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The role of the Kupffer cell in the infection of rodents by sporozoites of *Plasmodium:* uptake of sporozoites by perfused liver and the establishment of infection in vivo

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Summary

The uptake of *Plasmodium yoelii nigeriensis* sporozoites by isolated perfused rat liver was very rapid and efficient. 67% of the initial load was removed from the perfusion media in the first passage through the liver, and 95% after 15 min of perfusion. Much of the uptake was explained by mechanical trapping in the liver. Up to 75% of the sporozoite load was retained after 15 min both by heat killed liver and liver cooled to 4° C, therefore at least 20% of the sporozoite uptake in perfused normal livers was due to a biologically active process. In perfused normal livers, non-infective (heat-killed or trypsin-treated) sporozoites were taken up with an efficiency equal to infective sporozoite controls. However, a reduction in Kupffer cell number and activity, induced by silica treatment, resulted in a very significant decline in uptake of infective sporozoites by the perfused liver – and a parallel fall in the successful infection of the host by inoculated sporozoites in vivo. Since silica treatment produced no significant detectable pathological changes in hepatocytes, and infected blood passage results in a normal parasitaemia in silica treated animals it was concluded that the Kupffer cell was a component of the natural route of infection of the mammalian host by the majority of the infecting population of sporozoites of *Plasmodium yoelii nigeriensis.*

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Introduction

Observations on the circulation of sporozoites in the peripheral blood of the vertebrate host indicate a rapid loss of infectivity on transfusion to a clean host, i.e. a rapid clearance of sporozoites. Fairley et al. (1947) found a maximum persistence of infectivity of 30 min for *Plasmodium vivax* and 60 min for *P. falciparum* in human volunteers; Mulligan et al. (1949) – 30 min for *P. cynomolgi* in *Macaca;* Verhave et al. (1980) – 15 to 60 min for *P. berghei* in rats, and Canning et al. (1972) – 15 min for *P. yoelii* in mice. Following the rapid disappearance of the sporozoites from the peripheral circulation, immunofluorescent studies have demonstrated parasite material within the Kupffer cells of the liver after 2 h (Verhave et al., 1980) and in the hepatocyte after a similar period (El Nahal, unpublished). The most immature forms observed by classical cytological techniques were those of *P. cynomolgi* 24 h after inoculation (Bray, 1957). Despite these observations the passage of the sporozoite from the peripheral circulation into the hepatocyte, and the transformation of the sporozoite into the pre-erythrocytic schizont remains the single major undocumented phase of the malarial life-cycle.

The liver has an exceptional capacity to remove bacteria (Benacerraf et al., 1959) and viruses (Brunner et al., 1960) from the bloodstream. If it is assumed that sporozoites ingested by Kupffer cells are destroyed, this efficient phagocytosis and destruction of foreign material by the Kupffer cells conflicts dramatically with estimates that 80% of *P. cynomolgi* sporozoites given by intravenous inoculation successfully invade the hepatocyte (Krotoski, pers. comm.) and the fact that as few as 10 sporozoites of *P. vivax* will induce infections when inoculated intradermally (Shute et al., 1976). Conversely if the ingested sporozoite were to survive and infect the hepatocyte, phagocytosis by the Kupffer cell may be regarded as an efficient mechanism for the localization of the parasite in the target organ.

The ability of the liver to clear malarial parasites from the circulation has not been estimated directly. In this study we investigate both in vitro and in vivo the initial interaction between the sporozoite and the liver and evaluate the relative importance of the liver architecture, Kupffer cell function and sporozoite integrity in the process of sporozoite retention and uptake. Particularly we have compared the uptake of sporozoites in perfused livers and the efficiency of parasite infection, between groups of normal and silica treated mice. The specific regime of silica treatment chosen has been shown to produce a massive reduction in macrophage and lymphocyte number and activity (Allison et al., 1966; Van Loveren et al., 1977). Other pathological changes attributed to silica treatment include fibrosis but it is significant that no structural or functional changes have been described in hepatocytes – the site of exoerythrocytic schizont development (Vigliani and Pernis, 1961).

Methods

Animals. – 100–150 g Wistar rats and 30 g TO mice were maintained on standard laboratory diet. Silica treated animals (Van Loveren et al., 1977) were inoculated on 5 consecutive days with 7 mg silica of average particle size 0.012 μm (Sigma Chemicals) per 100 g body weight. Autoclaved silica was made up to 25 mg/ml in medium 199 (M 199) and was administered by intraperitoneal inoculation. Animals were used 10–14 days following the last injection.

Parasites. – *P. yoelii nigeriensis* was maintained by cyclic passage through TO mice and *Anopheles stephensi. P. cynomolgi* was obtained as sporozoite infections of *A. dirus* from Dr. Gwadz of the National Institutes of Health, Bethesda, Maryland.

Sporozoites of *P. y. nigeriensis* were obtained by sterile dissection of salivary glands. Isolated glands were suspended in M 199 + 10% foetal calf serum (FCS) at 0°C and homogenized by a single stroke of a glass/Teflon homogenizer. Sporozoites of *P. cynomolgi* were harvested from whole mosquitoes by renografin gradient techniques (Pacheco et al., 1979). Sporozoite numbers were determined by haemocytometry, allowing 20 min for the parasites to settle before counting. All sporozoite suspensions were maintained at 0°C prior to use.

Liver perfusion. – Rats were placed under deep anaesthesia by intraperitoneal injection of phenobarbitone (160 mg/kg Sagatal, May & Baker). The posterior vena cava was canulated and the liver flushed out with 100 ml of perfusate to remove blood from the severed hepatic portal vein. The liver was then excised, transferred to the perfusion apparatus (Krebs et al., 1973) and allowed to equilibrate for 30 min prior to the experiment. The perfusate used throughout the study was M 199 containing 10% FCS, 200 μg/ml streptomycin, 200 IU/ml penicillin and 20 mM Hepes buffer. The perfusate was maintained at 37 ± 0.1°C, equilibrated with 95% O_2 and 5% CO_2 and passed through the liver at flow rates of between 6 and 10 ml/min.

Sporozoite uptake was estimated by adding known numbers of sporozoites to the inflow reservoir, this initial load was then flushed through the liver and the effluent sampled after the first passage and after a further 15 min circulation. The sporozoite number was determined in each sample by haemocytometry and samples of the liver were fixed for microscopic examination.

