the caecum. It is apparent that at this dosage the over-all efficacy of the compounds against ascarids and hookworms was too high to permit an interpretation of the comparative efficacies of the individual drugs. In subsequent tests, therefore, the drugs were given at a lower dose rate in order to reveal a greater variation in the anthelmintic action of the compounds.

When the drugs were given at a dose rate of 0.1 cc. per pound of body weight (Table 2), the individual efficacies against ascarids and hookworms diminished sufficiently in most instances to permit reasonable comparisons among them.

TABLE 1.—*Data on the efficacy of some toluene compounds administered to dogs at the rate of 0.2 cc. per pound of body weight*

Dog No.	Drug	Parasites	Removed	Left	Efficacy (per cent)	Remarks
260	Toluene	Ascarids	25[a]	0	100	Ineffective against
		Hookworms	11	0	100	2 *Taenia;* mucus in
261		Whipworms	122	20	85	feces of 1 dog.
		Ascarids	66	0	100	2 ascarids, 3 hook-
262	o-Chlorotoluene	Hookworms	38	0	100	worms, and 1 whip-
		Whipworms	183	17	91	worm recovered before treatment.
		Ascarids	23	2	92	Mucus in feces.
263	m-Chlorotoluene	Hookworms	38	2	95	
		Whipworms	130	52	71	
		Ascarids	12	10	54	Mucus in feces.
264	p-Chlorotoluene	Hookworms	51	10	83	
		Whipworms	166	2	98	
		Ascarids	17	0	100	
265	o-Bromotoluene	Hookworms	141	5	96	
		Whipworms	374	5	98	
		Ascarids	8	2	80	
266	m-Bromotoluene	Hookworms	37	1	97	
		Whipworms	95	56	62	
		Ascarids	4	0	100	
267	p-Bromotoluene	Hookworms	12	0	100	
		Whipworms	31	0	100	
		Ascarids	.....	.....	.....	
268	o-Iodotoluene	Hookworms	112	1	99	
		Whipworms	13	35	27	
		Ascarids	1	0	100	Portion of feces
269	m-Iodotoluene	Hookworms	26	67	27	soft.
		Whipworms	12	157	7	
		Ascarids	8	2	80	2 ascarids recov-
270	p-Iodotoluene	Hookworms	40	49	44	ered before treat-
		Whipworms	62	2	96	ment.
		Ascarids	1	0	100	Small amount of
271	o-Fluorotoluene	Hookworms	76	0	100	mucus in feces.
		Whipworms	68	0	100	
		Ascarids	7	0	100	
272	m-Fluorotoluene	Hookworms	176	0	100	
		Whipworms	65	0	100	
		Ascarids	6	0	100	Portion of feces
273	p-Fluorotoluene	Hookworms	192	0	100	quite soft.
		Whipworms	210	0	100	
		Ascarids	.....	.....	.....	
274	2,6-Dichloro-	Hookworms	76	0	100	
	toluene	Whipworms	49	1	98	
		Ascarids	.....	.....	.....	Ineffective against
275	2,5-Dibromo-	Hookworms	46	72	38	1 *Taenia.*
	toluene	Whipworms	.....	.....	.....	

[a] One dog did not harbor ascarids.

Toluene removed 100 per cent of 61 ascarids from 2 dogs, 99 per cent (349) of 351 hookworms from 4 dogs, and 75 per cent (121) of 161 whipworms from 6 dogs. In 1 dog, hookworm eggs were found on fecal examination before treatment, but no worms were recovered after treatment and none were found at autopsy. The drug was ineffective against 10 *Taenia* and 3 *Dipylidium* in 1 dog.

TABLE 2.—*Data on the efficacy of some toluene compounds administered to dogs at the rate of 0.1 cc. per pound of body weight*

No. of dogs	Drug	Parasites	Removed	Left	Efficacy (per cent)	No. of tests	Remarks
6	Toluene	Ascarids	61	0	100	2	Ineffective against 10 *Taenia* and 3 *Dipylidium* in 1 dog.
		Hookworms	349	2	99	4	
		Whipworms	121	40	75	6	
5	o-Chlorotoluene	Ascarids	66	0	100	2	Ineffective against 1 *Taenia*.
		Hookworms	484	15	96	4	
		Whipworms	166	13	92	4	
5	m-Chlorotoluene	Ascarids	153	0	100	2	1 ascarid and 2 hookworms recovered before treatment.
		Hookworms	346	57	85	5	
		Whipworms	100	34	74	4	
5	p-Chlorotoluene	Ascarids	124	31	80	2	Mucus in feces of 1 dog; 1 ascarid and 3 hookworms recovered before treatment.
		Hookworms	442	138	65	5	
		Whipworms	75	61	55	5	
4	o-Bromotoluene	Ascarids	48	2	96	2	8 hookworms recovered before treatment.
		Hookworms	1595	109	93	4	
		Whipworms	121	98	55	4	
5	m-Bromotoluene	Ascarids	71	6	92	2	
		Hookworms	381	394	49	4	
		Whipworms	34	154	18	5	
4	p-Bromotoluene	Ascarids	24	4	85	2	1 hookworm recovered before treatment.
		Hookworms	1033	385	72	3	
		Whipworms	15	94	13	4	
3	o-Iodotoluene	Ascarids	21	14	60	1	
		Hookworms	123	576	17	3	
		Whipworms	112	51	68	3	

TABLE 2.—(*Continued*)

No. of dogs	Drug	Parasites	Removed	Left	Efficacy (per cent)	No. of tests	Remarks
3	m-Iodotoluene	Ascarids	9	10	47	1	One dog vomited.
		Hookworms	235	128	64	3	
		Whipworms	37	26	58	2	
4	p-Iodotoluene	Ascarids	82	19	81	2	1 ascarid and 4 hookworms recovered before treatment.
		Hookworms	1094	613	64	4	
		Whipworms	316	84	79	4	
3	o-Fluorotoluene	Ascarids	10	4	71	2	One dog vomited; 8 hookworms recovered before treatment.
		Hookworms	700	1031	40	2	
		Whipworms	149	145	50	3	
4	m-Fluorotoluene	Ascarids	45	0	100	2	2 hookworms recovered before treatment.
		Hookworms	728	245	74	3	
		Whipworms	43	131	24	4	
5	p-Fluorotoluene	Ascarids	17	1	94	2	One dog vomited; all hookworms at autopsy were immature and were in 1 dog.
		Hookworms	96	9	91	4	
		Whipworms	67	0	100	4	
5	2,6-Dichlorotoluene	Ascarids	6	4	60	2	Mucus in feces of 1 dog; 1 whipworm recovered before treatment.
		Hookworms	264	1811	12	5	
		Whipworms	42	190	18	5	
3	2,5-Dibromotoluene	Ascarids	2	14	12	1	Ineffective against 3 *Taenia* in 1 dog; 1 ascarid recovered before treatment.
		Hookworms	242	658	26	3	
		Whipworms	16	192	7	3	

