

**Studies on the Hookworm Larva as a Vector of Pathogenic Micro-Organisms: Ingestion of Bacteria by Hookworm Larvae.**

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Hookworm larvae in their early stages of development are intimately associated with materials heavily contaminated with pathogenic microorganisms. The present investigations are directed toward the possibility that these larvae might act as vectors in the dissemination of certain diseases related in their epidemiology to soil contamination.

The first requirement was to establish the nature of the association existing between larvae and micro-organisms. McCoy<sup>1</sup> has shown that hookworm larvae are able to grow to the infective stage on 22 out of 25 species of bacteria tested. He concludes that all of the evidence from his experiments indicates that living bacteria constitute the essential food utilized by hookworm larvae in developing to the infective stage.

The present work was undertaken to determine whether or not these bacteria could be ingested in their intact form. Certain evidence is presented to show that this is a physical possibility. Of less certainty is the demonstration of bacteria within various portions of the alimentary tract of immature larvae and within the sheath spaces of infective larvae.

The observations were made on living larvae by means of a technic in which the larvae may be studied under the oil immersion power of the microscope. The larvae therefore show no alteration or distortion of structure such as might be produced by death, staining or fixing of the specimens. The observations and measurements can readily be made during more or less prolonged periods of inactivity of the larva.

The technic depends upon the imprisonment of an actively swimming larva in a droplet of water surrounded and covered by cedar immersion oil. The larva is removed from the culture by means of a capillary pipette and transferred to a glass slide. The water medium is reduced to a tiny droplet slightly larger in diameter than the length of the larva by drawing off most of the drop with a cap-

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<sup>1</sup> McCoy, Oliver P., *Am. J. Hygiene*, 1929, **10**, 140.

illary pipette and then allowing evaporation to complete reduction to the desired size. At the proper instant a large drop of cedar oil is allowed to fall directly over the water droplet. A cover-slip completes the preparation.

If larvae are suspected of carrying bacteria it is necessary to demonstrate that they are physically capable of ingesting bodies of bacterial magnitude.

For this purpose *Necator americanus* larvae from patients in the Charity Hospital were examined from within a few hours after hatching to the time of their second ecdysis. The parts measured were the diameters at the base of the oral cup, the inlet and greatest width of the esophageal cylinder and the greatest observed distensibility of the canal of the esophagus, bulbus esophagi and intermediate body.

From this series of measurements it was found that in all of the structures named the dimensions were of such size as to accommodate bacteria of at least the size of *Bacillus coli*. It is believed that none of the flexible canals of the esophagus or bulb were seen at their greatest distensibility.

The second portion of the experiments consisted of attempts to visualize bacteria within the bodies of the larvae.

Larvae immediately after hatching were placed in cultures containing heavy suspensions of *Bacillus coli* or *Staphylococcus aureus* or were left in the original filtrate from the Baermann isolating apparatus. At intervals of several minutes, hours or days the larvae were removed and examined.

Three series of larvae were studied:

Series A consisted of 25 larvae which had been living since isolation in suspensions of colon bacilli and staphylococci. All larvae were examined within the first 4 days after hatching. All were active and their mouth parts still open.

Nine of the series (36%) showed motile bodies within the oral cavity, esophageal cylinder or lumen of the intestine. These bodies were round, oval or rod-shaped, smooth surfaced, darkly refractile and could not be visually differentiated from the colon bacilli and staphylococci seen immediately adjacent to them in the surrounding medium.

Series B. Eleven larvae immediately after hatching and isolation were placed in suspensions of *Bacillus coli*. All of the larvae matured in the suspensions and were examined at intervals of 1 to 31 days after reaching the infective sheathed stage. No bacteriform bodies were seen within the alimentary tract of any of this series,

but in 7 (63.6%) bodies similar to those seen in Series A were found within the space between the cuticle of the larvae and their sheaths.

Series C. Forty-three larvae were matured in the original filtrate from the Baermann isolator. This filtrate contained colon bacilli and staphylococci as determined by culture. The larvae were examined at intervals up to the sixteenth day after the second ecdysis. Twenty-two (51.1%) presented one or more bacteria-like bodies within their sheath spaces but none within their alimentary tracts. The average number of such particles was 12 per larva.

Of the total of 79 larvae of the 3 series 38 (48.1%) showed the presence of bodies of bacterial morphology and magnitude within some portion of their alimentary tracts or sheath spaces.

It cannot be positively asserted at this time that these bodies are actually bacterial or even viable.

Preliminary experiments have been performed with the purpose of liberating these bodies by amputation of the tail end of the sheath. Results have shown certain evidence that the particles in question are actually bacterial and can be cultured. This will be investigated further.

*Conclusions.* 1. The mouth parts and alimentary canal of immature larvae of *Necator americanus* are of such size as to accommodate particulate matter of bacterial magnitude. 2. Particles morphologically resembling colon bacilli and staphylococci have been seen within various portions of the alimentary canal of immature larvae of *Necator americanus* which had been living in a medium containing these micro-organisms. 3. Sheathed larvae of *Necator americanus* have been seen to carry bacteriform bodies within their sheath spaces for periods of from 1 to 31 days after the second ecdysis.

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### Observations on the Nutritive Value of Certain Fats.

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In connection with experiments concerned with the growth of rats on low-fat diets, observations have been made which emphasize the nutritive value of certain fats, and illustrate the marked variance in