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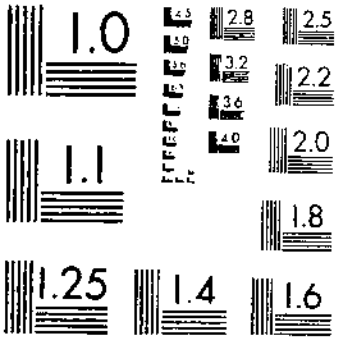

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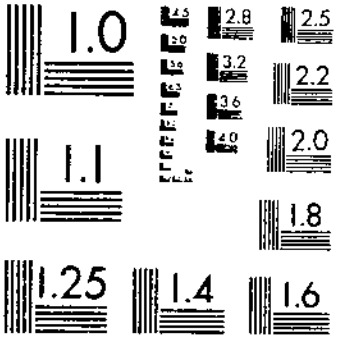

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Technical Bulletin No. 875 · September 1944

Effects of Experimental Nodular Worm (Oesophagostomum Columbianum) Infection in Sheep[1]

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INTRODUCTION

The cause of the nodules often found in the intestines of sheep was first discovered in the United States by Curtice in 1890 (*2*).[3] He demonstrated the presence of small worms in the nodules and showed that they were the immature stages of a parasitic nematode that he named *Oesophagostomum columbianum*, which inhabits the lumen of the large intestine. Curtice described the parasite and the symptoms and lesions of the "nodular disease" that it causes. He concluded that *O. columbianum* is the most injurious of the intestinal parasites of sheep and is the cause of heavy losses to the sheep industry through impairment of the health of sheep and damage to the wool. He noted, moreover, that the nodules characteristic of the disease rendered sheep intestines unfit for sausage casings.

Shortly after the publication of Curtice's account of this new disease, it was recognized as existing in South Africa and Australia. Later it was reported from many other sheep-raising countries and from practically all the farm-flock areas of the southern, eastern, and midwestern United States.

Although the disease was discovered more than 50 years ago, it has not been the subject of extensive experimental study. Most au-

[1] Submitted for publication June 29, 1944.
[2] Transferred to the Bureau of Entomology and Plant Quarantine of the Department on March 16, 1944.
[3] Numbers in parentheses refer to Literature Cited, p. 19.

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thors have agreed, in general, with Curtice's conclusions as to the serious nature of the disease, and some have suggested that nodular worms probably have been the cause of the abandonment of sheep raising in certain parts of the United States, Canada, South Africa, and Australia.

Much of the literature on the disease is of uncertain import because it consists of reports of damage caused by naturally acquired infections in sheep that were parasitized also with large numbers of other helminths. The experimental studies carried out in South Africa are an outstanding exception, however. Veglia (*15*) in that country worked out the life cycle and morphology of the parasite and published an extensive account of clinical and pathological findings in experimentally infected sheep (*16*). Fourie (*4*) subsequently made a study of the histopathology of the disease based on tissues obtained mainly from the sheep used in Veglia's studies.

The purposes of the present study, which was carried on at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., were as follows: To determine the effect of varying degrees of nodular worm infection on the parasitological findings in the lambs, their live weights, gross pathology, appearance of the wool and weights of pelts, and carcass yields.

MATERIALS AND METHODS

Sixteen lambs of mixed breeding were used in these experiments. The animals were born in clean stalls, between February 23 and March 9, 1941, to ewes that had been treated with therapeutic doses of phenothiazine the previous summer and again before parturition. On May 28 the lambs were weaned and separated on the basis of sex and were then transferred to clean, indoor pens bedded with straw. These pens opened into outdoor runways that were fully exposed almost all day to the sun. The control and experimentally infected sheep were kept in separate pens and runways; but it was necessary, because of limitation of space, to keep the rams and ewes of each of these two groups together during the first 9 days and the last 4 weeks of the experiment. Chance of reinfection was minimized by cleaning all dung out of the pens and replacing the bedding with clean straw three times each week. The lambs had access to rock salt and water at all times and were fed a measured ration of one-half pound of mixed grain (17.5 percent of cracked corn, 75 percent of whole oats, and 7.5 percent of bran) and 3 pounds of U. S. No. 2 alfalfa hay.

All the lambs had remained normal and had been gaining weight steadily up to the time the experiment was begun on October 7, 1941. At this time lambs of each sex were assigned alternately in order of birth to the following 4 groups, each of which contained 2 rams and 2 ewe lambs: Control group, not experimentally infected; and light-, medium-, and heavy-dose groups, each animal of which received 28 daily doses of 10, 100, and 1,000 larvae, respectively. The

daily doses of larvae were designed to simulate the effect of continual reinfection of lambs on pasture at 3 different levels of intake of infective material.

The larvae used for the infections were isolated from fruit jar cultures of the dung of a single sheep that had been experimentally infected with *Oesophagostomum columbianum*. They were isolated a few days before being fed to the lambs to allow time for the small number of *Strongyloides*[4] larvae, also present in the cultures, to die. The larvae used for infection came from 17- to 30-day cultures. The sheep were infected by pipetting the larvae into the back of the mouth.

Before, during, and after the period of daily administration of larvae to 12 of the sheep, the 16 sheep used in the experiment were under daily observation, and during the 17 weeks following the first infection detailed clinical examinations were made. During the experiment 9 egg counts were made from salt flotation of the parasite eggs present in one-half gram of feces obtained from the rectum.

Observations were made on the condition and weights of the carcasses and organs of each animal at the time of slaughter in the eighteenth week of the experiment. The carcass weights include the weights of the kidneys and diaphragm but not the weight of the shank bone of the legs. The alimentary tract of each sheep was preserved in 5-percent formalin for (1) later examination for gross pathological changes, (2) counts of the numbers of nodules present, and (3) screening for worms in the fourth stomach, small intestine, and large intestine.

RESULTS

Parasitological Findings

Preliminary fecal examinations were made on October 2, 5 days before the experiment was begun. Flotation preparations of feces revealed, in all the lambs, small numbers of *Strongyloides* eggs and very small numbers of coccidial oöcysts of several species and, in control ewe 21, two *Nematodirus*[4] eggs per gram of feces. Small numbers of eggs and coccidial oöcysts were continually observed throughout the experiment in the feces of most of the lambs and small numbers of *Strongyloides* eggs in the feces of all of them. Eggs of *Trichuris*[4] were occasionally found in the feces of lambs 19, 20, and 41 and *Nematodirus* eggs eventually appeared in the dung of all the lambs except Nos. 12, 23, 28, and 52. The number of eggs of these two species never exceeded six per gram.

