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Gizzard Lesions Associated with *Haemoproteus sacharovi* Infections of Pigeons[1]

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Abstract. Becker et al. (1956) described abnormally enlarged spleens and granular gizzards observed in several killed pigeons. Blood films made from these particular birds were diagnosed as positive for *Haemoproteus sacharovi*. Since mourning doves represent a host for this parasite, four infected doves were killed and selected tissues sectioned and examined in an attempt to locate sites of exoerythrocytic schizogony. None were observed although the spleens of these birds were markedly swollen and colored a mottled purplish-black. Concerning pigeons, however, granular gizzards were observed in each of eight *H. sacharovi* infected pigeons killed. Examination of sections of infected gizzard revealed areas of small, round, dark-staining bodies. These areas were bounded by membranous capsules closely resembling mature splenic megaloschizonts of *Leucocytozoon simondi*. The haemosporidian character of these schizonts was further strengthened by their failure to provoke a cellular response as long as the capsule remained intact. Once the schizonts ruptured, however, an immediate phagocytic response was observed. Attempts to transmit infection by inoculating young pigeons with macerated gizzard were unsuccessful. Thus, the evidence that gizzard lesions observed in pigeons infected with *H. sacharovi* are sites of exoerythrocytic development is suggestive rather than conclusive.

Becker and co-workers (1956, 1957) described naturally occurring *Plasmodium* and *Haemoproteus* infections in the common pigeon. Birds harboring these infections were obtained from a pigeon colony at Gilbert, Iowa. The occurrence of *Haemoproteus sacharovi* Novy and MacNeal in a number of these pigeons afforded an opportunity to investigate the habits of this parasitic haemosporidian.

If we assume that *H. sacharovi* follows a developmental course similar to that of *H. columbae* Kruse and *H. lophortyx* O'Roke, gametocyte development occurs within erythrocytes, sexual development and sporogony in invertebrate hosts and exoerythrocytic development within tissue cells. Observations of *H. sacharovi* gametocytes within erythrocytes of infected pigeons and mourning doves are well documented. In addition, it is generally accepted that the processes of fertilization and ookinete development are limited to the invertebrate hosts of the family Hippo-

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boscidae (louse flies). Huff (1931, 1932) reported the successful transmission of *H. sacharovi* from infected mourning doves to domestic pigeons, using laboratory-reared *Pseudolynchia maura.* Recent authors, however, (Baker, 1957; and Hanson et al., 1957) question louse flies as the sole disseminators of *Haemoproteus.* Fallis and Wood (1957) and Fallis and Bennett (1961) incriminate species of *Culicoides* (Ceratopogonidae) in the transmission of *Haemoproteus* and other parasites. The sites of exoerythrocytic development of *H. sacharovi* in tissues of mourning doves and pigeons, however, have yet to be described.

Becker et al. (1956) refer to abnormally enlarged spleens and granular gizzards observed in a number of killed pigeons. Some of the blood smears made from these particular birds were diagnosed as positive for *H. sacharovi.* Accordingly, histological sections of tissues removed from infected mourning doves and pigeons were examined to locate sites of exoerythrocytic schizogony of *H. sacharovi.*

Materials and Methods

Staining techniques employed for sections of avian tissue included Mallory's triple connective stain, Heidenhain's "Azan" triple stain, the Feulgen technique with fast green counterstain, and Delafield's haemotoxylin counterstained with eosin.

Fixation of tissues was usually accomplished with Bouin's, although 10% formalin, Zenker's and A.F.A. were also used.

With muscular tissue such as the gizzard, in which infiltration of paraffin would be expected to be difficult, the dioxan method was used. Even this technique was not found to be entirely satisfactory for obtaining unwrinkled sections. Soaking the paraffin block in a mixture of 95% ethanol and glycerine for a period of several hours proved helpful in obtaining smooth sections. Such glycerine-alcohol treated blocks permitted sections of 6 to 10μ to be obtained with little difficulty.