Kupffer cell function was assayed by determining the uptake of a colloidal suspension of carbon (India ink; Raymond Lamb) from the perfusate. The carbon suspension was added to the perfusate to give an initial concentration of 5% v/v and samples were taken at 5 min intervals for 30 min. The change in carbon concentration was determined spectrophotometrically by recording the OD_{960} in each sample.

Cytology. – Lobes from each perfused liver and from the livers of mice (42 h after the inoculation of sporozoites) used in the in vivo experiments were fixed in Carnoy's fluid. After embedding, wax sections were taken and stained by the Giemsa-colophonium technique (Bray and Garnham, 1962). The density of exoerythrocytic schizonts/mm³ was determined by counting the number of schizonts in a minimum of 250 sections and calculating the volume of tissue surveyed.

For electron microscopy livers were perfused with 2.5% glutaraldehyde in 0.18 M cacodylate buffer (pH 7.4) for 15 min, after which specimens for scanning microscopy were processed by the method of Motta et al. (1978). Specimens for transmission electron microscopy were treated by the technique of Langreth et al. (1978).

Results

A. Perfusion studies

The uptake of sporozoites of *P. y. nigeriensis* by perfused rat livers was very rapid (Table 1). 67% of the initial load was removed in the first passage through the liver, and 95% following 15 min repeated circulation. The significance of the following possible component activities in uptake was determined: a) mechani-

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Table 1. The uptake of malarial sporozoites by isolated rat livers in a closed circuit perfusion system

Liver (treatment)	Sporozoite (treatment) P. yoelii nigeriensis	Number of experiments	Sporozoite uptake (% initial load)	
			after 1 passage (range)	after 15 min circulation (range)
Normal	Normal salivary gland (S. G.)	4	78.52 (67.3–92.4)	94.43 (93.4–94.8)*
Normal	(Heat killed S. G.)	6	84.54 (45.6–99.36)	93.5 (70.7–100)
Normal	(Trypsin treated S. G.)	4	80.52 (62.9–93.5)	92.7 (85.8–98.9)
Normal	(Mixed oocyst/S. G.)	1	67.3	94.8
Normal	P. cynomolgi (Mixed oocyst/S. G.)	4	51.61 (35–67.4)	97.1 (91.38–100)
(Heat killed)	Normal (S. G.)	4	62.94 (52.6–71.1)	78.23 (69.4–81.48)
(Cooled at 4° C)	Normal (S. G.)	2	42.8 (41.7–43.9)	70.7 (62.2–79.2)
(Silica treated)	Normal (S. G.)	4	52.18 (33.3–60.2)	57.1 (38.9–75.4)*

* p < 0.02 Mann-Witney

Table 2. The effect of heat treatment and trypsin digestion on the infectivity of sporozoites of *Plasmodium yoelii nigeriensis* to mice

Sporozoite treatment	Time of treatment (min)	Mean number of mice infected (per group of 5)	Mean time to patency
Untreated (4° C)	60	5	3
Untreated (37° C)	30	5	5
	60	1	5
0.01% Trypsin (37° C)	15	4	7
	30	1	3
	60	0	–
Heat (45° C)	15	0	–
	30	0	–
	60	0	–
Heat (54° C)	15	0	–
	30	0	–
	60	0	–

cal retention and adsorption, b) active sporozoite penetration and c) phagocytosis by Kupffer cells.

a) *Mechanical retention.* – The possibility that sporozoites are retained within the liver by filtration and adsorption was examined in livers killed by heating or inactivated by cooling to 4° C.

Livers were killed in situ in the perfusion apparatus by heating the circulating perfusion medium to 54° C for 30 min after which the medium was replaced and the experiment conducted at 37° C. Sporozoites were killed separately by heating to 45° C for 30 min. Sporozoite infectivity and motility are completely suppressed by heating to 45° C for 15 min or longer (Table 2).

Heat killed livers retained the inert marker colloidal carbon, dead sporozoites, and viable sporozoites with markedly reduced efficiency compared to the normal liver. The retention figures following 15 min circulation were: 33% vs 78% for colloidal carbon (Fig. 1); 78% vs 94% for heat killed sporozoites (Table 1) and 56% vs 95% for viable sporozoites (unpublished observations).

