INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. X.

Experiments on the Length of Life of Infective Hookworm Larvae in Soils.*

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(Received for publication December 13, 1921.)

Historical account.

The conception of the length of life of the infective hookworm larvae in the soil which is widely accepted can be illustrated by a quotation from Nicoll (1917, p. 163): "These larvae (hookworm), when they reach this final stage have ceased to feed, and they can remain alive for months and even years under suitable conditions, i.e., where there is a sufficiency of moisture and not too great heat. In the laboratory they have been kept alive for over 18 months in plain water, at a temperature of about 60° F. It can hardly be doubted that they will live fully as long under natural conditions, unless it be that they are attacked and devoured by other animals, such as aquatic insects." The evidence which points to an extended period of life for the mature hookworm larvae comes chiefly from the work of the first seven investigators listed in Table 1. These observations (see Table 1) were made upon sheathed larvae, kept in moist feces or water under laboratory conditions in the temperate zone. Further, the inference has often been drawn, as in the above quotation, that if the larvae can remain alive for such periods of time under unnatural surroundings, their span of life would be as great or even greater, when in their natural environment.

The work of the last three investigators summarized in Table 1 needs further discussion, since their observations were made under natural conditions. Leichtenstern (1887, p. 669) found the larvae of *Ancylostoma duodenale* alive in the fecal mass from a brick-yard

* This paper is the tenth of the series on the hookworm investigations by the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, carried on with the cooperation of the International Health Board, Rockefeller Foundation.

latrine three months after the laborers had left the field. Stiles and Gardner (1910) found two motionless, but well-preserved sheathed hookworm larvae in sand 151 days after the sample had been taken from under a surface privy. In experiments by these investigators,

TABLE 1.

Showing results of previous authors' observations on length of life of hookworm larvae.

Authors.	Medium.	Environmental conditions.	Duration of life.
Leichtenstern, 1887	Water Moist feces	Laboratory temperature in Germany	7 months
Galli-Valerio, 1905	Moist feces	Laboratory temperature in Switzerland	13 months
Boycott, 1905.....	Moist feces	Laboratory temperature in London, England	More than 12 months
Loebker and Bruns, 1906.....	Water	By laboratory window, Germany	12 months
Oliver, 1910.....	Water	Laboratory temperature, England	15 months
Perroncito, 1913 ..	Water	Laboratory temperature, Italy	8–10 months
Nicoll, 1917.....	Water	60° F.	18 months
Leichtenstern, 1887	Fecal material	Abandoned latrine in brickyard, Germany.	3 months
Stiles and Gardner, 1910	Sand	Unheated laboratory during early fall, Wilmington, N. C.	A few alive up to 4 months
Baermann, 1917...	Soil covered with sod	In the open shade, Sumatra	6 months

where fecal material containing hookworm eggs was covered with sterilized sand, examinations made four and five months later disclosed no hookworm larvae. These experiments were carried on at unheated room temperature in the early fall at the United States Marine Hospital Station, Wilmington, North Carolina. Baermann (1917, p. 622) in his studies on the conditions of development and existence of hookworm larvae in the tropical climate of Sumatra found that the length of life varied under different conditions of moisture and shade. In one box, where a stool containing hookworm eggs was covered with earth by a sexton beetle and exposed to sun and rain without additional moistening, some slight development took place, but the larvae were

found to have died after three weeks. On shaded, sprinkled soil the larvae developed in large numbers, lived for 3 months and then died. In boxes planted with grass extensive development of larvae took place in both sun and shade. He found numerous larvae in the unshaded soil up to three months, and a few up to six months in the shaded. The results of these experiments on the length of life of hookworm larvae in the soil, with conditions as nearly natural as possible, gave a shorter period of life than the others in which the conditions were more unnatural, and suggest that soil does not remain infective as long as has been generally believed.

Leuckart (1868, p. 436), many years ago, pointed out that the mature larvae of *Ancylostoma caninum* (*Dochmius trignocephalus*) live at the expense of reserve food stored in the cells of the intestine during development, and as time passes these cells gradually loose their granules, until the worms become transparent. Looss (1911, p. 419) concluded that if the granules deposited in the body are reserve food stores, which are gradually reabsorbed during the later life of the larvae, a limit is set to the length of life, and death will come when this supply is exhausted. He believed that the length of life would depend on whether the supply of food was consumed slowly or rapidly, and since temperature influences to a high degree the motility of the mature larvae, they would remain alive longer at a low than a high temperature. If we accept this view we should expect the life of the larvae to be the shortest under those conditions of temperature which are most favorable to intense activity, as in the tropics. The results of my experiments carried on in Trinidad, British West Indies, and the field studies of Cort and Payne (1922*a* and *b*) support this view.