Oesophagostomum eggs were never found in the feces of any of the controls. They were first found after 5 or 6 weeks of infection in the feces of all 8 sheep given 10 or 100 larvae daily (table 1). They never appeared in the feces of 2 of the 4 sheep given 1,000 larvae a day and not until 7 and 8 weeks after infection in the other 2 sheep.

[4] Another genus of parasitic worms.

TABLE 1.—*Egg counts of Oesophagostomum columbianum per gram of feces on the 12 infected lambs during the experiment*

Group	Lamb No.	Sex	Number of eggs per gram of feces after—									Total number of eggs [1]	Number of eggs per larva administered [2]
			4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	10 weeks	12 weeks	13 weeks	16 weeks		
Light-dose (10 larvae per day).	31	Ram	0	0	38	60	92	8	362	4	94	678	2.4
	44	do	0	6	20	42	30	220	4	108	88	518	1.9
	18	Ewe	0	2	38	188	262	324	276	608	88	1,786	6.4
	23	do	0	2	10	128	270	248	1,200	372	56	2,202	8.2
Medium-dose (100 larvae per day).	35	Ram	0	0	140	318	690	14	56	26	34	1,278	.5
	46	do	0	28	382	690	300	436	1,500	308	662	4,306	1.5
	19	Ewe	0	32	38	392	276	202	94	72	158	1,264	.5
	28	do	0	0	306	814	102	38	438	86	336	2,120	.8
Heavy-dose (1,000 larvae per day).	37	Ram	0	0	0	2	0	0	0	0	0	2	.0001
	52	do	0	0	0	0	0	0	0	0	0	0	0.0
	20	Ewe	0	0	0	0	2	0	0	0	0	2	.0001
	93	do	0	0	0	0	0	0	0	0	0	0	0.0

[1] Average for the light-dose group, 1,319 eggs; medium-dose group, 2,242 eggs; heavy-dose group, 1 egg.
[2] Average for the light-dose group, 4.7; medium-dose group, 0.825; heavy-dose group, 0.0005.

In the light-dose group the egg counts increased gradually for some weeks and then decreased to low levels toward the end of the experiment. The egg counts for the medium-dose group had a sharper initial rise than those of the light-dose group, but they declined earlier and more abruptly. Secondary peaks of egg production by the worms occurred in 2 animals in this group. Three of the four sheep in this group were still passing considerable numbers of eggs at the time of the last count, 16 weeks after infection. As already stated, nodular worm eggs never appeared in the feces of 2 of the 4 lambs fed 1,000 larvae a day; and in the feces of the other 2 sheep, eggs were present in detectable numbers on only 1 examination.

The total number of *Oesophagostomum* eggs per gram of feces found on nine counts (table 1) was not directly proportional to the number of larvae fed. On the contrary, the administration of increasing numbers of larvae resulted in decreasing numbers of eggs.

The number of adult nodular worms recovered from the large intestine, as shown in table 2, did not increase in proportion to the number of larvae administered. The maximum number of worms recovered (267) is relatively small compared with the thousands of worms of other species frequently encountered in naturally acquired infections but is comparable with the numbers of nodular worms often found in such infections.

TABLE 2.—*Data on Oesophagostomum and other worms recovered during the experiment*

Group	Lamb No.	Sex	Total larvae administered in 28 daily doses	Total nodular worms recovered from large intestine	Larvae developing	Female nodular worms recovered	Eggs per gram of feces on last count, per female nodular worm	Other worms recovered		
								Strongyloides	*Nematodirus*	*Trichuris*
			Number	*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*
Control	41	Ram	0	0	.....	0	.....	1	15	1
	57	do	0	0	.....	0	.....	26	26	1
	12	Ewe	0	0	.....	0	.....	0	18	0
	21	do	0	0	.....	0	.....	3	25	0
Average			.....	0	.....	0	.....	7.5	21	0.5
Light-dose	31	Ram	280	122	43.6	89	1.1	0	12	0
	44	do	280	128	45.7	69	1.3	0	13	0
	18	Ewe	280	121	43.2	75	1.3	0	1	0
	23	do	280	92	32.9	59	.9	0	1	0
Average			.....	116	41.3	73	1.1	0	6.7	0
Medium-dose	36	Ram	2,800	44	1.6	35	1.0	1	3	0
	46	do	2,800	267	9.5	147	4.5	97	1	0
	19	Ewe	2,800	181	6.5	112	1.4	0	0	2
	28	do	2,800	201	7.2	130	2.6	0	4	0
Average			.....	173	6.2	106	2.4	24.5	2	0.5
Heavy-dose	37	Ram	28,000	1	.004	0	.....	78	9	0
	52	do	28,000	0	0	0	.....	0	7	0
	20	Ewe	28,000	4	.014	3	0	0	14	0
	93	do	28,000	4	.014	1	0	2	12	0
Average			.....	2	.008	1	0	20	10.5	0

Small numbers of *Strongyloides* and *Nematodirus* worms were recovered from the small intestine of most of the sheep, and in three of the animals one or two *Trichuris* were also recovered from the large intestine (table 2), but the numbers of worms of these three genera were not considered large enough to have produced any of the observed symptoms or gross pathology.

As measured by the number of nodular worms still present in the lumen of the large intestine at the time the sheep were slaughtered after 17 weeks of infection, the development and persistence of the parasite in the intestine were inversely related to the total number of larvae administered.

In every case the number of eggs per gram of feces on the last count, per female worm, was relatively low. There was no evidence, therefore, that egg production had been inhibited in the sheep receiving the larger doses of larvae.

Symptoms

Examination of the animals 5 days before the first larvae were administered showed that all were in excellent physical condition. Their appetites were good, and all of them had been gaining weight steadily and satisfactorily.

