CERTAIN FACTORS WHICH INFLUENCE ACTIVATION OF THE HEXACANTH EMBRYO OF THE FOWL TAPEWORM *RAILLIETINA CESTICILLUS*[1]

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While carrying out life history studies on the fowl tapeworm, *Raillietina cesticillus,* great variability was noted in the number of active six-hooked embryos found in different gravid proglottids collected from the feces of infected fowls. Proglottids, which are shed from the terminal portion of adult tapeworms, contain from 150 to 300 of these onchospheres or hexacanth embryos. In some preparations, made by teasing apart proglottids, all embryos remained quiescent, but in other preparations most of the embryos showed the remarkable breast-stroke-like activity which is typical of embryos in this stage of tapeworm life history.

Although the movements of the hexacanth embryos have been induced frequently in other species, (*Taenia taeniaeformis* by Bullock, Dunning, and Curtis 1934, *Taenia saginata* by Penfold, Penfold and Phillips 1937, *Dipylidium canium* by Venard 1938, and *Bertiella studeri* by Stunkard 1940) spontaneous hatching does not normally occur unless these larvae are first acted upon by enzymes or pressure. In *Raillietina cesticillus* the violent swinging action of the six hooks may result in rupture of both the embryophore and the outer uterine shell, thus freeing the embryo from surrounding membranes. The freed embryo normally works its way through the gut wall of a suitable species of beetle (Reid, Ackert, and Case, 1938). Within two to three weeks the onchosphere metamorphoses into a cysticercoid. If the beetle containing fully developed cysticercoids is eaten by a chicken, the freed larvae attach to the gut wall and grow to adult tapeworms.

A study of the factors inducing motility of the embryos was carried out for two reasons. First, an understanding of factors inducing motility would contribute to the knowledge of the life history of this organism and might help to explain the irregular infectivity of beetles under laboratory conditions. During attempts to produce cysticercoids for studies of the pathology or for treatment of this species, a low percentage of infestation has sometimes been encountered. A second reason for this study was to further enhance the use of these embryos for teaching purposes. Due to their large size and the transparent covering membranes, they make excellent demonstrations of this larval stage. However, inability to induce

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movement of the larvae has at times made them less effective.

A systematic study of the effects of (1) osmotic pressure changes, (2) pH concentration, and (3) temperature changes is reported upon in the present study.

Materials and Methods

Fowls were infected and proglottids were collected, according to methods described by Reid (1942). The freshly passed gravid proglottids were stored in fluids or kept moist on filter paper in covered Stender dishes before the count was made. To count the active or inactive onchospheres the proglottid was teased apart, placed in fluid on a glass slide, and covered with a cover glass. The slide was then sealed with Vaseline to prevent evaporation. The first 100 embryos were counted with the aid of a mechanical stage and classified according to the three categories: (1) living and motile; (2) living and non-motile; and (3) dead. If an embryo showed either active movements which might result in hatching or only slow lazy movements, it was classified as motile. Dead embryos could be distinguished by clouding of the normal hyaline protoplasm and disorientation of the hooks. Since considerable variability was found in the amount of motility, in later studies each proglottid was cut in half with one half serving as a control while the other half received the experimental treatment.

Effect of Osmotic Concentration Upon the Embryo

Numerous comparisons were made between the motility of onchospheres in one percent saline solution and those maintained in distilled water or two percent saline solution. No marked increase in motility was induced by any of these solutions. However, numerous embryos died after 30 minutes of exposure to distilled water. In a representative case, 90 percent of the embryos were living but non-motile after five minutes exposure and ten percent were dead. After 30 minutes, 47 percent of these same embryos were living but non-motile and 53 percent were dead. Two percent saline also had a deleterious effect upon the embryos, but it was not as rapid. In a typical trial, eight percent of the embryos were dead after five minutes, nine percent after 30 minutes, and 62 percent were dead after one hour. Embryos maintained in one percent sodium chloride were affected little, if at all, by the treatment. In a count, two percent of the onchospheres were dead after five minutes exposure and three percent after 30 minutes. This study showed that of the solutions tested, one percent saline is the optimum osmotic concentration in which to store the embryos, but that osmotic changes do not induce motility.

Effects of pH upon the Embyro

Since embryos are subjected to pH changes in the gut of the host, the sensitivity of embryos to three different solutions was tested. Using a one percent sodium chloride solution buffered to a pH of 3.6 by means of a 0.2M acetate buffer, no motility was induced in the embryos, but 64 percent were dead after five minutes exposure. All were dead at the end of seventeen minutes. One percent

saline solution buffered to a pH of 8.0 or to a pH of 6.0 by means of phosphate buffer induced no motility, but exposure for 30 minutes did not kill the embryos. It is concluded that a change in pH is not the factor which results in embryo activation.

Effects of Temperature upon the Onchosphere

Preliminary tests indicated that an increase from room temperature (21±3°C) to 37°C did not induce motility, but possibly increased the mortality rate on embryos exposed for two hours. However, a decrease from room temperature to 6-7°C for a few hours not only induced marked increase in motility, but fewer embryos died. A more thorough study of the effects of lowered temperature carried out by one of us (Nice, 1949) definitely established the effects of exposure to cold in inducing motility. By counting 100 embryos from each of ten half-proglottids which had been stored at 1°C for eighteen hours, an average of 78.7 (range 56-91) embryos were found active in each half-proglottid. In the other half-proglottids which were used as controls and stored at 21±3°C for eighteen hours, only 0.1 percent of the embryos were motile, while 96 percent were living but non-motile. None of the embryos stored in the cold were dead, but 3.9 percent of those kept at room temperature died.

Discussion

The cold-induced activity of these onchospheres may have adaptive significance in the life history of the parasite. Successful infection of the secondary host depends upon penetration of the gut wall of the beetle through activity of the embryo. Onchospheres in this species seldom live more than a day unless the temperature is cool. It has been shown by Wetzel (1934) and confirmed by Reid, Ackert, and Case (1938) that the proglottids of *Raillietina cesticillus* are shed in larger numbers late in the afternoon. Many of the beetle hosts are nocturnal in their feeding habits so that proglottids shed late in the day are eaten during the following night when a natural temperature drop occurs. For natural infections the nocturnal temperature could not drop as low as the 1 C° used in the experimental study, since the beetles themselves would be inactive at that temperature. However, the greatest numbers of onchospheres are consumed during that period when the temperature is lowest. Lack of temperature control after collecting proglottids for laboratory infections may account for some of the variability in infestation which has been encountered by various workers.

Summary

1. Decrease from room temperature (21±3 C°) to 1 C° induced activity in 78.7 percent of 1,000 embryos as compared to 0.1 percent motility found in 1,000 controls maintained at room temperature.

2. No other factors studied induced motility. A pH concentration of 3.6, distilled water, 2 percent sodium chloride solutions, and maintaining the embryos at room temperature or above increased the mortality rate of embryos, but pH ranges of 6.0 and 8.0 and one percent saline did not hasten death of the embryos.

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