Material and methods.

The hookworm larvae used in the experiments which will be described in this paper were taken from cultures five to eight days old, with the exception of those used in the series on the length of life of unsheathed larvae which were from an eighteen-day-old culture. The same technique was employed in counting and in preparing the soils as in the migration experiments previously described (Augustine, 1922*a*). Two sizes of containers were used, viz., (1) tin pudding pans, six inches in diameter and two inches in depth, and (2) three-ounce glass jars.

DATA ON LENGTH OF LIFE FROM MIGRATION EXPERIMENTS.

Early in my work on the migration of hookworm larvae in soils (Augustine, 1922*a*) I was struck by the great reduction in the numbers of larvae recovered from experiments which had run a few weeks. Table 2 gives the data on the reduction of hookworm larvae in ten of

TABLE 2.

Showing reduction in the numbers of hookworm larvae in the migration experiments.

No. Exp.	No. larvae placed in soil.	Type of soil.	Time larvae remained in soil.*	Larvae recovered.		
				Unsheathed.	Sheathed.	Total.
1	1,000	Moist clay loam	7 days	141	180	321
2	498	Moist sand	10 days	81	10	91
3	512	Moist clay loam	11 days	234	3	237
4	1,100	Moist clay loam	21 days	11	7	18
5	392	Moist clay loam sod	21 days	1	4	5
6	500	Moist red clay loam	22 days	6	3	9
7	361	Moist clay loam sod	30 days	1	1	2
8	500	Moist clay loam	35 days	2	3	5
9	426	Moist clay loam	37 days	0	1	1
10	650	Moist clay loam	42 days	0	1	1

these experiments. Although the experiments were made under favorable conditions, after a period of twenty-one days, there occurred a reduction in the numbers of larvae of over 90 per cent. Stimulated by these findings, experiments were planned to further test this point.

EXPERIMENTS ON LENGTH OF LIFE OF MATURE HOOKWORM LARVAE IN SOILS.

Table 3 gives data from a series of thirty experiments to determine the amount of mass reduction in a known number of mature hookworm larvae in three types of soil, viz., (1) clay loam, (2) sand, and (3) a clay loam soil, under favorable conditions of temperature and moisture. In these experiments the soil was placed to a depth of 1½ inches in thirty of the six-inch pans, ten pans with each type of soil. Three hundred active, sheathed hookworm larvae from cultures five and

* To give the total age of the larvae, at the conclusion of the experiment, the time of their development in the cultures (5 to 7 days) must be added to the time they remained in the soil.

seven days old were added to each pan. The pans were then placed in a shady place and the soil kept moist throughout the experiment. Examinations, by the isolation apparatus, of the soil from one pan of each type were made weekly for seven weeks and the larvae recovered counted. As the seventh examination gave negative findings for hookworm larvae the eighth examination was made three days later. As this also proved to be negative for all three soils the experiment was closed. The results of these experiments (Table 3) indicate a rapid

TABLE 3.

Showing mass reduction of hookworm larvae in 3 types of soil.

In each of the experiments 300 sheathed hookworm larvae, taken from cultures 5 to 7 days old, were placed in 6-inch pudding pans containing 1½ inches of soil. Experiments started July 5, 1921.

Exp. No.	Date of examination.	Clay loam soil.				Sandy soil.				Clay loam soil.			
		Larvae recovered.		Total.	Per cent. reduction.	Larvae recovered.		Total.	Per cent. reduction.	Larvae recovered.		Total.	Per cent. reduction.
		Unsheathed.	Sheathed.			Unsheathed.	Sheathed.			Unsheathed.	Sheathed.		
1	July 12	52	4	56	81.3	123	9	132	56	30	9	39	87
2	July 19	16	1	17	94.3	38	1	39	87	10	2	12	96
3	July 26	14	5	19	93.6	13	0	13	95.6	15	3	18	94
4	August 2	13	5	18	94	11	1	12	96	0	0	0	100
5	August 9	5	0	5	98.3	6	8	14	95.3	6	8	14	95.3
6	August 16	1	14	15	95	4	0	4	98.6	2	7	9	97
7	August 23	0	0	0	100	0	0	0	100	0	0	0	100
8	August 25	0	0	0	100	0	0	0	100	0	0	0	100
9 and 10	Not examined												