In livers cooled to 4° C energy dependent uptake of foreign particles will be suppressed, however attachment or adsorption by Fc type-, or some foreign-surface-receptors together with energy independent uptake may still proceed normally (Seljelid, 1980). Sporozoites cooled to 4° C are no longer motile (Vanderberg, 1975a). The clearance of sporozoites by cooled livers was depressed when compared to normals, being 43% after the first passage and 71% after 15 min perfusion (Table 1). When monitored continuously it can be seen that the uptake reached a plateau at about 70% of the initial sporozoite load within 10
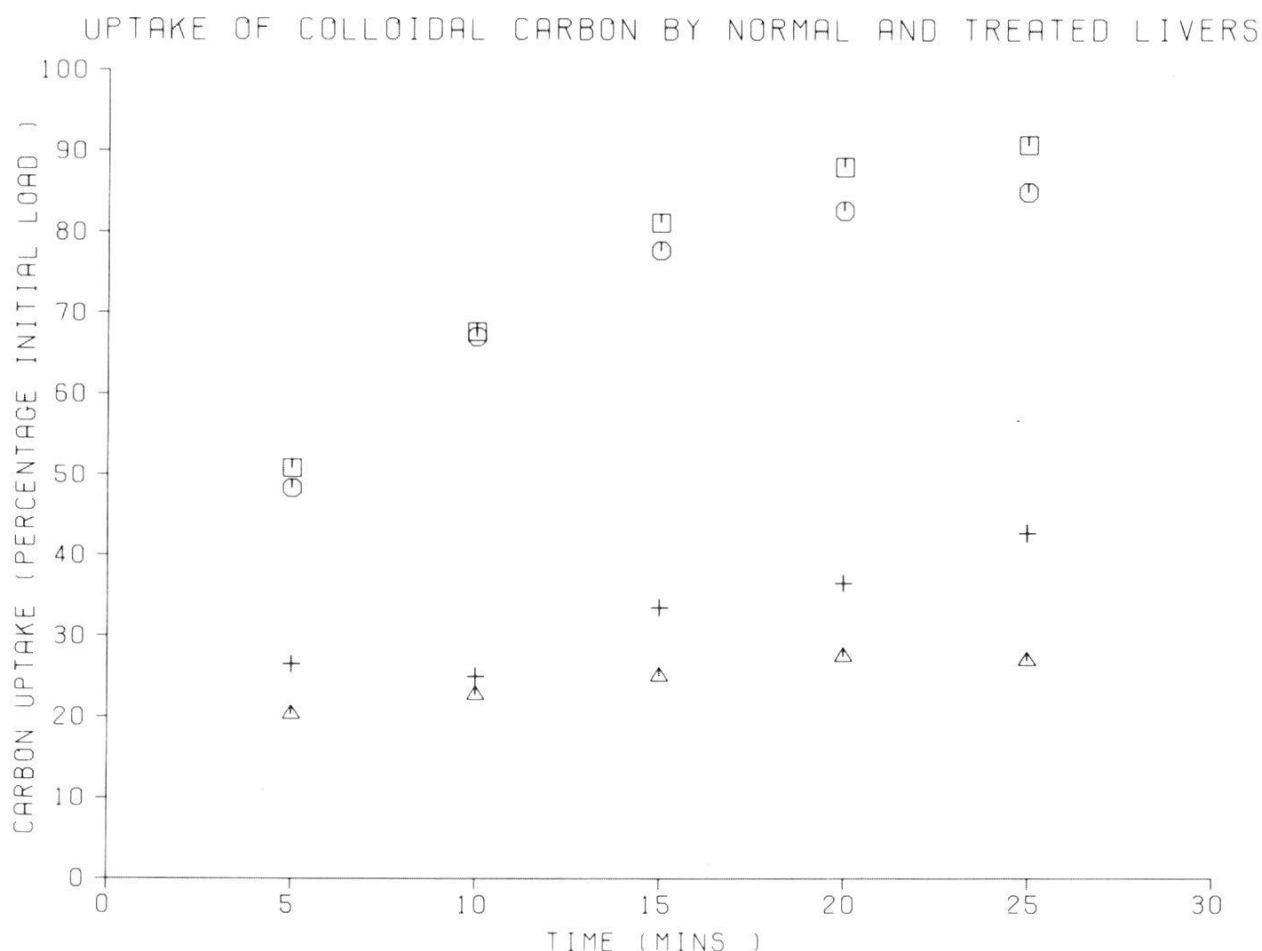
Fig. 1. The uptake of colloidal carbon by normal and treated rat livers in perfusion.
□ = percentage of initial carbon load taken up by isolated perfused normal liver.
○ = percentage of initial carbon load taken up by isolated perfused liver maintained at 4° C.
+ = percentage of initial carbon load taken up by isolated perfused liver previously heat treated for 30 mins at 54° C.
△ = percentage of initial carbon load taken up by isolated perfused silica treated liver.

min and this level was maintained for the remainder of the experiment (Fig. 2). The uptake of colloidal carbon by cooled livers was not significantly different after 15 min (78%) from normal (81%) (Fig. 1) and therefore contrasts markedly with the kinetics of sporozoite uptake.

From the combined observations on normal, heat-killed and cooled livers it appears that at least 20% of sporozoite uptake, probably more, must be due to the biological activity of either parasite or host.

b) *Active sporozoite invasion.* – The disappearance of normal sporozoites from the perfusate of viable livers was compared with that of the heat-killed parasites (Table 1). Surprisingly the uptake of the heat-killed sporozoites was found to be equal to that of normal infective sporozoites. Hence, the disappearance of sporozoites from the circulation is not directly related to their viability. Attempts were then made to determine the role of sporozoite infectivity as opposed to viability in parasite uptake in the liver.
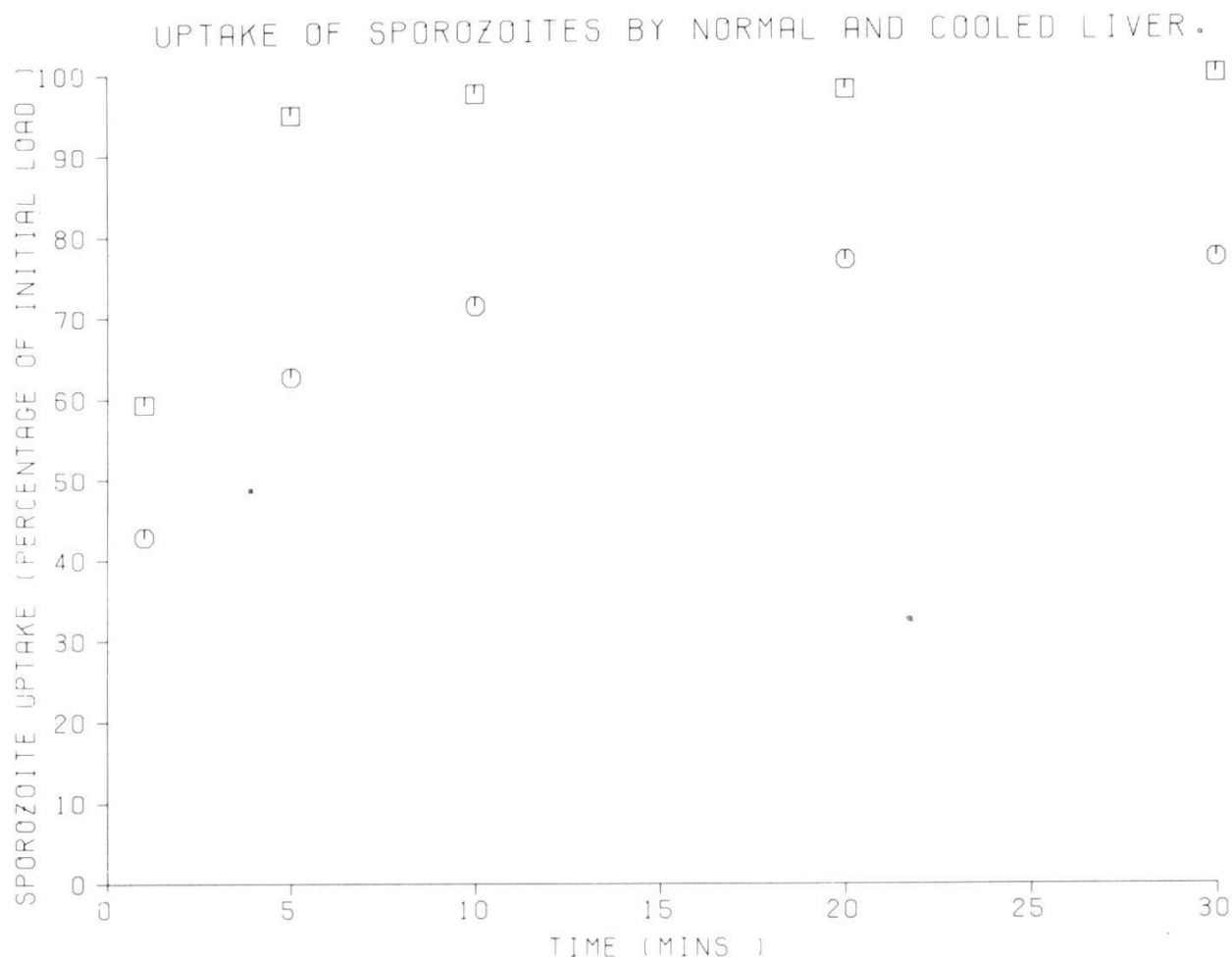
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Fig. 2. The uptake of *P. yoelii* sporozoites by normal and cooled (4°C) perfused rat liver.

