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Human Liver Biopsy in *P. falciparum* and *P. vivax* Malaria

A Light and Electron Microscopy Study*

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Bioptische Befunde der Leber bei menschlicher P.-falciparum- und P.-vivax-Malaria

Eine licht- und elektronenmikroskopische Studie

Zusammenfassung. Die Leberpunktate von 5 Patienten mit Plasmodium-vivax- und 1 Patienten mit Plasmodium-falciparum-Malaria zeigen nur unspezifische Veränderungen am endoplasmatischen Retikulum und den Mitochondrien der Leberzellen. An den Sinusoiden zugekehrten Leberzellgrenzen wird eine Verarmung und/oder Deformierung der Mikrovilli mit Schwellung der Endothelien gesehen.

Die Kupfferschen Sternzellen sind hyperplastisch und hypertrophisch und weisen eine lebhafte Phagozytosetätigkeit auf. Sie enthalten parasitisierte und parasitenfreie Erythrozyten. Es wird vermutet, daß die Alteration der Sinusoidgrenzen der Leberzellen einen verminderten Stoffaustausch zwischen Leberzellen und Blutstrom zur Folge hat.

In den Sinusoiden finden sich häufig miteinander verklumpte parasitisierte und parasitenfreie Erythrozyten. Sie werden als Ausdruck eines gestörten Koagulations- und/oder immunologischen Prozesses gewertet. Die örtlichen Zirkulationsstörungen sind nur Teil eines übergeordneten pathogenetischen Mechanismus, durch welchen ein hypoxischer hepatozellulärer Schaden bedingt wird.

Summary. Liver biopsy, in one patient with *P. falciparum* and in five patients with *P. vivax* malaria revealed non-specific hepatocellular damage chracterized by pathologic changes of the endoplasmic reticulum and mitochondria. Also the sinusoidal pole of hepatic cells was altered mainly by depletion of microvilli and/or distortion and swelling of the endothelial cells. Von Kupffer cells were hyperplastic and hypertrophic and exhibited considerable phagocytic activity for both parasitized and non-parasitized erythrocytes. It is believed that the altered sinusoidal pole causes a deficient exchange between the hepatic cells and the blood stream.

Clumped parasitized and non-parasitized erythrocytes were frequently observed in the sinusoids, a finding that could be interpreted as the manifestation of a disturbed coagulation process and/or an immunological mechanism in progress. This local circulatory disturbance is only part of a more general pathogenetic mechanism through which hepatocellular hypoxemic damage would be produced.

Several studies involving liver biopsies in patients with acute malaria have been reported (Brito *et al.*, 1962; Corcoran *et al.*, 1953; McMahon and Derauf, 1954; Telcharov and Todorowa, 1950; White and Doerner, 1954) but in only two has electron microscopy been done. One is a brief antemortem report of a single patient (Miwa and Tanikawa, 1965; Tanikawa, 1968) and the other is a report of light and electron microscopic observations of a liver obtained 15 minutes

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post mortem from a patient with falciparum malaria, blackwater fever, and a severe hemorrhagic pneumonitis (Rosen *et al.*, 1967).

The purpose of this paper is to report the light and electron microscopy findings in liver biopsies obtained from patients with malaria.

Material and Methods

Five patients with *P. vivax* and one with *P. falciparum* acute malaria were selected for this study. The pertinent clinical and laboratory data available up to the moment of the liver biopsy are given in the Table. Percutaneous liver biopsy was done with a Menghini needle in all six patients during the acute first attack (cases 2 and 3) or during relapse (cases 1, 4, 5 and 6). The patient with acute *P. falciparum* malaria was in coma and died 24 hours later from cerebral malaria. The five patients with *P. vivax* malaria were alert and well before and after the biopsy procedure.

Part of the fragment of liver was fixed in Helly's fluid, embedded in paraffin, and sectioned at 5 μ. The following stains were used: hematoxylin-eosin, periodic acid-Schiff (PAS) before and after diastase digestion, Wilder's reticulum, iron and Maximow's stain.

The remainder of the liver biopsy was cut into smaller fragments with a razor-blade, and fixed for two hours at 5° C in 1% solution of osmium tetroxide buffered in pH 7.4 with veronal acetate. To maintain isomolarity, 0.045 g of sucrose was added to each ml of solution. After dehydrating the fragments in an ascending series of ethyl alcohol, they were embedded in Araldyte. Ultrathin sections stained with uranyl acetate and lead citrate were mounted on copper grids and observed and photographed in a Zeiss EM 9 electron microscope.

