



**MAHIDOL
UNIVERSITY**

Pathology and Electron Microscopy in Severe Malaria

Department of Tropical Pathology

Faculty of Tropical Medicine

Mahidol University

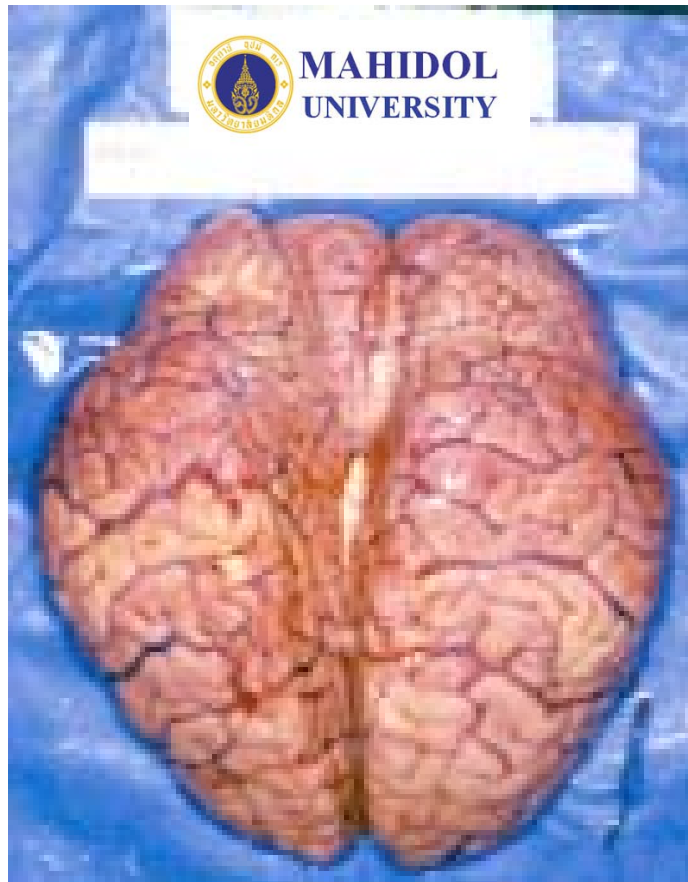
The pathophysiology of severe malaria is complex. Most severe and fatal malaria in humans is caused by *Plasmodium falciparum*, manifests clinically as a spectrum of disease ranging from asymptomatic or mild infection through severe to fatal disease.

Death can result from a variety of syndromes including

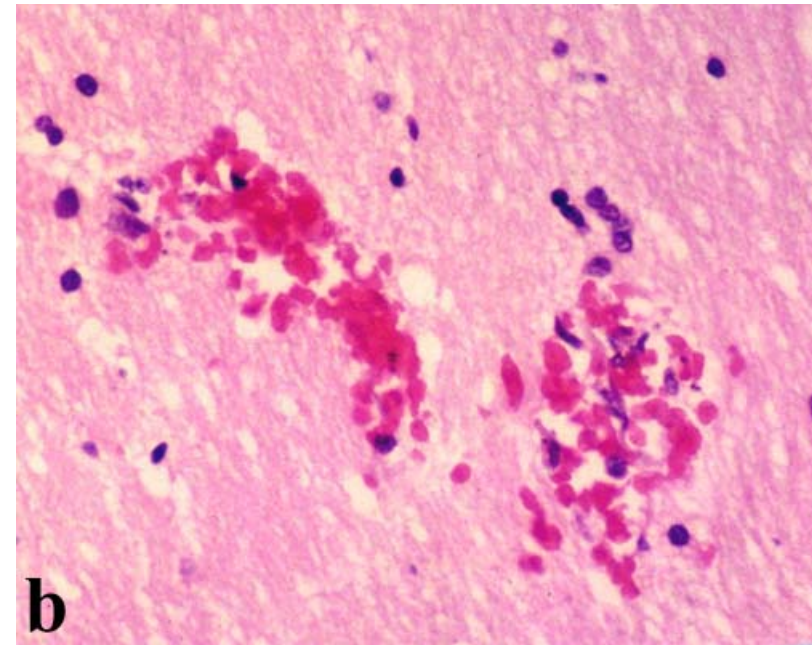
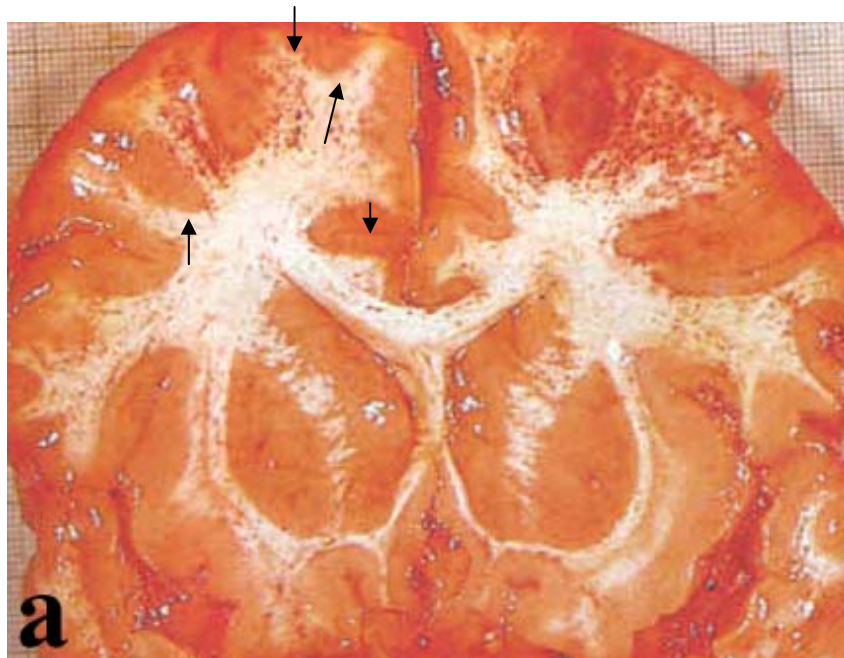
- renal failure,**
- severe anaemia,**
- shock,**
- multi-system organ failure**
- and cerebral malaria.**

Cerebral malaria is the most common clinical presentation and accounts for the majority of deaths in severe malaria (WHO, 2000).

**Severe malaria :
Swollen, oedematous,
congested brain**



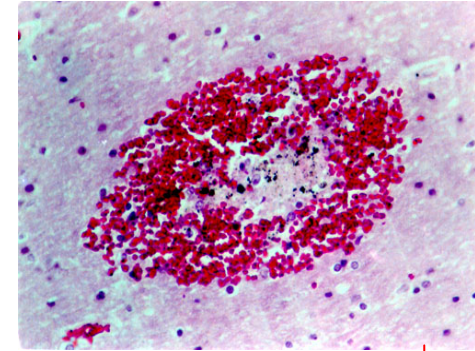
Many, but not all, post mortem brain in cases of fatal malaria has been described as oedematous (Riganti *et al.*, 1990). Cerebral oedema is detected less commonly during life, in two of ten unusually severe cases of cerebral malaria in Thailand, cerebral oedema developed as an agonal phenomenon (Looareesuwan *et al.*, 1983). Macpherson *et al.* (1985) found minimal evidence of oedema. Oo *et al.* (1987) found that cerebral oedema was variable in their study.



Petechial haemorrhages. a) Slicing brain from a fatal case of cerebral malaria shows petechial haemorrhages, mainly in the white mater. b) Two small haemorrhages in the white matter, around small vessels (H&E stain, x 400).

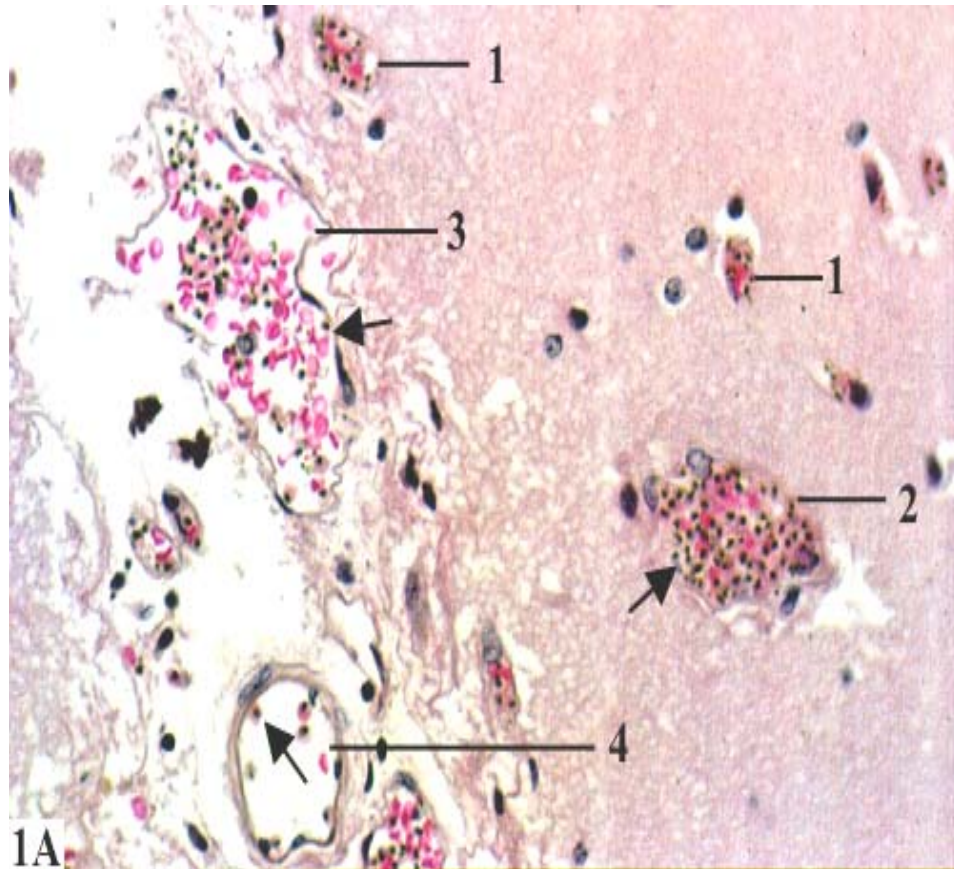
Brain, ring haemorrhages

(*Plasmodium falciparum*)



A cerebral capillary diapedesis which leading to formation of perivascular haemorrhage. Intravascular content including RBC and PRBC leak into the surrounding brain parenchymal tissue forming **ring haemorrhage**.

