THE EFFECT OF LIGHT AND OF DRYING ON INFECTIVE HOOKWORM LARVÆ.

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The Effect of Light.

THE prevailing view is that the light of day is not inimical to hookworm larvæ. Looss[1] discussed the matter fully and arrived at this conclusion after reviewing his own observations and those of earlier workers, some of whom had maintained the contrary. He maintained that any adverse action of sunlight was indirect and due to its "promotion of the processes of decomposition and decay" and raising the temperature. The band of more recent hookworm investigators seems to have accepted the conclusion of Looss and regard sunlight as not directly harmful to the larvæ.

The truth is that some of the earlier observers were right and that continuous exposure to even diffused daylight is rapidly fatal to infective larvæ of both the human species of hookworms.

The writer was led to experiment on the subject by a mysterious mortality of hookworm larvæ contained in watch glasses of water in a room where they were exposed to diffused light only; it had previously been the practice to keep them in a cupboard. For a time some new arrival among the vegetable or animal microorganisms in the water or some other unknown factor was suspected.

Some of the observations made will first be detailed, after which their apparent conflict with those of Looss and others will be discussed.

The writer's observations have been confined to "mature" larvæ in the infective stage, both sheathed and unsheathed, the former preponderating. But some of the experiments of the earlier observers described by Looss and even some of his own seem to indicate that light is injurious to ova and the younger larval stages as well; but Looss did not so interpret them.

As a test of life stimulation of the larvæ by bringing a warm needle near them under the dissecting microscope has been employed. Larvæ which do not respond by the slightest movement, are usually dead or dying, but a later examination has always been made and processes of disintegration looked for. The ages of the larvæ given below are reckoned from the approximate date of hatching, that is one day after culture. They were isolated after seven days' culture and kept in shallow glass vessels of water practically free from particles of earth or sand; the methods of culture and of isolation were those described in a previous paper.[2] All the larvæ used were kept in the dark except during the experiments. When exposures were made to direct sunlight, the maximum temperature reached by the water containing the larvæ at no time exceeded 35·50° C.; the day temperatures in the laboratory varied from about 23° to 26° C..

1. Two shallow vessels of thick glass with plate glass covers, each containing a mixture of larvæ of *Ancylostoma duodenale* and *Necator americanus*, sixteen days old, were placed near a closed window where there was plenty of light, but never direct sunlight. One vessel was within a closed box, the other exposed to the light on top of the box. Three days later every larva was dead and disintegrating in the vessel exposed to the light, the other contained plenty of vigorous larvæ of both species.

2. The last experiment was repeated with larvæ of *Ancylostoma duodenale,* twenty-seven days old, which had just been passed through sand and were in very clean distilled water. The result was the same.

3. Larvæ of *Ancylostoma duodenale,* eight days old, with plenty of "reserve granules" and larvæ of *Ancylostoma braziliense,* fifty-two days old, were exposed to strong sunlight in a watch glass resting on a white ground for two hours. None survived.

4. Larvæ of *Ancylostoma duodenale,* eight days old, were exposed to strong sunlight in a watch glass resting on a white ground for three-quarters of an hour. They were examined two days later, when nearly half were dead. Four days after the exposure all were examined; six were alive and sixty dead; no further mortality occurred among these six.

5. Larvæ of *Ancylostoma duodenale,* forty-one days old, were exposed to strong sunlight for two hours in a silica crucible lid containing a thin layer of wet, coarse yellow sand. No larvæ survived.

6. Larvæ of *Ancylostoma duodenale,* nineteen days old, were exposed in three test tubes to direct sunlight on a hazy afternoon. Tubes (*a*) and (*b*) were surrounded by four centimetres of water and were exposed for two and one and a half hours respectively; tube (*c*) was surrounded by one centimetre of saturated aqueous solution of picric acid and was exposed for two hours. Examined just after the exposure a few only of the larvæ from (*a*) responded to stimulation with the warm needle by feeble movement of the anterior end; some of those from (*b*) moved sluggishly, while those from (*c*) were lively and remained so at every subsequent examination. Next day no movement could be elicited from any larvæ from (*a*); some of those from (*b*) moved sluggishly. Four days after the exposure the larvæ from (*a*) were all decomposing; most of those from (*b*) were dead and the rest still sluggish. Eight days after exposure all but two larvæ from (*b*) were dead, but these seemed now to have recovered their vigour; every larva in (*c*) was alive.

7. Larvæ of *Ancylostoma duodenale,* sixty-one days old, were exposed on a roof for five days, three of which were cloudless. The light which reached them, had to traverse three centimetres of a saturated aqueous solution of picric acid and was somewhat concentrated by refraction at the surface of the cylindrical beaker containing it. The larvæ appeared none the worse at the end of the exposure and most of them were alive two weeks later.

