Hemobartonellosis and Eperythrozoonosis

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**Synonym.** Eperythrozoon spp. and Hemobartonella spp. of the order Rickettsiales, family Anaplasmataceae, are small (350 μm), pleomorphic, extracellular parasites infecting a wide variety of mammalian hosts (Moulder 1974).

**Gross Appearance**

The phagocytic elements of the spleen, liver, lymph nodes, and other organs appear to be primarily responsible for limiting the multiplication of these parasites. Splenic macrophages are especially important in suppressing the infections, and splenomegaly is often the first and only readily apparent gross pathologic lesion of latent infection. Following experimental injection with *E. coccoides* (Baker et al. 1971), peak splenic enlargement of 3-4 times normal occurs approximately 7 days after infection but diminishes rapidly during the ensuing week and reaches a plateau at 1.5–2 times normal by 21 days. This level of splenomegaly persisted for the remainder of a 42-day observation period. Splenomegaly is also a prominent gross pathologic sign of *H. mus* infection in rats; however, quantitative data are lacking.

**Microscopic Features**

*E. coccoides* has an annular or ring appearance in Romanowsky-stained blood smears (Fig. 100), and although this is known to be an artifact of fixation and staining, it remains a useful distinguishing feature of the organism. Extra-erythrocytic forms are seen frequently in the blood of infected mice (Fig. 100). In contrast, *H. mus* appears as solid coccoid elements arranged in clusters, chains, or singly on erythrocytes and is rarely seen free in plasma (Fig. 101). Both *Hemobartonella* and *Eperythrozoon* stain blue to polychromatophilic in Giemsa or Wright’s stained blood smears. Acridine orange staining increases the sensitivity of parasite identification, especially when their concentration is low (Cassell et al. 1979). When examined by dark-field fluorescence microscopy, stained organisms fluoresce yellow-green to red-orange, depending on the procedure of acridine orange staining used. Fluorescing structures such as Howell-Jolly bodies, reticulocytes, and platelet fragments may be confused with organisms. Positive identification of these organisms in peripheral blood smears is made difficult by their small size and resemblance to other erythrocyte inclusions unless massive parasitemias are induced. For example, basophilic stippled erythrocytes, which occasionally reach high concentrations in the peripheral blood of normal young rodents, are easily confused with these organisms.

The morphologic changes that occur in the spleens of pathogen-free mice infected with *E. coccoides* are dramatic. By post inoculation day 4 or 5, the splenic follicles are transformed into massive sheets of blasts and stem cells in which are scattered large macrophages containing cellular debris. These large sheets become rather diffuse, often extending into the splenic cords and thus obliterating the normal pattern of spleen morphology. By the 6th or 7th day, the normal pattern is partially reestablished as many of the actively dividing cells have differentiated into erythroid series cells and populate the cords in large numbers. By 10-14 days and later, little or no microscopic evidence of infection remains in the spleen except for an increased number of plasma cells. Increased numbers of Kupffer’s cells in the liver are noted early on, but after the 2nd week of infection their concentration is no greater than in normal liver (Baker et al. 1971; Cassell et al. 1979).
Ultrastructure

SEM and TEM examination of these organisms reveals that they are spherical, approximately 350–700 μm in diameter, without distinguishing internal structure, and are closely associated with indentations of the erythrocyte plasmalemma but discretely separated from it (Moulder 1974; Baker et al. 1971; Cassell et al. 1979; Tanaka et al. 1965).

Differential Diagnosis

Disease activation with subsequent detection of organisms in the peripheral blood has provided the most reliable procedure for detecting latent infections, and surgical splenectomy is the most consistent and potent activator of these infections. Mice infected with *E. coccoides* frequently develop massive parasitemias 2–4 days after splenectomy, but peak parasitemias are of short duration (12–24 h) and regress rapidly to undetectable levels. Mice usually develop only modest anemia and rarely die as the result of activation of the disease. In contrast, *H. muris* parasitemias occur 5–10 days after splenectomy of infected rats and do not reach the extreme magnitudes commonly seen in active *E. coccoides* infections. Infected adult rats frequently experience severe hemolytic episodes which may terminate fatally.

Uninfected, splenectomized animals provide the most sensitive test subjects for detecting these agents in various biological materials. The incubation period and intensity of parasitemias are dose-dependent, and occasionally serial animal passage is necessary to raise the concentration of organisms to levels that are readily detectable. Uninfected mice, with spleens intact, will develop patent parasitemias if sufficient numbers of *E. coccoides* are given, while latently infected mice frequently fail to show patent parasitemia even when reinfected with a large number of organisms. In contrast, unless they are splenectomized, infected rats virtually never develop patent parasitemia, even when enormous numbers of *H. muris* organisms are given.

A variety of procedures, in addition to splenectomy, that compromise the reticuloendothelial system result in activation of latent *Hemobartonella* and *Eperythrozoon* infections. These include: chemical suppression of reticuloendothelial function (Stuart 1962), polonium injury of the spleen (Scott and Stannard 1954), and whole