☐ = percentage of initial load of *P. cynomolgi* sporozoites taken up by isolated perfused normal liver.

○ = percentage of initial load of *P. y. nigeriensis* sporozoites taken up by isolated perfused liver maintained at 4°C.

Sporozoites of *P. gallinaceum* may be rendered non-infective, whilst still remaining motile and viable by brief incubation in dilute trypsin (Turner, 1980). We therefore incubated sporozoites in 0.01% trypsin in M 199, and in M 199 alone for 15, 30 and 60 min, and assessed the infectivity and motility of the washed sporozoites. The infectivity results are shown in Table 2, and indicate that the trypsin treatment results in a significantly faster loss of infectivity than in the control group, though after 30 min incubation these poorly infective sporozoites were still motile. The uptake of sporozoites, incubated for 30 min in trypsin, by normal perfused liver was indistinguishable from that of the control group (Table 1).

Additional but indirect supportive evidence that the infectivity of sporozoites is not relevant to their disappearance within the liver was obtained by comparing the uptake of pure "salivary gland" sporozoites with those pooled from homogenized mosquitoes (i.e. both oocyst and salivary gland forms). Vanderberg (1975b) had shown that salivary gland sporozoites may be up to

Table 3. The effect of silica treatment of rats on the prevalence of infection, time to patency, and exoerythrocytic schizont density of *Plasmodium yoelii nigeriensis;* and the Kupffer cell/hepatocyte ratio in the host liver