The efficacies of the substitution products were too variable to permit extensive generalizations. This is clear from the data in table 2 and the information in figure 1. However, three of the compounds, namely, o- and m-chlorotoluene, and m-fluorotoluene, were equally effective against ascarids, and o- and m-bromotoluene, and p-fluorotoluene removed more than 90 per cent of these worms. The variation in efficacy of the several compounds against hookworms was even more distinct. None of the substitution products was as effective as toluene, and only three, namely, o-chlorotoluene, o-bromotoluene, and p-fluorotoluene, removed 90 per cent or more of these worms. The discrepancies in the efficacy of some of the compounds at the different dose rates may have been due to individual variation or to the limited data at the larger dosage.

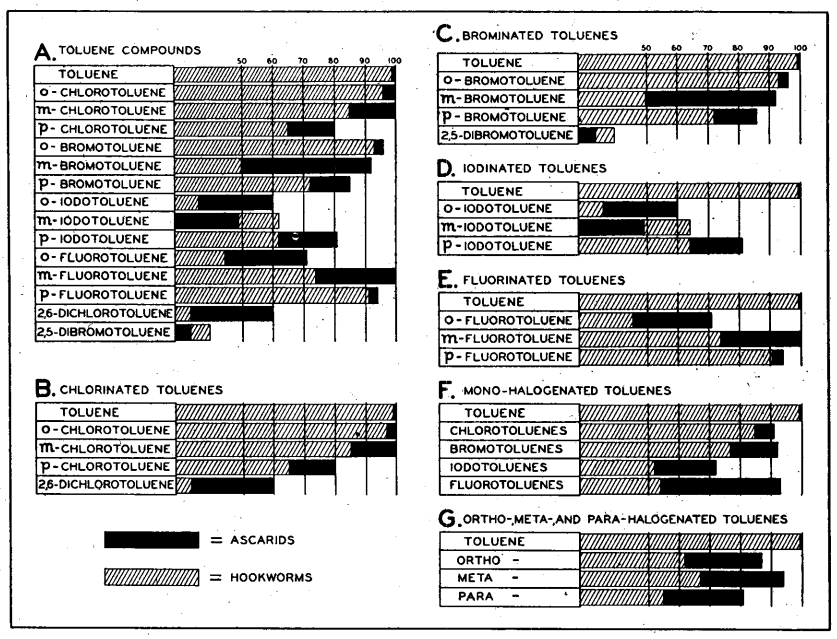

FIG. 1. Percentage efficacies of toluene compounds administered to dogs at the rate of 0.1 cc. per pound of body weight.

In general, the compounds were well tolerated in the dosages employed. Vomition occurred in less than 4 per cent of the dogs, and this was unaccompanied by nausea, depression, or inappetence. Some of the dogs exhibited evidence of intestinal irritation as mucus was found in the feces after treatment. It is known, however, that dogs heavily parasitized with hookworms occasionally pass rather large amounts of mucoid fecal material. At autopsy, the gross lesions, when present, were confined essentially to a slight to moderate congestion of the liver, kidneys, and/or small intestine.

A compilation of the aggregate efficacies of toluene, the mono-halogenated compounds, and the 2 di-substituted products is given in table 3, and a comparison of the anthelmintic action of the ortho-, meta-, and para-compounds is given in table 4. In the aggregate, toluene removed 100 per cent of 61 ascarids, 99 per cent (349) of 351 hookworms from 4 dogs, and 75 per cent (121) of 161 whipworms from 6 dogs; the chlorotoluenes removed 91 per cent (343) of 374 ascarids

TABLE 3.—*Data on the efficacy of halogenated toluenes in dogs*

		Parasites		Efficacy (per cent)	No. of tests
		Removed	Left		
Toluene	Ascarids	61	0	100	2
	Hookworms	349	2	99	4
	Whipworms	121	40	75	6
Chlorotoluenes	Ascarids	343	31	91	6
	Hookworms	1272	210	85	14
	Whipworms	341	108	75	13
Bromotoluenes	Ascarids	143	12	92	6
	Hookworms	3009	888	77	11
	Whipworms	170	346	32	13
Iodotoluenes	Ascarids	112	43	72	4
	Hookworms	1452	1317	52	10
	Whipworms	465	161	74	9
Fluorotoluenes	Ascarids	72	5	93	6
	Hookworms	1524	1285	54	9
	Whipworms	259	276	48	11
Dichlorotoluene	Ascarids	6	4	60	2
	Hookworms	264	1811	12	5
	Whipworms	42	190	18	5
Dibromotoluene	Ascarids	2	14	12	1
	Hookworms	242	658	26	3
	Whipworms	16	192	7	3

from 6 dogs, 85 per cent (1272) of 1482 hookworms from 14 dogs, and 75 per cent (341) of 449 whipworms from 13 dogs; the bromotoluenes removed 92 per cent (143) of 155 ascarids from 6 dogs, 77 per cent (3009) of 3897 hookworms from 11 dogs, and 32 per cent (170) of 516 whipworms from 13 dogs; the iodotoluenes removed 72 per cent (112) of 155 ascarids from 4 dogs, 52 per cent (1452) of 2769 hookworms from 10 dogs, and 74 per cent (465) of 626 whipworms from 9 dogs; and the fluorotoluenes removed 93 per cent (72) of 77 ascarids from 6 dogs, 54 per cent (1524) of 1809 hookworms from 9 dogs, and 48 per cent (259) of 535 whipworms from 11 dogs. The dichlorotoluene removed 60 per cent (6) of 10 ascarids from 2 dogs, and 12 per cent (264) of 2075 hookworms and 18 per cent

TABLE 4.—*Data on the efficacy of ortho-, meta-, and para-halogenated toluenes in dogs*