8. Larvæ of *Ancylostoma duodenale,* twenty-seven days old, were exposed in the same manner as in the last experiment, except that the light filter used was three centimetres of a 2% solution of sulphate

of quinine in 0·6% sulphuric acid. The period was four and a half days, of which the first three were cloudless. Examined after two days most of the larvæ responded sluggishly or not all to stimulation with the warm needle; at the end of the four and a half days all were dead and disintegrating.

9. Larvæ of *Ancylostoma duodenale,* twenty-six days old, were exposed to strong sunlight through eight centimetres of water and eleven millimetres of museum jar glass. The period was one and three-quarter hours and the light on the larvæ was somewhat concentrated by refraction. Examined immediately afterwards and four hours later, none made any response to stimulation. The next evening all were certainly dead.

Other observations were made on necator larvæ besides the single one here recorded and it is certain that they share with larvæ of *Ancylostoma duodenale* a susceptibility to light; it was not determined which species is most resistant.

Larvæ, kept in complete darkness in water, suffer practically no mortality for a long time; after four weeks or so it may be difficult to find a single dead specimen; when they are kept in a poorly lighted cupboard, deaths begin to occur at a much earlier period. Looss states that a percentage of larvæ always dies off in the first few days and mentions that his cultures were not protected from light.

These observations prove that a continuous exposure for two hours in clean water to direct sunlight in Townsville is sufficient to kill all mature larvæ of *Ancylostoma duodenale.* Larvæ of *Necator americanus* are similarly affected and both species are killed by a few days' exposure to diffused daylight in a well lighted room. Indications have been obtained that young infective larvæ are rather more resistant than those weeks old. The active part of the solar spectrum is not completely absorbed by passage through water or ordinary glass of the thickness recorded, but is absorbed by aqueous picric acid solution and incompletely by quinine sulphate solution. If the exposure to sunlight has been a little below or a little above the lethal dose, the larvæ may in the former case require days to regain an apparently normal condition and in the latter may linger on for days before they finally die.

The literature of this subject has been consulted as far as possible. None of the hookworm investigations published after the date of the work of Looss[1] which have been seen, appears to attribute a lethal effect to light due to its direct action apart from desiccation and heating. But only abstracts of the important work of Baermann[3] have been available. In the experiments of Nicoll[4] it appears that the sunlight could not have reached the majority of the organisms and the effect found was probably due to heating, as the author himself appears to suggest.

Looss in his discussion of earlier work, most of which has not been accessible, mentions the opinion of Tenholt in 1896 that light is adverse to eggs and larvæ; of Bruns in 1904 that three days' intense sunlight kills eggs and that diffused light will also in time kill eggs and especially the early larval stage and of Lambinet in 1901 that the movements of mature larvæ exposed to the sun become slower and eventually cease and that most of the larvæ are dead in from twenty-four to forty-eight hours.

For the contrary opinion he quotes a longer list and himself supports it emphatically. He argues that his cultures throve, although exposed to plenty of diffused light, but elsewhere states that they were always made with charcoal. Incidentally the possibility may be suggested that the favouring effect of charcoal on cultures made by his method may be due as much to its absorption of light as to its effect on putrefactive processes. In experiments in which development failed to occur in cultures exposed to the sun, Looss attributes the result to the elevation of temperature and promotion of decomposition. He maintains that some experiments, made on a microscope stage with cultures partly illuminated, presumably by daylight, through the substage condenser, were conclusive that light has no action; the possibility of the absorption of harmful ultra-violet radiation by some component of the optical glasses is not considered.

But Looss as well as the more recent investigators might seem to be on strong ground in emphasizing the fact that larvæ will live in soil exposed to sunlight, for instance, European brickfields and irrigated land in Egypt and in pointing out that infective larvæ have a habit of extending themselves singly or in bundles into the air from prominent particles on the surface of a culture. They may also climb the sides of glass vessels and such objects as fragments of dead grass, provided a film of moisture is present. It is said that they may behave in this way both in darkness and in strong light and that there is no evidence that they either seek or avoid the light. From the fact that larvæ do not avoid the light, it is assumed by Looss and others that it cannot be harmful to them.

The publications of the investigators associated with the Rockefeller Foundation contain references to infective larvæ, usually necators, living in ground exposed to direct sunlight and give interesting details of the aerotropic habit under natural conditions and in strong light. Shaded ground is considered more favourable to the development and survival of larvæ than that exposed to the sun, but it is contended that this is not due to any direct adverse action of sunlight, but to its drying action on the soil.