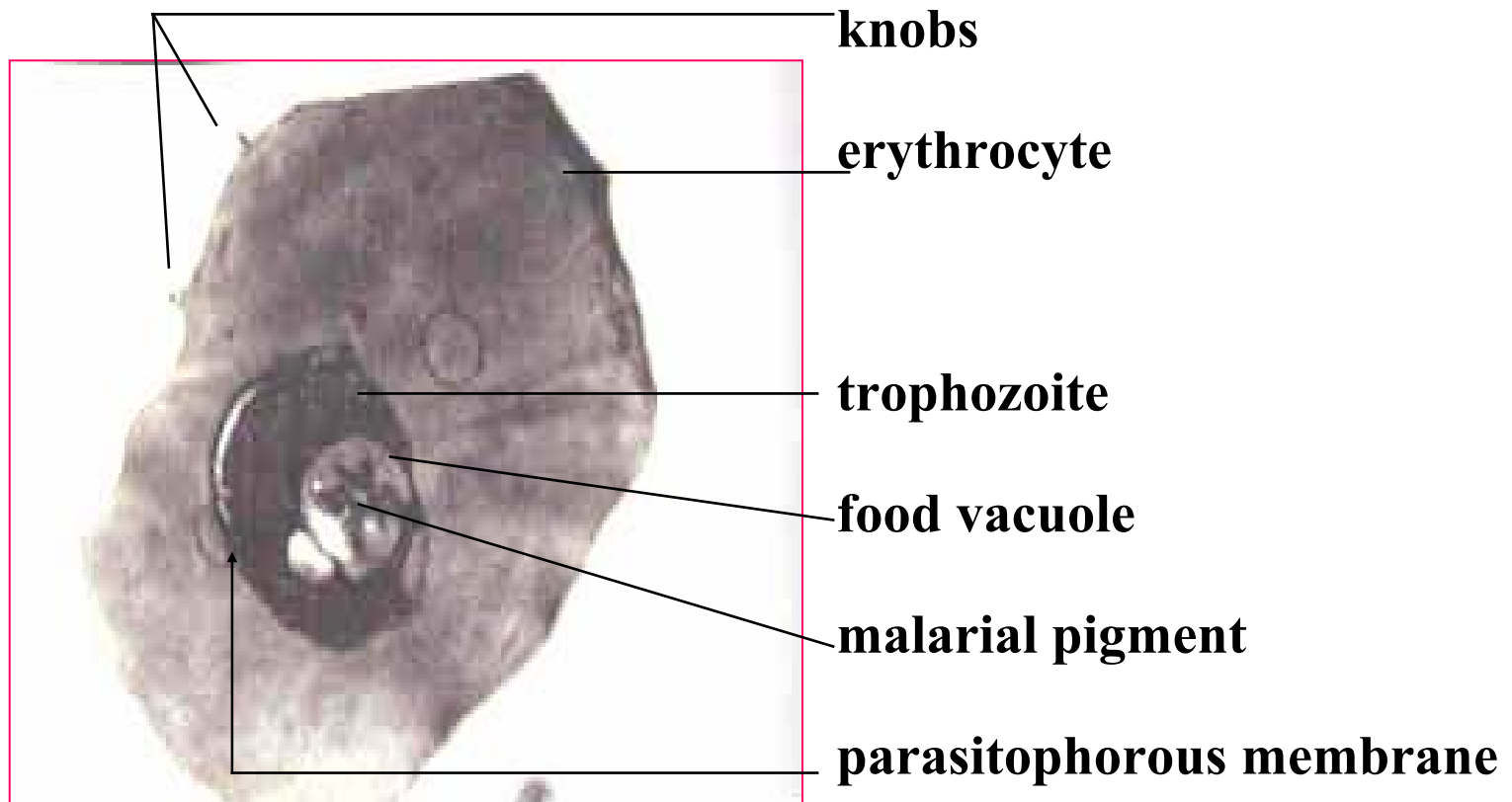
Cerebral vascular sequestration : *Falciparum malaria*



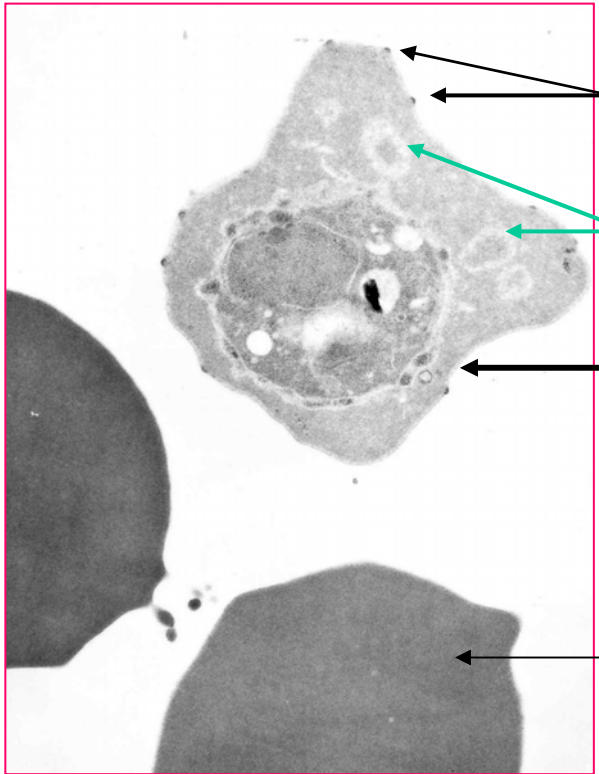
A section of brain tissue showing cerebral capillaries (1) and cerebral venule (2) are packing with parasitized red blood cells (PRBC) (arrows). A small vein (3) is seen with a number of PRBC and aggregation of red blood cells (RBC) in the center of the lumen. Few RBC and PRBC are seen in the lumen of a small artery (4). The PRBC appear attach to the endothelial cells (arrows) (H&E stain, x 200).

Plasmodium life cycle and pathogenesis

Mid trophozoite



Late trophozoite, *Plasmodium falciparum*

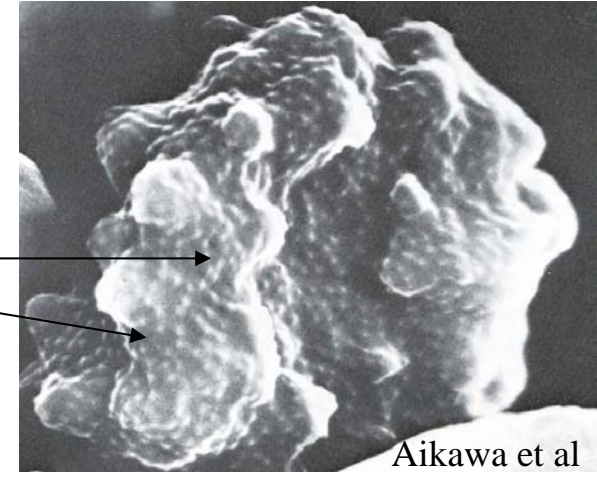


knobs

Maurer's clefts (dots)

parasitized red blood cell

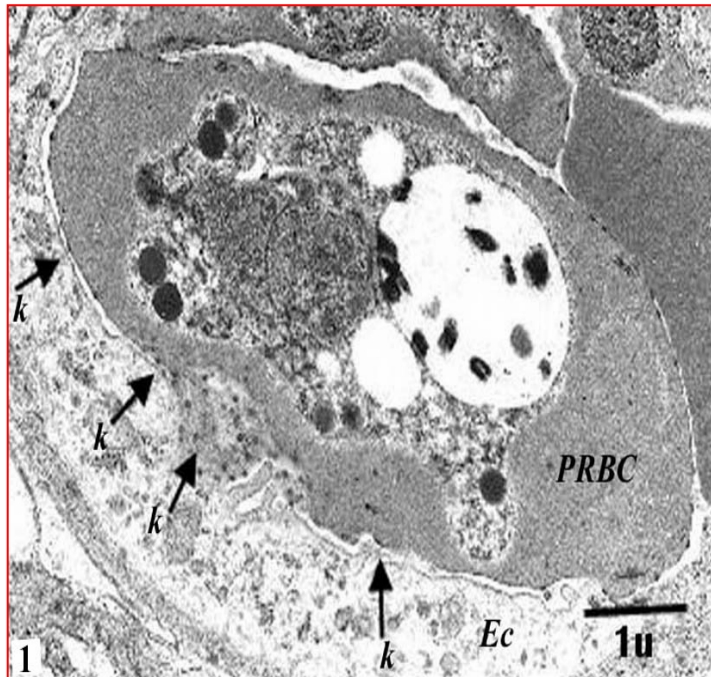
red blood cells



Aikawa et al

Cytoadherence

(*Plasmodium falciparum*)

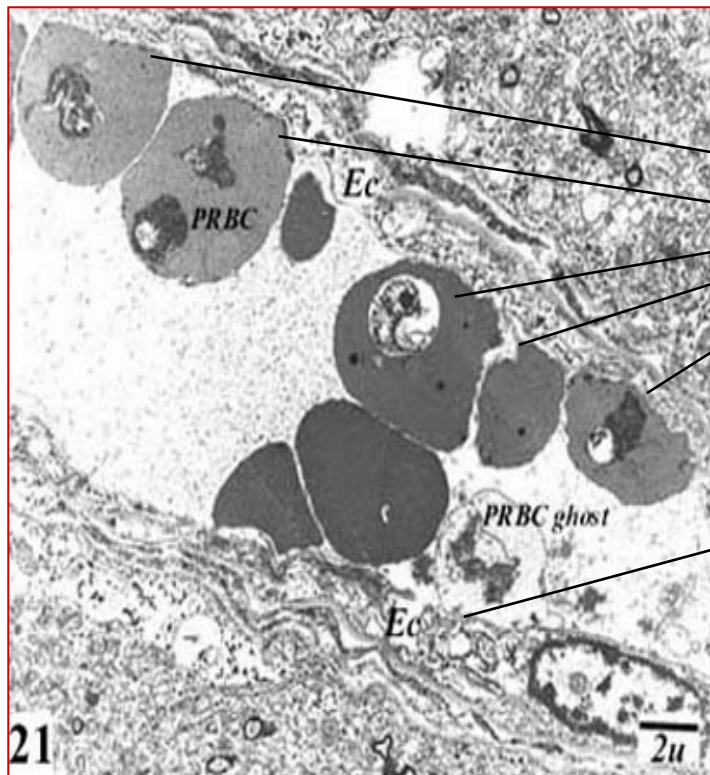


Cytoadherence of a parasitized red blood cell (PRBC) to the endothelial cell (Ec) of a cerebral microvessel, is mediated by **contacts between electron dense knobs on its surface** (arrows).