Results

Attempts were made to locate the site of exoerythrocytic development of *H. sacharovi* in infected mourning doves. During the course of the study, 41 doves were trapped and their blood was examined for haemosporidian parasites. Thirty-one of these birds were found to be infected, with 21 harboring *H. sacharovi* infections. To be sure that birds selected for this study were infected with *H. sacharovi* only, isodiagnostic techniques (Sergent, 1920) were used to eliminate the possibility of latent *Plasmodium* infections. One dove proved to be positive for *Plasmodium,* although erythrocytic stages were not demonstrable in its blood. Eventually seven birds, apparently harboring *H. sacharovi* only,

were selected and their blood carefully examined every other day for a period of 60 days. Three of these birds were found to harbor *Leucocytozoon marchouxi* infections that had not been detected previously. The four remaining doves harbored only *H. sacharovi*.

One dove was killed at the time developing gametocytes were demonstrable in its blood. The spleen of this bird was swollen and colored a mottled purplish-black. It was very fragile and ruptured easily when being removed from the killed animal. Pieces of the liver, lungs, kidney, spleen, gizzard, heart and brain were removed, fixed, sectioned, stained and examined. Sections of the lung were examined with extreme care, for it is known that asexual development *H. columbae* occurs within cells of the capillaries of this organ. Nothing was seen to indicate the lung or other tissues as sites of exoerythrocytic development.

Two more doves were killed following the onset of a relapse. As in the previous bird, the spleens were purplish-black. Pieces of liver, lungs, kidneys, spleens, hearts, gizzards and brains were removed, fixed, sectioned, stained and examined. No areas of asexual development were seen.

The remaining dove was killed after being under observation for 120 days. Rare, mature gametocytes were observed in its blood at the time of sacrifice. Tissues were removed, sectioned, and examined. The spleen was swollen, fragile and a dense purplish-black in color. Examination of sectioned material revealed nothing new concerning the site of exoerythrocytic schizogony.

Becker et al (1956), in describing the natural occurrence of *Plasmodium* and *Haemoproteus* infections in pigeons, also referred to abnormally enlarged spleens and granular gizzards observed in a number of freshly killed pigeons. Further investigations have shown these gizzard lesions to be present in each of the eight *H. sacharovi* infected pigeons killed.

Accordingly, pieces of gizzard were removed from freshly killed pigeons known to harbor patent *H. sacharovi* infections. Gross examination and observation of a cut surface of an infected gizzard revealed small dark and yellowish spots scattered throughout the organ (Fig. 1).

Examination of sections of infected gizzard revealed the presence of masses of small, round, clearly defined, dark-staining bodies. These areas were bounded by apparently membranous capsules of varying thickness (Figs. 2 and 3). These areas closely resembled mature splenic megaloschizonts encapsulated within connective tissue membranes described by Cowan (1955) in *Leucocytozoon simondi* infected ducks. Other encapsulated areas, filled with erythrocytes, were also observed (Fig. 4). The host

