

## A STUDY ON THE PATHOGENESIS OF HUMAN CEREBRAL MALARIA AND CEREBRAL BABESIOSIS

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*Cerebral complications are important, but poorly understood pathological features of infections caused by some species of Plasmodium and Babesia. Patients dying from P. falciparum were classified as cerebral or non-cerebral cases according to the cerebral malaria coma scale. Light microscopy revealed that cerebral microvessels of cerebral malaria patients were filled with a mixture of parasitized and unparasitized erythrocytes, with 94% of the vessels showing parasitized red blood cell (PRBC) sequestration. Some degree of PRBC sequestration was also found in non-cerebral malaria patients, but the percentage of microvessels with sequestered PRBC was only 13%. Electron microscopy demonstrated knobs on the membrane of PRBC that formed focal junctions with the capillary endothelium. A number of host cell molecules such as CD36, thrombospondin (TSP) and intercellular adhesion molecule 1 (ICAM-1) may function as endothelial cell surface receptors for P. falciparum-infected erythrocytes. Affinity labeling of CD36 and TSP to the PRBC surface showed these molecules specifically bind to the knobs. Babesia bovis infected erythrocytes produce projections of the erythrocyte membrane that are similar to knobs. When brain tissue from B. bovis-infected cattle was examined, cerebral capillaries were packed with PRBC. Infected erythrocytes formed focal attachments with cerebral endothelial cells at the site of these knob-like projections. These findings indicate that cerebral pathology caused by B. bovis is similar to human cerebral malaria. A search for cytoadherence proteins in the endothelial cells of cattle may lead to a better understanding of the pathogenesis of cerebral babesiosis.*

Key words: human cerebral malaria – cerebral babesiosis – pathogenesis – Plasmodium – Babesia - rhesus monkey

Sequestration of parasitized erythrocytes (PRBC) in microvessels is one of the major pathological changes seen in severe falciparum malaria (Aikawa, 1988; Aikawa et al., 1990; Pongponratn et al., 1991). The disruption of blood flow caused by PRBC sequestration contributes to the pathological changes in various organs and to organ failure. The severity of falciparum malaria appears to be proportional to the degree of PRBC sequestration in microvessels of various organs, particularly the brain.

We examined 39 falciparum malaria autopsy cases from the Hospital for Tropical Diseases, Mahidol University, Bangkok, Thailand and classified them as cerebral malaria (CM) or non-cerebral malaria (NCM) cases by employing the cerebral malaria coma scale (Riganti et al., 1990). Based on this scale, we compared the PRBC sequestration rate between CM and NCM in various organs including the brain, heart, lungs and small intestines. In addition, we discuss various cytoadherence proteins in relation to PRBC sequestration in microvessels.

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Cattle infected with *Babesia bovis* often die from cerebral babesiosis (Aikawa et al., 1985). The pathogenesis of cerebral babesiosis appears to be similar to that of cerebral malaria. We performed light and electron microscopy on brains of cattle that died from cerebral

babesiosis and compared pathological findings with those of cerebral malaria.

#### THE PATHOLOGY OF THE CENTRAL NERVOUS SYSTEM IN CEREBRAL MALARIA AND NON-CEREBRAL MALARIA

The World Health Organization malaria action programme states that for a diagnosis of cerebral malaria, there must be unrousable coma, exclusion of other encephalopathies and confirmation of *P. falciparum* infection (Riganti et al., 1990). In the past, these criteria have not been strictly applied, resulting in conflicting reports on the pathological findings of cerebral malaria, particularly the importance of PRBC sequestration in cerebral microvessels.

Warrell et al. in 1988 established the cerebral malaria coma scale (Table). When clinical findings on admission showed a coma scale of two or more, the patients were designated as having cerebral malaria. Based on this coma scale and the strict diagnostic criteria of WHO, we identified 24 cerebral malaria and 15 non-cerebral malaria patients from the Hospital for Tropical Diseases, Mahidol University, Bangkok, Thailand.

In order to determine the presence of cerebral edema, we examined the presence of uncal herniation in postmortem brains. Uncal herniation was found in 70% of cerebral malaria brains and 60% of non-cerebral malaria brains. Since no statistical difference ( $p > 0.005$ ) was found, the significance of cerebral edema in cerebral malaria is unknown.