*The first impact of EM on the research into the pathology of malaria was to reveal the **electron-dense knob regions** on some strains of PRBC. **Ultrastructural studies by Luse and Miller (1971), found an anatomical basis for the phenomenon of cytoadherence.***

Sequestration

(*Plasmodium falciparum*)



**This process
is termed
sequestration**

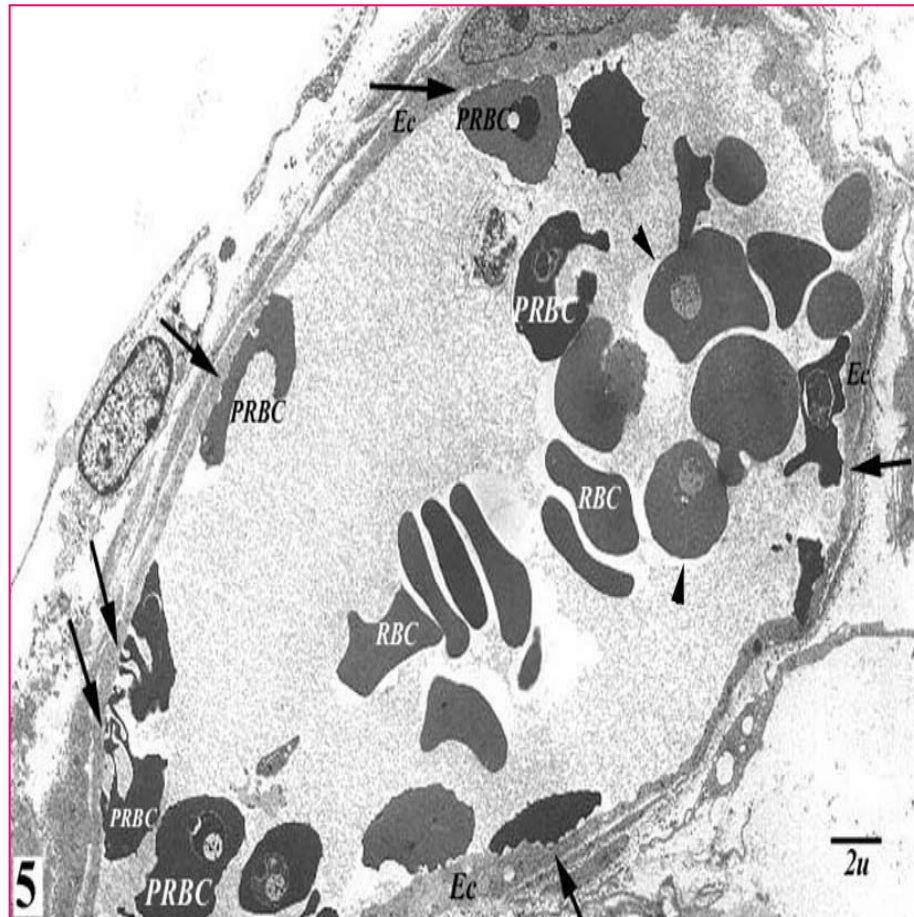
Sequestration of PRBC in the Brain



Dr. Gareth Turner
Oxford University

Microvascular obstruction

Multiple contacts : Cytoadherence in a larger blood vessel (*Plasmodium falciparum*)



Folding of the PRBC membranes is noted in many cells which appear firmly adherent to the endothelium of a larger blood vessel via **multiple contacts** (arrows). The normal RBC and early K-PRBC are mostly seen in the center of the lumen.

Red blood cell deformability

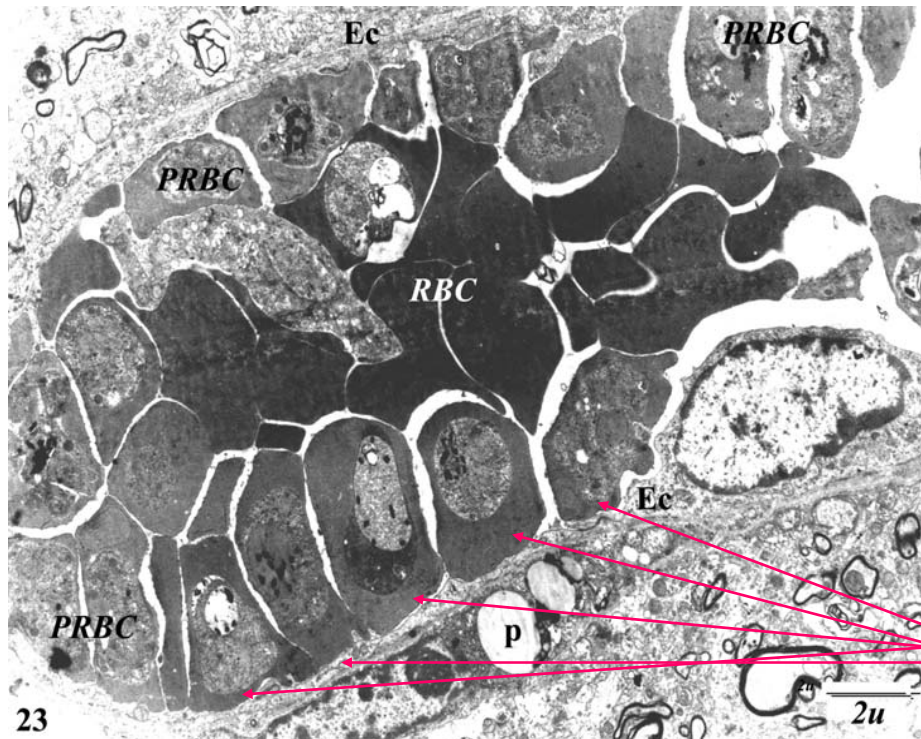


A PRBC (*Plasmodium falciparum*) is seen in a **congested cerebral capillary**. The normal red blood cells (RBC) showing amoeboid movement passing the PRBC which adheres to the endothelial cell (Ec).

Uninfected red blood cells (uRBC) also show changes to membrane deformability. Release of haemazoin from ruptured PRBC causes oxidative damage to uRBC membrane causing rigidity (less deformability) (Dondorp et al 2003 Redox Rep)

Marked sequestration

(*Plasmodium falciparum*)



A **congested cerebral venule** showing **marked sequestration** of parasitized red blood cells (PRBC). The venule is tightly packed with PRBC showing **margination** to the endothelial cells (Ec).

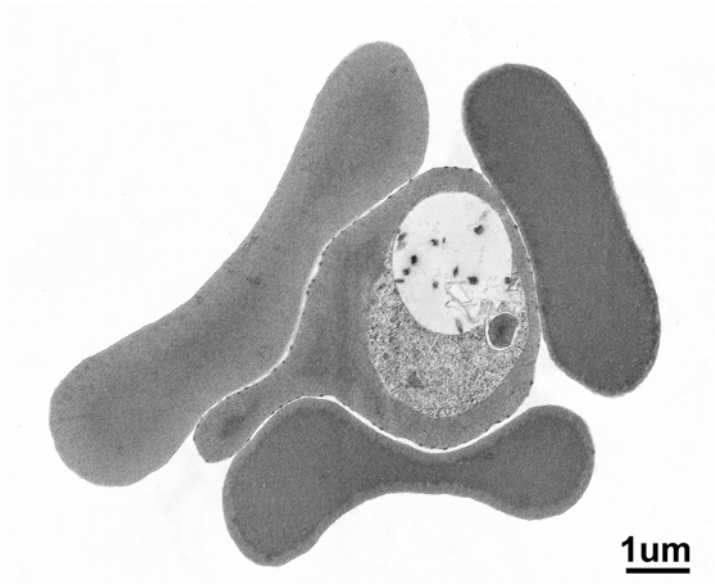
Additional causes of reduce microcirculatory flow

Receptor Mediated Adhesion

- PRBC bind to a number of host cells
 - Endothelial cells (sequestration)
 - Uninfected red blood cells (rosetting)
 - Platelets
 - Other PRBC and uRBC (autoagglutination)
 - Leukocytes (macrophages, dendritic cells)
- These adhesive interactions play an important role in causing disease

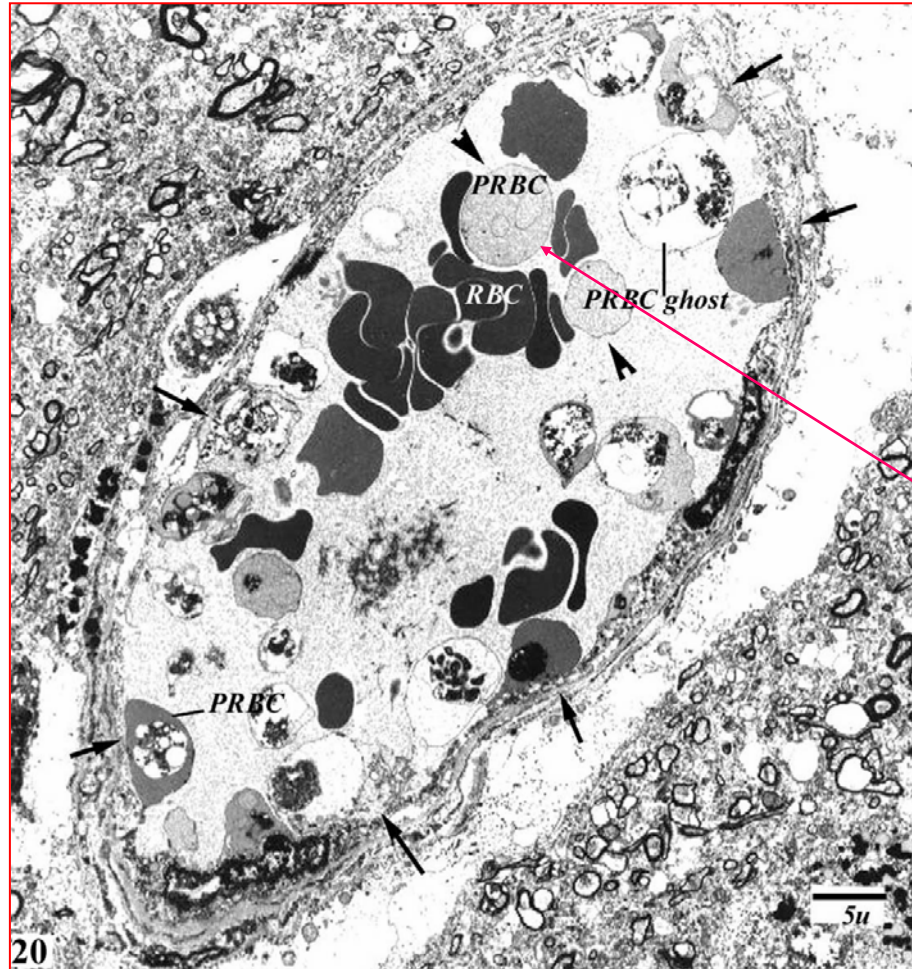
Rosette formation

(*Plasmodium falciparum*)