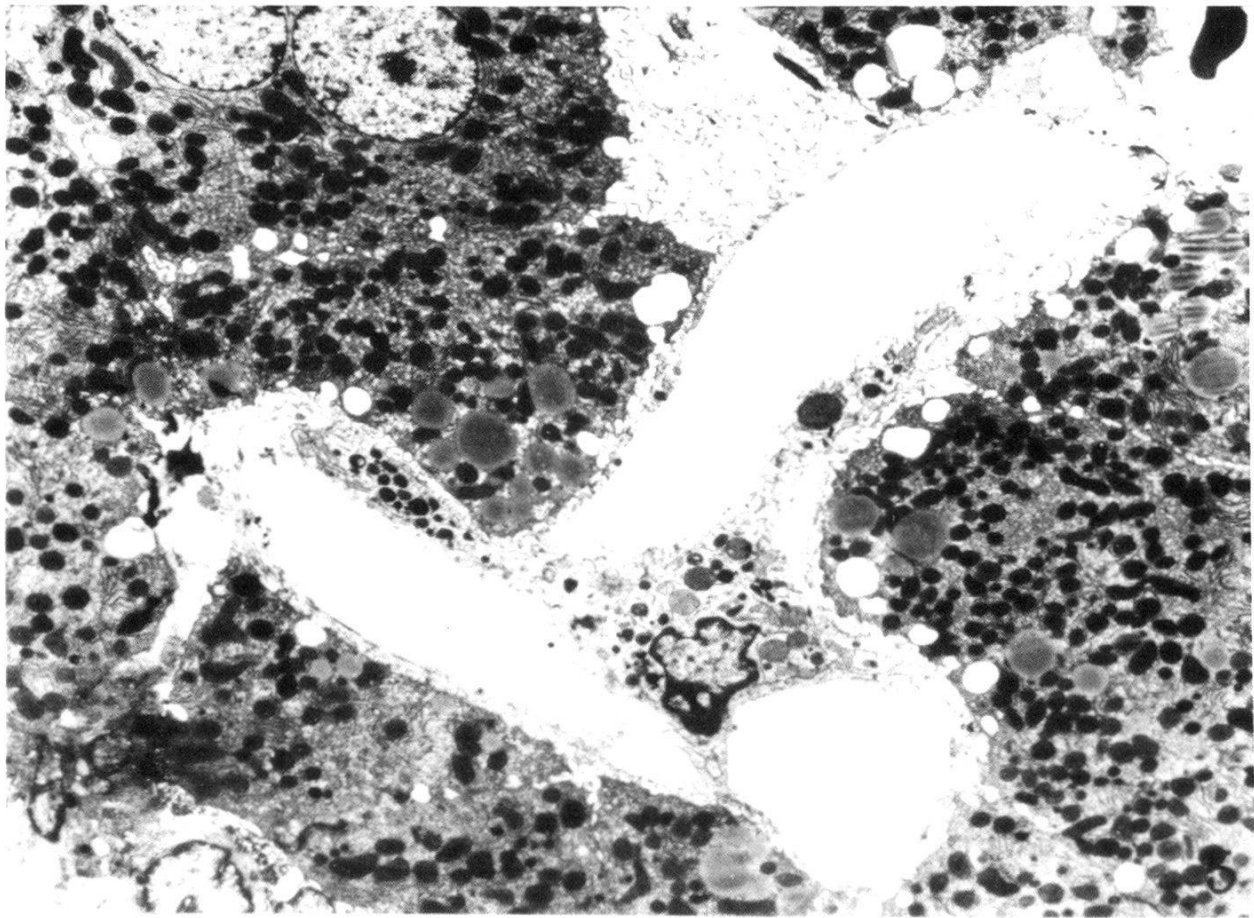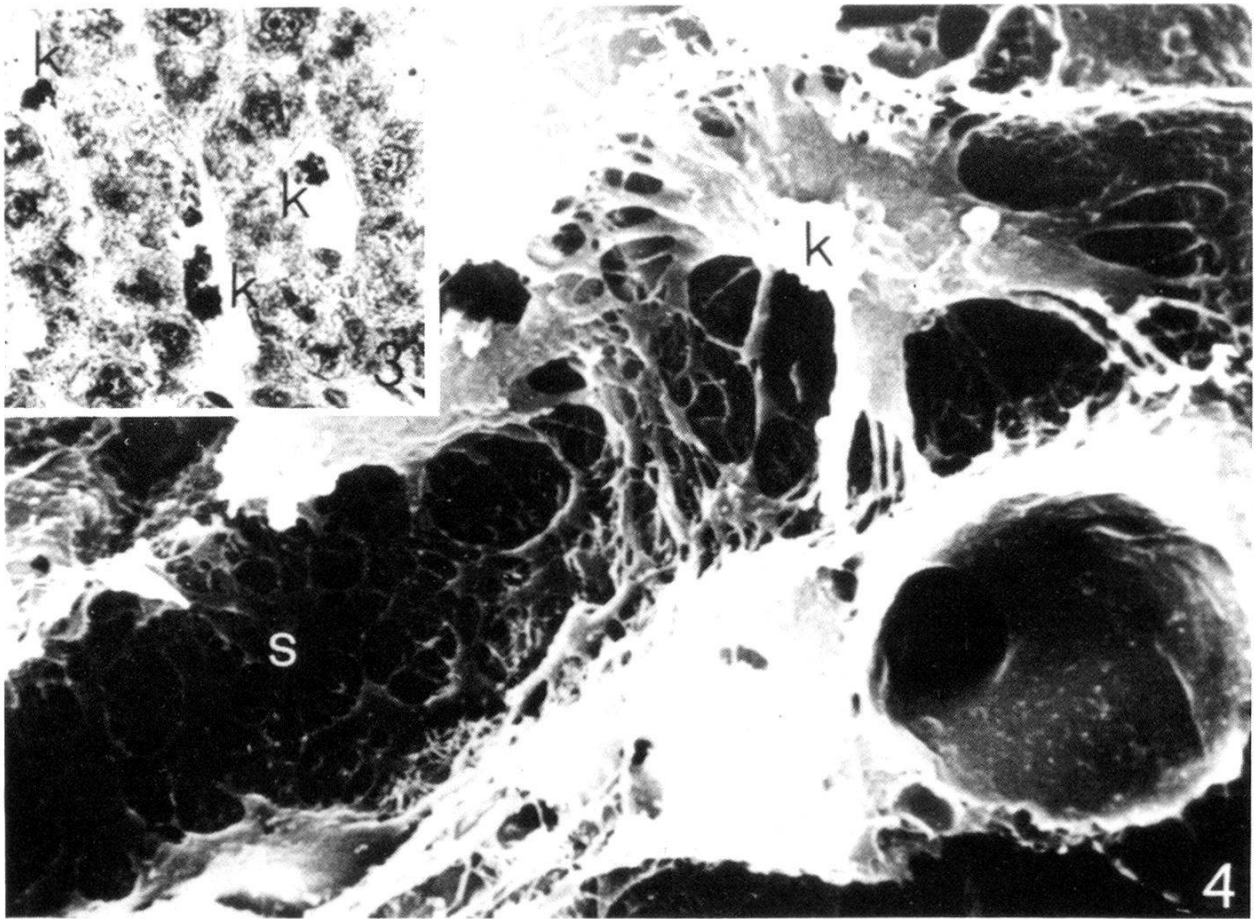|		Normal		Silica treated	
Kupffer cell density (% total cells in liver)		21.4 ± 0.7*		12.1 ± 0.7*	
Density of exoerythrocytic schizonts (parasites/mm³)		1.134		0.055	
Sporozoite infectivity	Inoculated dose	Number infected _____ Number inoculated	Days to patency	Number infected _____ Number inoculated	Days to patency
Expt. A	500	4/4	5.5.5.5	3/4	3.5.5
	1000	3/3	3.3.3	1/3	5
	1500	3/3	3.3.3	2/3	5.5
Expt. B	500	0/5	–	1/5	6
	1000	4/5	4.5.5.5	1/5	6
	1500	2/5	4.5	2/5	5.9

* t-test p >0.01

1000 times more infective than the oocyst forms. However, our data (Table 1) shows the uptake of the mixed sporozoite population by the perfused liver to be the same as that of sporozoites derived purely from the salivary glands. Further the uptake of a species of mammalian malaria parasite not infective to rats (*P. cynomolgi bastianellii*) by perfused rats livers was also examined. It was again observed that the kinetics of uptake (Fig. 2, Table 1) were similar to those of the homologous species.

c) *Phagocytosis by Kupffer cells. Comparison of uptake of sporozoites and carbon by normal or silica treated perfused livers.* – Cytological examination of livers 10–14 days after silica treatment at both light and electron microscopic levels (Figs. 6–11) revealed significant changes compared to control preparations (Figs. 3–5). The Kupffer cell:hepatocyte ratio, as determined by light microscopy, was significantly higher in normal liver than in the silica treated group (Table 3). This observation was confirmed by both scanning (SEM) (Figs. 4, 6) and transmission electron microscopy (TEM) (Figs. 5, 7). TEM studies

Fig. 3. Light micrograph of liver perfused in vitro with colloidal carbon. Carbon is concentrated in Kupffer cells (K) in sinusoids. Magn. ×570.

Fig. 4. Scanning electron micrograph of fractured normal liver. Large Stellate Kupffer cell (K) is seen lying on surface of highly fenestrated sinusoid endothelium (S). Magn. ×5,340.

Fig. 5. Transmission electron micrograph of Kupffer cell stretched across the lumen of the sinusoid. Magn. ×2,748.