Two liver biopsies from patients with duodenal ulcer that were obtained during gastrectomy served as control and were studied in a similar manner.

Results

1. *Light Microscopy Findings.* Pathological findings were essentially similar in *P. vivax* and *P. falciparum* malaria, but more intense in the latter. Sinusoids usually appeared dilated and von Kupffer cells were hyperplastic and hypertrophic with engulfed malarial pigment. In five of the six cases including the one of *P. falciparum* malaria, granules of iron were also seen in a few von Kupffer cells. The sinusoidal lining was seen as a thin continuous line, dotted with black granules of malarial pigment. Disse's space was enlarged. Inside the sinusoids clumps of red blood cells were seen, sometimes with neutrophils, mononuclear cells and plasma cells. Although the fixation used was not entirely adequate for preservation of glycogen, hepatic cells, chiefly those located at the center of the lobules with reduced basophilic cytoplasm, appeared frequently but not always to be depleted of glycogen. This finding was seen in four of the six cases, including the one of *P. falciparum* malaria, where it was marked. After diastase digestion, PAS positive granules were demonstrated in the hypertrophic von Kupffer cells. Hepatic cells with two nuclei and a condensed basophilic cytoplasm were also seen among those with a light cytoplasm.

Lipofuscin pigment was observed in five of the cases and was especially prominent in *P. falciparum* malaria. Isolated hepatic cells in five of the six cases exhibited iron granules in their cytoplasm, including *P. falciparum*. Iron also was evident in the one case without lipofuscin pigment.

Portal spaces were found in only three cases of *P. vivax* malaria, and in two of them they were edematous with dilated small vessels. The inflammatory infiltrate was mainly of histiocytes.

2. *Electron Microscopy Findings.* The findings in *P. falciparum* and *P. vivax* malaria were essentially the same, but more marked in the former. Endothelial cells were swollen, their cytoplasm partially overlapping (Fig. 1) and a basal membrane-like material sometimes appeared at the points of contact between them. Von Kupffer cells were hyperplastic and hypertrophic, with many autophagic vacuoles and osmiophilic bodies that varied in size and were usually delimited

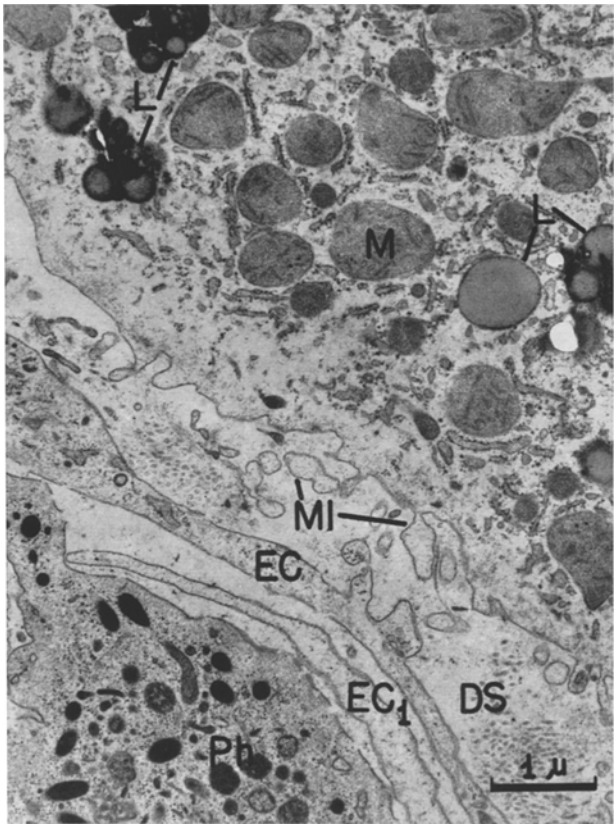


Fig. 1 (*P. falciparum* malaria): Overlap of swollen endothelial cells (*EC*) cytoplasms. Part of a phagocyte, probably a granulocyte (*Ph*) is seen inside the sinusoidal lumen. Hepatic cell show marked glycogen depletion, and scanty edematous microvilli (*MI*)