dying off of the larvae within the first two weeks of the experiment. If the time of development in the cultures is added, it is seen that in three weeks after the eggs left the host 90 per cent. of the larvae which developed to the infective stage had perished. The significant points brought out in this series (Table 3) are (1) the high reduction occurring within two or three weeks, (2) the fairly constant number of larvae remaining alive up to seven weeks, and (3) a complete dying

out after that time. It would therefore appear that the activities of the larvae are at first intense, which is followed almost immediately by a high death rate.

TABLE 4.

Showing mass reduction of hookworm larvae in a clay loam and sandy soil.

In all these experiments the 3-oz. glass jars were used, filled ¾ full with soil.

Exp. No.	All experiments started July 19. Date of examination.	Clay loam soil. Number of larvae placed in soil.	Larvae recovered. Unsheathed.	Sheathed.	Total.	Per cent. reduction.	Sand. Number of larvae placed in soil.	Larvae recovered. Unsheathed.	Sheathed.	Total.	Per cent. reduction.
1	July 22	300	128	161	289	3.6	300	83	159	242	19.3
2	July 25	300	115	144	259	13.6	311	99	96	195	37.2
3	July 28	300	69	54	123	59.0	321	90	67	157	51.0
4	July 31	300	42	29	71	76.3	308	107	70	177	42.5
5	Aug. 3	300	59	29	88	70.6	303	29	9	38	87.4
6	Aug. 6	300	33	12	45	85.0	316	48	3	51	83.8
7	Aug. 9	300	40	7	47	84.3	304	40	15	55	81.9
8	Aug. 12	300	5	0	5	98.3	331	38	0	38	88.8
9	Aug. 15	300	13	5	18	94.0	325	26	1	27	91.6
10	Aug. 18	300	3	0	3	99.0	304	21	1	22	92.1

Table 4 gives the results of a series of experiments similar to those summarized in Table 3, except that the examinations were made every third day and the containers were the three-ounce glass jars. As the data show, the greatest reduction occurred about two weeks after the experiment was started, or about three weeks after the deposition of the eggs.

THE RELATION OF LOSS OF SHEATH TO LENGTH OF LIFE.

As has been previously stated (Cort, Augustine, et al., 1922) a large proportion of the mature hookworm larvae lose their sheaths in the soil. This discovery immediately introduces a new factor into the study of the length of life of the infective hookworm larvae. Three questions present themselves, viz., (1) Does the loss of the protective sheath directly shorten the life of the larvae? (2) Does the loss of sheath make the larvae more susceptible to unfavorable conditions of

environment and thus shorten their life under certain conditions? and (3) Does the loss of sheath render the larvae non-infective, which would be the equivalent of terminating their lives?

TABLE 5.

On the mass reduction of hookworm larvae in loam soil from cane field.

Ten experiments started July 18, with 100 unsheathed larvae in each. All larvae were obtained from same culture, 18 days old.

Experiment number.	Date of examination.	Number of larvae recovered.	Per cent. reduction.
1	July 21	87	13
2	July 24	52	48
3	July 27	51	49
4	July 30	15	85
5	August 2	13	87
6	August 5	4	96
7	August 8	7	93
8	August 11	0	..
9	August 12	0	..
10	August 12	0	..

Table 5 gives the data on a series of experiments on the length of life of unsheathed hookworm larvae in clay loam soil under favorable conditions. The larvae used in these experiments were isolated from soil on which a stool containing hookworm eggs had been placed eighteen days before. Of the 1,300 larvae obtained only 10 were found enclosed in sheaths. The experiments were made under the same environment as those with the sheathed forms. Examinations were made every third day as long as the results were positive. After the first negative appeared, which was 24 days after the experiments were started, or 42 days after the stool had been placed on the soil, the remaining jars were examined on the day following. These were likewise negative. While this series of experiments indicates that the life of the unsheathed hookworm larvae may be similar to that of the sheathed forms it must be taken into consideration that there undoubtedly had occurred a great reduction in the numbers of larvae before isolating them from the culture, and that the larvae used in the experiment were the survivors of this reduction.