		Parasites		Efficacy (per cent)	No. of tests
		Removed	Left		
Toluene	Ascarids	61	0	100	2
	Hookworms	349	2	99	4
	Whipworms	121	40	75	6
Ortho-	Ascarids	145	20	87	7
	Hookworms	2902	1731	62	13
	Whipworms	548	307	64	14
Meta-	Ascarids	278	16	94	7
	Hookworms	1690	824	67	15
	Whipworms	214	345	38	15
Para-	Ascarids	247	55	81	8
	Hookworms	2665	1145	55	16
	Whipworms	473	239	66	17

TABLE 5.—*Physical properties of some halogenated toluenes*

Name	Constitutional formulae	Physical state	Water sol.	Melt. point (° C.)	Boil. point (° C.)
Toluene	$CH_3C_6H_5$	Colorless liquid	v.sl.sol.	− 95°	109.5–110.5°
o-Chlorotoluene	$CH_3C_6H_4Cl$	" "	Insol.	− 34 to − 36°	157–159°
m-Chlorotoluene	"	" "	"	− 48°	160–162°
p-Chlorotoluene	"	" "	"	7–7.8°	162°
o-Bromotoluene	$CH_3C_6H_4Br$	" "	"	− 26°	58–59°/10 mm.
m-Bromotoluene	"	" "	"	− 40°	66–69°/16 mm.
p-Bromotoluene	"	" "	"	25–26°	184°
o-Iodotoluene	$CH_3C_6H_4I$	" "	"	.....	204–207°
m-Iodotoluene	"	" "	"	.....	92–94°/15 mm.
p-Iodotoluene	"	Colorless leaflets	"	34–35°	211.5°
o-Fluorotoluene	$CH_3C_6H_4F$	Colorless liquid	"	− 80°	113–114°
m-Fluorotoluene	"	" "	"	− 111°	115–116°
p-Fluorotoluene	"	" "	"	.....	116–117°
2,6-Dichlorotoluene	$CH_3C_6H_3Cl_2$	Light amber liquid	"	.....	193–198°
2,5-Dibromotoluene	$CH_3C_6H_3Br_2$	Dark amber liquid	"	.....	105–107°/9 mm.

(42) of 232 whipworms from 5 dogs; and the dibromotoluene removed 12 per cent (2) of 16 ascarids from 1 dog, and 26 per cent (242) of 900 hookworms and 7 per cent (16) of 208 whipworms from 3 dogs.

The ortho-compounds removed, in the aggregate, 87 per cent (145) of 165 ascarids from 7 dogs, 62 per cent (2902) of 4633 hookworms from 13 dogs, and 64 per cent (548) of 855 whipworms from 14 dogs; the meta-compounds removed 94 per cent (278) of 294 ascarids from 7 dogs, and 67 per cent (1690) of 2514 hookworms and 38 per cent (214) of 559 whipworms from 15 dogs; and the para-compounds removed 81 per cent (247) of 302 ascarids from 8 dogs, 55 per cent (2665) of 3810 hookworms from 16 dogs, and 66 per cent (473) of 712 whipworms from 17 dogs.

The constitutional formulae and certain physical properties of the compounds are given in table 5. Most of the compounds tested are colorless liquids, although the two di-substituted products are amber-colored. One of the compounds, p-iodotoluene, occurs as colorless leaflets at room temperature, and another, p-bromotoluene, solidifies at temperatures slightly below ordinary room temperature. Both of these substances liquefy promptly when heated, and this facilitates the administration of the compounds to the test animals. All of the compounds tested are relatively insoluble in water, and their melting points and boiling points vary considerably. The boiling points of the various isomers, however, are reasonably uniform. No correlations between these physical properties and the anthelmintic action of the compounds were apparent.

DISCUSSION

Toluene, the basic compound in the series, exhibited marked anthelmintic action against ascarids and hookworms in dogs. The drug was fairly effective against whipworms, but the number of tests was too limited to permit a satisfactory evaluation of its trichuricidal action. In the dosages employed in this study, the efficacy of the drug against ascarids and hookworms was not exceeded by any of the halogenated derivatives (Fig. 1, A). However, three of the derivatives, viz., ortho- and meta-chlorotoluene, and meta-fluorotoluene, exhibited comparable ascaricidal action; but further testing at a lower dose rate would probably show a variation in the comparative ascaricidal action of the four compounds. None of the derivatives achieved comparable anthelmintic action against hookworms, although o-chlorotoluene, o-bromotoluene, and p-fluorotoluene removed significant numbers of these parasites.

Although the data are probably too limited, at least in some instances, to permit highly significant comparisons, the apparent indications are of considerable interest. The introduction of halogen atoms into the nucleus of the toluene ring usually caused a reduction in the anthelmintic action of the compound (Fig. 1, B–E), the reduction varying with the substituent, with its position in the ring, and with the number of substituents introduced. A single chlorine atom affixed in the ortho- or meta-positions apparently did not affect the ascaricidal action of the compound, but a significant reduction occurred when the substituent was affixed in the para-position. In regard to the action of the drugs against hookworms, the effect of a single chlorine atom in the ortho-position was relatively inconsequential. A significant reduction in anthelmintic action occurred, however, when the substituent was affixed in the meta-position, and a marked reduction occurred with the para-isomer. The introduction of two chlorine atoms into the toluene ring resulted in a conspicuous reduction in the anthelmintic action of the compound against both ascarids and hookworms.

A single bromine atom affixed in the ortho-, meta-, or para-positions of the toluene ring resulted in a moderate, progressive reduction in ascaricidal action.

With respect to hookworms, only a slight reduction in efficacy occurred when the substituent was affixed in the ortho-position; but significant reductions occurred with the meta- and para-isomers, the former being most profoundly affected. The introduction of two bromine atoms into the ring resulted in a marked reduction in the efficacy of the drug against both ascarids and hookworms.

An iodine atom affixed in the ortho-, meta-, or para-positions in the toluene ring resulted in a marked reduction in the efficacy of the compounds against both ascarids and hookworms. A di-substituted product was not available for testing, but the anthelmintic action of such a compound probably would be insignificant.

The substitution of a fluorine atom at the ortho-position of the toluene ring resulted in a significant reduction in the efficacy of the drug against both ascarids and hookworms. The ascaricidal action of the meta-isomer, however, was comparable to that of toluene, but the efficacy of the drug against hookworms was significantly less than that of the parent substance. The anthelmintic action of the para-isomer against ascarids and hookworms compared rather favorably with that of toluene. Although a di-substituted compound was not available for testing, the findings suggest that the anthelmintic action of such a substance probably would be less than that of the mono-substituted products.