Light microscopy revealed that cerebral microvessels of all cerebral malaria patients were filled with a mixture of parasitized and

unparasitized erythrocytes (Fig. 1). PRBC sequestration was quantified by the examination of 300 cerebral microvessels from each patient and expressed as the percentage of cerebral microvessels with sequestered PRBC. The percentage of PRBC sequestration in cerebral microvessels ranged from 57 to 100% and on average, 94% of cerebral microvessels showed PRBC sequestration. On the other hand, among non-cerebral malaria patients, the percentage of cerebral microvessels with sequestered PRBC was 13% on average. The high rate of PRBC sequestration appeared to be associated with high parasitemia (Pongponratn et al., 1991).

Electron microscopy has previously demonstrated multiple electron dense knobs protruding from the membranes of PRBC (Aikawa, 1988; Luse & Miller, 1971). These knobs formed focal junctions with endothelial cells and adjacent erythrocytes (Fig. 2), and this phenomenon appears to be a major event in the process responsible for PRBC sequestration.

Ring Hemorrhages were seen in 96% of cerebral malarial patients, while they were found in 33% of non-cerebral malaria patients. A statistical significance was seen between these two groups ( $p < 0.005$ ). This finding clearly indicates that ring hemorrhages occur frequently in cerebral malaria brains. The microvessels located in the center of a ring hemorrhage were often blocked with PRBC.

Our study clearly demonstrated that PRBC sequestration in cerebral microvessels is the major pathological change which distinguishes cerebral from non-cerebral falciparum malaria. Also, we demonstrated that the cerebral malaria coma scale developed by Warrell et al. (1988) correlates well with the degree of PRBC sequestration in cerebral microvessels.

TABLE

Coma scale for cerebral malaria

0:	Rousable to full consciousness.
1:	Impaired consciousness but purposeful response to stimuli.
2:	Unrousable, motor response non-localizing.
3:	Unresponsive, tendon reflexes intact.
4:	Unresponsive, areflexic.

(From Warrell et al., 1988. *Lancet*, ii: 534).

#### THE PATHOLOGY OF THE HEART, LUNGS AND SMALL INTESTINES IN CEREBRAL AND NON-CEREBRAL FALCIPARUM MALARIA

The heart of cerebral malaria patients showed fatty degeneration, focal necrosis of cardiac muscle and sub-pericardial hemorrhages (Pongponratn et al., 1991). On the other hand, changes in the hearts of non-cerebral malaria patients were minimal. PRBC sequestration was seen in myocardial microvessels of both CM and NCM cases. However, an

