BRIEF NOTE

Studies on Feline Haemobartonellosis

III. Scanning Electron Microscopy of *Haemobartonella felis*

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The morphologic features of *Haemobartonella felis* (*H. felis*) in blood films after Giemsa or Wright stain are well known, that is; they appear as a purple rod, coccoid or ring bodies attached to the erythrocytes [1, 3, 5, 6]. Transmission electron microscopy has revealed that the organisms were surrounded by a single or double membrane, having an internal structure consisting of granular, electron-dense materials embedded in electron-lucid substances [2, 6, 7]. Recently, the scanning electron microscopic features of *H. felis* parasitizing erythrocytes from a naturally infected anemic cat were reported by Jain and Keeton [5].

This brief note contains the results of observation of the three-dimensional morphology of *H. felis*, which have not been reported by this time.

Materials and methods: A healthy female cat, 2-year-old, was injected intraperitoneally with 1 ml of the blood from

an anemic cat naturally infected with *H. felis*. Blood sample was collected by venesection of the auricular vein on the 17th day of inoculation. At that time, the erythrocytes were highly parasitized with *H. felis*. Five drops of the blood were added to 20 ml of cold 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. After fixation for 2 hours at 4°C, the erythrocytes were washed with 0.1 M phosphate buffer, dehydrated in an ascending series of acetone (50 to 100%), and resuspended in absolute acetone. A small amount of the cell suspension was dropped and air-dried rapidly on a thin coverslip. Small pieces of coverslip films were then cut, stuck to aluminum stubs, and coated with a thin layer of silver and gold. Some of the coverslip films were etched by ion with the method of Fujita et al. [4]. The preparations were examined at 30-degree angle with a scanning electron microscope, Hitachi HHS-2R, at an accelerating voltage of

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Fig. 1. Blood smear showing *Haemobartonella felis* on the erythrocytes. Giemsa stain. $\times 2000$.
 Figs. 2-4. Discoid-shaped organisms are seen singly, in pairs, in a row and in a cluster attached to the erythrocytes. Fig. 2, $\times 10000$; Fig. 3, $\times 15000$; Fig. 4, $\times 12500$.
 Fig. 5. The surface of the erythrocytes on which the organism is attached are indented. By ion-etching method. $\times 10000$.
 Fig. 6. A coccoid body (c) and discoid-shaped organisms. Discoid organisms appear as in pairs. Notice a discoid organism (arrow) showing binary fission. $\times 9000$.

25 kV.

Results and discussion: The organisms on Giemsa stained preparations were predominantly discoid and some were coccoid or ring in form, and purple or faint violet in color. The purple organisms were smaller than those stained faint violet (Fig. 1). In scanning electron microscopy, the organisms, about 0.5 to $0.6\mu\text{m}$ in diameter, were mostly discoid with a shal-

low concavity at the centre of the bodies (Figs. 2-4, 6). Coccoid or lemon-shaped ones, about 0.2 to $0.3\mu\text{m}$ in diameter, were also observed (Fig. 6). The organisms were attached to the erythrocyte singly, in a pair, in a row, or in a cluster, and the surface areas of the erythrocyte on which the organism was attached appeared to be indented (Fig. 5). Most of the discoid-shaped organisms appeared to be attached to the

erythrocyte in pairs, respectively (Figs. 3, 4, 6). The organisms at a stage of binary fission was seldom observed (Fig. 6).

Jain and Keeton revealed that *H. felis* was of conical forms with variably tapered free ends, coccoid forms or rod-shaped forms in scanning electron microscopy, and that some affected erythrocytes had small shallow pock-marks, which were thought to represent lesions produced by parasitic adherence.

In the present observation, most of the organisms were discoid and some were coccoid, and no rod-shaped organisms were observed. And also, such lesions like shallow pock-marks were not seen on the erythrocyte. Discoid-shaped organisms and the organisms showing binary fission were not observed by Jain and Keeton. Discoid-shaped organisms appeared to be attached to the erythrocyte in pairs, which seemed to be the result of binary fission of the organism. In the present study, the blood sample was collected from an experimentally infected cat at a period when almost all the erythrocyte were parasitized with *H. felis*. From the fact, it appears that the proliferation of the organisms occurred explosively just before our sampling, and that the discoid-shaped organisms may be the vegetative form of *H. felis*.

Summary: The morphologic features of *H. felis* were studied with a scanning elec-

tron microscope, using highly parasitized blood from an experimentally infected cat. The organisms were mostly discoid, 0.5 to 0.6 μ m in diameter, with a shallow-concavity at the centre of the body, and coccoid or lemon-shaped ones, 0.2 to 0.3 μ m in diameter, were also observed. The organisms were attached to the erythrocyte singly, in a pair or in small clusters. Most of the discoid-shaped organisms appeared to be attached to the erythrocyte in pairs. Some organisms appeared to be at a stage of binary fission on the erythrocyte surface.

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References

- [1] Bedford, P. G. C. (1970). *Vet. Rec.*, **87**, 305-310.
- [2] Demaree, R. S., and Nessmith, W. B. (1972). *Amer. J. vet. Res.*, **33**, 1303-1308.
- [3] Flint, J. C., Roepke, M. H., and Jensen, R. (1958). *Amer. J. vet. Res.*, **19**, 164-168.
- [4] Fujita, T., Nagatani, T., and Hattori, A. (1974). *Arch. histol. jap.*, **36**, 195-204.
- [5] Jain, N. C., and Keeton, K. S. (1973). *Amer. J. vet. Res.*, **34**, 697-700.
- [6] Small, E., and Ristic, M. (1967). *Amer. J. vet. Res.*, **28**, 845-851.
- [7] Venable, J. H., and Ewing, S. A. (1968). *J. Parasit.*, **54**, 259-268.