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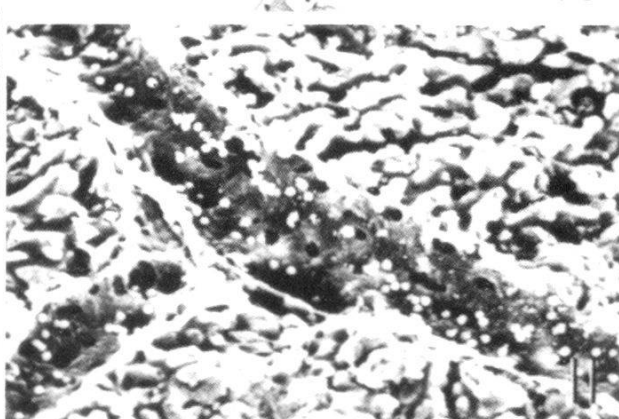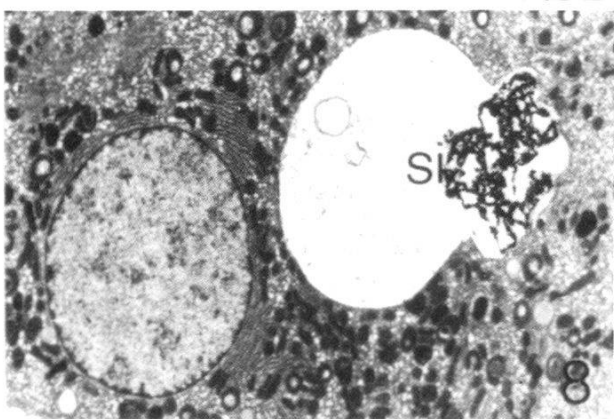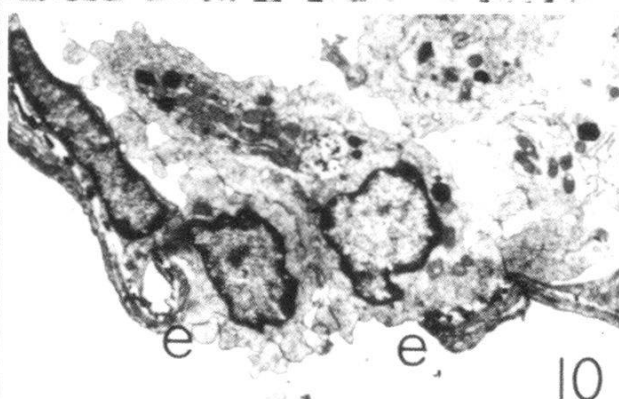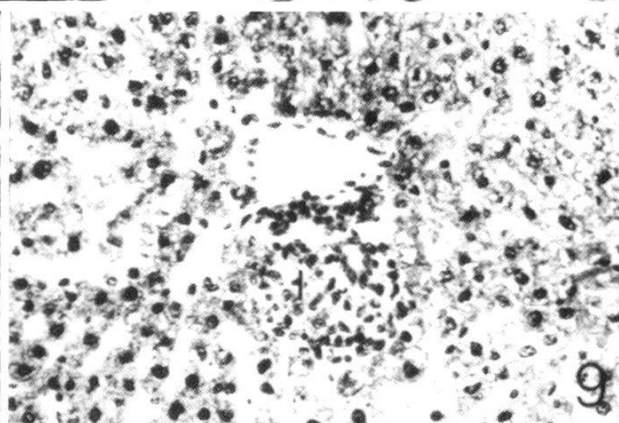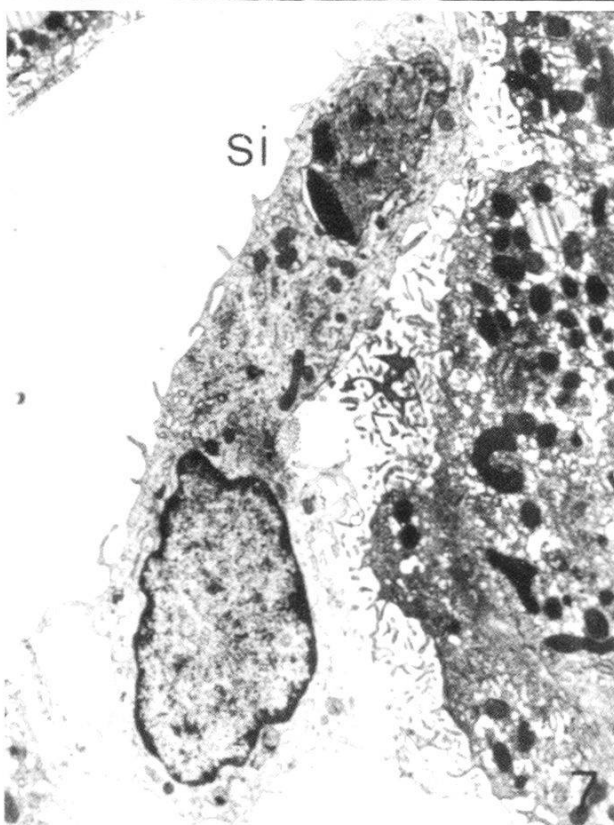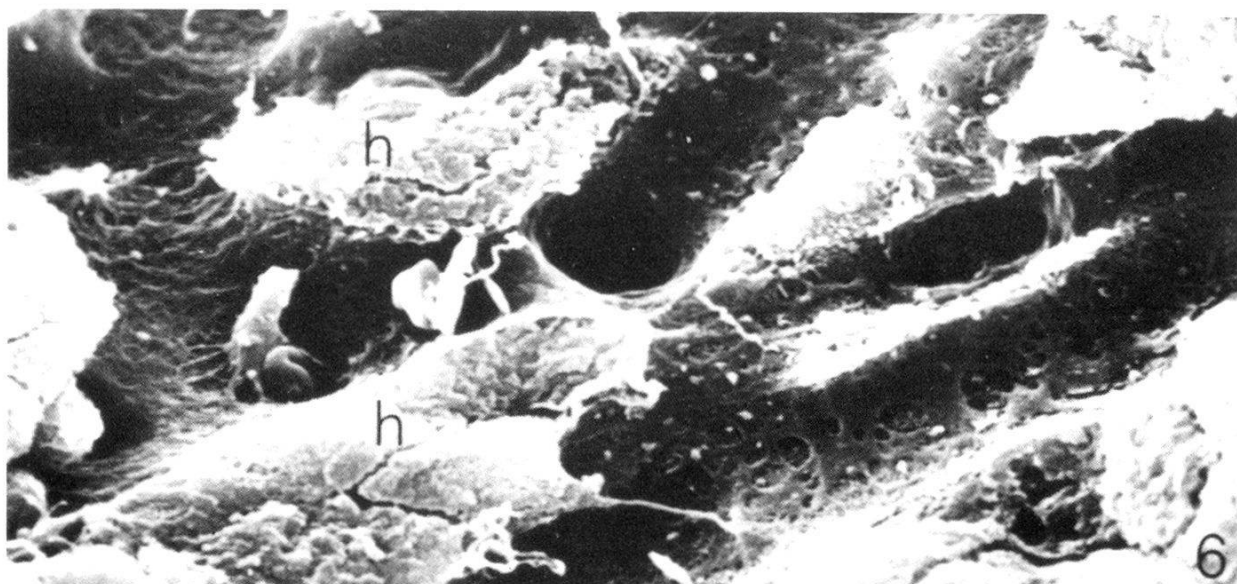
further revealed the presence of silica in vacuoles within the majority of the Kupffer cells remaining in the treated livers (Fig. 7). Silica was very occasionally detected within hepatocytes which were otherwise of normal appearance (Fig. 8). Nonetheless most aspects of treated livers were normal, e. g. the fenestrated structure of the sinusoid endothelium was unchanged (Figs. 4, 6) and hepatocyte microvilli were seen to extend through the fenestrations into the sinusoid lumen. The endothelial lining of the larger blood vessels, e. g. the collecting veins, was complete, but in the silica treated group some endothelial damage was indicated by the inflammatory invasion of the perivascular space (Figs. 9, 10) and margination of leucocytes (Fig. 11).