Table

Case no.	Sex	Race	Age (years)	*Plasmodium*	Clinical data			
					Duration of the clinical history	jaundice[a]	enlargement of spleen	enlargement of liver
1 3498	♂	mulatto	28	*P. vivax*	2 months	++	7 cm	10 cm
2 3515	♂	white	43	*P. vivax*	5 days	+	—	4 cm
3 3553	♂	white	63	*P. falciparum*	13 days	—	—	—
4 3528	♂	mulatto	17	*P. vivax*	4 months	—	4 cm	—
5 3644	♀	mulatto	15	*P. vivax*	3 months	—	6 cm	6 cm
6 3657	♀	mulatto	11	*P. vivax*	5 months	—	2 cm	6 cm

n.a. = non available.

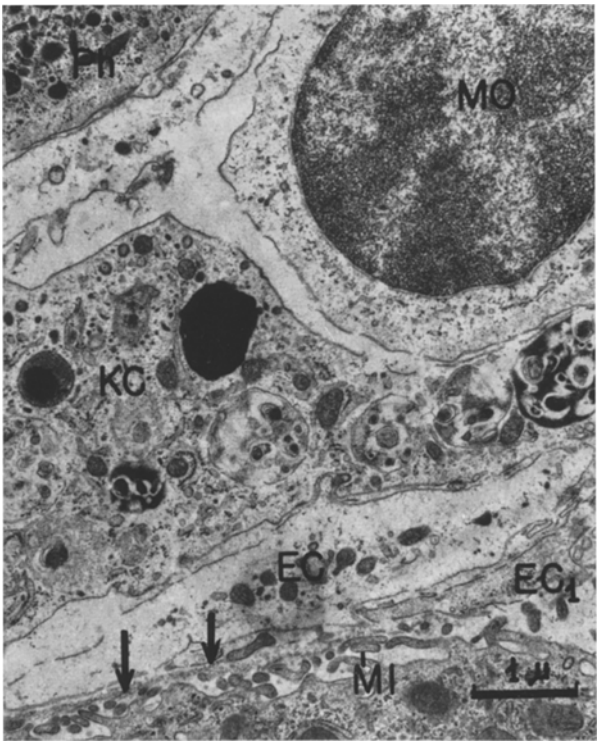
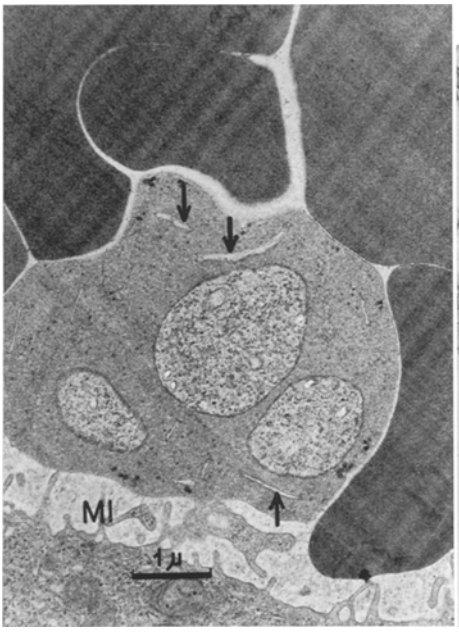


Fig. 2 (*P. falciparum* malaria): Enlarged Kupffer cell (*KC*) with dense and laminated bodies, surrounded by a single membrane, interpreted as malarial pigment. A mononuclear cell (*MO*) is also seen inside the sinusoid. Swollen endothelial cells (*EC* and EC_1) are seen partially overlapping and presenting beneath them a faint linear deposit interpreted as a basement membrane-like material (arrows). Microvilli (*MI*) are preserved

Table

Laboratory data				
hemoglobin (g/100 ml)	leucocytes (mm^3)	total bilirubin (mg/100 ml)	glutamic oxalacetic transaminase-SF unit normal 8—40	Mucoprotein (mg/100 ml) normal 2—5
12.3 (77%)	4.800	7.1	128	3.4
13.7 (86%)	3.900	0.7	61	4.9
14.5 (100%)	12.000	n.a.	n.a.	n.a.
11.4 (71%)	7.300	0.3	12	4.0
8.4 (53%)	5.100	n.a.	n.a.	n.a.
13 (81%)	9.000	0.4	12	4.16

[a] Intensity of the jaundice graded from + to ++++.