In comparing the aggregate efficacies of the mono-halogenated compounds (Fig. 1, F), it is evident that the variation in the ascaricidal action of the chloro-, bromo-, and fluoro-toluenes is relatively insignificant, and that their aggregate efficacies compare favorably with the ascaricidal action of toluene. On the other hand, the aggregate efficacy of the iodotoluenes is considerably less than that of toluene and of the other halogenated products. It is noteworthy that the ascaricidal action of the fluorotoluenes, collectively and individually, is quite marked, although the ortho-isomer is somewhat less effective than the meta- and para-compounds. This circumstance is especially interesting in view of the recent report (Habermann, Enzie, and Foster, 1945, Amer. Jour. Vet. Res. 6: 131–144) with respect to the ascaricidal action of sodium fluoride in swine. These findings indicate that fluorine-containing compounds are worthy of further consideration in the search for potential ascaricides. With respect to hookworms, the variation in the anthelmintic action of the different groups is more clearly defined. The aggregate efficacy of the chlorotoluenes was higher than that of the other halogenated compounds, although individually they were not uniformly more effective than some of the other compounds. The bromotoluenes were only slightly less effective than the chlorinated products, but the aggregate efficacy of the fluorotoluenes and iodotoluenes was comparatively insignificant. In the last two groups, however, individual compounds exhibited rather marked anthelmintic action.

A comparison of the anthelmintic action of the ortho-, meta-, and para-compounds (Fig. 1, G) suggests that the most favorable site for the substitution of halogen atoms is at the meta-position, and that the para-position is least favorable in this respect. In considering the individual efficacies of the compounds, however, it is evident that their action does not conform to this pattern.

ABSTRACT SUMMARY AND CONCLUSIONS

In limited anthelmintic studies with toluene and some of its halogen substitution products, namely, the ortho-, meta-, and para-isomers of chloro-, bromo-, iodo-, and fluoro-toluene, and 2 di-substituted derivatives, namely, 2,6-dichlorotoluene and 2,5-dibromotoluene, it was found that the introduction of halogens into the ring usually caused a reduction in efficacy. In general, the dimunition was more pronounced with hookworms than with ascarids, and it was considerably greater with the di-substituted products than with the corresponding mono-halogenated compounds. At a dose rate of 0.1 cc. per pound of body weight, toluene removed 100

per cent of 61 ascarids from 2 dogs, 99 per cent (349) of 351 hookworms from 4 dogs, and 75 per cent (121) of 161 whipworms from 6 dogs. The most effective substitution product, o-chlorotoluene, removed 100 per cent of 66 ascarids from 2 dogs, and 96 per cent (484) of 499 hookworms and 92 per cent (166) of 179 whipworms from 4 dogs. Comparable ascaricidal action was exhibited by m-chlorotoluene and m-fluorotoluene, and o-bromotoluene and p-fluorotoluene removed significant numbers of hookworms. In the aggregate, the chloro-, bromo-, and fluorotoluenes were about equally effective against ascarids; the iodotoluenes, however, were significantly less effective than the other halogenated compounds. With respect to hookworms, the chlorotoluenes were most effective, followed in order by the bromo-, fluoro-, and iodo-toluenes. In considering the aggregate efficacy of the mono-halogenated compounds, the meta-position appeared to be the most favorable site for halogenation, and the para-position appeared to be least favorable in this respect.

Diagnosis of gastrointestinal nematode parasitism of sheep by differential egg counts. K. C. KATES, U. S. Bureau of Animal Industry.

Gastrointestinal parasitism of sheep is of common occurrence in the United States, affected sheep usually harboring several different genera and species of helminths. These helminths differ markedly in their effect on the host, and a reasonably accurate knowledge of both kinds and numbers of helminths present is, therefore, desirable. At present two methods of securing that knowledge, at least in part, are available. One consists in slaughtering some affected animals in a flock and collecting and counting the helminths present in the alimentary tract. This method is accurate so far as the individual animal examined at necropsy is concerned, but it is only indicative with respect to the remaining animals in the flock. The other method consists in the examination of droppings, for helminth eggs, of a number of animals in the flock. This method may have two advantages over the slaughter method in that (a) no animals are lost by slaughter, (b) many more animals can be examined and, therefore, a better estimate of the helminth population of the flock as a whole can be obtained.

Qualitative differential diagnosis of gastrointestinal parasitism in sheep by fecal examination for eggs has been shown to be a practical procedure, with some limitations, by Shorb (1939, 1940) and Kates and Shorb (1943). Little information is available, however, concerning the practical application of differential egg counts to quantitative diagnosis of helminths in sheep. The problem of determination of genera and species of adult helminths present in the alimentary tract by fecal examinations for eggs is the relatively simple one of differentiating the eggs of the various genera or species by means of structural, color, and size differences. Accuracy of these determinations is limited mainly by the presence or absence of recognizable differences in eggs of various species. In contrast, in any attempt to correlate differential egg counts with the number of parasites present in the host, certain complicating factors must be taken into consideration in order properly to evaluate the data. Some of these factors are:[1]

(1) Differences in egg-producing capacity of various species of helminths.

(2) Variations in egg production within the species because of variations in size of infestation, diet of host, age of parasites, age of host, etc.

(3) Variations in resistance of host to various species of helminths.

(4) Sex ratio of parasites.

(5) Physical condition of stool.

[1] I am indebted for some helpful suggestions in this connection to a manuscript of an address delivered by Dr. A. O. Foster before the Medical Association of the Isthmian Canal Zone on August 4, 1938, entitled: "Factors affecting egg–worm ratios in hookworm infections."

The purpose of this paper is to present data showing some aspects of the utility and limitations of differential egg counts in the quantitative determination of gastrointestinal nematode populations of lambs. These data were collected at the Zoological Division, Agricultural Research Center, U. S. Department of Agriculture, Beltsville, Md., over a considerable period and the observations recorded were incidental to other investigations on sheep parasites. Although a special situation is represented here, it is thought that it may be representative of some situations encountered in the field.