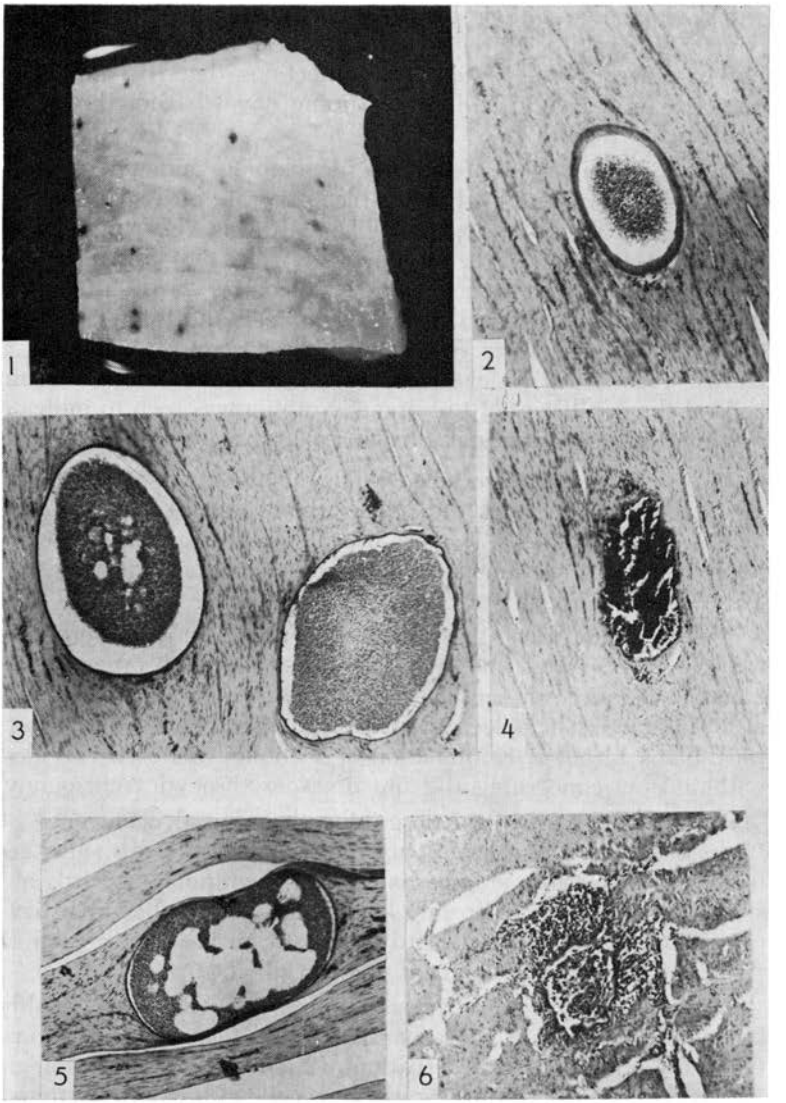

Figure 1. Cut surface of an infected gizzard removed from a four week old pigeon.
Figures 2 and 3. Sections of an infected gizzard revealing encapsulated masses of small round clearly defined dark staining bodies. These are thought to represent schizonts of *H. sacharovi*.
Figure 4. A ruptured schizont filled with erythrocytes. Note crenated capsular wall.
Figure 5. An intact schizont illustrating the lack of a cellular host response.
Figure 6. A ruptured schizont illustrating a marked cellular host response to its presence.

apparently did not react to the presence of these cysts so long as the cyst wall remained intact (Fig. 5). A host response is obvious once the cyst wall ruptures, however (Fig. 6).

These lesions have also been noted in killed squabs, whose blood, examined several days previously, was parasite-free. Consequently, although it is suspected that these areas represent the site of exoerythrocytic development of *H. sacharovi* in pigeons, the evidence although suggestive is not conclusive.

Since Gonder (1915), O'Roke (1930), and Lastra and Coatney (1950) have all reported successful transmission of *Haemoproteus* infections using injections of macerated tissue or tissue transplants, similar techniques were employed during this study.

Since the lungs are generally considered to be the site of exoerythrocytic development of *Haemoproteus*, these organs and pieces of gizzard were removed from freshly killed pigeons known to harbor *H. sacharovi* infections. Several small pieces of lung were transplanted to the pectoral muscles of anesthetized pigeons. Four laboratory-reared pigeons used in these experiments failed to develop patent infections, however. Pieces of infected gizzard were similarly transplanted to a single laboratory-reared pigeon. Four other birds were inoculated with infected gizzard macerated in normal saline. The five birds failed to develop patent infections of *H. sacharovi*.