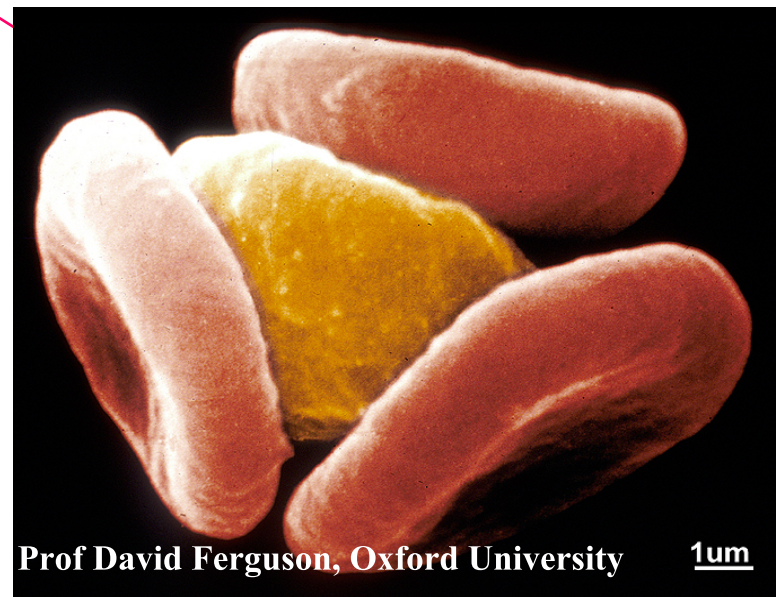


Rosetting of PRBC to uninfected red blood cells *in vitro* has been proposed as a possible additional factor in causing microvascular obstruction *in vitro* (David *et al.*, 1988; Udomsangpetch *et al.*, 1989).

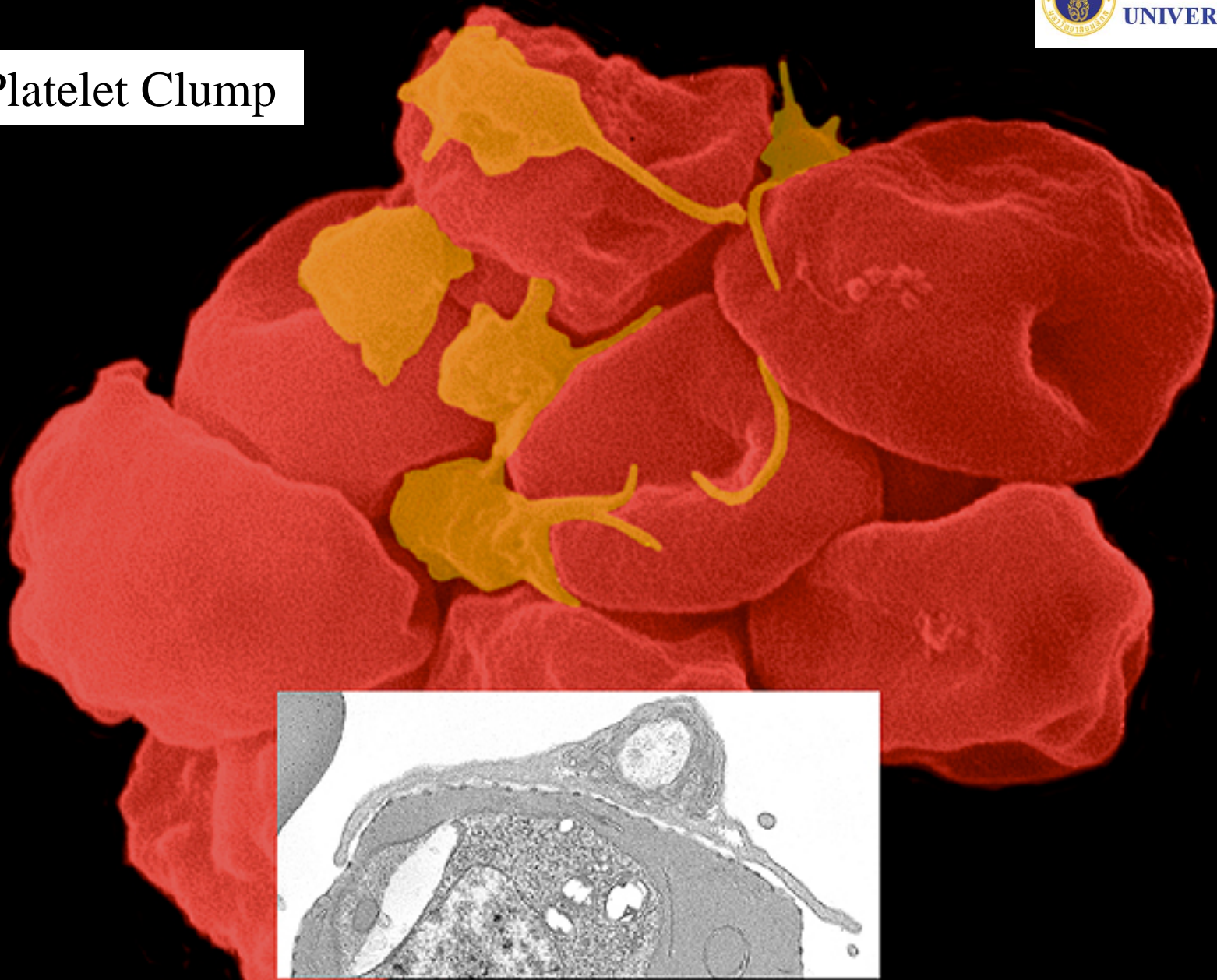
Aggregation in a large caliber cerebral vessel (*Plasmodium falciparum*)



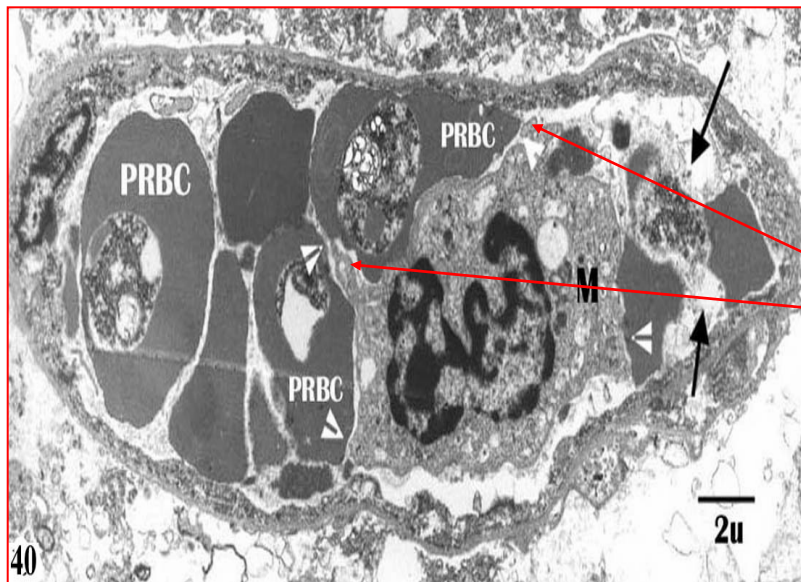
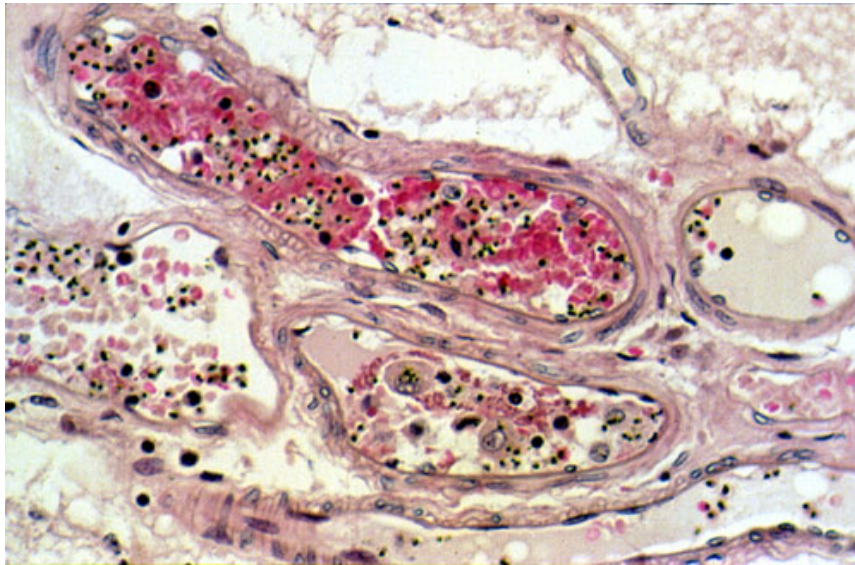
Note an aggregate of RBC and PRBC in the lumen, some PRBC is surrounded by RBC (arrow head), this probably represent rosette formation



A Platelet Clump



Prof David Ferguson, Oxford University

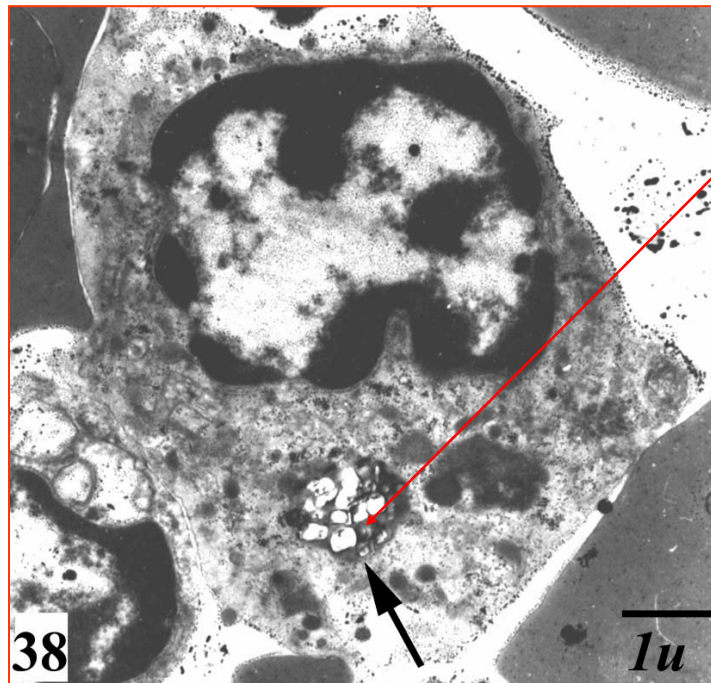


Phagocytosis

Intermixed with the parasitized red blood cells (PRBC), the cytoplasmic processes of a monocyte (M) are engulfing the PRBC (arrow heads)