METHODS

Over a period of about 2½ years 59 lambs, parasite-free except for light infestations with *Strongyloides* and coccidia, were used as test animals on pastures contaminated with parasite eggs and larvae. All lambs, which varied somewhat in age at the time of exposure to infection, were handled in the same manner. Lambs in groups of two were placed on small infested pastures for 4-day periods each, then removed to clean pens which were elevated about 2 feet from the ground. These pens were cleaned thoroughly each day while occupied. Subsequent to exposure on the plots, the lambs remained in the pens for 5 weeks to allow the majority of parasites that presumably had been acquired to reach maturity. The lambs were then killed and autopsied, and all parasites in the alimentary tract were collected and counted. At the time of autopsy a fecal sample of a dozen or more pellets was taken from each lamb. Differential egg counts were made on duplicate pellets. Average-sized pellets, rather than a measured quantity of feces, were used as the unit of feces in each case, because a pellet is a convenient quantity of feces to use for egg flotations in the field. Generally, in this series of lambs, average-sized pellets varied in weight from 0.2 to 0.4 gram each, a gram of stool thus being represented by 2.5 to 5 pellets, depending upon the size of the lamb. It has been our experience, that of Stoll (1929), and that of Shorb *et al.* (1941), that a direct relationship usually exists between lamb size and pellet size, egg counts per gram, and egg counts per pellet, and is a fairly consistent relationship in animals producing normal stools. In animals producing soft or watery stools correction factors reflecting the resultant dilution of helminth eggs may be used. Such correction factors were not employed in this study, as none of the lambs had unformed stools at the time of autopsy.

Differential egg counts were made in each case by the brine direct centrifugal flotation method, as described by Stoll (1930). By careful use of this method it was found that well over 90 per cent of parasite ova were concentrated on the first coverslip. In most cases egg counts on duplicate pellets did not vary more than 10 per cent. The duplicate counts were averaged for the final count. When the number of eggs per pellet was very large a higher dilution of the stool was made before flotation was done.

RESULTS AND DISCUSSION

The parasites recovered post mortem from all lambs belonged mainly to six genera, namely, *Haemonchus, Trichostrongylus, Oesophagostomum, Nematodirus, Cooperia,* and *Ostertagia.* Two genera were represented by one species each, namely, *Haemonchus contortus* and *Cooperia curticei.* The species of *Trichostrongylus* were mainly *T. colubriformis,* with a small percentage of *T. axei;* of *Oesophagostomum,* mainly *O. columbianum,* with a small number of *O. venulosum;* of *Nematodirus* mainly *N. spathiger,* with a small number of *N. filicollis;* and the genus *Ostertagia* was a mixture of *O. circumcincta* and *O. trifurcata.*

Before the data can be properly evaluated, it is important to consider the factor of time after infection in relation to the different prepatent periods of the helminths under discussion. Egg production by the parasites of certain genera may

not have reached a maximum at the time the counts were made. The following shows prepatent periods of some of the species under discussion.

Parasite	*Prepatent Period*	*Authority*
Haemonchus contortus	20 days	Veglia (1915), Andrews (1942)
Trichostrongylus colubriformis	17–21 days	Andrews (1939a)
Oesophagostomum columbianum	35 days	Sarles (1944)
Nematodirus filicollis	4 weeks	Boulenger (1915)
Cooperia curticei	15 days	Andrews (1939b)
Ostertagia circumcincta	17 days	Threlkeld (1934)

It is clear from this tabulation that, with the possible exception of *Oesophagostomum* spp., the various helminths had ample time (4 days' exposure on pasture and 5 weeks in pens) to reach a sizable, if not maximum, egg production. The egg counts of *Oesophagostomum* spp. are probably lower than normally would be expected if the parasites had a longer developmental period (Sarles, 1944). In the case of the other parasites, it is possible that some may not have reached their period of maximum egg production at the end of a 5-week period.

RELATIONSHIP BETWEEN TOTAL EGG COUNTS AND NEMATODE POPULATIONS, ALL GENERA OF PARASITES INCLUDED

a. Individual lambs.—Little information of value can be elicited by attempts to correlate total nematode populations, including all genera of worms present, and total egg counts in individual lambs. This is well illustrated in the case of lambs 17, 19, 24, and 27, in table 1. These lambs had very low total egg counts yet relatively high total worm counts. Most of the parasites in these lambs were *Nematodirus* spp., which produce relatively few eggs. Egg counts in this case are of little value, being subject to great variation.

b. *All lambs.*—In table 1 the nematode populations of all 59 lambs and their comparable egg counts per pellet of feces are totaled. The ratio of total eggs per pellet to total worms recovered post mortem is that of about 1 to 1.4. This ratio represents a relatively close correlation between totaled egg counts and totaled nematode populations, but there is little reason to suppose that such a ratio can be of general application as it would change markedly if, for example, the majority of parasites present were *Nematodirus* spp. instead of *Haemonchus contortus.*

RELATIONSHIP BETWEEN DIFFERENTIAL EGG COUNTS AND NEMATODE POPULATIONS

a. *Individual lambs.*—Data in table 1 show considerable variation, in some instances, in the generic egg–worm ratios, between the various lambs. For example, in lamb 1 the ratio of *Haemonchus* eggs per pellet to worms recovered post mortem is about 11 to 9, while in lamb 2 the ratio is about 1 to 15. In more than half of the lambs listed, however, the egg–worm ratio for *Haemonchus* is approximately 1 to 1. Except for the smaller number of eggs produced per worm in other genera, this variability in egg–worm ratios is characteristic when individual lambs are compared.

b. *All lambs.*—In table 1 the eggs per pellet of feces and number of parasites recovered post mortem are totaled for all lambs for each genus of nematode, and their respective egg–worm ratios indicated. The egg–worm ratios vary from approximately 1 to 1 for *Haemonchus* to 1 to 18.1 for *Nematodirus* spp., the ratios for the other genera falling between these two extremes. These ratios show the general relationship between the egg-producing capacities of the parasites and illustrate the importance of such information in the interpretation of differential egg counts.

