ANATOMICAL DISTRIBUTION OF DEVELOPING TROPHOZOITES AND SCHIZONTS OF *PLASMODIUM VIVAX* IN *AOTUS LEMURINUS LEMURINUS* AND *SAIMIRI SCIUREUS*

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Anatomical Distribution of Developing Trophozoites and Schizonts of *Plasmodium vivax* in *Aotus lemurinus lemurinus* and *Saimiri sciureus*

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ABSTRACT: Organ distribution of developing trophozoite- and schizont-infected erythrocytes of *Plasmodium vivax* and night monkeys, *Aotus lemurinus lemurinus*, and squirrel monkeys, *Saimiri sciureus*, was determined. The primary site for the infection in both species was the splenic vasculature. Secondary organ involvement differed between hosts although some overlapping did occur.

The ability of various species of plasmodia to complete their asexual development within the vasculature of various internal organs of the host has been documented (Desowitz et al., 1969; Miller, 1969; Miller and Fremount, 1970; Miller et al., 1971; Fremount and Rossan, 1974; Fremount and Miller, 1975). Because mortality due to vivax malaria is rare, few attempts have been made to follow its course anatomically, though there is much information concerning its clinical pathology and effects of the chronic disease. The present study was undertaken to elucidate the preferred sites of sequestration and obtain a quantitative comparison in 2 animal models at parasitemias comparable to those found in chronic cases in humans.

Four night monkeys, *Aotus lemurinus lemurinus*, and 4 squirrel monkeys, *Saimiri sciureus*, were inoculated intravenously or intraperitoneally with *Plasmodium vivax*, Achiote strain. The inoculum size for night monkeys ranged from 2.7 to 5.7 × 10^6 parasites, and each squirrel monkey was inoculated with 10.4 × 10^6 parasites. To determine the time of maximum parasite sequestration, Giemsa-stained blood smears were prepared 3 times daily at 0800, 1200, and 1500 hr using the method of Earle and Perez (1932). When peripheral parasitemias reached about 1% the animals were killed with Sernylan®. The abdominal and thoracic cavities were opened, and blood films and organ crushes were prepared from the left lobe of the liver, the right lobe of the lung, the right cardiac ventricle, the left kidney, spleen, and left cerebral cortex, as well as bone marrow from the left femur. The number of parasitized erythrocytes per 5,000 erythrocytes was determined for each organ and from a thin film of peripheral blood taken from the ear. Five slides from each organ were examined, and intracellular and free parasites were counted. Statistical analysis of the data was performed with a test of equality using the analysis of means applied to the percent defective (infected erythrocytes).

In squirrel monkeys and the night monkeys the maturing parasites showed a predilection for the splenic vasculature. Secondary organ in-

TABLE I. Total parasitemia of each stage of parasite expressed as a mean percentage of total parasites in organ crushes from 4 night monkeys, *Aotus lemurinus lemurinus*, and 4 squirrel monkeys, *Saimiri sciureus*, infected with *Plasmodium vivax*.

Organs	Total parasitemia		Trophozoites		Schizonts	
	N M*	S M*	N M	S M	N M	S M
Peripheral blood	1.22	1.15	74.60	49.48	7.14	3.98
Ventricle	1.38	1.02	59.21	45.85	29.33	1.42
Spleen	1.19	0.93	58.02	58.63	35.39	13.08
Lung	1.16	0.83	58.85	48.53	28.04	4.67
Bone marrow	0.95	0.62	61.42	54.33	29.44	3.93
Skeletal muscle	0.57	0.60	51.58	60.86	29.31	2.60
Kidney	0.86	0.59	60.00	56.19	28.85	3.30
Brain	0.60	0.52	73.17	46.22	15.44	0.94
Liver	1.54	0.41	58.04	53.53	29.33	9.21

* N M, night monkey; S M, squirrel monkey.

volvement differed between hosts, although some overlapping did occur (Table I). (The vessels of the heart were involved but not to the extent found in *Plasmodium falciparum* infections [Miller, 1969].)