The writer has made but few observations on the phenomenon of aerotropism and has never seen its most striking manifestations. Several observers mention that it is more marked in the larvæ of *Ancylostoma caninum* than in those of the human hookworms, but it has often been observed in cultures of *Necator americanus* larvæ and the observations of Looss were probably made on those of *Ancylostoma duodenale.*

The writer believes that under natural conditions in the soil the direct action of light is probably an important agent in shortening the life of infective hookworm larvæ. It is admitted that this conclusion does not necessarily follow from the obser-

vations here recorded; further work is required to determine whether discontinuous illumination is as harmful as continuous, for instance, short periods of exposure to light separated by long ones of darkness and also whether larvæ in earth under natural conditions die out sooner when exposed to daylight than when kept in obscurity, in conditions otherwise similar. The experiments of Baermann seem to indicate that they do, although they have been interpreted otherwise.

On the other hand the facts at present known concerning survival in soil exposed to the sun and the aerotropic habit certainly do not warrant the generally accepted inference that light is without effect. It is known that the larvæ live near the surface of the ground and sometimes come out and expose themselves fully to strong light; it is not known how often they do this nor for how long at a time. At a depth of a few millimetres below the surface of most soils there would be very little light.

It does not necessarily follow from the fact that larvæ sometimes expose themselves to light that it is harmless to them; it may well shorten their lives in the soil just as their activity of movement in warm climates is known to do; but both practices may increase the chances of a few larvæ reaching the skin of the host.

The factors concerned in the aerotropic habit are incompletely understood; surface moisture and a suitable temperature are necessary conditions. It may be an instinctive reaching out for the mammalian host. It has been suggested that it may be an effort to escape when the conditions in the culture are unfavourable. Radiant heat may possibly play a part; infective hookworm larvæ are very strikingly thermotropic; the warmth of the sun may lure them forth to their destruction.

The very different results obtained by different investigators of the longevity of hookworm larvæ in earth cultures, both natural and artificial, is rather striking. For instance, while some earlier observers found them to live many months, Augustine[5] concluded that the extent of their life is limited to about six weeks, a conclusion which was once more disputed by later investigators. The conditions as to light probably partly account for these discrepancies. The experiments of Augustine were planned to reproduce natural conditions and the vessels containing earth and larvæ were exposed out of doors to the tropical light of Trinidad, sometimes to direct sunlight. He found that the larvæ came to the moistened surface in numbers and rapidly died off in a few weeks.

The Effect of Drying on Infective Hookworm Larvæ.

In the course of other work on infective hookworm larvæ some observations were made on their power to resist desiccation and a few of them are recorded below.

Quite conflicting statements exist on this subject. Looss[1] says that the larvæ succumb as soon as the fluid inside the sheath has evaporated and the larvæ are shrivelled. His observations were made on larve of *Ancylostoma duodenale* in the very dry climate of Egypt. The prevailing view, based mainly on observations on larvæ of *Necator americanus,* is in agreement with Looss that once the larvæ are dry they die almost immediately. The writer also has found that infective larvæ of *Ancylostoma duodenale* can survive drying for short periods only, though for more than a few minutes, in the climate of Townsville. But in apparently dry earth some of them may survive for a few days. The larvæ of *Necator americanus* seem to be less resistant to drying, as was found by Svensson.

The majority of the larvæ used in all the following experiments were sheathed.

1. Some larvæ of *Ancylostoma duodenale* in clean water, seventeen days old, were placed after dark on a small piece of filter paper supported on a needle. At 9 p.m. the paper seemed quite dry. At 9.40 it was placed in water and at 10 p.m. some of the larvæ when detached from the paper and examined under the microscope were seen to be shrunken within their sheaths and air was present round them. Eleven of these larvæ were left in the field of the microscope and at 10.25 they had imbibed water and the air had disappeared. At this time some of them were beginning to move feebly. These eleven larvæ were pipetted into another vessel. Next morning six of them were dead and five alive, though sluggish. In the vessel containing the rest there were also many living and many dead. Some days later a minority of the larvæ was alive, but these seemed quite active. The evening of the experiment was a dry one; the dry and wet bulb readings at 3 p.m. were 23·8° and 18° C. (75° and 64·5° F.).

2. A large drop of clean water containing numerous infective larvæ of *Ancylostoma duodenale* and a smaller number of necators was made to hang from a platinum loop which was then allowed to dry. An hour and a quarter after the completion of drying it was placed in water. Two days later only two larvæ were alive, both vigorous and both ancylostomes. At the time of this experiment the dry and wet bulb readings were 25·7° and 21·2° C. (80·2° and 70·2° F.).

3. Infective larvæ of *Ancylostoma duodenale* and larvæ of *Ancylostoma braziliense,* fifty-one days old, in clean water were placed on pieces of filter paper, where they remained for two and a half hours after the papers seemed dry. The dry and wet bulb readings at the time were 22·7° and 17·3° C. (73° and 63·2° F.). None of the *Ancylostoma braziliense* survived, but about 5% of the *Ancylostoma duodenale* recovered.