TABLE 1.—*Total parasites recovered post mortem (both sexes) and differential egg counts. Post mortem examinations and egg counts made 5 weeks after infection. Infection accomplished by 4-day exposure on infested pasture. Lambs arranged in table according to size of infestation in descending order*

Lamb No.	Total eggs & parasites		*Haemonchus* (*contortus*)		*Trichostrongylus* spp.		*Oesophagostomum* spp.		*Nematodirus* spp.		*Cooperia* (*curticei*)		*Ostertagia* spp.	
	EPP[a]	WPM[b]	EPP	WPM	EPP	WPM	EPP	WPM	EPP	WPM	EPP	WPM	EPP	WPM
1	12158	9520	11890	9150	63	96	1	4	35	132	72	48	97	90
2	830	8882	454	7443	116	685	58	101	0	5	162	435	40	213
3	2565	4963	1898	1634	148	1841	135	54	0	25	189	1336	195	73
4	2416	4455	2180	2460	120	922	26	60	0	0	90	1010	0	3
5	3253	4271	2929	2533	162	726	10	121	2	50	150	835	0	6
6	2129	4120	1928	3250	109	670	72	16	0	2	15	150	5	32
7	5335	3970	5283	3719	30	121	3	3	0	3	15	101	4	23
8	1445	3158	981	1934	322	858	24	57	0	6	68	208	50	95
9	2361	3010	2310	2910	18	30	1	4	1	6	13	30	18	30
10	625	2692	554	1808	32	475	1	22	1	16	33	361	4	10
11	2488	2463	2444	2114	24	196	2	3	0	0	6	116	12	34
12	3301	2309	3275	2230	4	30	8	9	0	3	4	23	10	14
13	2534	2307	2482	2150	10	90	22	20	0	2	14	30	6	15
14	2049	2238	2019	2109	4	49	12	11	0	4	2	43	12	22
15	626	2177	394	212	105	191	0	1	85	1700	32	36	10	37
16	750	1770	516	667	122	799	6	15	4	11	62	240	40	38
17	81	1427	4	4	20	97	0	0	53	1276	4	50	0	0
18	1084	1326	1014	1005	32	130	0	0	0	0	30	170	8	21
19	31	1122	4	4	14	218	0	0	12	853	1	46	0	1
20	1314	1069	1248	891	28	75	17	3	4	45	12	45	5	10
21	1041	1001	1002	917	29	50	0	0	0	0	6	20	4	14
22	1227	905	1129	487	41	200	0	0	2	30	52	180	3	8
23	440	857	19	20	0	5	0	0	20	104	0	0	401	728
24	74	749	4	5	25	65	0	0	36	654	3	14	6	11
25	396	730	347	452	29	165	0	0	0	35	18	75	2	3
26	362	655	20	10	0	2	0	0	17	77	0	0	325	566
27	16	644	0	0	5	24	0	0	11	619	0	1	0	0
28	1413	612	1362	338	33	110	0	0	5	128	5	20	8	16
29	1144	518	1121	440	16	50	1	2	0	2	3	20	3	4

TABLE 1.—*Continued*

Lamb No.	Total eggs & parasites		*Haemonchus* (*contortus*)		*Trichostrongylus* spp.		*Oesophagostomum* spp.		*Nematodirus* spp.		*Cooperia* (*curticei*)		*Ostertagia* spp.	
	EPP[a]	WPM[b]	EPP	WPM	EPP	WPM	EPP	WPM	EPP	WPM	EPP	WPM	EPP	WPM
30	77	433	0	0	0	0	0	0	5	43	0	0	72	390
31	618	417	587	159	9	29	0	0	12	198	10	30	0	1
32	196	370	192	318	4	36	0	5	0	8	0	2	0	1
33	573	357	535	223	36	122	0	2	0	0	1	6	1	4
34	30	284	3	8	3	8	0	1	18	231	6	36	0	0
35	208	262	171	190	4	19	0	0	4	24	26	24	3	5
36	357	253	327	142	19	50	0	0	1	35	5	16	5	10
37	317	249	306	216	0	17	0	0	0	3	6	9	5	4
38	115	232	100	66	0	0	0	0	14	161	0	0	1	5
39	248	230	181	51	34	78	0	0	4	50	27	45	2	6
40	20	202	0	0	15	53	0	0	2	133	0	1	3	15
41	44	181	2	3	24	92	0	0	10	82	0	0	8	4
42	310	177	305	156	3	8	0	0	0	3	2	10	0	0
43	31	165	0	0	0	7	0	0	6	74	0	0	25	84
44	22	163	0	1	4	24	0	0	18	137	0	1	0	0
45	127	155	115	40	0	2	0	0	5	106	0	0	7	7
46	663	146	658	123	2	10	0	0	0	2	3	11	0	0
47	16	105	0	0	12	36	0	0	3	62	0	1	1	6
48	144	103	128	33	5	26	0	3	1	12	8	26	2	3
49	14	82	0	0	14	77	0	0	0	5	0	0	0	0
50	41	68	29	12	7	11	0	0	3	39	0	0	2	6
51	9	29	1	2	4	7	0	0	4	20	0	0	0	0
52	40	25	40	18	0	2	0	0	0	3	0	2	0	0
53	9	23	4	8	5	6	0	0	0	9	0	0	0	0
54	69	15	67	8	0	0	0	0	0	2	2	5	0	0
55	27	13	23	9	0	0	0	0	0	0	4	4	0	0
56	4	11	1	3	3	3	0	0	0	5	0	0	0	0
57	19	4	19	4	0	0	0	0	0	0	0	0	0	0
58	0	1	0	1	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals	57836	78657	52605	52690	1868	9693	399	517	398	7235	1161	5872	1405	2668
Average egg–worm ratios	1 to 1.4		1 to 1		1 to 5.1		1 to 1.2		1 to 18.1		1 to 5		1 to 1.8	

[a] EPP, eggs per pellet.
[b] WPM, worms post mortem.

FACTORS OF IMPORTANCE INFLUENCING DIFFERENTIAL EGG COUNTS AND EGG–WORM RATIOS

a. *Variations in egg production of different genera.*—In making estimates of nematode populations by means of differential egg counts in sheep, variations in egg production in the different genera may lead to errors of interpretation. If these variations can be relatively standardized, better estimates of nematode populations in a flock can be made, on the basis of egg counts alone. This study indicates that *Haemonchus contortus, Oesophagostomum* spp. and *Ostertagia* spp, produce more eggs per worm than *Trichostrongylus* spp. and *Cooperia curticei; Nematodirus* spp. is the least productive of the six genera. Although no data were obtained concerning egg–worm ratios of *Bunostomum trigonocephalum* and *Chabertia ovina*, studies of Lucker and Neumayer (1946) and Kauzal (1933) show these two species belong in the high egg-producing group. Therefore, these parasites must be considered when making differential egg counts, although seldom present in large numbers in lambs or older animals and thus are not as important as the more commonly occurring pathogenic species.