The physiological effect of silica treatment on liver function was assessed by comparing the uptake of colloidal carbon in normal and silica treated livers in vitro (Fig. 1). The retention of colloidal carbon was greatly reduced although not totally abolished in the silica treated group. Cytological examination of control livers perfused with carbon revealed that most of the retained carbon was located in the few remaining Kupffer cells (Fig. 3) and suggested therefore that the reduced carbon uptake was due to the inhibition of Kupffer cell number and function. It was concluded that the greatest single effect of silica treatment on liver structure and function was the loss of Kupffer cell number and activity. The uptake of sporozoites by perfused livers taken from silica treated rats was significantly lower than that of the controls. Approximately 52% of the initial load was removed in the first passage but only 57% after 15 min of circulation (Table 1). The integrity of the phagocytic Kupffer cell population therefore seems essential for the normal uptake of sporozoites by the liver.

B. The effects of silica treatment upon the infection of mice in vivo by sporozoites of Plasmodium y. nigeriensis

If it is assumed that phagocytosis by the Kupffer cell was, for the majority of sporozoites, the first step in the process of invasion, a loss of Kupffer cell function would result in a *reduction* in infection. By contrast a loss of Kupffer cell function would cause an *increase* in infection if the Kupffer cells were des-

Fig. 6. Scanning electron micrograph of silica treated liver. Note normal sinusoid endothelium and hepatocyte chains (H), and the absence of Kupffer cells. Magn. ×2,750.

Fig. 7. Kupffer cell in sinusoid of silica treated liver with large phagosome containing silica particles (Si). Magn. ×5,506.

Fig. 8. Transmission electron micrograph of rare hepatocyte containing silica (Si) in vacuole. Cytoplasm and nucleus otherwise of normal morphology. Magn. ×3,736.

Fig. 9. Light micrograph of silica treated cell showing inflammation and leucocyte invasion of perivascular space (i). Magn. ×161.

Fig. 10. Transmission electron micrograph of leucocytes in lesion of endothelial wall of collecting vein (E) in silica treated liver. Magn. ×5,744.

Fig. 11. Scanning electron micrograph showing margination of leucocytes in large collecting vein of silica treated liver. Magn. ×1,100.

troying large numbers of injected sporozoites. The success of sporozoite infection in mice was determined by measuring a) the number of animals in a given experimental group which became infected, b) the time to patency of the infected animals and c) the density of the exoerythrocytic schizonts in infected livers 42 h after infection.

In assays (a) and (b), groups of normal and silica treated mice were inoculated intraperitoneally with 0.5, 1.0 and 1.5 times the minimum infective dose of 1000 sporozoites (unpublished observations) (Table 3). The number of mice which became infected in the experimental groups was with one exception lower than in control groups. Additionally, the time to patency in the infected animals was longer in silica treated mice than in controls. These results indicate that silica treatment reduces the success of infection of mice by *P. y. nigeriensis* sporozoites.

A direct assessment of sporozoite infectivity was obtained by determining the density of exoerythrocytic schizonts in normal and silica treated animals 42 h after a large (4.5×10^7) infecting dose of sporozoites (Table 3). These results demonstrate a massive 95% reduction in the density of e. e. schizonts in the silica treated livers. In marked contrast silica treated mice inoculated with blood stages were found to be equally sensitive to infection when compared to normal controls.

Discussion

The objective of this work was to determine whether the sporozoites of mammalian malarial parasites are ingested by phagocytic Kupffer cells within the liver. If so, how efficient was this uptake and what relevance does this event have in vivo to the natural route of infection of the hepatocyte – where the parasite undergoes exoerythrocytic schizogony.

Sporozoite uptake by Kupffer cells

Observations to date indicating that sporozoites may be removed from the blood by Kupffer cells are limited. Sporozoites, infective upon subinoculation, persist in the peripheral circulation for only a very brief period of 15–60 min (Fairley et al., 1947; Mulligan et al., 1949; Canning et al., 1972). This time is, however, significantly extended in mice whose reticuloendothelial system is impaired by pretreatment with silica (Verhave et al., 1980). These rates of clearance are similar to those recorded for other "foreign particles", e. g. bacteria and viruses, where this highly efficient phagocytosis will rapidly remove 80–90% of the initial load (Benacerraf et al., 1959; Brunner et al., 1960). Uptake may be mediated by complement, immunoglobulin, an ill defined "foreign surface" activation (Seljelid, 1980) or by lectin-like binding to sugar moieties. Cytological evidence for the phagocytosis of malaria parasites by Kupffer cells has been

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shown for merozoites (Aikawa and Antonovych, 1964; Terzakis et al., 1979) and possibly also for sporozoites (Verhave et al., 1980).

In the present study the liver perfusion system of Krebs et al. (1973) has proved a most versatile tool with which to examine the interaction of the malarial sporozoite with intact livers. It has shown that even on the first pass of the parasites a significant mechanical trapping of the large sporozoite occurred in the blood vessels of inactive (heat killed, or cooled) livers, additionally it revealed a biologically active retention of parasites in the organ. The latter appeared to depend solely upon the viability of the host tissue and not the viability of the sporozoite. We find this observation surprising in view of the numerous demonstrations of active host cell invasion by sporozoan merozoites and sporozoites (Jensen and Edgar, 1976; Miller et al., 1979; Werk and Bommer, 1980). Our inability to detect a significant active invasion by the parasite may be explained by the low natural infectivity of the rodent malaria sporozoite to laboratory rodent hosts; between 80 and 1000 sporozoites of *P. berghei* or *P. yoelii* are commonly reported as being the minimum dose required to ensure an infection (Killick-Kendrick, 1973; Vanderberg, 1975b; Pacheco et al., 1979). Active parasite invasion at this frequency (i.e. 1/80–1/1000) would not make detectable differences to the observed rate of sporozoite uptake by perfused livers.