The only symptoms observed in the first week after infection were loss of weight and elevation of temperature of lamb 37. In the second week all the sheep of the heavy-dose group showed definite clinical evidence of systemic disturbance. They were less active than the other animals, either lying down a great part of the time or standing apart in an attitude of depression with the head down and the back arched. In the third week and continuing through the sixth week, these sheep continued to lose weight and the above-mentioned symptoms became more pronounced (fig. 1). The infected animals began to suffer from diarrhea and passed liquid, foul-smelling feces, the appetite was greatly diminished, the animals became weak, and small nodules could be detected on rectal examination. These symptoms increased in severity until about the seventh week. At this time the animals stopped losing weight, but owing to the damage caused by the infection their weight loss was never completely regained, their clinical appearance did not improve materially, and they continued to be in very poor condition until the end of the experiment.

The symptoms shown by the light- and medium-dose groups were similar to those observed in the heavy-dose group, differing mainly in time of onset and severity. The medium-dose group lagged behind the control group in weight gains from about the fourth week until the end of the experiment. The medium-dose animals began to show evidence of intestinal disturbance about 4 weeks after infection, and diarrhea appeared during the fifth week. During the remainder of the experiment the symptoms were similar to those shown by the heavy-dose group, and at the end of the experiment the sheep in the medium-dose group were considerably lighter than those in the control group. The light-dose group did not show any marked clinical evidence of infection during the experiment, except for a slight diarrhea during the last month. The final weights of the two ewes in this group were, however, below the weights of the ewes in the control group.

Rectal temperatures of control and infected sheep were compared on 14 days during the 17 weeks of the experiment. Significant deviations from the temperatures of the control animals occurred only in the 4 sheep fed 1,000 larvae per day and consisted in an elevation of 2° F. This increased temperature was present in some sheep at the end of the first week of infection and persisted more or less throughout the experiment.

Changes in Live Weight

Although the 16 sheep were alternately allocated by age to the 4 groups, this procedure did not result in groups or pairs of sheep of the same sex within groups that were of the same weight at the time of weaning or at the beginning of the experiment (table 3). At both of these times the rams and ewes in the control and heavy-dose groups had a lower average weight than the sheep of the same sex in the light-dose and medium-dose groups. However, the advantage in weight

that the sheep of the last two groups thus had before infection only made more striking their failure to maintain this advantage over the control sheep. The changes in weight of the sheep appeared to be

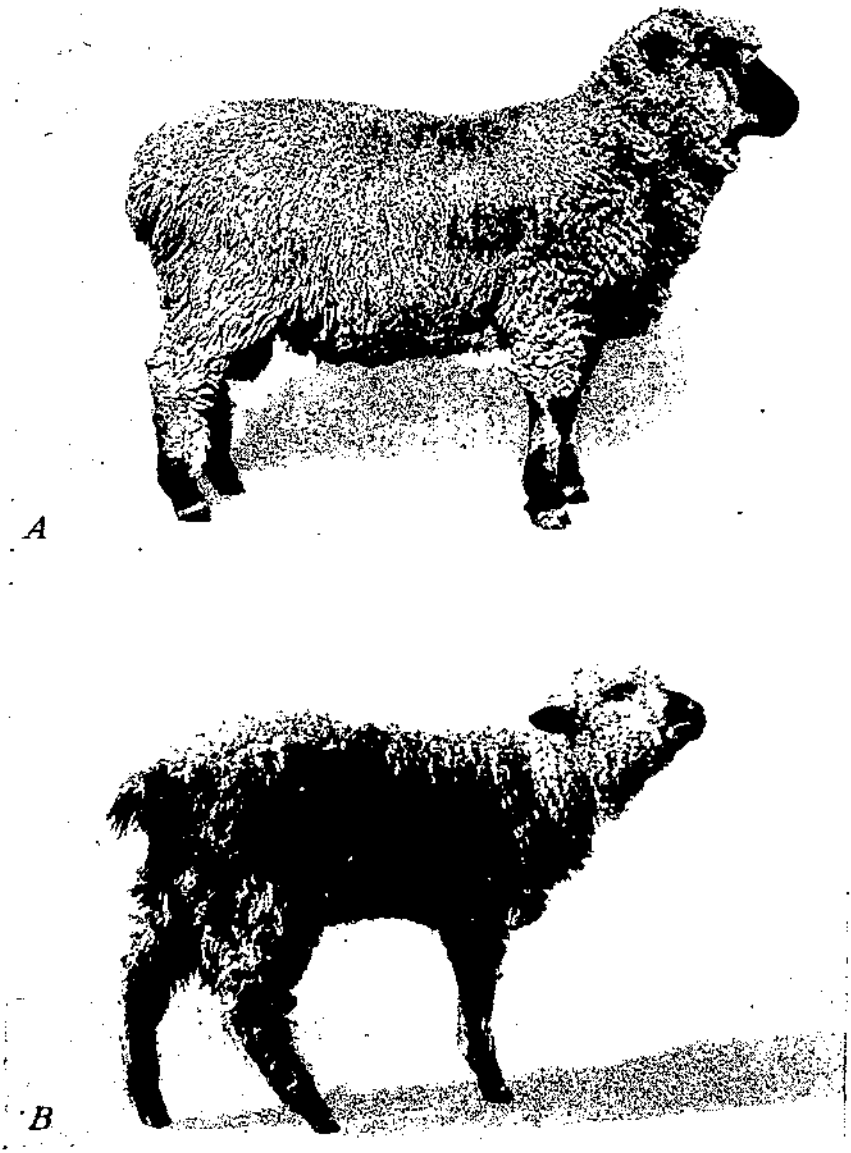

FIGURE 1.—Comparative appearance, in the sixth week of the experiment, of uninfected control ram 41 (*A*), and of ram 52 (*B*), which had received 1,000 nodular worm larvae per day for 28 days. The infected animal illustrates the typical "straining" attitude frequently assumed by sheep severely afflicted with nodular worm disease. Also typical are the short, rough, dry coat, the dull and drawn appearance of the face, and the generally stunted and emaciated condition of the animal.

directly related to the number of larvae administered. This relationship was most striking at the end of the seventh week, when the differences between the various groups were greatest. This evidence of decreased gain in weight with increasing infection is also reflected in the net changes in weight during the entire 17 weeks of the experiment (table 3).