Somewhat similar observations were made by Kauzal (1933), who employed differential larval counts from cultures of feces obtained from sheep which were later examined post mortem. Egg production of *H. contortus* was found to be 5 to 10 times higher than of *Trichostrongylus* spp. and *Ostertagia* spp. combined, and from 50 to 100 times greater than *Nematodirus* spp. As differential larval counts from fecal cultures were employed in that study, the larva–worm ratios had probably a wider spread than egg–worm ratios would have under similar conditions. Gordon (1933a) reported that a lamb having an egg count of 10,000 per gram actually contained 10,000 *Trichostrongylus* spp. determined by post-mortem examination. This is a 1 to 1 ratio, using eggs per gram as the unit of feces counted. This ratio compares favorably with the 1 to 5.1 egg–worm ratio for *Trichostrongylus* spp. reported herein (table 1) when eggs per pellet were counted, a gram of stool consisting of several pellets. Gordon (1933b) also reported the results of an egg–worm correlation in mixed infections in sheep. He found that under certain conditions *H. contortus* may produce almost 100 times as many eggs as *Trichostrongylus* spp. and that *Ostertagia* spp. may be at least as poor in egg production as *Trichostrongylus* spp. These results were obtained in mixed infections where the number of *H. contortus* was small and the number of *Trichostrongylus* spp. and *Ostertagia* spp. relatively large. The relative size of the infestations (see b below) was an important variable factor in this case.

b. *Size of infestation.*—In table 2 the relationship between size of infestation and egg–worm ratio is recorded for each genus. When the infestation was relatively light, the calculated numbers of eggs per worm were higher, and when the infestations were relatively heavy, the calculated numbers of eggs per worm were lower for each genus. This well illustrates the phenomenon that egg production per female worm decreases as the total number of parasites increases (Andrews (1936), Gordon (1933b) and others).

c. *Variations in host's resistance.*—This is an important factor in the interpretation of egg counts and is well illustrated in nodular worm infections. Sarles (1944) reported that when large daily doses of *O. columbianum* larvae were given to lambs over a period of 28 days, maturation of the parasites was inhibited and the expected egg production did not occur. Thus, egg counts did not reflect the intensity of nodular-worm disease present in the animals, the disease being caused by the larvae and young worms in the gut wall. The development of host resistance in heavy infections prevented the majority of larvae from reaching maturity. Therefore, the average egg–worm ratio of *Oesophagostomum* spp. of 1 to 1.2 reported herein (table 1) may not be very significant for practical pur-

TABLE 2.—*Correlation between size of infestation and egg–worm ratio*

Parasites	Size of infestation	Number of lambs	Totals		Egg–worm ratio
			EPP[a]	WPM[b]	EPP-WPM
Haemonchus (*contortus*)	a. More than 500 per lamb	18	44,407	48,924	1 – 1.1
	b. Less than 500 per lamb	34	8,198	3,766	1 – 0.45
Trichostrongylus spp.	a. More than 500 per lamb	7	1,099	6,501	1 – 6
	b. Less than 500 per lamb	45	769	3,192	1 – 4.1
Oesophagostomum spp.	a. More than 100 per lamb	2	68	222	1 – 3.2
	b. Less than 100 per lamb	20	331	295	1 – 0.9
Nematodirus spp.	a. More than 500 per lamb	5	197	5,102	1 – 25.8
	b. Less than 500 per lamb	45	201	2,133	1 – 10.6
Cooperia (*curticei*)	a. More than 200 per lamb	7	754	4,425	1 – 5.8
	b. Less than 200 per lamb	37	407	1,447	1 – 3.5
Ostertagia spp.	a. More than 200 per lamb	4	838	1,897	1 – 2.2
	b. Less than 200 per lamb	39	567	771	1 – 1.3

[a] EPP, eggs per pellet.
[b] WPM, worms post mortem.

poses, except, perhaps, in light infections. It should also be noted that 35 to 39 days is insufficient time for *Oesophagostomum* spp. to attain a state of maximum egg production.

d. *Sex ratios.*—The sex ratios of the various helminths recovered from this series of lambs were found to be quite variable. For practical reasons only the total number of nematodes is given. In a small number of lambs the numbers of male and female nematodes for each genus were about equal, but usually 10 to 20 per cent more females than males were present. In a few instances, where only small numbers of worms of certain genera were found, only one sex was represented.

Marked variations in sex ratios which profoundly affect the egg counts may occur. Andrews (1942), for instance, reported a case of *H. contortus* infection in which 1,474 male and only 92 female worms were present. Egg counts in such individual cases would be of little value for quantitative estimation of nematode populations. Variations in egg counts caused by variations in sex ratios may be minimized by averaging counts made on a fair number of animals from the flock under observation.

e. *Other factors to consider in differential egg counts.*—Some of the variable factors affecting differential egg counts discussed in this report have been briefly considered in light of the data presented. Other factors, not of great significance to this study, may be of importance. These include diet of host, age of parasites, age of hosts, condition of hosts, and physical condition of feces. Further studies are indicated on the use of differential egg counts for quantitative diagnosis of helminth parasitism of sheep and lambs in farm and range flocks. Such studies would add to our knowledge of the utility of this method and aid in the interpretation of the many variable factors involved.

LIMITATIONS AND UTILITY OF DIFFERENTIAL EGG COUNTS FOR DIAGNOSIS OF INTERNAL PARASITISM IN SHEEP

So many variables enter into the problem of proper interpretation of differential egg counts that the chances of error are great when the diagnostician is concerned with individual animals. In many cases it may be possible to make reasonably accurate estimates of nematode populations of individual animals by the egg count method, but too much confidence cannot be placed in results obtained. The method is of more value, however, if applied to a reasonable number of animals in a flock, and the counts averaged and interpreted on the basis of the variables already discussed. Thus, it would be possible to determine, without sacrifice of animals for post-mortem examination, the relative densities of infection of the various genera and/or species of helminths of major pathogenic importance. When one or two animals from a flock are killed for post-mortem examination for parasites, the information thus obtained may be less accurate, for the flock as a whole, than that obtained from fecal examinations of a larger number of representative animals. The accuracy of this information will depend on the ability of the diagnostician to differentiate the eggs of the various parasites commonly found in sheep in the locality where the work is done and to consider the variable factors that may influence the egg count.

