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Retention of *Plasmodium berghei* sporozoites within perfused mouse livers*

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Summary

A mouse liver perfusion model was adapted to evaluate the efficiency of the liver in retaining *Plasmodium berghei* sporozoites. Specific numbers of sporozoites were perfused into each liver via a portal vein cannula. The number of sporozoites in the perfusate effluent were counted and the percent sporozoite retention calculated. Over 95% of sporozoites suspended in medium with plasma were retained in a normal liver following a single passage. Sporozoites were seen in sinusoids of perfused livers using scanning electron microscopy. This liver perfusion model offers a valuable method to help clarify sporozoite interactions with elements of the liver.

Key words: malaria; *Plasmodium berghei;* sporozoite; schizont; *Anopheles stephensi;* hepatic perfusion; scanning electron microscopy; hepatic sinusoids.

Introduction

Sporozoites of *Plasmodium berghei* are known to develop into exoerythrocytic (EE) stages in parenchymal cells of the liver, although mechanisms by

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which they become sequestered within the liver and eventually reach and enter hepatocytes are poorly understood. A recent in vitro study by Strome et al. (1979) has shown that, given the opportunity, sporozoites of *P. berghei* can also enter non-hepatic cells and develop into EE stages. It is well established that various species of avian malaria produce EE stages in both fibroblastic cells and cells from the reticuloendothelial system. These latter observations suggest that entry of sporozoites into hepatocytes to initiate the EE development may simply be the result of extensive trapping of sporozoites by the liver.

The objective of this study was to evaluate the efficiency of the liver in trapping *P. berghei* sporozoites. To accomplish this, we modified a liver perfusion system originally used to observe the clearance of bacteria and yeasts by mouse and rat livers (Moon et al., 1975; Sawyer et al., 1976). This model offers advantages over other systems in that it can be experimentally manipulated under essentially physiological conditions and it yields quantitative data.

Materials and Methods

Animals

NIH/Nmri female mice weighing 22–25 g were used in this study. The animals were maintained under standard laboratory conditions with food and water available ad libitum.

Sporozoites

The Anka strain of *Plasmodium berghei* was used for this study. *Anopheles stephensi* mosquitoes, infected 21 days previously by feeding on mice infected with *P. berghei*, were cut into two halves at the junction of the abdomen and thorax and the halves were processed separately. Each was ground in a mortar and pestle in the presence of 1 ml of either fresh defibrinated mouse plasma, heat inactivated plasma, or medium 199 (M-199). Sporozoites were then separated using the renografin gradient technique described by Pacheco et al. (1979) employing a discontinuous gradient. The number of sporozoites resulting was counted on a hemocytometer and appropriate dilutions of 500,000 to 8,100,000 parasites/ml were made.

Mice were injected with 200 units sodium heparin and 1.5 mg pentobarbital sodium and their livers were exposed. An afferent cannula was inserted into the portal vein and an efferent cannula into the vena cava through the right atrium. The livers were washed with M-199 through the afferent cannula until the effluent was clear of red blood cells. One ml of the appropriate medium containing suspended sporozoites was then slowly perfused into the liver via the portal vein cannula. This was immediately followed by 25 ml of M-199 either alone or containing 5% (V/V) normal or heat inactivated mouse plasma. The effluent was collected from the efferent cannula into a graduated cylinder and centrifuged at 6,000 *g* for 12 min. The supernate was drawn off, the pellet of sporozoites resuspended in 1 ml of M-199, and the number of sporozoites in the effluent counted on a hemocytometer.

Scanning electron microscopy

Following sporozoite perfusion and wash, 10 ml of 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) was infused into the livers. They were removed from the animals, cut into small pieces, and processed for microscopy (Moon et al., 1975; Friedman and Moon, 1977; Danforth et al., 1980).

Table 1. Retention of sporozoites of *Plasmodium berghei* by isolated mouse livers following perfusion

Number of replicate experiments	Source of sporozoites in mosquito	Suspension medium	Number of sporozoites		% retention mean ± S.D.
			in perfusate	in effluent (mean)	
6	thorax	M-199 + 50% normal plasma	500,000	7,416	98.5 ± 1.2
6	abdomen	M-199 + 50% normal plasma	500,000	9,708	98.0 ± 0.6
6	thorax	M-199 + 50% heat inactivated plasma	500,000	2,375	99.5 ± 0.2
6	abdomen	M-199 + 50% heat inactivated plasma	500,000	3,292	99.3 ± 0.5
1	both thorax and abdomen	M-199 alone	500,000	97,500	80.5
1	both thorax and abdomen	M-199 + 50% normal serum	500,000	32,500	93.5