TABLE 3.—*Weights of sheep and rates of change in weight before and during the experiment*

Group	Lamb No.	Sex	Weight of sheep— When weaned at 3 months old	Weight of sheep— At beginning of experiment when 8 months old	Weight of sheep— At end of experiment when 12 months old	Net gain (+) or loss (−) in weight during 17 weeks of experiment	Average weekly gain (+) or loss (−) in weight during— 19 weeks before experiment began	First 7 weeks of experiment	Last 10 weeks of experiment	Entire 17 weeks of experiment
			Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Control	41	Ram	40	81	123	+42	+1.8	+2.4	+2.5	+2.5
	57	do	47	75	127	+52	+1.5	+2.6	+3.4	+3.1
	12	Ewe	43	70	105	+35	+1.4	+2.3	+1.9	+2.1
	21	do	37	64	98	+34	+1.4	+2.6	+1.6	+2.0
Average			43	73	113	+40	+1.5	+2.5	+2.3	+2.4
Light-dose (10 larvae per day)	31	Ram	49	80	122	+42	+1.6	+2.7	+2.3	+2.5
	44	do	48	84	130	+46	+1.9	+3.0	+2.5	+2.7
	18	Ewe	44	76	97	+21	+1.7	+1.6	+1.0	+1.2
	23	do	41	67	80	+13	+1.4	+.6	+.9	+.8
Average			45	77	107	+30	+1.7	+2.0	+1.7	+1.8
Medium-dose (100 larvae per day)	36	Ram	49	86	101	+15	+1.9	+1.6	+.4	+.9
	40	do	41	73	82	+9	+1.7	+.7	+.4	+.5
	19	Ewe	45	77	95	+18	+1.7	+1.4	+.8	+1.1
	28	do	40	72	86	+14	+1.7	+1.3	+.5	+.8
Average			44	77	91	+14	+1.7	+1.3	+.5	+.8
Heavy-dose (1,000 larvae per day)	37	Ram	50	90	61	−29	+2.1	−4.1	0	−1.7
	52	do	29	62	59	−3	+1.7	−3.0	+1.8	−.2
	20	Ewe	43	76	68	−8	+1.7	−3.6	+1.7	−.5
	93	do	37	64	45	−19	+1.4	−2.9	+.1	−1.1
Average			40	73	58	−13	+1.7	−3.4	+.9	−.9

The rams and ewes given 1,000 larvae a day were most affected. Ram 37 lost weight even in the first week after infection, and all 4 sheep infected at this rate progressively lost weight in the first few weeks. Ram 37 lost 32 pounds (35.6 percent) in 10 weeks; ram 52 lost 23 pounds (37.1 percent) in 6 weeks; ewe 20 lost 25 pounds (33.0 percent) in 7 weeks; and ewe 93 lost 20 pounds (31.2 percent) in 7 weeks. Ram 37 and ewe 93 failed to regain any significant part of this lost weight at the end of the experiment, but the other 2 sheep began to gain weight from the fifth to eleventh week after infection, although neither succeeded in regaining all that they had lost. On the other hand, the sheep given 10 and 100 larvae a day continued to gain weight after infection, although less rapidly than the control sheep and less satisfactorily toward the end of the experiment. The terminal lag in the weight curves was more marked and was manifest earlier in the medium-dose group than in the light-dose group.

The effect of the degree of infection on the weight of the sheep is also shown, and in an especially striking manner, by the weekly change in weight during 3 periods of observation (table 3). These

data show that during the 19 weeks before the experiment began all the sheep gained weight at a fairly comparable rate. However, different results were obtained during the first 7 weeks after the experiment began. Although during this period the control sheep gained at an even greater average rate than in the preliminary period, the 2 ewes given 10 larvae a day and the 4 sheep given 100 larvae a day made a smaller rate of gain than the control sheep of the same sex, and all 4 sheep given 1,000 larvae a day experienced large weekly losses in weight. In the last 10 weeks of the experiment, all the sheep except 1 made some weekly gain in weight. The rate of gain was, in general, greatest in the control sheep and in the others was inversely related to the number of larvae administered. In the medium-dose and heavy-dose groups, sex difference in rate of gain was not manifested as in the control and light-dose groups. The effect of increasing doses of larvae on the rate of gain was still apparent when the weekly changes in weight were averaged for the entire 17-week period of the experiment.

Hematocrit Readings

Hematocrit determinations (table 4) were made at three different times during the experiment on blood samples taken from the jugular vein. At 37 days after infection a slight indication of anemia was seen in the slightly subnormal average hematocrit readings for the medium-dose group. At this stage of infection the average reading for the heavy-dose group was slightly higher than that for the control sheep, possibly owing to dehydration resulting from constant diarrhea. At 64 days the average hematocrit readings were lower for the medium-dose and heavy-dose sheep than for the controls, and there was an apparent tendency for the average reading to decrease with increasing dosage. This tendency was even more marked after 111 days of infection.

Table 4.—*Hematocrit readings at 3 intervals after the experiment began*

Group	Lamb No.	Sex	Hematocrit reading (percent packed red cells) after—		
			37 days	64 days	111 days
Control	41	Ram	38.0	33.0	34.5
	57	do	40.5	37.5	37.0
	12	Ewe	40.0	38.0	42.0
	21	do	42.5	44.5	44.0
Average			40.3	38.3	39.4
Light-dose (10 larvae per day)	31	Ram	39.5	33.0	35.7
	44	do	40.0	40.2	40.3
	18	Ewe	40.5	39.0	33.0
	23	do	40.0	35.8	37.5
Average			40.0	37.7	38.1
Medium-dose (100 larvae per day)	36	Ram	33.0	28.6	29.5
	46	do	38.0	32.0	35.7
	19	Ewe	37.5	39.0	36.5
	28	do	37.2	36.0	34.0
Average			36.4	33.7	33.9
Heavy-dose (1,000 larvae per day)	37	Ram	45.0	34.8	22.3
	52	do	41.0	28.5	21.5
	20	Ewe	44.0	31.0	28.5
	33	do	40.0	27.0	22.7
Average			42.5	30.3	23.7

Gross Pathology

As shown in table 6, the weights of the organs tended to decrease as the number of larvae administered increased. The extent of intestinal involvement and the numbers of nodules in the walls of the intestines (table 5) were directly related to the number of larvae given.