SUMMARY

Data and discussion are presented on the limitations and utility of the differential egg-count method for use in qualitative and quantitative diagnosis of internal parasitism of sheep.

1. Marked variation was found in total egg counts (all genera of nematodes) and in differential egg counts in relation to nematode populations when counts from individual animals were compared.

2. In a series of 59 lambs the total number of eggs per pellet of feces was 57,836 (all genera) and the total worms recovered post mortem (all genera) was 78,675, an egg–worm ratio of 1 to 1.4. This ratio is not necessarily applicable where conditions are dissimilar to those described in this paper.

3. Although egg–worm ratios varied considerably when similar genera of helminths in different lambs were compared, more than half of the egg–worm ratios in this case were reasonably close to the average egg–worm ratio for each genus for all 59 lambs.

4. The average egg–worm ratios for each genus of helminth derived from totaled data from all 59 lambs, are as follows: *Haemonchus* (*contortus*), 1 to 1; *Trichostrongylus* spp., 1 to 5.1; *Oesophagostomum* spp., 1 to 1.2; *Nematodirus* spp., 1 to 18.1; *Cooperia* (*curticei*), 1 to 5; *Ostertagia* spp., 1 to 1.8. These ratios were obtained under certain specified conditions and are of more value in illustrating the variation in egg productivity of the various nematode genera, than in serving as bases for calculating nematode populations in sheep from egg count data obtained under different conditions.

5. Data are presented to illustrate the phenomenon that egg production decreases as the total number of nematodes (taken genus by genus) increases.

6. The differential egg count method may prove of value for quantitative estimates of internal helminth infections in sheep, if such counts are made on a fairly large number of animals from a flock and the data conservatively interpreted with due regard to important variable factors.

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Preliminary note on the successful breeding of an intermediate host of the Asiatic blood fluke.[1] CHARLES H. HILL.[2]

The snail, *Schistosomophora quadrasi,* is one of the intermediate hosts for *Schistosoma japonica* and is important as a laboratory animal in the biological phases of Asiatic schistosomiasis. This mollusc is small and difficult to collect and identify in the field. Furthermore, naturally infected individuals shedding infective larvae (cercariae) of the parasite when brought to this country were observed either to cease shedding cercariae or the number shed was sharply diminished. Attempts to infect these snails, using miracidia hatched from eggs from dog feces, resulted in failures.

This situation was the opposite to that observed in connection with studies on the trematode *Schistosoma mansoni.* The intermediate host of *S. mansoni,* the snail *Australorbis glabratus* which is indigenous to the West Indies and South America, proved to be an excellent intermediate host of this parasite under laboratory conditions. This snail could be raised easily in filtered river water to which was added calcium carbonate (Baker's) and using iceberg lettuce as food. Eggs of *A. glabratus* were produced in large numbers and, under considered management, many hatched and the young snails raised to maturity. Young snails were easily infected when miracidia hatched from eggs of *S. mansoni* from the feces of infected hamsters were used. Thousands of cercariae of *S. mansoni* were shed daily by these snails and were employed in experimental work.

The writer attempted to transfer the experience thus gained in cultivating the snail *Australorbis glabratus* to the problem of producing large numbers of young *Schistosomophora quadrasi.* Following this method it was hoped that an unlimited supply of easily infected molluscs which would produce large quantities of cercariae of *S. japonica* could be obtained. Since there was an acute need of adequate quantities of the cercariae of *S. japonica* for use in various phases of research on Asiatic schistosomiasis, many attempts were made to reproduce natural conditions which would either stimulate copulation or produce eggs and young individuals of the mollusc in question. Various combinations of water, chemicals, soil and growing plants were tried during the winter months and in the spring one of these attempts was successful. It was not determined, however, whether the success was due to a combination of factors in the culture medium or to a seasonal reproductive cycle.

During the winter months a large culture dish was set aside to be used in one of the attempts to secure eggs or young of *S. quadrasi.* This dish contained filtered river water and several large lumps of black bog soil obtained from a swamp in Michigan; fibrous vegetable matter was plentiful in these lumps of soil. Dried and cured maple leaves were added to the culture and these were replenished as needed (Krull, 1937). Calcium carbonate (Baker's) was added from time to time and monthly some of the water was carefully withdrawn and fresh, filtered river

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[2] Lieutenant, H(S), U. S. Naval Reserve (Inactive).

water was added. The water withdrawn was examined for snails or eggs and later discarded after boiling to prevent any possible contamination of local waters. The pH of the water of the culture was always on the alkaline side (about pH 7.4 to 7.5).

When the culture was first made, 10 infected snails were added. Two of these died within a month. During a period of 4 months, 2 lots of snails were added to the original number and a total of 28 were present at the end of the experiment. Eggs were never observed even after examining microscopically many samples of soil, leaves and debris. Recently, the egg has been found on Leyte, P. I., and the breeding habits of the adult have been described (Abbott, 1946). Many adult snails were usually found on the maple leaves either feeding or at rest. A few, however, were always seen among the root-like fibers in the lumps of bog soil or in the little recesses of the soil itself.

In the spring (April, 1945), in spite of the fact that no eggs were found, the first lot of young snails appeared crawling up the side of the culture dish. These young snails resembled the adults in the dish in almost every respect except in habits. In contrast to the adults, the young snails spent most of their time out of the water on the sides of the culture dish. A few could always be found, however, crawling over the surface of the maple leaves in the bottom of the culture. Several were carefully scraped from the sides of the dish and submitted alive to Drs. Paul Bartsch and J. P. E. Morrison of the U. S. National Museum for identification. Both of these specialists identified the young individuals as *Schistosomophora quadrasi* (3 specimens are catalogued as U.S.N.M. No. 543541). During the following month many lots of young snails emerged after which the number of new individuals gradually tapered off. The writer was then ordered to other duty and unfortunately the termination of the experiment could not be observed.

The observations reported herein suggest that *Schistosomophora quadrasi* can be maintained and will produce young under laboratory conditions and possibly under natural conditions in some parts of this country but whether reproduction occurs in one or two seasons or is continuous, or whether the young snails can be infected with miracidia and yield cercariae of *Schistosoma japonica* was not determined due to the conditions previously mentioned.

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