Phagocytosed malarial pigment

(*Plasmodium falciparum*)

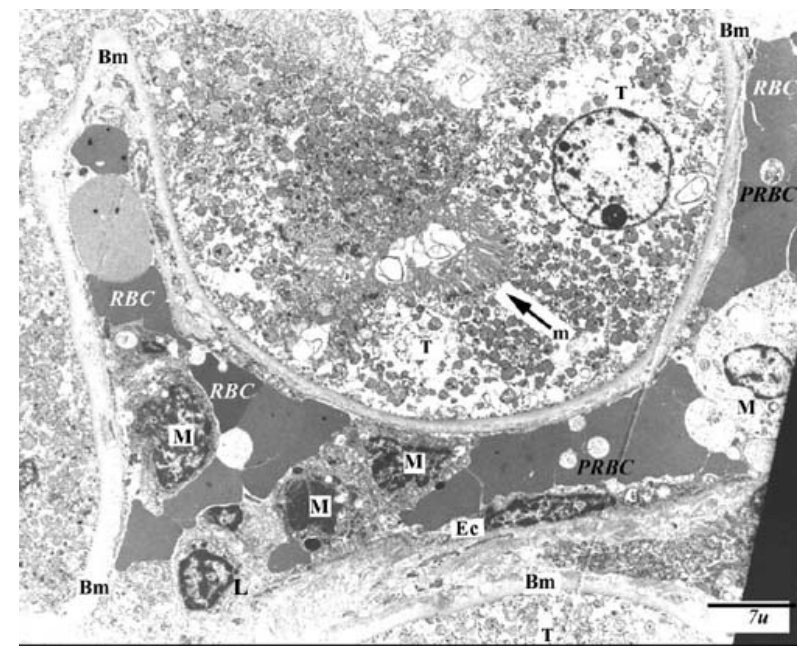
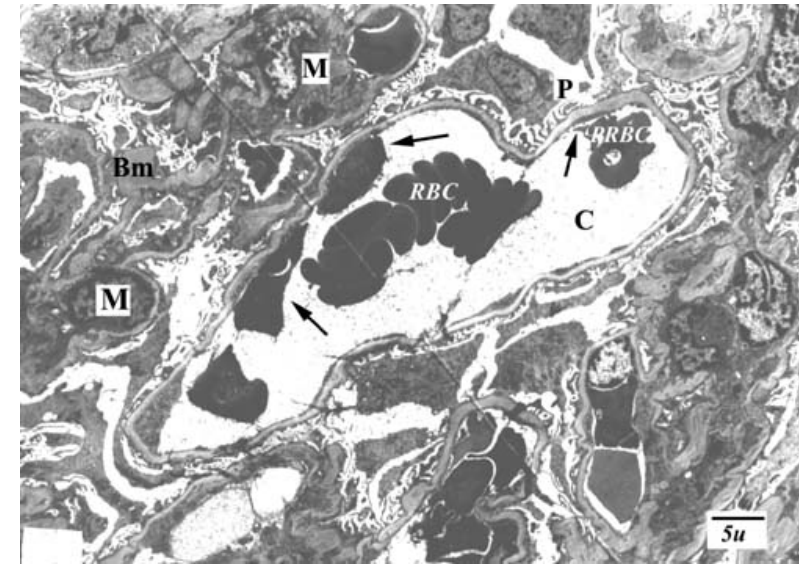
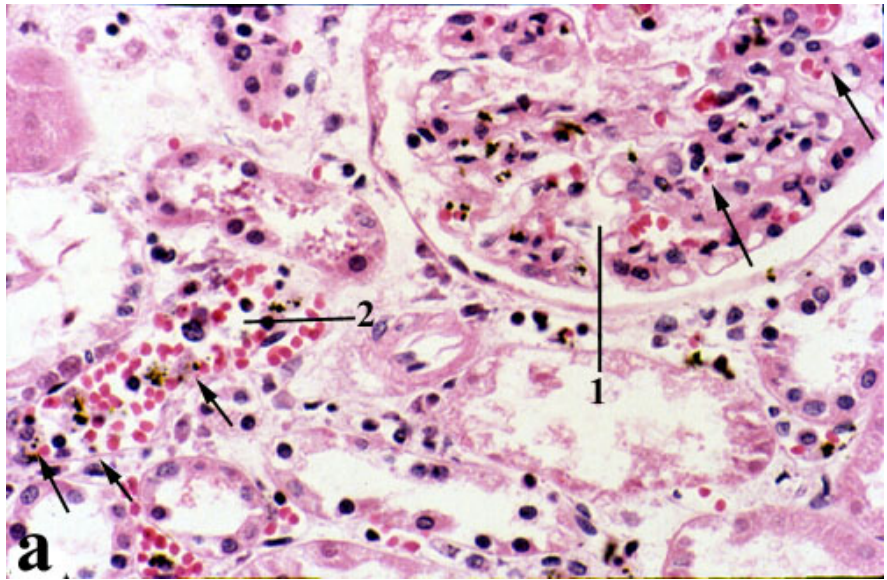


Malarial pigment(arrow) was always found in phagolysosomes mainly in mononuclear cells.

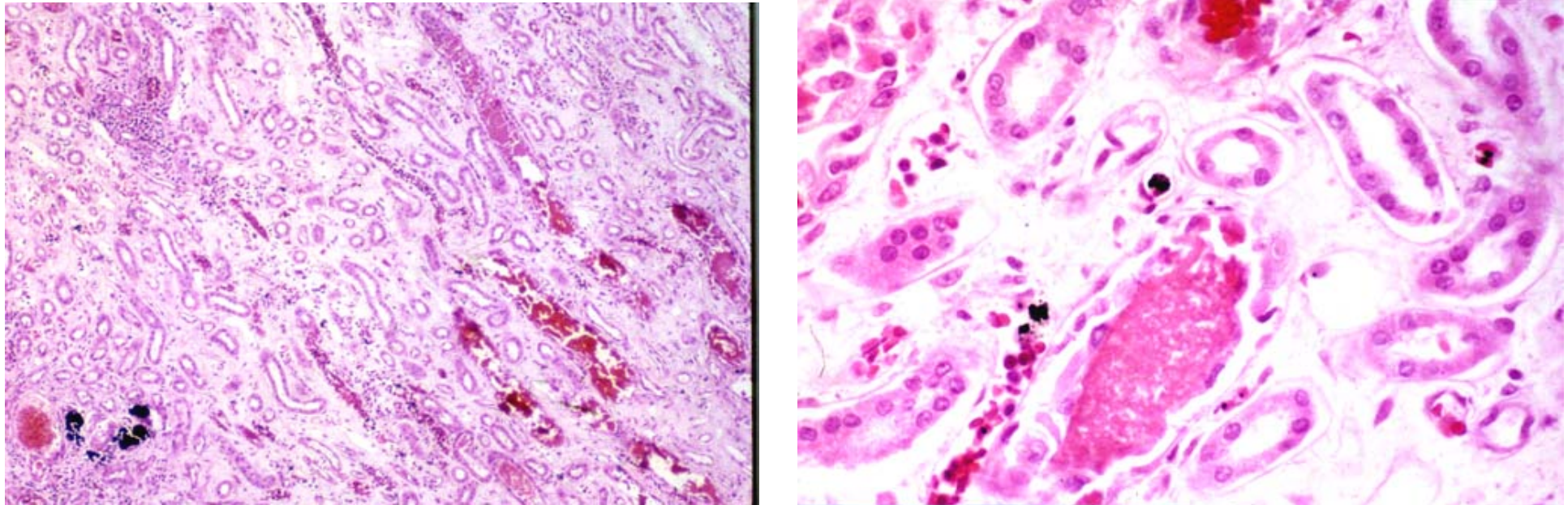
Previous studies have tended to concentrate on deaths from cerebral malaria and examine pathological changes in the brain.

There have also been reported that other complications such as **pulmonary oedema and **renal failure** are as common as **coma** in this patient population.**

PRBC sequestration in the kidney

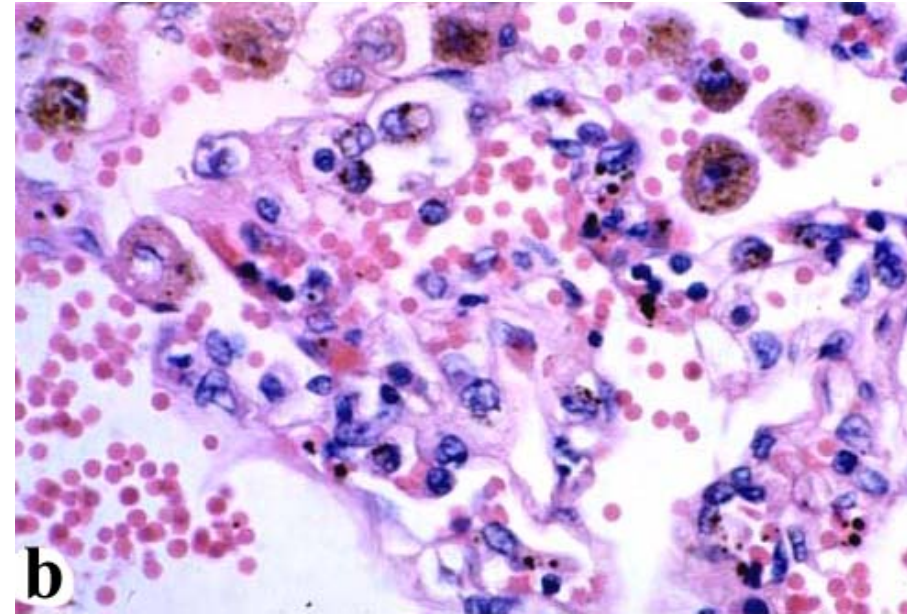
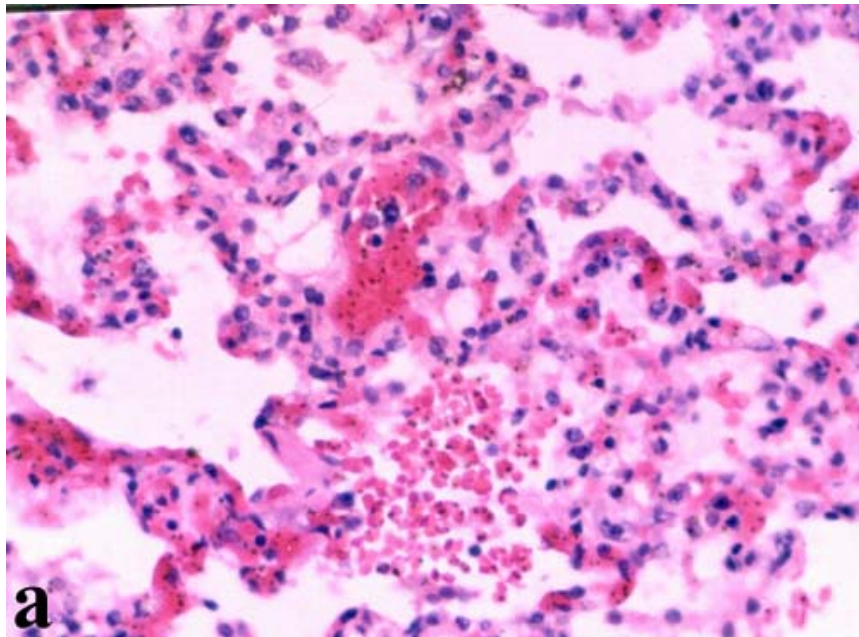