Table 5.—*Data on nodular lesions in tissues* [1]

Group	Sheep No.	Sex	Nodules and remnants of nodules in—		Proportion of total lesions that were nodules	Nodular lesions in extraintestinal tissues [2]						
			Small intestine	Large intestine		Omentum	Liver	Spleen	Rumen	Lungs	Mesentery	Mesenteric lymph nodes
			No.	*No.*	*Pct.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Light-dose (10 larvae per day).	31	Ram	99	18	12.0	0	0	0	0	1	0	0
	44	do	6	5	36.4	0	0	0	0	0	0	0
	18	Ewe	50	28	32.1	0	0	0	0	0	+	0
	23	do	28	22	44.0	0	0	0	0	0	0	0
Average			46	18	31.1	0	0	0	0	0.3		
Medium-dose (100 larvae per day).	36	Ram	640	726	58.4	4	3	0	0	1	0	0
	46	do	554	743	63.0	2	10	0	0	0	+	+
	19	Ewe	458	463	62.3	2	0	0	0	0	+	0
	28	do	240	463	76.0	6	2	0	0	0	0	0
Average			475	599	65.1	2	3.7	0	0	0.3		
Heavy-dose (1,000 larvae per day).	37	Ram	4,452	[3] 1,414	[3] 25.6	10	[4] 6	0	0	0	+	++
	52	do	2,855	[3] 688	[3] 18.4	6	8	0	3	0	0	++
	20	Ewe	100	713	80.4	8	3	0	0	0	++	++
	93	do	1,700	[3] 1,132	[3] 40.4	5	[4] 3	0	0	0	+	+
Average			2,277	[3] 987	[3] 21.2	7.3	5	0	0.7	0		

[1] No lesions were found in any of the controls.
[2] + indicates that nodular lesions were present but no exact count of them was made.
[3] Figure is undoubtedly low as it was impossible to count more than the superficial nodules in the greatly thickened intestinal wall.
[4] One of these nodules was at the apex of a small infarcted area at the edge of the liver.

With two exceptions, there were no lesions, nodules, or pathological changes of any kind in the four control sheep. Each of these animals had much fat in the omentum, and the kidneys were entirely covered with fat. Considerable fat was also present in the mesentery and about the pericardium. All parts of the intestines were normal in appearance, free from adhesions, and thin-walled (fig. 2, C).

The sheep given 280 larvae were all in fairly good condition at slaughter. Only a few large nodules and a few old brown remnants of nodules were scattered sparsely along the small and large intestines. The crown (the coiled part of the small colon) and the posterior part of the large intestine were abnormally pale and devoid of feces. The first coil of the crown was usually slightly dilated and was roughened on the peritoneal surface, and small hemorrhages were present in the slightly thickened and wrinkled mucosa.

In the sheep given 2,800 larvae, much larger numbers of nodules were present in the small intestine, and some of the nodules had resulted in local constriction of the intestine. Nodules were especially numerous in the terminal part of the ileum and about the ileocecal valve. Great

numbers of large nodules were scattered along the walls of the cecum, colon, and crown (fig. 2, *A*), and the intestinal wall in these places was abnormally thickened. A few nodules were found all along the large intestine below the crown and also in the walls of the rectum. The cecum and colon were paler and smaller than normal in some of these sheep. Extraintestinal nodules were encountered in the omentum,

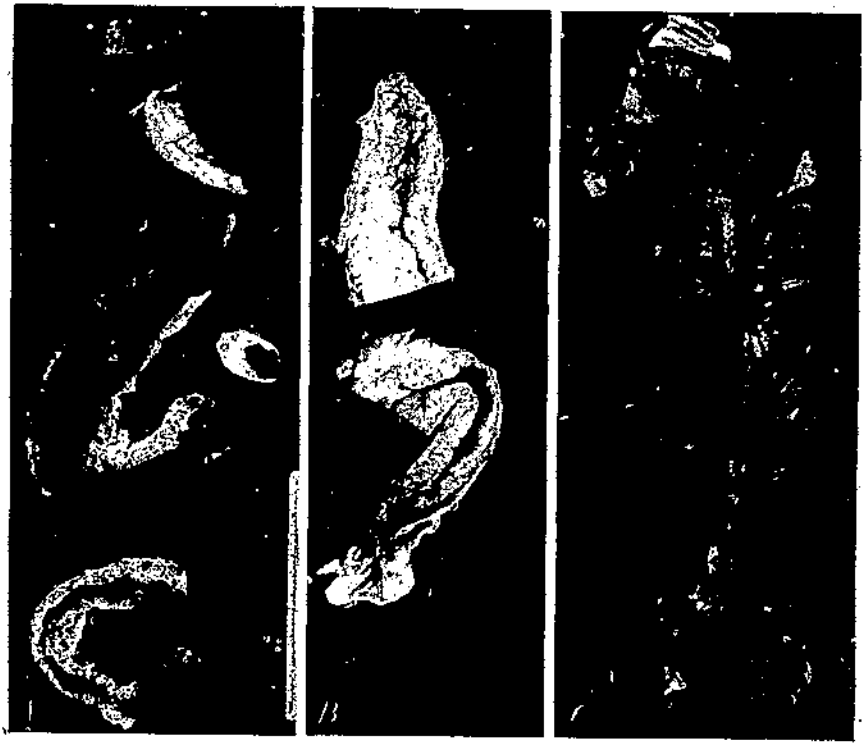

FIGURE 2.—Nodular involvement of the cecum and large colon (upper and lower half of *A*, respectively) in moderately severe nodular worm disease and of these parts of the large intestine (upper and lower half of *B*) in a severe form of the disease, as compared with their normal appearance (*C*) in one of the uninfected control sheep.

mesentery, and liver, as shown in table 5. In some of the sheep, the mesentery nearest the nodular portions of the large intestine had an abnormal, milky appearance, and adhesions were present binding the colon to the coils of the crown and to each other. The colon and first coil of the crown usually contained many large, greenish nodules concentrated mostly on the mesenteric side of the intestine (fig. 2, *A*). The first half of the first coil of the crown was dilated and thickened. The mucosa of this portion of the crown, which was apparently the main site of localization of adult nodular worms, was wrinkled and covered with thick mucus. There were red patches along the mucosa and scattered deep, petechial hemorrhages. In several instances small ulcerated areas were present in the immediate vicinity of nodular worms that were deeply embedded between the folds of the mucosa. The lymph nodes adjacent to the crown and colon were markedly en-

larged. A small amount of fluid was usually present in the abdominal cavity.