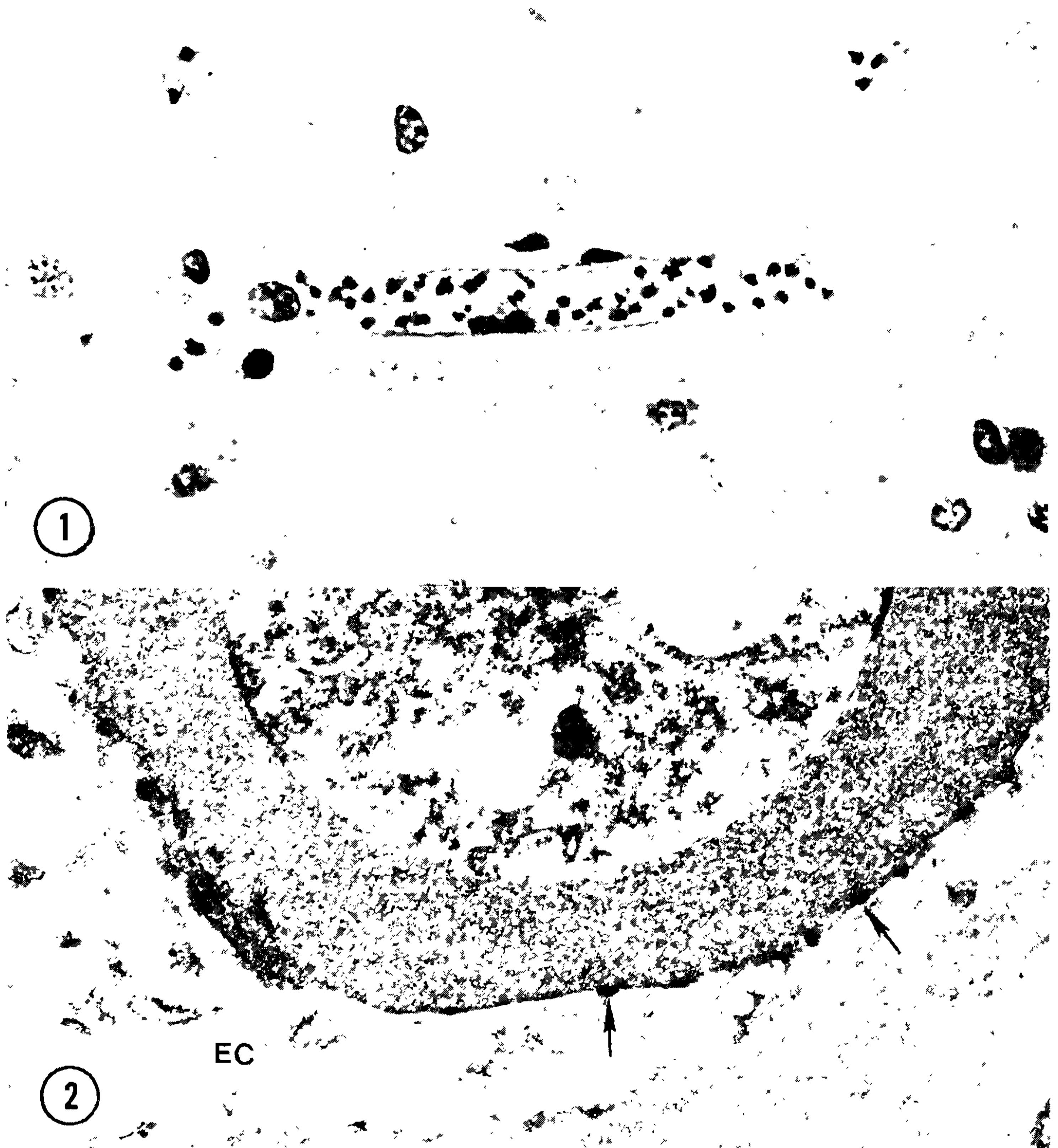


Fig. 1: a cerebral microvessel from a cerebral malaria patient showing PRBC sequestration. (H&E Stain). Fig. 2: electron micrograph showing adhesions (arrow) between knobs and endothelial cells (EC) of a cerebral microvessel from a cerebral malaria patient. X 35,000. (From Pongponratn et al., 1991. *Am J. Trop. Med. Hyg.*, 44: 168-175).

average of 56% of these vessels showed PRBC sequestration in cerebral malaria patients, while myocardial vessels showed only 19% PRBC sequestration on average in the NCM cases.

The lungs of cerebral malaria patients were edematous (Deaton, 1970), congested and marked by diffuse thickening of alveolar septa. The thickening of the alveolar septa was associated with an accumulation of PRBC within microvessels. On average, PRBC sequestration was found in 51% of CM lung vessels. Among

non-cerebral falciparum malaria patients, pulmonary edema and congestion and the thickening of alveolar septa were also observed. PRBC sequestration was seen in 5% of blood vessels of lungs of the NCM group.

In the intestinal mucosal and villous capillaries of cerebral malaria patients, an average of 61% showed PRBC sequestration. Submucosal hemorrhages were seen in two cases. On the other hand, an average of 15% of mucosal and villous vessels showed PRBC sequestra-