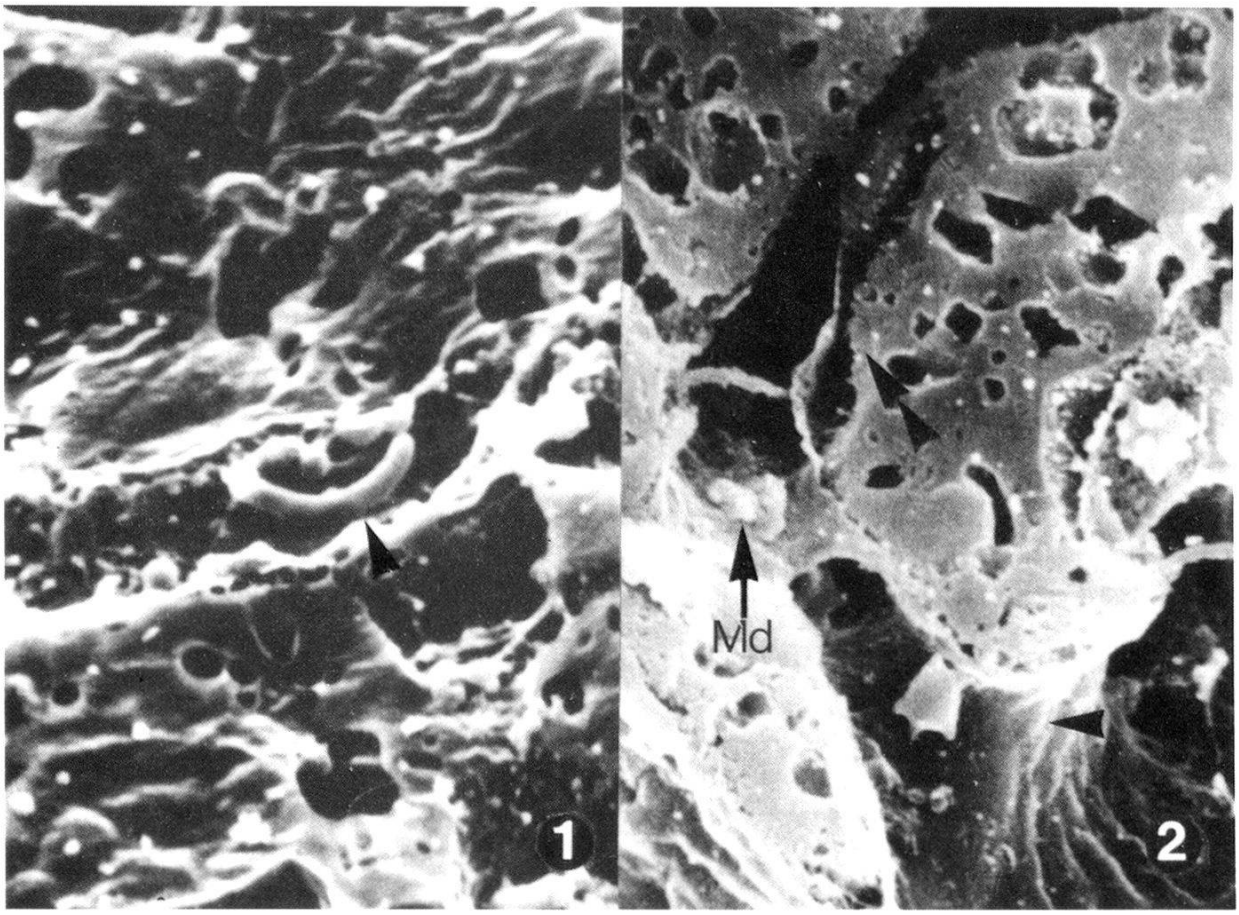
Table 2. Hepatic trapping of larger numbers of sporozoites in normal or heat inactivated (HI) mouse plasma

Type of sporozoites	Number of sporozoites perfused into liver	Number of sporozoites recovered from effluent	Percentage of sporozoites trapping
Thoracic in 50% plasma	2,112,500	97,500	95.4%
Abdominal in 50% plasma	3,060,000	67,500	97.8%
Thoracic in 50% HI plasma	4,712,000	67,500	98.6%
Abdominal in 50% HI plasma	8,187,000	290,000	96.5%

Results

An average of 95 to 99.5% of the *P. berghei* sporozoites perfused through isolated livers of mice are retained during a single passage (Table 1). Within the limits examined, neither retention efficiency nor capacity appeared to be related to the source of sporozoites from the mosquito thorax or abdomen, the presence or absence of complement, or the total number of sporozoites perfused (Tables 1 and 2). Preliminary data indicated that retention was diminished with the elimination of plasma from the perfusion medium (Table 1).