Black Water Fever : Kidney

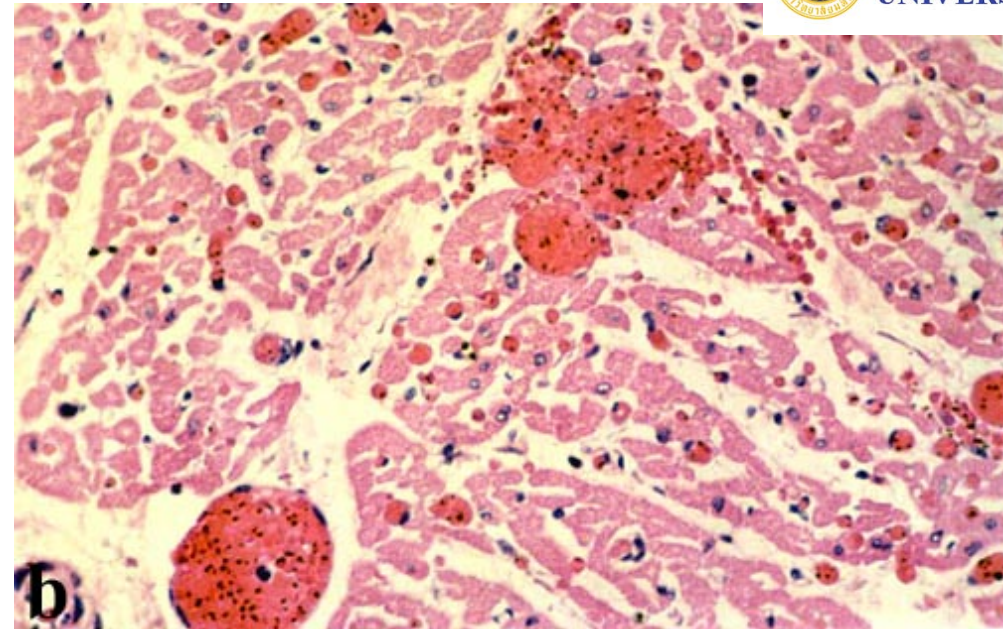
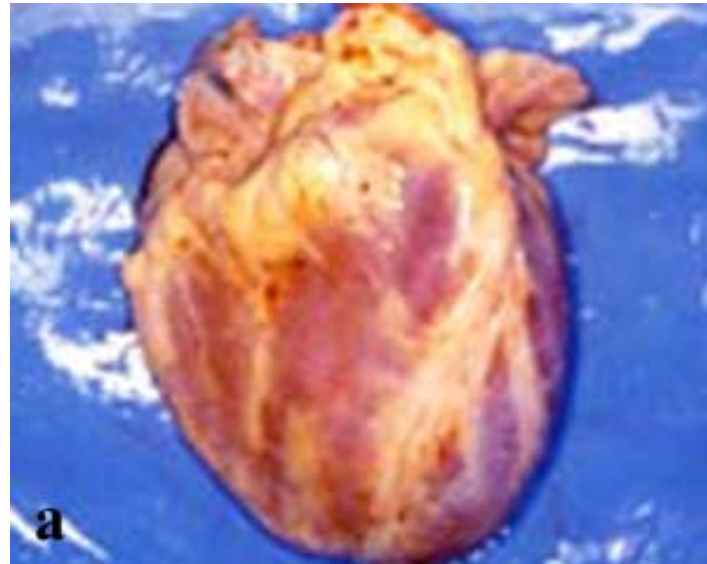


Within the degenerated renal tubules, observe the haemoglobin cast as well as the frank red blood cells. These are the result of massive hemolysis. Note dark brown pigments in the interstitial blood vessels (H&E stain, x 400).

Lungs in falciparum malaria

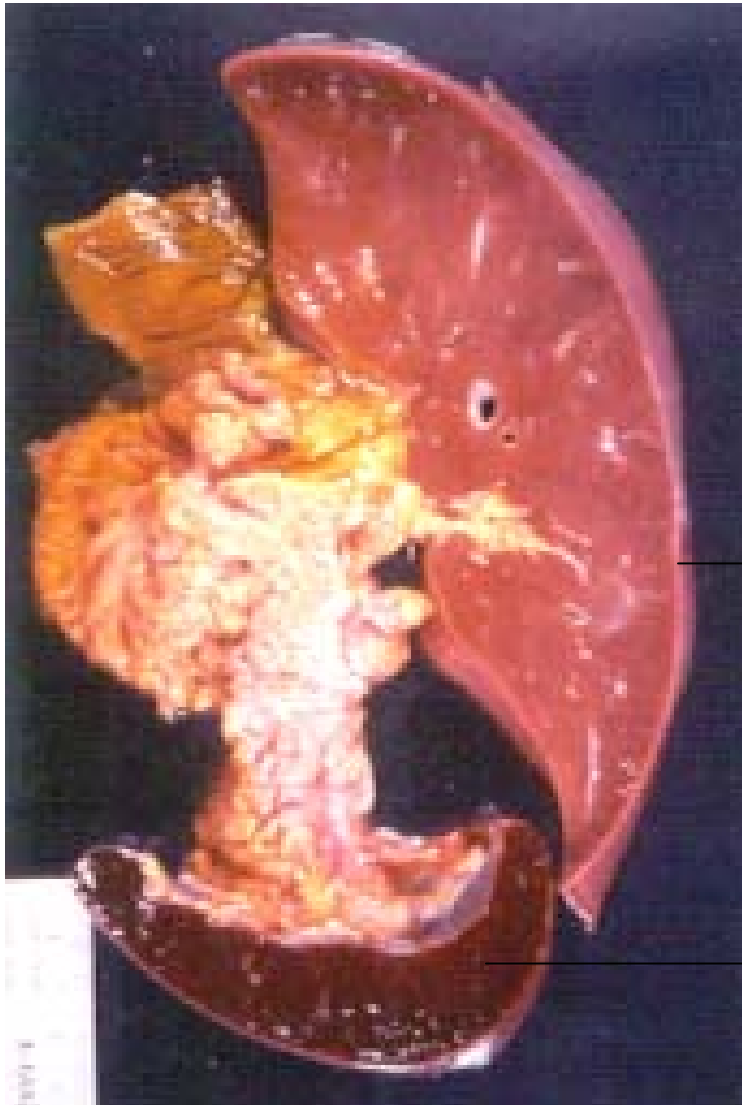


a) In addition to oedema fluid, the alveoli are filled with PRBC, RBC, neutrophils and pigment-laden macrophages. Parasitized red blood cells (PRBC) sequester in the septal capillaries and small blood vessels in the lung (H&E stain, x 200). **b)** A good number of pigment-laden alveolar macrophages are always seen (H&E stain, x 400).



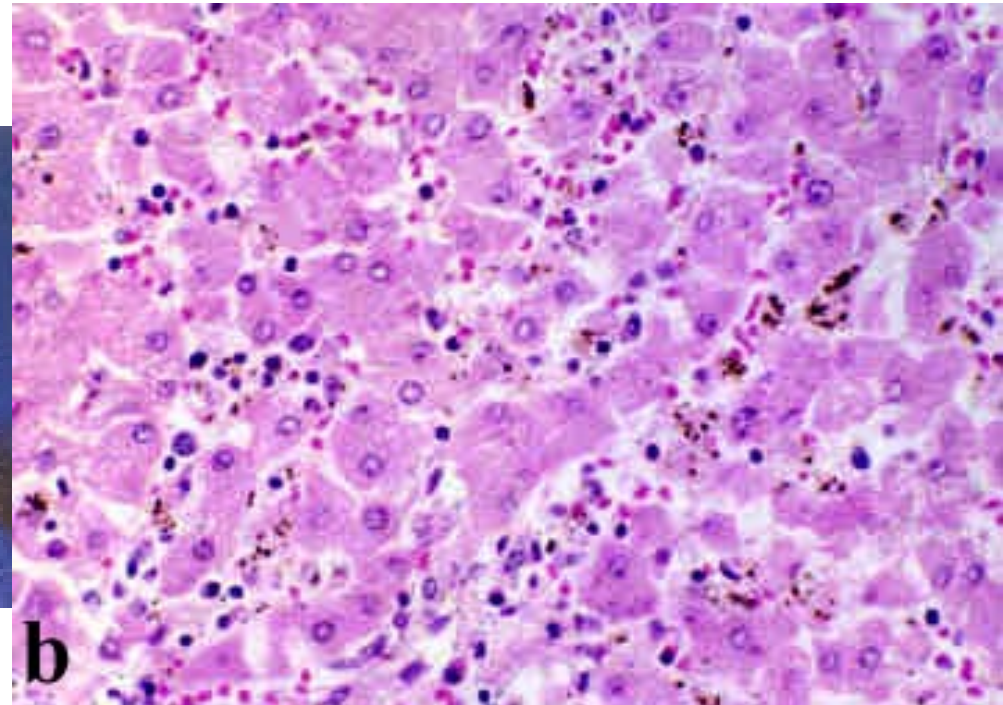
Heart in falciparum malaria. a) Petechial haemorrhage of epicardial surface. b) Congestion of myocardial microvessels with PRBC, RBC, pigment laden macrophages and mononuclear cells (H&E stain, x 200).

Despite intense sequestration in the myocardial vessels, the heart's pump function is remarkably well preserved in severe malaria.