The sheep given 28,000 larvae were extremely abnormal in many respects. Their obviously weak, stunted, and emaciated appearance was found at slaughter to be associated with an extreme degree of emaciation of the whole body and with striking pathological changes in the abdominal organs. In each of these 4 sheep, the omentum and liver were abnormally small, as shown in table 6, and the normal accumulations of fat about the kidneys and in the omentum were absent. The abdominal contents of all these sheep had a peculiarly grayish color and an unpleasant, slightly decomposed odor. From 25 to 50 cc. of fluid was present in the abdominal cavity. The omentum contained scattered nodules, as indicated in table 5, and the very thin film of fat on this organ was of an abnormal, milky appearance. In some cases the omentum was reduced to a parchmentlike membrane; in others it was tightly bound by adhesions to the extremely nodular intestines.

The outer surfaces of the cecum and colon were studded with large nodules and covered with adhesion strands. In ram 52 (fig. 2, *B*) the nodular involvement of the intestines was especially severe; all parts of the large intestine were so completely bound up into a hard, round mass by adhesions between the parts of the large intestine, mesentery, and omentum that only the tip of the cecum was visible.

In these severe cases of the disease the mesenteries near the cecum, colon, and rectum contained greatly enlarged solitary lymph nodes, and scattered through these nodes as well as in the main cord of mesenteric lymph nodes there were green nodules and brown calcified remnants of nodules. Nodules were also present in the livers of three sheep in the heavy-dose group, sometimes at the apex of a small infarct extending to the edge of the liver. The site of the most extreme pathological change was clearly in the intestines, especially the large intestine. Nodules and calcified remnants of nodules were scattered in great numbers along the gut from the duodenum to the anus and were especially concentrated in the greatly thickened portions of the intestinal wall of the terminal part of the ileum, ileocecal valve region, cecum, colon, and rectum. In all these regions there had occurred a localized scarring, hardening, and thickening of the intestinal wall with resultant constriction of the lumen of the intestine to form a narrow tube lined with protruding nodules. The cecum and colon were greatly reduced in length and capacity. In ram 52 the cecum had become reduced to a small sack, and the large colon had become transformed into a narrow and sharply bent tube, with both regions so thickened and hardened as to render them practically functionless (fig. 2, *B*).

The very small number of worms in the large intestine of the sheep of the heavy-dose group was not associated with the type of local lesions found in the sheep of the other two infected groups. The part of the large intestine below the crown usually contained considerable mucus and many nodules. Only small amounts of feces were present in the last few feet of the large intestine of the most heavily dosed sheep and were usually localized in an abnormally dilated portion of the large intestine above the rectum. The rectum was empty and reduced to crevices between bulging nodules. The wall of the rectum was greatly thickened, hardened, and filled with white, fibrous tissue,

forming a firm, tumorlike mass of densely encapsulated green nodules and calcified remnants of nodules.

The lesions in the small intestines were mostly small, calcified remnants of nodules, but in the large intestines they were predominantly large, green nodules surrounded by a thick connective tissue capsule. The nodules were sometimes concentrated in bands or rings in the cecum and colon.

Appearance of Wool and Weights of Pelts

During the experiment it was noticed that the wool on the infected sheep had become much dirtier than that on the controls, and in the heavy-dose group it was shorter, more irregular, and devoid of yolk (fig. 3). At the end of the experiment, "breaks" were found in the

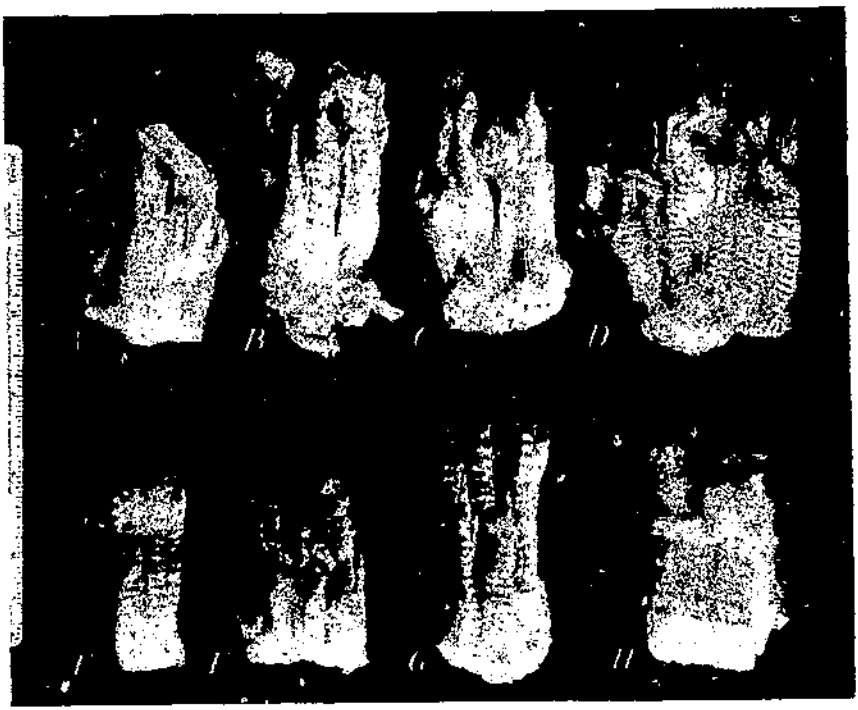

FIGURE 3.—Samples of shoulder wool from uninfected sheep (*A, B, C, D*) and heavily infected sheep (*E, F, G, H*). Note the stunted, irregular, and dry appearance of the wool from the heavily infected sheep and the occurrence of "breaks" at the middle of sample *E* and the base of sample *H*.

wool fibers of all four sheep of the heavy-dose group and also in two of the medium-dose group. When the sheep were slaughtered and skinned additional and related effects were found. The average pelt weight for the four groups decreased with the number of larvae administered (table 6), a fact that was apparently due partly to decreased thickness of the skin and partly to decreased amounts of wool and subcutaneous fat.