There was no evidence of flow rate inhibition or liver swelling in any experiment following perfusion of sporozoite suspensions. This suggests that the



Figs. 1 and 2. Scanning electron micrographs of perfused mouse liver tissue after introduction of *P. berghei* sporozoites.

Fig. 1. Sporozoite of *P. berghei* (arrow) present in the sinusoid of perfused liver (1,700 ×).

Fig. 2. Perfused liver demonstrating endothelial cells (single arrow) and Kupffer cells (double arrow). A small amount of mosquito debris (Md) is present in the sinusoids (2,000 ×).

retention capacity of the organ had not yet been reached. Reversing direction of flow of the perfusate so that 25 ml of M-199 flowed back into the liver via the superior vena cava and exited through the portal vein, freed less than 2% of the trapped sporozoites. This suggests that the trapped organisms were tightly bound within the liver, and not simply retained because of mechanical clogging of liver sinusoids by either sporozoites or associated mosquito debris.

Scanning electron micrographs of livers following perfusion revealed sporozoites in the sinusoid (Fig. 1). In this instance the sporozoite appears to be associated with an endothelial cell and not with a Kuppfer cell. The difference in appearance between Kupffer cells and endothelial cells is illustrated in Fig. 2.

Although large numbers of sporozoites were used in perfusion inocula and the retention factor was high, it was difficult to find sporozoites in situ for observation by scanning electron microscopy. Presumably, this results from the size of the organ and the large number of sinusoids present. In addition to sporozoites, a small amount of mosquito debris was also observed in the sinusoids (Fig. 2). It is noteworthy that the architecture of the liver following perfusion

with sporozoite suspensions appeared normal in all respects when studied by scanning electron microscopy (Motta and Porter, 1974).

Discussion

The results of this study demonstrate that a very large percentage of *P. berghei* sporozoites are retained in a normal perfused mouse liver following a single passage. This represents an even higher degree of clearance efficiency of sporozoites than has been reported in intact animals (Nussenzweig et al., 1972). In the latter investigation the gradual decrease in the number of circulating *P. berghei* sporozoites over a 1-hour period led the authors to postulate that some sporozoites may pass through the liver as many as 600 times before being retained by the organ.

The lack of difference between normal plasma and heat-inactivated plasma for perfusion suggests that the presence of an active complement system in the mouse plasma does not significantly alter sporozoite retention by a perfused liver. Furthermore, the infectivity of a sporozoite does not seem to be related to its ability to attach to cells of the hepatic sinusoid, since no differences in percent retention occurred between sporozoites obtained from the mosquito thorax compared to those from the abdomen. Vanderberg (1974) has reported that oocyst sporozoites have only a slight degree of infectivity compared with that of salivary gland sporozoites.

Recent studies have shown that normal mouse serum added to incubation medium enhances sporozoite motility and infectivity (Spitalny, 1973; Vanderberg, 1974) and produces greater attachment of sporozoites to macrophages in vitro (Danforth et al., 1980). When sporozoites were perfused in M-199 in complete absence of plasma, average retention by the liver was reduced to approximately 80%. Whether or not plasma alters the surface of the sporozoite as indicated for serum protein (Schulman et al., 1980), resulting in qualitative or quantitative changes in the nature of the interaction of sporozoites with sinusoidal cells, warrants further study.

Exactly how and where the sporozoites are retained in the liver must also await further investigation. Preliminary attempts to study this question by scanning electron microscopy have identified sporozoites in the sinusoids of perfused livers that were not apparently associated with Kupffer cells (Fig. 1). A recent study has shown that *P. berghei* sporozoites in normal mouse serum do attach to and enter Kupffer cells (Danforth et al., 1980). In the same report it was also shown that sporozoites suspended in medium containing normal serum enter peritoneal mouse macrophages but, unlike the Kupffer cell, the peritoneal macrophage is destroyed by the sporozoite. When suspended in medium containing immune serum, however, the parasites were destroyed by the macrophage. This has led to speculation that Kupffer cell-sporozoite interaction in a normal animal may function as a concentrating mechanism for

sporozoites, retaining them in the organ where exoerythrocytic development subsequently takes place. Verhave et al. (1980) postulated that, in vivo, cells of the liver sinusoids, including Kupffer cells, are responsible for concentrating the parasites in the vicinity of parenchymal cells.

Our data support the notion that hepatic sinusoids are effective concentrators of sporozoites in vivo. Results of studies on hepatic clearance of *Salmonella typhimurium* and *Candida albicans* using the same perfusion techniques have clearly established that both Kupffer cells and endothelial cells play a similar role in liver retention of microorganisms (Sawyer et al., 1976; Friedman and Moon, 1977). It is becoming increasingly apparent that initial clearance may involve a number of sinusoidal cell types. We believe that liver perfusion offers a valuable model which will help clarify the nature of the interaction of malarial sporozoites within the liver.

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