Ultrastructure of erythrocytes from *Aotus trivirgatus* and *Saimiri sciureus* monkeys infected by *Plasmodium vivax*

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**Abstract.** Erythrocytes from *Aotus* and *Saimiri* monkeys parasitized by *Plasmodium vivax* show dramatic changes starting during the early stages of parasite development. Invaginations of the erythrocyte membrane, caveolae, are found during all parasite development stages. Up to six vesicles can be fused with one caveola, forming a caveola-vesicle complex. As the parasite grows, large accumulations of these vesicles can be seen within the erythrocyte cytoplasm. In addition to these caveolae-vesicle complexes, knob-like structures appear on the erythrocyte surface that are similar to those seen on the host-cell surface of *P. falciparum*-infected red cells. Extensive membrane-bound clefts spread throughout the erythrocytic cytoplasm, sometimes forming stacks or large whorls. The density of the red cell cytoplasm begins to decrease at an early stage of parasite development. All of these changes may be responsible for an increased fragility of the *P. vivax*-infected red cell from *Aotus* or *Saimiri* monkeys. Moreover, the large amount of parasite material that is released during rupture of the red cell may account for the high fever paroxysms that are characteristic of *P. vivax* malaria infection.

The malarial parasite *Plasmodium vivax* is one of the major scourges of mankind, causing high morbidities of benign tertian malaria in the tropics and subtropics. Although it is not as deadly as *P. falciparum*, *P. vivax* nevertheless causes a most debilitating disease characterized by intermittent high fever paroxysms (Boyd 1949). An understanding of the biology and pathogenicity of this parasite is of great interest, particularly in view of future drug and vaccine developments.

**Materials and methods**

Blood from two splenectomized *Aotus lemurinus griseimembra* was used. These animals had previously been infected with *Plasmodium falciparum* during an active immunization experiment (Trager et al. 1983). They were infected with blood from another *Aotus* monkey by inoculation of approximately $2 \times 10^6$ parasites into the saphenous vein. The parasite strain that was used originated from CDC (South Vietnam Palo Alto strain) and was passed through five animals before the monkeys in the present study were inoculated. The infection rate in these *Aotus* monkeys seldom rose above 1%. Blood from three splenectomized *Saimiri sciureus* monkeys of the Bolivian phenotype was inoculated into the saphenous vein of experimental animals along with cryopreserved material obtained from the American Type Culture Collection (Rockville, Md., Chesson strain, ATCC 30060). The history of simian adaptation of this material is not known.

All blood was collected by venipuncture into preservative-free heparin after monkeys had been restrained by the injection of 10 mg/kg ketamine. Parasitemia was monitored by frequent examination of Giemsa-stained thin blood films. Blood cells were additionally stained with brilliant cresyl blue (Retic-Set, Curtin Matheson Scientific, Inc.) for identification of reticulocytes.

Under gentle stirring, infected blood was slowly added to a fixative consisting of 2% (v/v) glutaraldehyde, 4% (w/v) sucrose, and 20 mM CaCl$_2$, in 0.1 M Na-cacodylate-HCl buffer at pH 7.3, on ice. In some experiments the cacodylate buffer was replaced by 50 mM PIPES buffer. The cells were postfixed in 1% (w/v) OsO$_4$, stained en bloc with uranyl acetate, dehydrated in ethanol, and embedded in Epon 812 via propylene oxide. Stained sections were examined with a Philips 300 or a JEOL 1200EXII electron microscope. Tracer studies were done using microperoxidase (Karnovsky and Rice 1969) and kationized ferritin (Miles-Yeda; see Kawakami and Hirano 1986).

**Results**

**Ultrastructure of the parasite**

The ultrastructure of the parasite *Plasmodium vivax* is only slightly different from the reported fine structure...
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