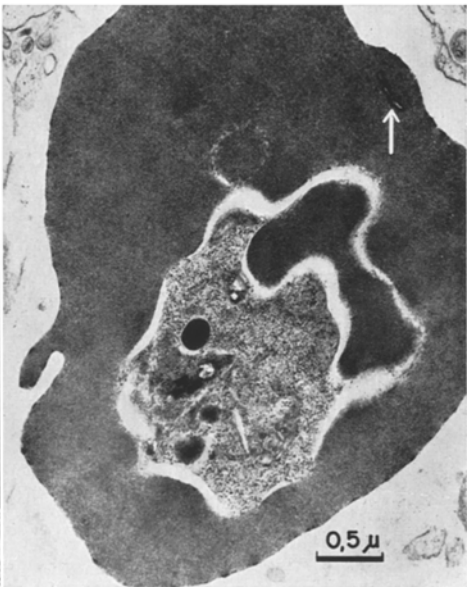


Fig. 3 Fig. 4

Fig. 3 (*P. vivax* malaria): Agglutinated erythrocytes inside the sinusoidal lumina, one of them parasitized and having a light stroma. Arrows designates the clefts

Fig. 4 (*P. falciparum* malaria): Parasitized erythrocyte showing an irregular contour. White arrow designates a cleft in the erythrocyte stroma

by single or double membranes. These bodies were identified as malarial pigment. As Rosen *et al.* (1967) pointed out, they were sometimes composed of osmiophilic material in which rectangular or trapezoidal areas could be seen. Sometimes the osmiophilic body was made up of laminated structures or multiple membranes (Fig. 2).

Chiefly in *P. falciparum* malaria the von Kupffer cells showed engulfed altered parasitized and non-parasitized erythrocytes. The sinusoids contained several types of cells, some identified as plasma cells, lymphocytes and granulocytes. Occasionally, platelets were also seen both among the cells mentioned or engulfed by von Kupffer cells.

Malaria erythrocytes were agglutinated mainly in the cases of *P. vivax* (Fig. 3). Parasitized erythrocytes were frequently seen in *P. falciparum* malaria. The malarial parasites, evidently immature trophozoites, were characterized by abundant ribosomes, cytoplasmic membranes and a peripheral double membrane. They were essentially as described by Trager *et al.* (1966) and Ladda *et al.* (1966). Clefts were observed in the erythrocytes, interpreted as cytoplasmic prolongations of the parasite (Trager *et al.*, 1966; Aikawa *et al.*, 1966) (Fig. 3). Frequently the parasitized red blood cells had a lightly stained stroma, but this aspect could be seen in apparently non-parasitized erythrocytes. Their contours were irregular but no peripheral vacuolization, as described by Trager *et al.* (1966), was noted (Fig. 4).

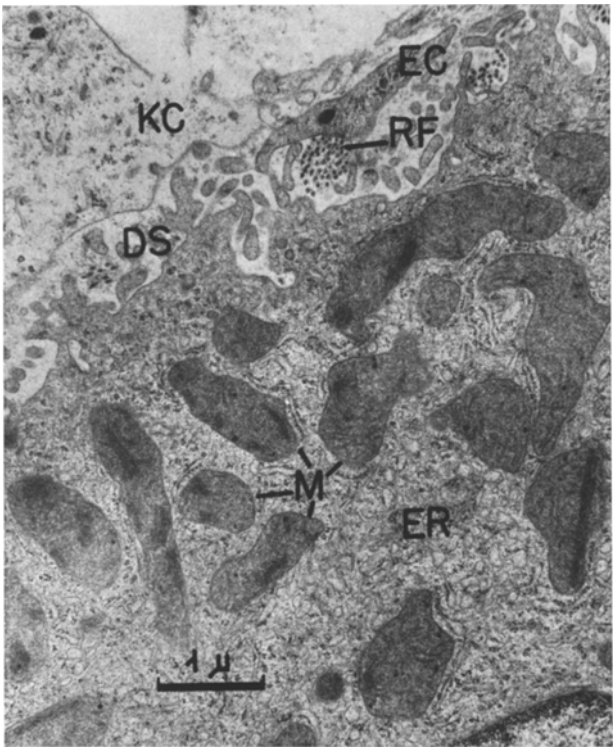


Fig. 5 (*P. vivax* malaria): Hepatic cells showing mitochondria (*M*) irregular in size and shape, and exhibiting crystalline bodies in their matrix. Agranular endoplasmic reticulum (*ER*) with dilated cisternae predominates. There is marked glycogen depletion. *EC* designates a endothelial cell cytoplasm, *RF* reticulum fibers inside Disse's space (*DS*) and *KC* a hypertrophic Kupffer cell