The parasitemia between the organs of infected night monkeys was not significantly different ($P < 0.05$). The ventricle, lung, and liver showed a high percentage of parasites per total number of red blood cells; however, there were relatively high proportions of schizonts in all the organs, particularly the spleen (Table I).

The difference in parasitemia between organs of infected squirrel monkeys was significant ($P < 0.05$). Although the peripheral blood, vena cava, and ventricle demonstrated the greatest number of parasites, schizont parasitemia was significantly increased in the spleen (Table I).

The primary anatomical sites of asexual development of *P. vivax* in squirrel and night monkeys are identical. In *Aotus* and *Saimiri* the spleen had a higher proportion of schizont-infected erythrocytes. The schizont concentration in the spleen of squirrel monkeys was more dramatic than that of night monkeys (Table I). The differences noted in their secondary involvement (Table I) are probably due to the exhaustion of the primary sites with subsequent overflow of parasitized red cells to other organs and occurs as a random event.

The anatomical distribution of *P. vivax* differs from that of falciparum malarias, all of which have a predilection for the cardiac vasculature. Moreover, the caveola–vesicle complex noted in the infected erythrocyte membrane coincides more closely to that produced by *Plasmodium knowlesi* (Fremount and Miller, 1975). Ultrastructural studies of *P. vivax* have shown that the infected cells lack the knoblike membrane abnormality present in infected cells of the falciparum complex.

The trapping phenomenon in the organ vasculature is not due to any single factor but rather to a combination of biochemical and mechanical events. Because red cells are required to change shape as they flow through the circulation, they must be deformable. The biconcave shape of normal erythrocytes is well suited to this need. Because the surface area of the biconcave disc is larger than necessary to enclose its volume, a portion of its surface area also remains available to accommodate deformation. The limit of deformability depends upon the surface area-to-volume ratio of the red cell. It has been shown that erythrocytes lose their deformability when infected by a malarial parasite (Schuffner and Esseveld, 1936; Miller et al., 1972). Because of the intracellular position of the malarial parasite, the membrane of the erythrocyte no longer remains pliable and cannot pass through the smaller vessels. Miller et al. (1972) found a reduction in the deformability of infected red blood cells and suggested that either the internal viscosity of the infected erythrocytes increases and/or the membrane flexibility decreases in cells containing these stages of the parasite. This may account for the presence of a greater percentage of erythrocytes containing trophozoites and schizonts concentrating in certain organs of the host. In *P. vivax* infections enlargement of the red blood cell commonly occurs and measurably contributes to the decreased deformability of infected erythrocytes. In addition, this decreased deformability presents a membrane defect that is recognized readily by the spleen.

The spleen normally participates in the re-

moval of effete corpuscles and is particularly efficient at trapping and destroying erythrocytes with minimal defects. Schnitzer et al. (1972, 1973) described the pitting process as it occurred in the spleen of *P. knowlesi*-infected rhesus monkeys and presented morphologic evidence that suggested this organ is capable of removing that part of the cell containing the parasite and returning the unparasitized portion to the circulation as a spherocyte. Although this may be possible with erythrocytes containing ring forms or young trophozoites, it is not likely to occur with fully developed trophozoites or schizonts because they occupy most of the interior of the red cell, making them less deformable.

In an ultrastructural study of *Plasmodium fragile*, Fremount and Miller (1975) showed that the knoblike protrusions on the infected red cell were in close apposition to the vascular endothelium. The association between the knoblike foci on the membranes of *P. fragile*-, *P. falciparum*-, and *Plasmodium coatneyi*-infected erythrocytes and vascular sequestration implicates these foci in adhesion of infected erythrocytes to endothelium.

The mechanism involved in removal of the infected cells by the spleen is not understood fully, but it may be hypothesized that decreased red cell deformability together with immunologically altered red cells may contribute significantly to the trapping phenomenon as it occurs in *P. vivax* infections.

Roberts et al. (1985), in in vitro studies of *P. falciparum*-infected red cells, demonstrated the role of a soluble protein thrombospondin as a possible mediator for vascular sequestration.

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