While no experiments were made to test the comparative resistance of the unsheathed and the sheathed larvae, it was found that upon the

addition of a few drops of dilute formalin (See Cort, Ackert, et al., 1922, p. 5) to the preparation before counting, the unsheathed larvae quickly succumbed, while the sheathed forms were stimulated to greater activity, and could resist the formalin for a considerable time. This observation indicates, as would be naturally inferred, that unsheathed larvae are less resistant to unfavorable conditions than sheathed. This would then lead to a more rapid dying of the unsheathed larvae whenever conditions became unfavorable. Since our present knowledge of the resistance of the mature hookworm larvae to various conditions is based entirely upon studies of the sheathed forms it will be necessary to repeat much of this work with the unsheathed larvae, to determine what actually happens under natural conditions.

That sheathed hookworm larvae complete their second ecdysis, while penetrating the skin, and become adults in the intestines of man has been conclusively demonstrated by a number of investigators. Whether the loosing of the sheath in the soil renders the larvae noninfective, as far as man is concerned, which would be equivalent to their death, is not yet known. To gain information on this point unsheathed larvae were applied to the shaven belly of a male white rat, sixteen months old. Upon examination with a binocular microscope the larvae were seen to quickly spread themselves over the surface of the skin as far as the moisture extended. As the moisture around the larvae evaporated penetrating movements were started. The anterior ends of the worms would be lifted into the air and then turned toward the skin again, touching here and there as if seeking some port of entry. In one particular instance a larva was seen to leave the shaven area, to settle at the base of a hair and push one third of its body into the hair follicle. Then after a moment's rest the posterior end swung up parallel with the hair and with several slow twists the worm moved further into the skin and again rested. This movement was repeated several times until the tail of the worm vanished beneath the surface of the skin. Two other unsheathed larvae were seen to penetrate the skin within the shaven area. The entire process took less than a half hour. One hour later water was applied to the infected spot and upon microscopical examination no larvae were found. While this observation only shows that unsheathed hookworm larvae can penetrate mammalian skin, it seems very probable that they can also reach the intestine and grow to maturity. Further experiments are needed to clear up this point.

Discussion of results.

This experimental evidence on the length of life of the infective hookworm larvae in the soil is supported by the field observations of Cort and Payne (1922*a*, p. 138). In one instance soil pollution was found near the edge of a cane field and soil samples from this area showed it to be an intense center of infestation. This area was visited frequently at later intervals, but no further evidence of soil pollution was found. Soil samples, taken three weeks later from the same place, yielded but nine hookworm larvae, and later examinations showed a complete dying out of the larvae. More striking still is their study of the mass reduction of hookworm larvae in a chosen area of this field which, before educational work, sanitation, and treatment were started, was found to have a very heavy infestation with hookworm larvae. In less than three weeks after the reduction of soil pollution, which immediately followed the introduction of control measures, a second series of examinations was made. The results of these examinations showed a reduction of over 90 per cent. from the first series. Subsequent examinations of samples taken from this area showed still further reductions. In about six weeks after pollution of the soil had been stopped this once heavily infested strip of cane field had become practically free from hookworm larvae. Similar results were obtained during their studies on the sources of hookworm infestation in a cacao grove (Cort and Payne, 1922*b*). Although soil pollution was not checked samples of soil taken from grossly polluted areas, six weeks after treatment, showed a marked reduction in the number of infective hookworm larvae present.

The results of these laboratory experiments and field observations show that the life of the hookworm larvae in the soils of Trinidad is limited to a few weeks. Whether this finding is constant under a variety of conditions is not known, and experiments are planned to test this point. These results are very different from those obtained with sheathed larvae under laboratory conditions at lower temperatures. It would seem that the differences in these results can be explained by differences in the conditions of the experiments. The conditions of the experiments of the early workers (see Table 1), in which larvae were kept alive for such long periods, were such that the activities of the larvae were greatly reduced and the protective sheaths retained. In my experiments in Trinidad the daily range of temperature during the course of the work was from about 74° F. to 94° F., a temperature

which, if other conditions were favorable, tended to keep the larvae active. Further it was found that a large proportion of the larvae lost their sheaths in the soil. While it is not possible from the information at hand to find any direct correlation between the process of unsheathing and the death rate under favorable conditions, it was found that the unsheathed larvae are more susceptible when in unfavorable environments. As the unsheathing of the larvae appears to be a continuous process, as long as the soil remains infective, the newly unsheathed larvae would be constantly killed when becoming exposed to diverse conditions, even though sheathed larvae were able to withstand the diversity. It can be concluded, therefore, that conditions favorable to intense activity and to the loss of the sheath will tend to shorten the life of mature hookworm larvae in the soil.