4. In this and the two following experiments the earth used was of loose character, devoid of clayey quality and not rich in humus.

About fifty larvæ of *Ancylostoma duodenale,* a month old, were placed in a quantity of wet earth which was left on a piece of paper in the laboratory. Three days later it looked and felt quite dry to the centre and could be crumbled into a dry dust. It was left for two days more when a sample of it was found to lose 0·5% of its weight on prolonged drying at 105° C.. It was then moistened and later placed in the isolation apparatus. One living and active larva was obtained.

In this experiment the earth contained no added organic matter; in the two following old cultures were used which had been made with earth and emulsified fæces.

5. A culture of *Ancylostoma duodenale*, made a month previously, the larvæ in which had never been numerous, was allowed to dry on a sheet of paper. It took seven days to become thoroughly dry throughout and was left for a further three days, when a sample of it lost 1·1% of its weight at 105° C.. It was then wetted and twenty-four living larvæ obtained. The mean dry and wet bulb readings at 3 p.m. during this and the preceding experiment were about 25° and 20·5° C. (77° and 69° F.).

6. Part of a ten-day-old culture which had yielded numerous larvæ of *Ancylostoma duodenale* and *Necator americanus* in the ratio of seven to one, was exposed to the air and appeared thoroughly dry throughout after four days. Twenty-four hours later a part of it, on being wetted, yielded thousands of larvæ; two hundred on examination proved all to be ancylostomes. Five days after it had become dry a further portion after wetting yielded five ancylostome larvæ. The remainder, twelve days after drying, yielded no larvæ. The weather was rainless throughout and the mean dry and wet bulb readings at 3 p.m. were 28·5° and 23° C. (83·3° and 73·4° F.).

Two of these observations suggest that *Ancylostoma braziliense* probably does not differ much from the human hookworms as regards the powers of the larvæ to withstand light and drying. No observations have been made on the larvæ of the other common hookworm of cats and dogs *Ancylostoma caninum*. The larvæ of these species are of interest both on account of the difficulties they may cause in the examination of soil and because one of them is now suspected by American investigators to be the cause of a form of creeping disease in Florida. The photographs and description of the condition leave little doubt that it is the same as a creeping affection of the feet common in Townsville and other parts of North Queensland and popularly known as "sand worm." The writer has made attempts to produce the condition experimentally with larvæ of *Ancylostoma braziliense*, but so far without success. However, *Ancylostoma caninum* seems the more likely of the two to be responsible since *Ancylostoma braziliense* can parasitize man in a normal manner.

Earlier in this paper mention was made of the use of a warm needle to stimulate infective hookworm larvæ. Special apparatus has been devised by Fulleborn and others for the purpose of employing the thermotropism of hookworm and *Strongyloides* larvæ to separate them from free living larvæ of the earth. The warm needle is a very simple instrument by means of which the presence of a few hookworm larvæ among a number of free living larvæ may be detected without recourse to morphological characters or the higher powers of the microscope. A needle with a metal handle is best, since it does not need such frequent reheating in the flame and loss of warmth is at once noticed. The larvæ are examined under a dissecting microscope at the same time that the point of the needle is dipped into the water containing them and passed near to all parts of the collection. Hookworm larvæ which respond to the approach of the needle by a violent increase in activity, are at once detected and can if necessary be pipetted on to a slide for further examination; the free living larvæ make little response.

An observation has just been completed on the effect of discontinuous illumination on hookworm larvæ. Some larvæ of *Ancylostoma duodenale*, ten days old, were exposed in a watch glass resting on white paper to strong sunlight for six minutes each day. The temperature was kept below 31° C.. At all other times they were kept in the dark. Examined just before the fifth exposure, only a few living larvæ were found and these were feeble. Twenty-four hours after the fifth exposure all but one were quite dead and that was nearly dead. The total exposure which killed, was thus half an hour, certainly not a longer time than would have been required if it had been given continuously.

Summary and Conclusion.

It has been shown that, contrary to the accepted view, the light of day rapidly injures and kills infective hookworm larvæ of both the human species; the practical implications are discussed.

Infective hookworm larvæ do not die at once in earth which has been dried in the atmosphere of Townsville; some of them survive for several days; the larvæ of *Ancylostoma duodenale* survive longer than those of necator.

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Reports of Cases.

LARVA MIGRANS (MYIASIS LINEARIS) OCCURRING IN THE TERRITORY OF NEW GUINEA.

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LARVA MIGRANS or *myiasis linearis* is the generic name given to a condition first described by Lee in 1874 and