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OBSERVATIONS ON THE DEVELOPMENT AND SIGNIFICANCE OF *Leucocytozoon simondi* MEGALOSCHIZONTS AND A TECHNIQUE FOR THEIR ISOLATION*

The role of the megaloschizont in *Leucocytozoon simondi* (Mathis and Leger) infections has not been explained. Chernin (1952, J. Parasitol., 38: 499-508), Fallis et al. (1951) Canad. J. Zool., 29: 305-328), Kocan et al. (1967, J. Protozool., 14: in press), and Desser (1967, J. Protozool., 14: 244-254) have all indicated that most of the megaloschizonts are lost between 1 and 2 weeks after infection. This corresponds closely with the first appearance of elongate gametocytes and has led to the belief that the megaloschizont is the precursor of the elongate gametocytes.

Two experiments were designed to determine: 1) what stage of the parasite gives rise to the megaloschizont and 2) what stage(s) arise from the megaloschizont. The first experiment consisted of infecting three Pekin ducklings with sporozoites from infected black flies and 4 days later 4 ml of blood from each of these was transfused (I.V.) to three uninfected ducklings. The only stages found in the blood at this time are 1 - 2 μ intraerythrocytic merozoites. Both groups were then followed by blood smear examination. On the 8th, 9th, and 10th day following the sporozoite infection, one duckling from each group was killed and the spleens examined for megaloschizonts.

The second experiment involved the isolation of large numbers of megaloschizonts and the removal of all host cells which might be infected with some other stage of the parasite. This was accomplished by removing the spleens of infected ducks on the 10th day postexposure, just prior to megaloschizont loss, and mincing them in Hank's Balanced Salt Solution. This material was then poured through four layers of gauze into a conical centrifuge tube and allowed to settle undisturbed. The free megaloschizonts, being very dense, settled rapidly to the bottom allowing the remainder of the host cells, still in suspension, to be decanted. After several resuspensions the megaloschizonts were microscopically clean of any host cells (Fig. 1). About ¼ ml of packed megaloschizonts were collected this way. Megaloschizonts can be collected in this way from days 8 through 10 postinfection. Prior to the 8th day they remain too closely associated with host tissue to be purified. Since we were interested in what arose from this stage, the 10th day was chosen because the megaloschizont ruptures at about this period.

Once the megaloschizonts were purified and found to be free of all cellular material other than megaloschizonts, they were resuspended and divided into two equal aliquots. One of these was injected intraperitoneally into a 3-day-old Pekin. The other was placed in a tissue grinder and the megaloschizonts were gently disrupted. This suspension was injected intravenously into another 3-day-old Pekin.

The results of the first experiment showed that identical parasitemias developed in both the sporozoite infected birds and the transfused birds except that the latter had fewer parasites per volume of blood than the former. They both showed the first mature round gametocytes on the same day (7 days after sporozoite injection). Megaloschizonts were also present in the spleens of both groups upon autopsy, the transfused group appearing to have fewer than the other group.

It is evident that the precursor to both the round gametocytes, or immature forms of these, and megaloschizonts are present in the circulation prior to the appearance of mature gametocytes. There was apparently no alteration of the

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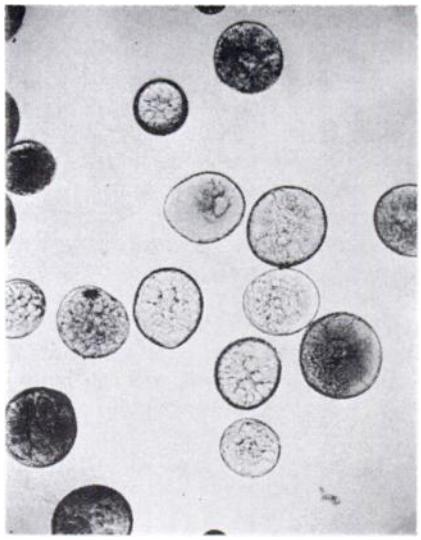

FIGURE 1. *Suspension of* Leucocytozoon simondi *megaloschizonts free of host cells.*

transfused stages since parasite development proceeded simultaneously in both groups. This is in agreement with Dresser (1967, J. Protozool., 14: 244-254), who transfused blood from a newly infected duck to an already patent duck and got an increase in round gametocytes followed by elongate gametocytes and with Fallis et al. (1956, Canad. J. Zool., 34: 389-404) who did the same with uninfected recipients. Having killed our ducks prior to the 11th day, the expected day of elongate gametocyte appearance (Kocan & Clark, 1966, J. Parasitol., 52: 962-966), we have no way of knowing if they would have occurred at all. Since megaloschizonts were present, it is possible that they would have produced elongate gametocytes if the birds had not been killed.

The experiment to determine what developed from megaloschizonts was negative. Neither the duckling receiving whole megaloschizonts nor the one receiving disrupted megaloschizonts showed any circulating parasites for 7 days following inoculation.

Different results might have been obtained if a more precise timing of transfer were employed, or the manipulation of the megaloschizonts might have altered them sufficiently to render them noninfectious. It is also possible that megaloschizonts are not the source of elongate gametocytes and that their disappearance in the 11th day post infection, and the simultaneous appearance of elongate gametocytes, is only a coincidence. As Wehr (1962, Avian Diseases, 6: 195-210) pointed out, elongate gametocytes are present in turkeys infected with *L. smithi* but no megaloschizonts have been found.

Whatever the reason for the negative results, it is hard to imagine that the megaloschizont does not play some major role in the development of *L. simondi*. Further research is needed to clarify the role of the megaloschizont in the life cycle of *Leucocytozoon*.

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PEROSIS IN CANADA GEESE
(Branta canadensis)

Perosis (slipped tendon), a condition characterized primarily by deformities of the leg bones, is commonly encountered in young chickens and turkeys maintained on rations either deficient in one or more essential nutrients or containing them in improper balance. A lack of manganese is a common cause in poultry (Norris and Scott, 1965. *In* Biester and Schwarte (ed.) Diseases of Poultry:144-180. Fifth Ed. Iowa State Univ. Press, Ames; Siegmund (ed.), 1967. *In*