SUMMARY.

1. From the observations of various investigators on hookworm larvae under unnatural conditions the opinion has become current that they live for months or even years in the soil, under favorable conditions of temperature and moisture.

2. Laboratory experiments carried on in Trinidad, British West Indies, from May to September, 1921, show that a rapid reduction in the number of hookworm larvae occurs in soils of various types, and that the extent of their life is limited to about six weeks.

3. The completion of the second ecdysis of the infective hookworm larvae in the soil presents a new factor in determining their length of life, which was not found to directly shorten the life of the larvae under favorable conditions, but renders them more susceptible when in unfavorable environments.

4. Experimental evidence indicates that the loss of sheath does not render the mature larvae non-infective which would be the equivalent of their death.

5. The conclusion can be drawn that environmental conditions, such as tropical temperatures which tend to increase the activity of the mature hookworm larvae, will shorten their lives by the more rapid using up of the stored food material.

LITERATURE CITED.

AUGUSTINE, D. L.

1922*a*. Investigations on the control of hookworm disease. VIII. Experiments on the migration of hookworm larvae in soils. AMER. JOUR. HYG., II, 162–171.

1922*b*. Investigations on the control of hookworm disease. IX. On the position of the infective hookworm larvae in the soil. AMER. JOUR. HYG., II, 172–176.

BAERMANN, G.

1917. Über Ankylostomiasis, deren Ausbreitungsbedingungen durch die Bodeninfection und deren Bekämpfung. Geneesk. Tijdschr. Nederl. Indie, Batavia, LVII, 579–669.

BOYCOTT, A. E.

1905. Ankylostomiasis. Transact. Epidemiological Soc., London, M. S. XXIV, 113–142.

CORT, W. W., ACKERT, J. E., AUGUSTINE, D. L., AND PAYNE, F. K.

1922. Investigations on the control of hookworm disease. II. The description of an apparatus for isolating hookworm larvae from soil. AMER. JOUR. HYG., II, 1–16.

CORT, W. W., AUGUSTINE, D. L., ACKERT, J. E., PAYNE, F. K., AND PAYNE, G. C.

1922. Investigations on the control of hookworm disease. III. A discussion of the finding of unsheathed hookworm larvae in the soil. Ibid., 17–25.

CORT, W. W., AND PAYNE, G. C.

1922*a*. Investigations on the control of hookworm disease. VI. A study on the effect of hookworm control measures on soil pollution and infestation in a sugar estate. AMER. JOUR. HYG., II, 107–148.

1922*b*. Investigations on the control of hookworm disease. VII. An epidemiologic study of hookworm disease in a cacao estate. AMER. JOUR. HYG., II, 149–161.

GALLI-VALERIO, B.

1905. Notes de Parasitologie et de technique Parasitologique, Centrbl. Bact., etc., XXXIX, 230–247.

LEICHTENSTERN, O.

1877. Einiges über Ankylostoma duodenale. Deutsche med. Wchschr., XXIX, 669–672.

LEUCKART, R.

1868. Die menschlichen Parasiten und die von ihnen herrührenden Krankheiten. Leipzig und Heidelberg, II, 766.

LÖBKER UND BRUNS, H.

1906. Über das Wesen und die Verbreitung der Wurmkrankheit (Ankylostomiasis), mit besonderer Berücksichtigung ihres Auftretens in deutschen Bergwerken. Arb. a. d. Kais. Gesundh. Amt. Berlin, Bd. XXIII, 421–524.

LOOSS, A.

1911. The anatomy and life-history of Anchylostoma duodenale, Dub. Part II. The development in the free state. Ministry of Education, Cairo, Egypt. Records of the School of Medicine, IV, 613.

NICOLL, WM.

1917. Observations on the influence of salt and other agents in modifying the larval development of the hookworms: Ankylostoma duodenale and Necator americanus. Parasitology, Cambridge (Eng.), IX, 157–189.