Normal
liver

spleen

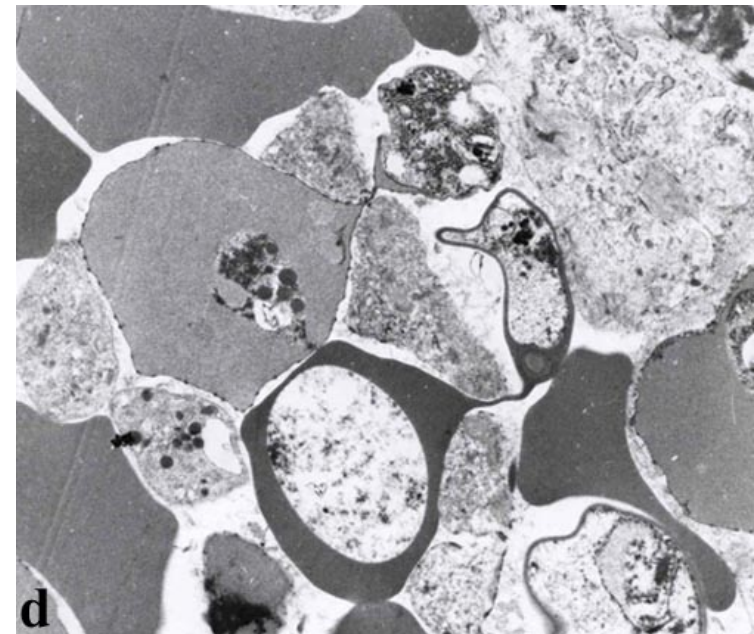
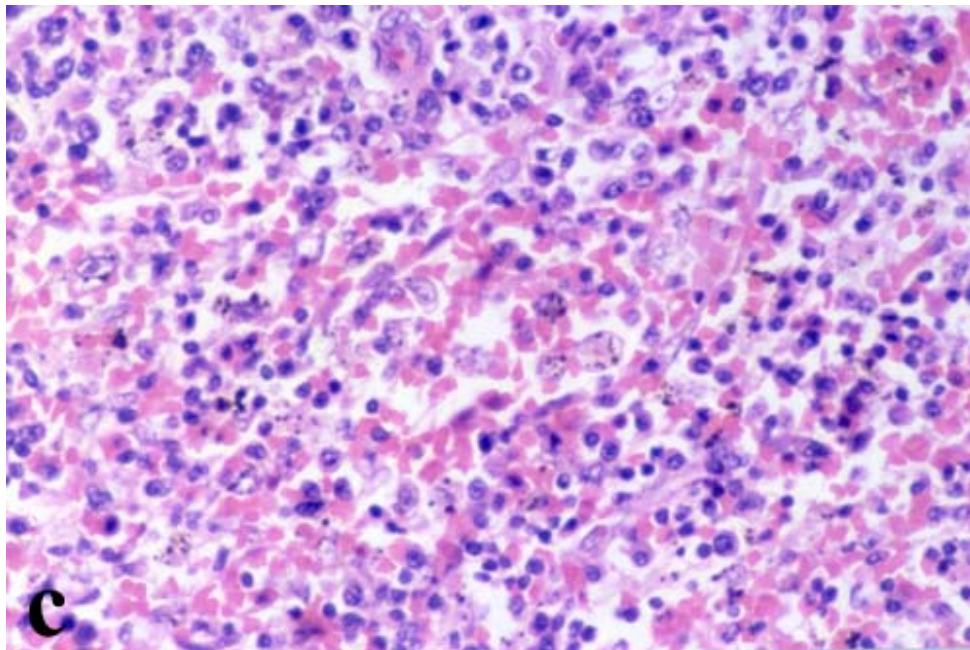
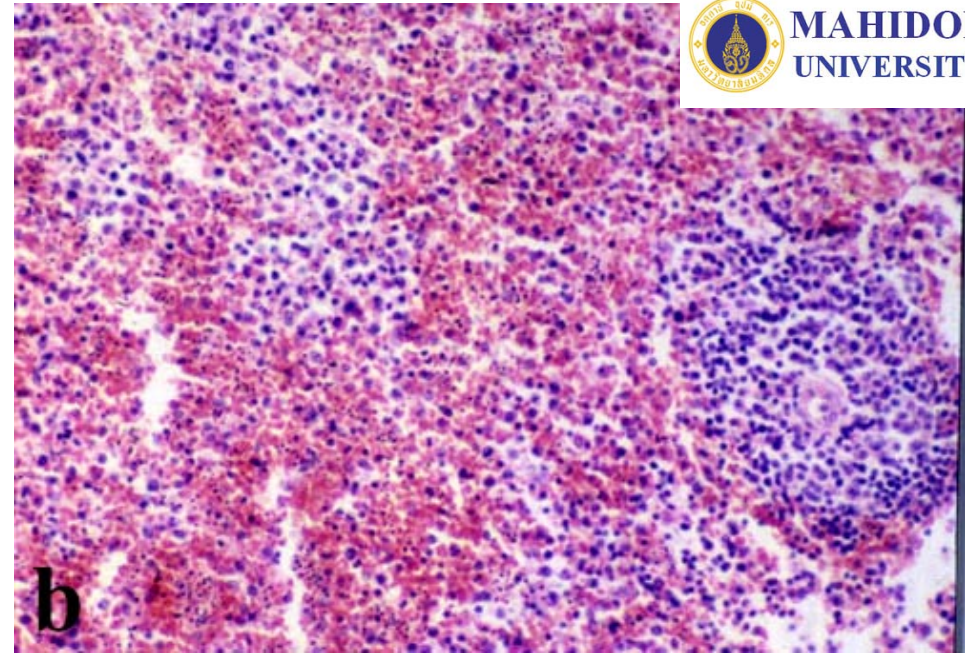


Falciparum malaria, liver. a) The liver is enlarged and oedematous, and coloured brown, as a result of deposition of malaria pigment.

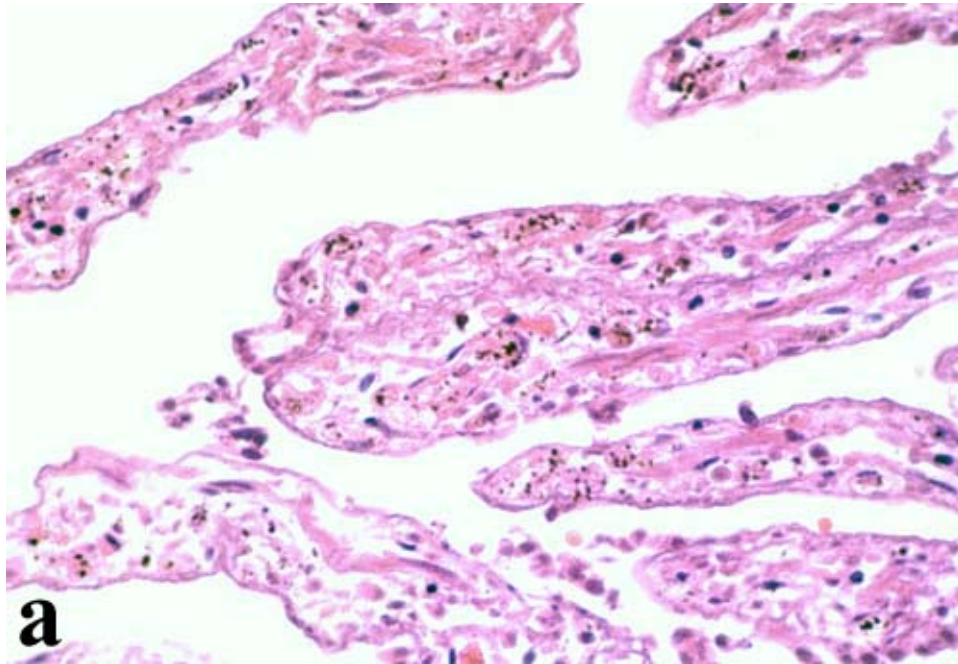
b) Hepatic sinusoids are dilated and congested with hypertrophied Kupffer's cells-laden malaria pigment, variable mononuclear cells, and PRBC (H&E stain, x 400).



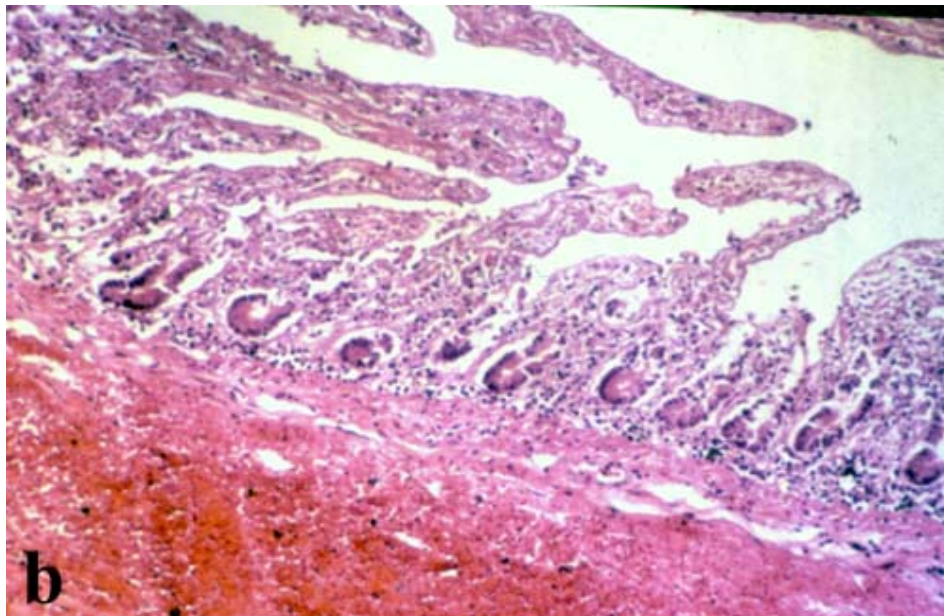
Spleen in falciparum malaria



Small intestine in malaria.