The sinusoidal pole of some hepatocytes, chiefly beneath hypertrophic von Kupffer cells, appeared partially devoid of microvilli or these were edematous. These changes were marked in *P. falciparum* malaria. (Fig. 1).

Groups of normal hepatic cells alternated with pathological ones. Chiefly in *P. falciparum* malaria, the latter were depleted of glycogen and ribonucleic granules. The agranular endoplasmic reticulum predominated in the altered hepatic cells of both malarias and their cisternae appeared dilated (Fig. 5). Mitochondria in some cells varied in size and shape. The so-called "fibrillary degeneration" or "crystalline structures" were observed in groups of mitochondria, chiefly in cases of *P. vivax* malaria (Fig. 5).

Lipofuscin pigment was frequently observed, mainly in *P. falciparum* malaria usually near the biliary pole (Fig. 6), together with more coarse electron-dense granules which were delimited by a single membrane and identified as ferritin. It is worth mentioning that some granules, presumed to be lipofuscin, exhibited the rectangular or trapezoidal areas seen in the malarial pigment. Cytosomes and cytosegresomes were more frequent in damaged cells, were located near bile

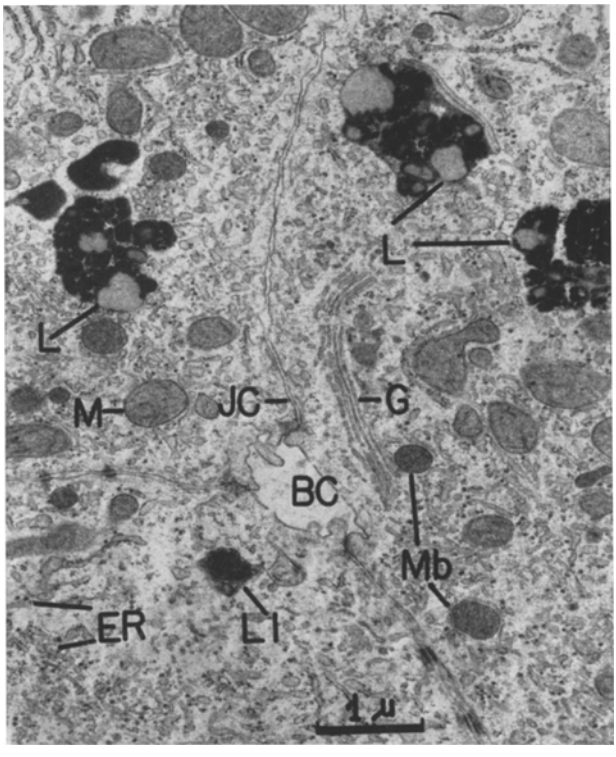


Fig. 6 (*P. falciparum* malaria): Bile capillary (*BC*) with few distorted microvilli. Hepatic cells showing lipofuscin (*L*) pigment, microbodies (*Mb*), marked glycogen depletion and a predominance of the agranular reticulum. Mitochondria (*M*) are normal and the junctional complexes (*JC*) are preserved. *LI* designates a lysosome

capillaries, and were associated with an apparent increase in number of microbodies.

The bile capillary was seldom partially or totally devoid of microvilli (Fig. 6). The few microvilli left were either normal or edematous. In the lumina of bile capillaries an electron-dense laminated material was occasionally observed. These findings were not frequently observed but were more common in *P. falciparum* malaria.

The Golgi apparatus was either normal or had somewhat dilated vesicles containing a round electron-dense material in the lumina.