TABLE 6.—*Weights of organs, carcasses, and pelts of the 4 groups of sheep*

Group	Sheep No.	Sex	Weight of—					Weight of dressed carcass	Carcass yield	Shrinkage in carcass weight in cooler after—		Weight of pelt
			Omentum	Liver	Heart	Testes	Spleen			8 weeks	12 weeks	
			Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Lbs.*	*Pct.*	*Pct.*	*Pct.*	*Lbs.*
Control	41	Ram	995	780	200	315	65	50	48	12.7	15.7	15.7
	57	do	1,145	885	190	305	40	55	43.3	12.7	------	20.3
	12	Ewe	1,450	500	150	------	46	50.5	48.1	13.9	17.3	16
	21	do	1,020	535	145	------	40	49.5	50.5	12.1	15.1	13.3
Average	----	----------	1,153	690	171	310	48	53.5	47.5	12.9	16.0	16.3
Light-dose (10 larvae per day).	31	Ram	855	690	195	332	49	53.3	43.7	14.1	17.4	17
	44	do	1,320	851	175	225	65	62.3	47.9	14.9	------	16.3
	18	Ewe	813	495	153	------	60	46.5	47.5	16.1	19.3	14.3
	23	do	1,145	410	120	------	39	40.5	50.6	19.1	23.5	11.3
Average	----	----------	1,035	611	163	279	53	50.7	47.5	16.1	20.1	14.7
Medium-dose (100 larvae per day).	36	Ram	485	565	160	405	45	44	43.6	17.1	22.2	11.7
	46	do	736	557	156	205	38	35.3	42.7	17	22.7	12.3
	19	Ewe	1,040	545	130	------	47	43	45.3	15.1	22.7	12.5
	26	do	955	458	145	------	43	39.3	45.7	21	26.1	14.5
Average	----	----------	804	531	148	305	43	40.4	44.3	17.5	23.4	12.7
Heavy-dose (1,000 larvae per day).	37	Ram	130	538	160	127	------	23.3	38.2	30.1	------	9.5
	52	do	278	606	130	207	58	23.3	39.5	37.2	46.8	7
	20	Ewe	500	532	140	------	46	26.5	39.0	24.5	------	10.3
	93	do	70	345	95	------	20	15.3	34.0	36.1	44.3	7.5
Average	----	----------	245	505	131	167	41	22.1	37.6	32.0	45.5	8.6

MEAT FINDINGS

Figure 4 shows the comparative size, shape, and condition of representative carcasses from the sheep of the four groups. The carcasses of the control sheep were all large, well filled out, and thickly covered with fat. The light-dose group had carcasses that were fairly large and well filled out, but those of the medium-dose group were definitely smaller, and the superficial muscles were noticeably thin and pale. The carcasses of the heavy-dose group were all markedly shorter and narrower than those of the other sheep and had only a very thin covering of fat or none at all. In several of these carcasses the ribs showed through the very pale and filmlike thoracic muscles. Taken as a whole, the carcasses decreased in size and in amount of lean and fat as the number of larvae fed increased, the trend being most marked in the sheep that received 2,800 or more larvae.

The same effect was seen in the average weights of the dressed carcasses of each parasitized group (table 6) as compared with those of the controls. The weights of the carcasses of the medium- and heavy-dose groups were markedly less than those of the light-dose group.

Not only did the sizes and weights of the dressed carcasses decrease progressively with increasing dosage of larvae, but the percentage of carcass yield was likewise less in the two most heavily infected groups than in the controls, as shown also in table 6. Furthermore, the degree of shrinkage of the carcasses during 8 weeks of storage in

the cooler was markedly greater in the heavy-dose group and somewhat greater in the medium- and light-dose groups than in the controls. The effect was even more striking after 12 weeks of storage.

After a few days of storage, the emaciated carcasses of rams 37 and 52 and of ewe 93 were of an abnormal, pale-pink color, gradually oozed a sliny, unpleasant-smelling fluid, and failed to harden in the cooler. These carcasses were at first wet, flaccid, and unwholesome in

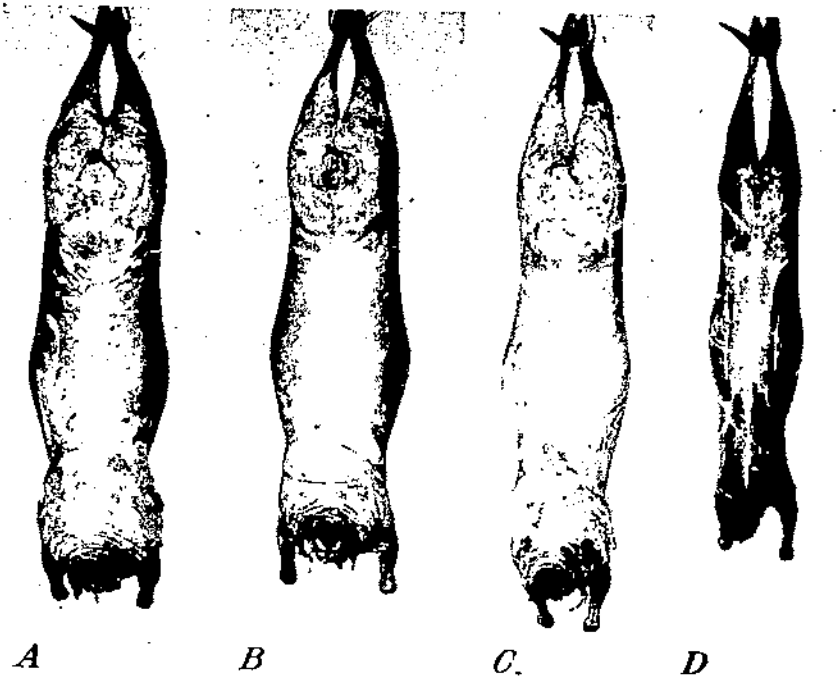

FIGURE 4.—Representative carcasses from an uninfected control ewe (*A*) and from ewes fed 280 (*B*), 2,800 (*C*), and 28,000 (*D*) nodular worm larvae.

appearance, odor, and feel. After several weeks in the cooler the thinner portions, such as the ribs and flanks, dried out and became dark red and almost as firm and dry as leather.

Figure 5 illustrates the effect of the infection on the size, shape, and composition of chops taken from the twelfth rib of sheep of each of the four groups. With an increase in the number of larvae fed, the chops decreased in size, the eye muscle became smaller and flatter, and the fat less and less abundant until it disappeared altogether.