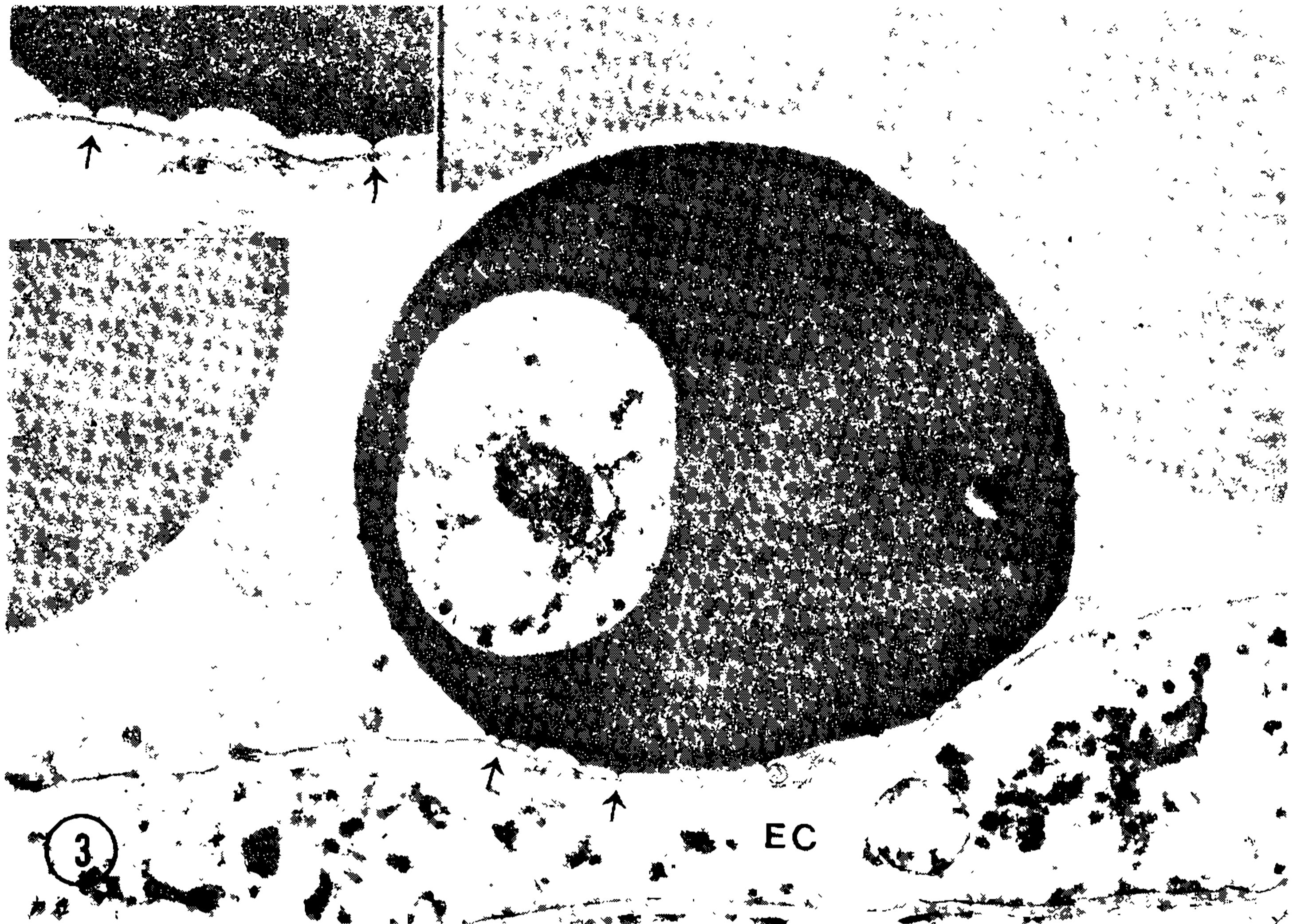


Fig. 3: electron micrograph showing adhesion (arrow) between stellate protrusions and endothelial cells (EC) of cerebral microvessels from cerebral babesiosis cattle. X 19,000. Inset: a higher magnification micrograph showing adhesion (arrow) between protrusions and the endothelial cell. X 36,000

tion in non-cerebral falciparum malaria patients.

Our study, therefore, indicates that cerebral malaria patients showed a high degree of PRBC sequestration in small blood vessels of the internal organs (Fig. 4). The comparison between cerebral malaria and non-cerebral falciparum malaria patients clearly showed that the cerebral malaria group had a higher average percent-

age of PRBC sequestration than the non-cerebral falciparum malaria patients in the brain, heart, lungs and small intestine. This suggests that the high degree of sequestration is responsible for the development of severe cerebral malaria (Pongponratn et al., 1991). Severe falciparum malaria alters the normal function of many tissues and organs due to impairment of blood flow resulting from PRBC sequestration in microvessels.