Comments

The hepatocellular damage seen in *P. falciparum* and *P. vivax* was essentially similar and non-specific, but was generally more marked in the former. Light microscopy did not show centrolobular necrosis as reported in necropsied human cases and experimental malaria (Andrews, 1948; Maegraith, 1948; Jervis *et al.*, 1968), but revealed that the more damaged cells were at the center of the lobules. Such a preferential site of injury has been interpreted over the years by Maegraith

and his school (1948) to be the result of hypoxia due to severe anemia, impeded outflow through the hepatic vein and more recently, reduced blood flow in the portal venous system (Skirrow *et al*, 1964).

Ultrastructural studies revealed alteration of mitochondria in both malarias, chiefly in *P. vivax* malaria. On the other hand, depletion of glycogen and ribonucleic granules were more prominent in acute *P.falciparum* malaria. Proliferated agranular endoplasmic reticulum was seen in both malarias, but lipofuscin pigment, nowadays interpreted as the end-result of autophagic digestion (Schaffner, 1963) was a more prominent finding in *P. falciparum* malaria.

These differences are probably due to the acuteness and severity of *P. falciparum* malaria as compared with the more benign and protracted course of *P. vivax* malaria. The crystalline bodies in mitochondria are apparently lesions which take more time to develop since they were more frequent in the last type of malaria.

According to Fletcher and Maegraith (1966), damage to mitochondria explains the active inhibition of cellular respiration and oxidative phosphorylation seen in mammalian malaria. Soluble substances are present in the plasma of infected animals and in some cases of human *P. falciparum* malaria that inhibit the respiration and active phosphorylation of isolated normal liver cell-mitochondria.

Bhamarapravati (Desowitz, 1967) found that bilirubin transport was in some way disturbed in malaria, possibly by a reticuloendothelial blockade which impeded the blood flow or by derangement of the microvilli. Maegraith and his research group (Desowitz, 1967) revealed that fat transport in the liver cell is disturbed in simian and human malaria. There is a demonstrable damage to mitochondria and endoplasmic reticulum in simian malaria, a finding also demonstrable in our human cases.

In human acute malaria we found prominent pathological changes at the sinusoidal pole end of the hepatic cell where swollen endothelial and hyperplastic and hypertrophic Kupffer cells were present with focally altered microvilli. The overlap of swollen cytoplasm of endothelial cells, sometimes with a deposition of a basal membrane-like material also contributed to alter the exchange between the circulating blood stream and the hepatic cell.

Our findings, therefore, essentially agree with the observations of Bhamarapravati and Maegraith although for us, endoplasmic reticulum pathology predominated over the mitochondrial damage. Furthermore, on a purely morphological basis, considering the alterations of the bile capillary microvilli, a moderate degree of impairment of bile excretion could be expected chiefly in acute *P. falciparum* malaria.

A clinico-pathologic correlation was not possible in our cases because adequate laboratory data was not available. However, the two patients with jaundice had high levels of transaminase, but lower than those seen in virus hepatitis, as previously pointed out by Bhamarapravati (Desowitz, 1967).

Our case of *P. falciparum* malaria did not show the plasma membrane disruptions and the extensive vacuolization of sinusoidal cells described by Rosen *et al.* (1967). Such changes probably represent post mortem ultrastructural artefacts. Malarial pigment, however, appeared as it was formerly characterized in their reports (Rosen *et al.*, 1967 and 1968).

Another point deserving comment is the clumping of erythrocytes in the sinusoids, seen in both malarias. This aspect has long been reported in Knisely's observations "in vivo" in experimental and human malaria (Knisely, 1941 and 1943). It occurs chiefly in the brain (Maegraith, 1948). The process depends for its development on the escape of abnormal amounts of protein and fluid across the endothelial cell membrane rendered more permeable by multiple factors, such as anoxia and/or increased concentration of active kinins (Fletcher and Maegraith, 1966). However, increased permeability is not noticeable in the liver where sinusoids normally allow the passage of certain amounts of protein so that the liver cells are bathed in fluid with a high protein content. It is possible, then, that the immunological mechanism that plays a definite role in malaria anemia (Dixon, 1966) and even the accelerated intravascular coagulation also seen in human and experimental malaria (Dennis *et al.*, 1966) can be held responsible for the erythrocyte agglutination in the sinusoids.

In any circumstance, a local disturbance of the blood flow impedes the exchanges between hepatic cells and the circulating media, and is one of the multiple causes of the hepatocellular damage seen in malaria.

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