Discussion

Lesions observed in the gizzards of pigeons harboring patent infections of *H. sacharovi* are suspected to be the site of exoerythrocytic development of these organisms in pigeons. Several reasons may be advanced to substantiate this statement. First, these lesions were observed in gizzards in every sacrificed pigeon harboring gametocytes of *H. sacharovi*. Second, there is a similarity of appearance between the small encapsulated areas observed in infected gizzards and those mature megaloschizonts described by Cowan (1955). Admittedly, Cowan described splenic schizonts of *Leucocytozoon simondi*. However, the two genera, *Haemoproteus* and *Leucocytozoon*, are generally considered to belong to the same family. Accordingly, the appearance of exoerythrocytic schizonts may be similar. Finally, the lack of cellular response to the presence of developing schizonts in characteristic of haemosporidian asexual development. Shortt and Garnham (1948), Shortt et al. (1951), and Garnham and Bray (1956) referred to this phenomenon in relation to pre-erythrocytic schizonts of *P. falciparum*, *P. vivax* and *P. cynomolgi*. The schizonts were "silent" and provoked no response. Once the schizonts ruptured, however, an immediate phagocytic response was observed. A similar pattern is demonstrable in sections of infected gizzard.

Unfortunately, injections and transplants of infected gizzard into pigeon hosts failed to induce infection of *H. sacharovi*. Thus,

the evidence that these lesions represent sites of exoerythrocytic schizogony of *H. sacharovi* remains circumstantial.

Although considerable information may be accumulated concerning developmental stages of *H. sacharovi*, discovery of the invertebrate host(s) responsible for its transmission would permit verification of these data by enabling bird-to-bird transfer to be carried out in the laboratory. Thus, clarification of this asexual phase of *H. sacharovi* infections remains dependent upon discovery of the definitive host.

Summary

1. The area for exoerythrocytic development of *H. sacharovi* in the mourning dove was not found.
2. Attempts to transmit *H. sacharovi* by use of tissue transplants and inoculations of macerated lung tissue were unsuccessful.
3. Attempts to transmit *H. sacharovi* to pigeons by use of tissue transplants and inoculation of macerated granular gizzards of infected pigeons were also unsuccessful.
4. Gizzard lesions observed in pigeons infected with *H. sacharovi* are suspected to be the site of exoerythrocytic development of this parasite in pigeons. The evidence while suggestive, is not conclusive.

Literature Cited

Baker, J. R. 1957. Protozool. 4: 204-208.
Becker, E. R., W. F. Hollander and W. H. Pattillo. 1956. Parasit. 42: 474-478.
———, ——— and J. N. Farmer. 1957. Proc. Iowa Acad. Sci. 64: 648-649.
Cowan, A. B. 1955. J. Protozool. 2: 158-167.
Fallis, A. M. and D. M. Wood. 1957. Canad. J. Zool. 35: 425-435.
——— and G. F. Bennett. 1961. Mosquito News 21: 21-28.
Garnham, P. C. and R. S. Bray. 1956. Rev. Bras. Malar. Doencas Tropicas 8: 151-160.
Gonder, R. 1915. Arch. Protistenk. 35: 319-323.
Hanson, H. C., N. D. Levine, C. W. Kossack, S. Kantor and L. J. Stannard. 1957. J. Parasit. 43: 180-193.
Huff, C. G. 1931. J. Parasit. 18: 122.
———. 1932. Am. J. Hyg. 16: 618-623.
Lastra, I. and G. R. Coatney. 1950. J. Nat. Malar. Soc. 9: 151-152.
O'Roke, E. C. 1930. Univ. Calif. Pub. Zool. 36: 1-50.
Sergent, E. T. 1920. Compt. Rend. Soc. Biol. 83: 1063-1064.
Shortt, H. E., N. H. Farley, G. Covell, P. G. Shute and P. C. Garnham. 1951. Trans. Roy. Soc. Trop. Med. Hyg. 44: 405-419.
——— and P. C. Garnham. 1948. Trans. Roy. Soc. Trop. Med. Hyg. 41: 785-795.