CYTOADHERENCE PROTEINS AND PRBC SEQUESTRATION IN MICROVESSELS

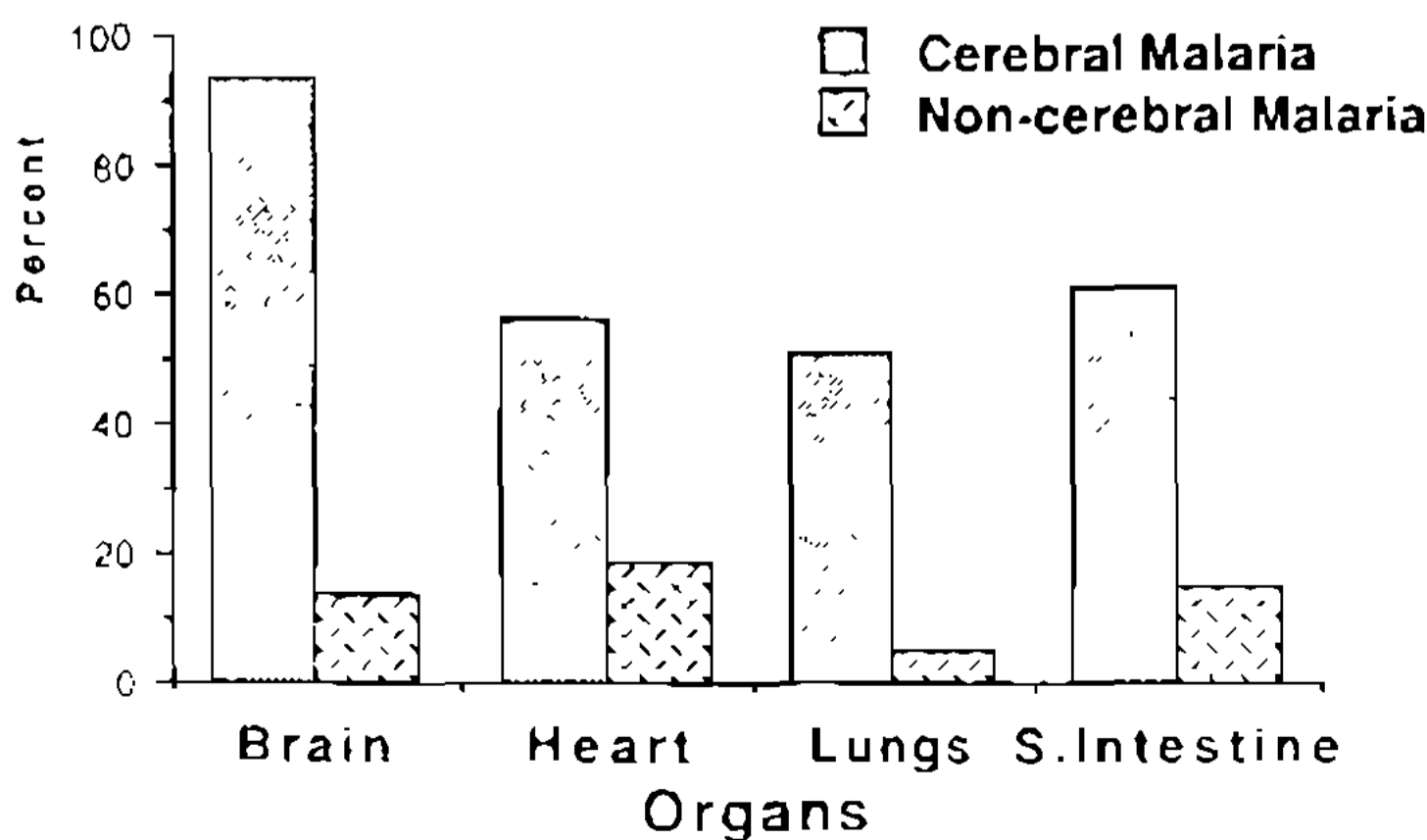


Fig. 4: parasitized red blood cell sequestration in cerebral malaria and non-cerebral malaria. (From Pongponratn et al., 1991. *Am. J. Trop. Med. Hyg.*, 44: 168-175).

Multiple electron dense knobs protrude from the membrane of infected erythrocytes. These knobs of PRBC attach to the endothelial cells of microvessels resulting in PRBC sequestration. At least three malarial proteins are associated with knobs: Pf EMP1 (EMP = erythrocyte membrane protein), Pf EMP2 and Pf HRP1 (HRP = histidine rich protein). Another malarial protein Pf HRP2 has been shown to be secreted from intact infected erythrocytes into culture medium (Howard et al., 1986). Howard et al. (personal communication) recently indicated that Pf EMP1 possesses the cytoadherence property and Pf EMP2 and Pf

HRP1 are responsible for the formation of knobs.

Recently, several investigators suggested that host cell molecules such as CD36 (Barnwell et al., 1989), thrombospondin (TSP) and ICAM-1 (Berendt et al., 1989) may function as the endothelial cell surface receptors for *P. falciparum* - infected erythrocytes. Immunohistochemistry demonstrated the presence of CD36, TSP and ICAM-1 in the endothelium of cerebral microvessels (Aikawa et al., 1990). This indicates that these molecules can act separately or together as molecules responsible for the sequestration of PRBC *in vivo*. Our preliminary data showed that these molecules can also specifically bind to knobs of PRBC by immunoelectron microscopy.