DISCUSSION

The results of the present experiment confirm in general the composite picture of the symptoms and pathology of nodular worm infection observed in the United States by Curtice (*2*), Jacob (*8*), Dalrymple (*3*), Mote (*10*), Bell and Edgington (*1*), and Threlkeld (*14*); in Canada by Swales (*12*); in South Africa by Hutcheon (*6*),

Walker (*17*), Theiler (*13*), Veglia (*16*), Mönnig (*9*), and Fourie (*4*); and in Australia by Irving (*7*), Roberts (*11*), and Gordon (*5*).

There were no deaths among the experimental animals, and intussusception with its associated stretching (reksiekte), perforation of

FIGURE 5.—Twelfth-rib chops from the ewe carcasses shown in figure 4 (*A* to *D*) and from ram carcasses of comparable groups (*E* to *H*): *A* and *E*, from uninfected control sheep; *B* and *F*, from sheep fed 280 larvae; *C* and *G*, from sheep fed 2,800 larvae; and *D* and *H*, from sheep fed 28,000 larvae.

the intestine with resultant peritonitis, and ulcerative peritonitis—all reported as complications of nodular worm disease by South African workers—were not observed. The clinical and pathological findings for infected sheep varied somewhat among individuals of the same

sex and of different sexes that had been treated alike but were in general directly related to the number of larvae administered.

The sheep used in this experiment were 8 months of age and had a considerable amount of flesh and wool when first infected early in October, a whole season later than the time at which lambs usually first pick up the infection on pasture. Despite this advanced age the lambs were susceptible to the infection and in general suffered from it according to the numbers of larvae that they received. It seems likely that younger lambs exposed to similar doses of larvae under natural conditions of daily infection on pasture would be even more susceptible, since such lambs would probably not be receiving grain and would usually be affected with many other kinds of parasites. Nodular involvement of the intestine to the degree found in the sheep of the light- and medium-dose groups is commonly encountered in the fall in lambs from farm flocks kept on permanent pasture. The extreme form of the disease observed in the heavy-dose group, however, is not so common, although it appears to be similar to that observed by Swales (*12*) in eastern Canada and by Threlkeld (*14*) in Virginia during the winter after the first grazing season and in animals chronically suffering from several seasons of exposure to reinfection.

The large size obtained by all the sheep of the control group and the large amount of muscle and fat in their carcasses establish the fact that the ration fed was nutritious and conducive to development of a fairly high degree of finish. However, the infected sheep which had access to an equal amount of the same ration as the controls were not able to utilize the grain and hay so efficiently, and their deficiency in this respect was on the whole proportional to the number of larvae administered.

The damage produced by the parasite was primarily localized in the intestinal tract, especially in those parts of the small and large intestines where some degree of stasis occurs. This local damage and the nodules produced resulted secondarily in a dystrophy that is probably the basis of the more generalized symptoms. Far more serious structural and functional damage seemed to result from the nodular reaction characteristic of the disease and from the attempts of the host tissues to wall off and dispose of the parasite and the nodule contents around it than from the local damage and irritation produced either by the penetration of the larvae into the intestinal wall or by the local irritation caused by the mature worms in the posterior colon. However, the latter may well be concerned to some degree in producing the diarrhea, increased mucus secretion, slight anemia, and poor condition observed.

There was no evidence of the development of an acquired immunity to the over-all effects of nodular worm infection, since the severity of the disease appeared to be directly related to the number of larvae administered and was most damaging in the sheep that at slaughter harbored the fewest adult worms. Indications of an acquired immunity were seen in the following: Failure of egg production to increase proportionately to the number of larvae administered, the occurrence of an early and sudden drop in the rapidly rising egg counts of the sheep of the medium-dose group, and failure of the egg-

count curves to stay at a high level in the light-dose group (table 1); the small number of worms present at slaughter in the heavy-dose group and the decreasing percentage of larvae developing into worms as the dosage increased (table 2); and the increased percentage of larvae causing nodules in some of the sheep that received 2,800 or more larvae (table 5). The last-mentioned indication probably served to prevent normal development of the parasite, even though it resulted at the same time in serious nodular complications.

SUMMARY AND CONCLUSIONS

A study was made of the clinical and pathological effects produced by the administration of 28 daily doses of 10, 100, and 1,000 *Oesophagostomum columbianum*, or nodular worm, larvae to lambs. Two rams and two ewe lambs were used in each group, as well as in a control group of uninfected lambs. The animals were approximately 8 months of age when first infected, were of mixed breeding, and were fed an adequate ration of hay and grain. The work was carried on at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., in 1941.

Parasitological data indicated that the establishment and persistence of adult nodular worm infection in the intestines of the sheep became decreasingly less as the number of larvae administered increased. The daily ingestion of the larvae produced marked symptoms and pathological changes, the severity of which was determined both by the number of larvae administered and the directly related number of nodules in the walls of the intestines rather than by the number of mature parasites in the lumen of the intestine. Symptoms included loss of weight, decreased rate of gain in weight, slight elevation of rectal temperature, inactivity and unresponsiveness, diarrhea, loss of appetite, weakness, emaciation, stunting, and anemia.

Except for general emaciation and depletion of fat reserves the pathological changes were chiefly intestinal. They included nodular involvement of all parts of the small and large intestines; nodular constrictions, thickenings, and adhesions in those regions where some stasis occurs (terminal ileum, cecum, colon, crown, and rectum); extraintestinal nodules in the liver, omentum, and in the greatly enlarged mesenteric lymph nodes; local lesions in the form of small, deep hemorrhages and small ulcerations in the reddened and thickened mucosa of the dilated and reddened first coil of the crown, where the adult nodular worms were found in greatest numbers.

Nodular worm infection reduced the weight of the live sheep, weights of the pelt and organs, the amount of lean and fat in the carcass and chops, the dressing-out percent, and increased the degree of shrinkage of the carcass on storage. The wool from severely infected sheep was short and dry and showed breaks in the fibers.

It is concluded that the debilitating effects of the disease are mainly due to interference with normal intestinal functions produced by the nodular involvement of the intestinal wall that follows penetration of the larval stage of the parasite.

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U. S. GOVERNMENT PRINTING OFFICE: 1944

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