The observation that livers from silica treated rats (in which the only cytological change observed was that Kupffer cell population was severely depleted) failed to retain sporozoites with the same efficiency as normal liver following 15 min circulation is strong evidence that it is the phagocytosis of sporozoites by the Kupffer cell which accounts for the biologically active retention of sporozoites by the perfused livers. The present perfusion data further suggests that sporozoite viability, host species specificity, infectivity, maturity and surface coat integrity have little significance in determining sporozoite uptake by isolated perfused liver under the conditions used in these experiments. However, in vivo studies emphasise the essential role of each of these parameters in determining sporozoite infectivity to its host (Vanderberg, 1975b; Cochrane et al., 1976; Turner, 1980). These observations therefore suggest that if the uptake of sporozoites by Kupffer cells is a common step in the route of infection in vivo, then the host specific nature of infection does not reside in the particular recognition of the parasite surface by the phagocyte.

The relevance of phagocytosis of sporozoites by Kupffer cells to the events of host infection in vivo

Although we have been unable to detect sporozoites within Kupffer cells microscopically it can be inferred from the perfusion studies above, that sporozoites are taken up by Kupffer cells in vitro. Danforth et al. (1980) have similarly demonstrated that mouse peritoneal macrophages in vitro will also ingest sporozoites of *P. berghei*, and that the sporozoites persist within the phagocyte

for many hours. The dilemma to be resolved is whether during the events of infection in vivo the sporozoite is necessarily bound to and/or taken up by the Kupffer cell and subsequently escapes into the adjacent hepatocyte; or if the sporozoite specifically recognises the hepatocyte and invades it directly (while those sporozoites that are inevitably ingested by the Kupffer cell are destroyed).

This study has shown not only that perfused livers from silica treated rats are less capable of removing sporozoites from the circulation, but that silica treated mice are less susceptible to infection, showing in particular a massive 95% reduction in the density of exoerythrocytic schizonts compared to controls given the same sporozoite inoculum. The fall in exoerythrocytic schizont density was very large compared to the observed reduction in phagocytosis. This probably resulted from the inherent problem that the high "background" of mechanical retention in the perfused livers reduced our ability to discriminate the full extent of phagocytosis in vitro. Verhave et al. (1980) have shown a smaller but significant (63%) reduction in exoerythrocytic schizont density following a less well defined silica treatment programme. The implication of these observations in vivo combined with the perfusion experiments is that the majority of the infecting population of sporozoites of *Plasmodium yoelii nigeriensis* pass through the Kupffer cell prior to its entry into and development within the hepatocyte. This study does not, however, rule out the possibility that a small proportion of inoculated sporozoites may invade the hepatocyte directly.

Despite the evidence presented here some will interpret the work of Shute et al. (1976) which showed that as few as ten sporozoites of *P. vivax* if inoculated intradermally will induce an infection in humans, and the work of Krotoski (unpublished) showing that 80% of an intravenous inoculum of *P. cynomolgi* sporozoites will form e. e. schizonts, as evidence for a specific directed invasion of hepatocytes by sporozoites. If this interpretation were correct it would be necessary to ask:

1. How do such a high proportion of *P. vivax* and *P. cynomolgi* sporozoites specifically evade the efficient sequestration and/or phagocytic activity of reticuloendothelial cells observed in the present study? Such evasion must on current evidence be a "parasite-active" event, which in view of the poor motility of the sporozoite (Vanderberg, 1975a) is unlikely.

2. Why does silica treatment of susceptible hosts result in such a dramatic reduction in e. e. density? The observed reduction in phagocytosis of the sporozoite results in a prolonged circulation time, and hence a greater probability of direct invasion of the hepatocyte (Verhave et al., 1980). It becomes necessary to argue that under normal circumstances not only that there are specific receptors present on the hepatocyte plasmalemma recognised by the sporozoite but that these hypothetical receptors are coincidentally removed by silica treatment. Kessel et al. (1963) have stated that silica has "no toxicity" for freshly isolated liver cells, and that "the mere intracellular location of silica is not sufficient to cause cytotoxicity for cells other than macrophages". We noted that the very

limited occurrence of silica containing vacuoles in hepatocytes did not result in any other detectable ultrastructural changes in these cells. Hence, specific changes induced by silica in the hepatocyte plasmalemma or hepatocyte metabolism which would limit e.e. development are, on this limited evidence, considered unlikely.

By contrast all these major questions are resolved if it is assumed, as suggested here, that the majority of sporozoites of mammalian malarias pass through the Kupffer cell into the hepatocyte. In which case the outstanding questions are:

1. How does the sporozoite avoid destruction within the Kupffer cell and escape into the hepatocyte? This problem is clearly surmountable as avian malarial parasites undergo a full exoerythrocytic schizogony within phagocytic cells (Huff and Coulston, 1944), and other sporozoa, e.g. *Toxoplasma*, clearly survive in a similar environment. Escape from host cells presents little problem to the malarial sporozoite when in the salivary gland of the mosquito, and the coccidians *Eimeria* and *Toxoplasma* can be shown to escape host cells with consummate ease (Jensen and Edgar, 1976; Werk and Bommer, 1980). It might therefore be anticipated that escape of some malarial sporozoites from the Kupffer cell is inevitable.

2. Why is host infection largely a host specific event? Here the recent evidence of Danforth et al. (1980) and Schulman et al. (1980) may be relevant. The latter authors showed that malarial sporozoites would adsorb serum components of susceptible, but not insusceptible, hosts; and Danforth et al. (1980) suggested that sporozoites were adsorbed and taken up by peritoneal macrophages with greater efficiency in the presence of homologous host serum than serum from a heterologous host species. Hence it may be suspected that the specificity of host infection results from the binding of host serum components by infective, but not non-infective, sporozoites and that this binding enhances the uptake of the sporozoites by the Kupffer cells thus "ensuring" an infection even at very low sporozoite inocula. The work of Tonkin (1947) and Porter et al. (1952) has been interpreted as evidence for just such an enhancement (Schulman et al., 1980). Confirmation of this suggestion is, however, required before specific conclusions can be drawn.

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