#### THE PATHOLOGY OF CEREBRAL BABESIOSIS

*Babesia bovis* is one of the causative agents of cerebral babesiosis. Light microscopy of the brain of cattle infected with *B. bovis* showed that *B. bovis*-infected erythrocytes sequestered in cerebral microvessels. Electron microscopy demonstrated that PRBC showed many stellate protrusions similar to knobs seen in *P. falciparum*-infected erythrocytes (Wright, 1972). These protrusions adhered to the endothelial cells of cerebral microvessels (Fig. 3). In addition, they adhered to adjacent erythrocytes by stellate projections within cerebral microvessels. These observations indicate that PRBC sequestration is also one of the major pathological findings of cerebral babesiosis as is seen in cerebral malaria. Therefore, search for cytoadherence proteins such as CD36, TSP and ICAM-1 may be involved in PRBC sequestration in cerebral microvessels in cerebral babesiosis. In addition, ligands on the stellate protrusions on PRBC should be identified in order to understand the pathogenesis of cerebral babesiosis as well as to prevent and/or treat the disease.

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#### REFERENCES

- AIKAWA, M., 1988. Human cerebral Malaria. *Am. J. Trop. Med. Hyg.*, 39: 3-10.
- AIKAWA, M.; ISEKI, M.; BARNWELL, J.W.; TAYLOR, D.; Oo, M.M. & HOWARD, R.J., 1990. The pathology of human cerebral malaria. *Am. J. Trop. Med. Hyg.*, 43: 30-37.
- AIKAWA, M.; RABBEGE, J.; UNI, S.; RISTIC, M. & NUTLER, L.H., 1985. Structural alteration of the membrane of erythrocytes infected with *Babesia bovis*. *Am. J. Trop. Med. Hyg.*, 34: 45-49.
- BARNWELL, J.W.; ASCH, A.S.; NACHMAN, R.L.; YAMADA, M.; AIKAWA, M. & INGRAVALLO, P., 1989. A human 88 kDa membrane glycoprotein (CD 36) functions *in vitro* as a receptor for a cytoadherence ligand of *Plasmodium falciparum* - infected erythrocytes. *J. Clin. Invest.*, 84: 765-772.
- BERENDT, A.R.; SIMMONS, D.L.; TANSEY, J.; NEWBOLD, C.I. & MARCH, K., 1989. Intercellular adhesion molecule - 1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. *Nature*, 341: 57-59.
- DEATON, J.G., 1970. Fatal pulmonary edema as a complication of acute falciparum malaria. *Am. J. Trop. Med. Hyg.*, 19: 196-201.
- HOWARD, R.J.; UNI, S.; AIKAWA, M.; ALEY, S.B.; LEECH, J.H.; LEW, A.M.; WELLEMS, T.E.; RENER, J. & TAYLOR, D.W., 1986. Secretion of malarial histidine-rich protein (Pf HRP-II) from *Plasmodium falciparum* infected erythrocytes. *J. Cell Biol.*, 103: 1269-1277.
- LUSE, S.A. & MILLER, L.H., 1971. *Plasmodium falciparum* malaria: ultrastructure of parasitized erythrocytes in cardiac vessels. *Am. J. Trop. Med. Hyg.*, 20: 655-660.
- PONGPONRATN, E.; RIGANTI, M.; PUNPOOWONG, B. & AIKAWA, M., 1991. Microvascular sequestration of parasitized erythrocytes in human falciparum malaria: A pathological study. *Am. J. Trop. Med. Hyg.*, 44: 168-175.
- RIGANTI, M.; PONGPONRATN, E.; TEGOSHI, T.; LOOAREESUWAN, S.; PUNPOOWONG, B. & AIKAWA, M., 1990. Human cerebral malaria in Thailand: a clinico-pathological correlation. *Immunol. Letters*, 25: 199-206.
- WARRELL, D.A.; WHITE, N.J.; VEALL, N.; LOOAREESUWAN, S.; CHANTHAVANICH, P.; PHILLIPS, R.E.; KARBWANG, J. & PONGPAEW, P., 1988. Cerebral anaerobia glycolysis and reduced cerebral oxygen transport in human cerebral malaria. *Lancet*, ii: 534.
- WRIGHT, I.G., 1972. An electron microscopic study of intravascular agglutination in the cerebral cortex due to *Babesia argentina* infection. *Inter. J. Parasitol.*, 2: 209-215.