

Title: SITES OF DEEP VASCULAR SCHIZOGONY IN PLASMODIUM COATNEYI MALARIA.

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Objective Several species of plasmodia complete their asexual development in the vasculature of the internal organs. Very little is known regarding the cause of this phenomenon or the exact sites at which schizogony takes place. Until the discovery of Plasmodium coatneyi by Eyles et al., (1962) there was no convenient simian malaria model with which to study deep vascular schizogony. In a preliminary investigation, Garnham (1965) found that schizogony of P. coatneyi occurs principally in the capillaries of the heart. This present investigation was undertaken in order to make a more extensive search for the sites of deep vascular schizogony and to obtain a quantitative comparison between these sites.

Description Three monkeys were used in this experiment, a splenectomized Pakistani Macaca mulatta (PK 13), an intact Thai M. mulatta (MS 56) and a splenectomized M. irus (MS 48). All were infected by intravenous inoculation of P. coatneyi parasitized blood. The course of infection in various species and geographic strains of monkeys has been described in a previous paper (Desowitz et al., 1968). The animals were studied immediately prior to the second or third sporulation. An investigation by Miller et al., (1968) on hypovolaemia indicated that maximal schizogonic development in our strain of P. coatneyi took place at about 11 AM and accordingly this time was selected to carry out the present experiments. A thin blood film was made from a finger stick, the animal anaesthetized with Combital and another peripheral blood film made when it was asleep. The animal was opened and blood films and crush smears from various parts of the vasculature and tissues made as shown in Table 1. Tissues were placed in Bouin's fixative, Zenker's fixative (AFIP), and buffered neutral 10% formalin. Blocks were embedded in paraffin and sections cut at 5 microns. Hematoxylin and eosin and Giemsa staining techniques were employed for general morphology. An allochrome connective tissue method (Lillie) was used to identify fibrin thrombi. Arterial elastic membranes were demonstrated by Verhoeff's iodine ironhematoxylin method.

Enumeration of the parasites in the Giemsa-stained blood films was made by counting the number of parasitized erythrocytes in ten thousand cells. For the tissue smears, the percentage of parasitaemia in, at least, one thousand erythrocytes was obtained. The growth stage of each parasite, i.e., trophozoite or schizont, was also recorded.

Progress In no instance was there any difference in peripheral blood parasitaemia before and after anaesthesia. Nor was there any significant difference in parasitaemia between peripheral blood (finger prick) and in blood from mesenteric vein, aorta, femoral artery, coronary artery, hepatic artery and pulmonary artery. The results from the various tissues studied are summarized in Table 1. It will be seen from this table that in all monkeys studied the major site of sequestration is the ventricular myocardium. Examination of the fixed sectioned material further showed schizogonic development to be concentrated in the capillary vessels and sinusoidal venous spaces of the ventricular myocardium (Pl. 1, fig. 1). No

TABLE I. The distribution of schizonts and trophozoites in blood and tissue crushes from 3 monkeys infected with *P. coatneyi*

Source of blood film or tissue crush	Monkey		
	MS 56 Parasitaemia % ratio of schizont: trophozoite	PK 13 Parasitaemia % ratio of schizont: trophozoite	MS 48 Parasitaemia % ratio of schizont: trophozoite
Finger prick	0.05	3.6	0.2
Mesenteric Vein	0.05	4.5	0.5
Ventricle	9.2	25.4	3.8
Atrium	1.4	7.1	0.4
Lung	0.6	9.3	1.7
Liver	0.4	15.5	1.1
Kidney	0	6.8	0.4
Spleen	2.9	—	—
Pancreas	1.2	3.6	—
Diaphragm	0.7	—	0.1
Bone Marrow	0.03	10.1	0.2
Cerebrum	0.5	7.8	0
Adrenal gland	0.06	3.7	0.8
Skeletal muscle	—	6.4	—

myocardial necrosis or extensive formation of fibrin thrombi was present. The extramural coronary arteries, the perforating arteriolar rami, and their accompanying veins contained only occasional schizonts (Pl. 1., fig. 2).



Fig. 1 Schizonts lining myocardial capillaries (arrow). No schizonts are visible in the perforating artery and vein. Note the absence of inflammatory cells. Hematoxylin and eosin  $\times 550$ .

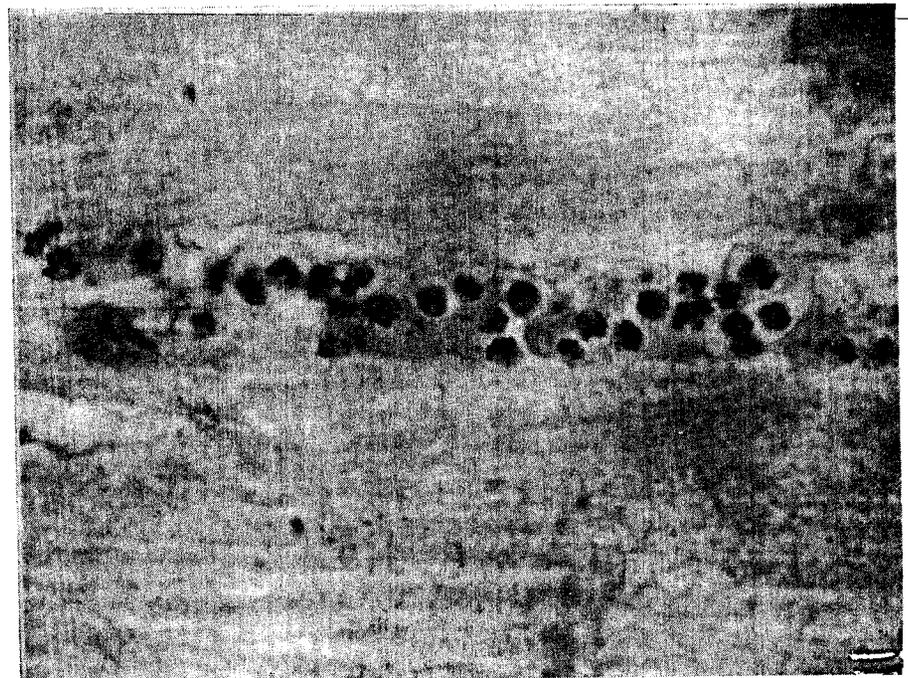


Fig. 2 Schizonts and erythrocytes packing myocardial capillary. Fibrin thrombi were not demonstrated. Hematoxylin and eosin  $\times 1620$ .

The pulmonary capillaries and hepatic sinusoids were also found to be sites of deep vascular schizogony. However, the degree of schizont concentration in these organs, as compared to the ventricle, varied from animal to animal. In PK 13 and MS 48 the number of schizonts in the liver and lung was about one half to one third that in the ventricle while in MS 56 it was less than one tenth. In the one intact animal (MS 56) the spleen evidenced an increased concentration of schizonts but many were engulfed by phagocytes and most parasites appeared to be partially destroyed.

In PK 13 and MS 56 the parasitaemia was slightly increased, as compared to the peripheral blood, in other tissues, such as the pancreas, skeletal muscle and brain. However the parasites at these sites were, like those in the peripheral blood, predominately trophozoites.

Summary Three rhesus monkeys were experimentally infected with P. coatneyi. At the time of maximal schizogonic development they were killed and blood films, tissue crushes and sections made in order to locate the sites of deep vascular schizogony. It was found that the major site of schizont concentration was the capillaries of the ventricular myocardium. There was also sequestration of schizonts in the microvasculature of the liver, lung, and spleen but not to the same extent as in the myocardium.