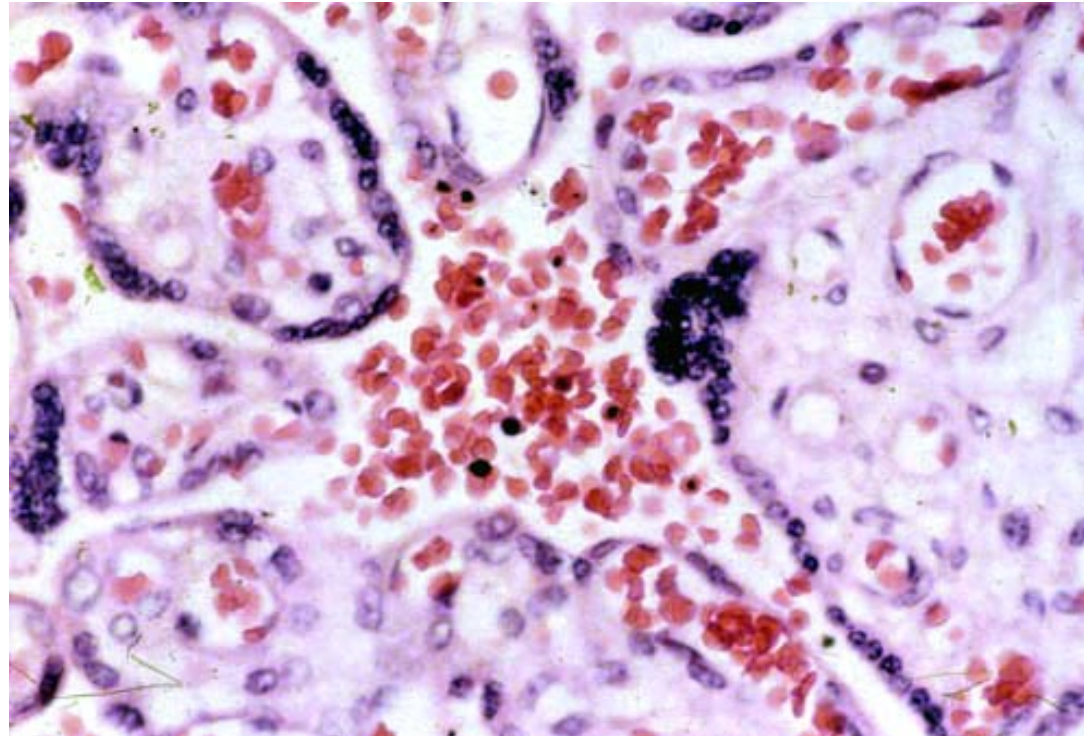


a) PRBC sequestration are seen in the small intestine, predominantly within the lamina propria capillaries (H&E stain, x 200).



b) Submucosal haemorrhages (H&E stain, x 100).

Placenta, falciparum malaria

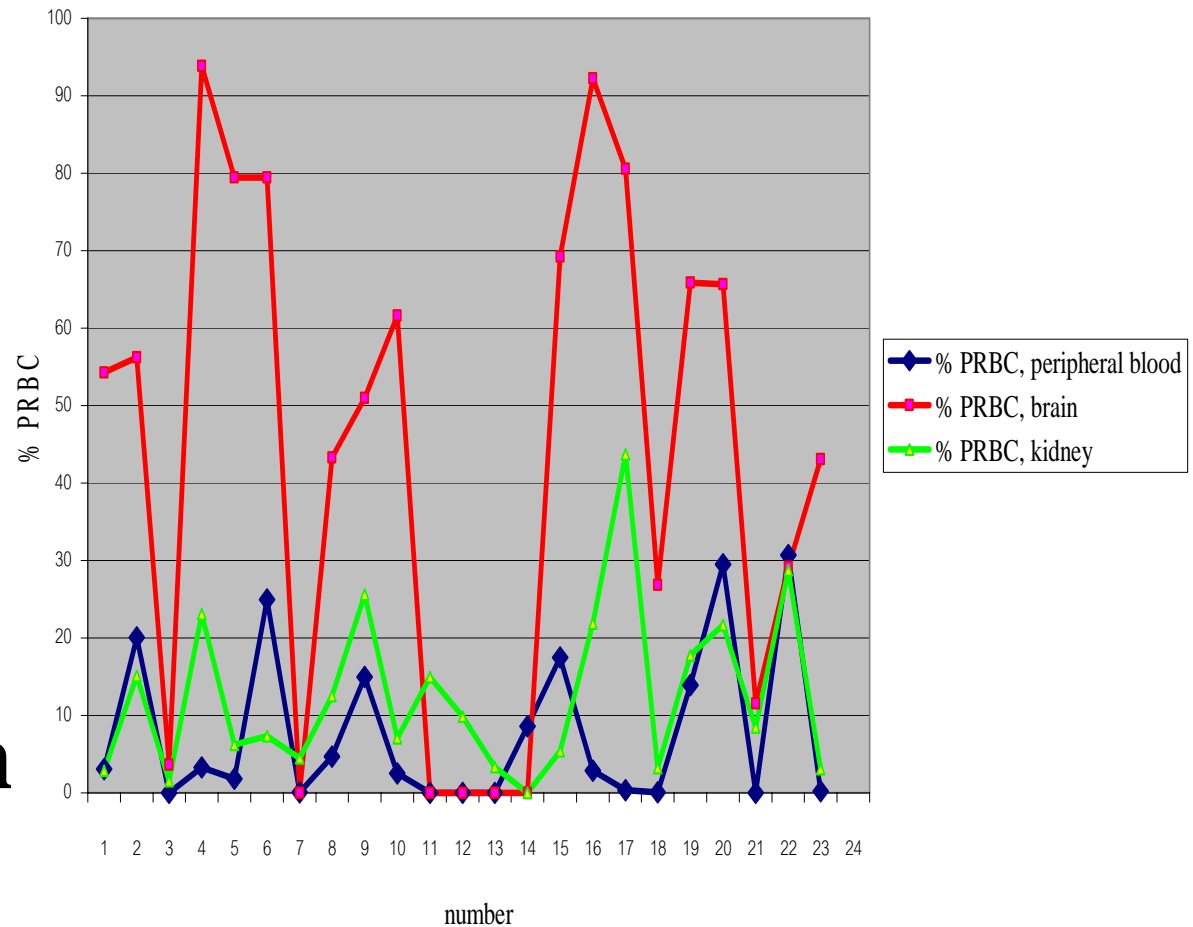


There is heavy parasitism of red blood cells in the maternal sinuses accompanied by monocytosis containing haemozoin pigment. Transplacental infection (congenital malaria) is uncommon. (H&E stain, x 400).

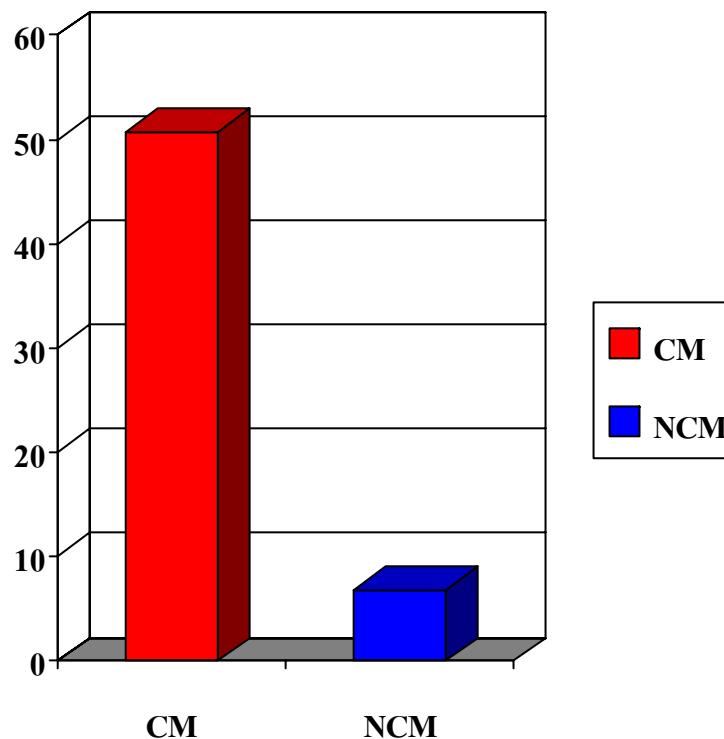
Comparison of % PRBC (*P. falciparum*)

Sequestration in the **brains**, **kidneys** and **peripheral blood**

Sequestration Index of the brain is higher than the kidney and there is no correlation between the two.

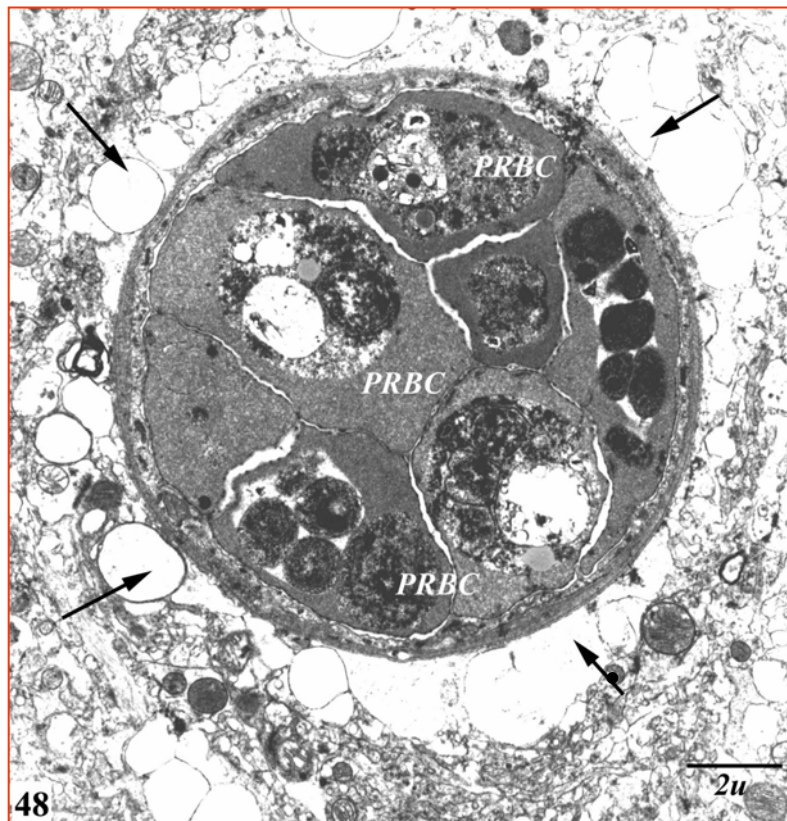


Sequestration Index (S.I.) of the brain (*Plasmodium falciparum*)



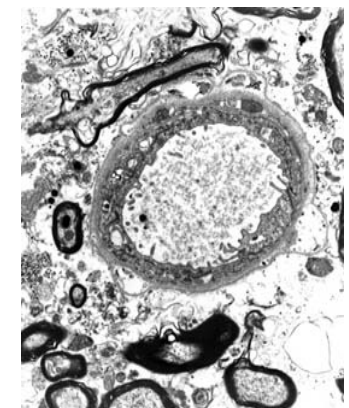
It reveals that S.I. of the PRBC in the brain in **CM (50.66)** is significantly higher ($p=0.042$) than **NCM (6.88)** groups.

PRBC sequestration is a critical initiating event in the genesis of cerebral malaria.



The results of the study show significantly more PRBC sequestration in the brain of CM patients compared to NCM cases

(*Plasmodium falciparum*)



Empty blood vessel

There has been some debate as to the role of sequestration in causing disease within a particular organ such as the brain in cerebral malaria .

Some authors believe that malaria infection can cause generalized vital organ dysfunction as a result of the release of systemic cytokines such as tumour necrosis factor alpha , or local release of mediators such as nitric oxide (NO).

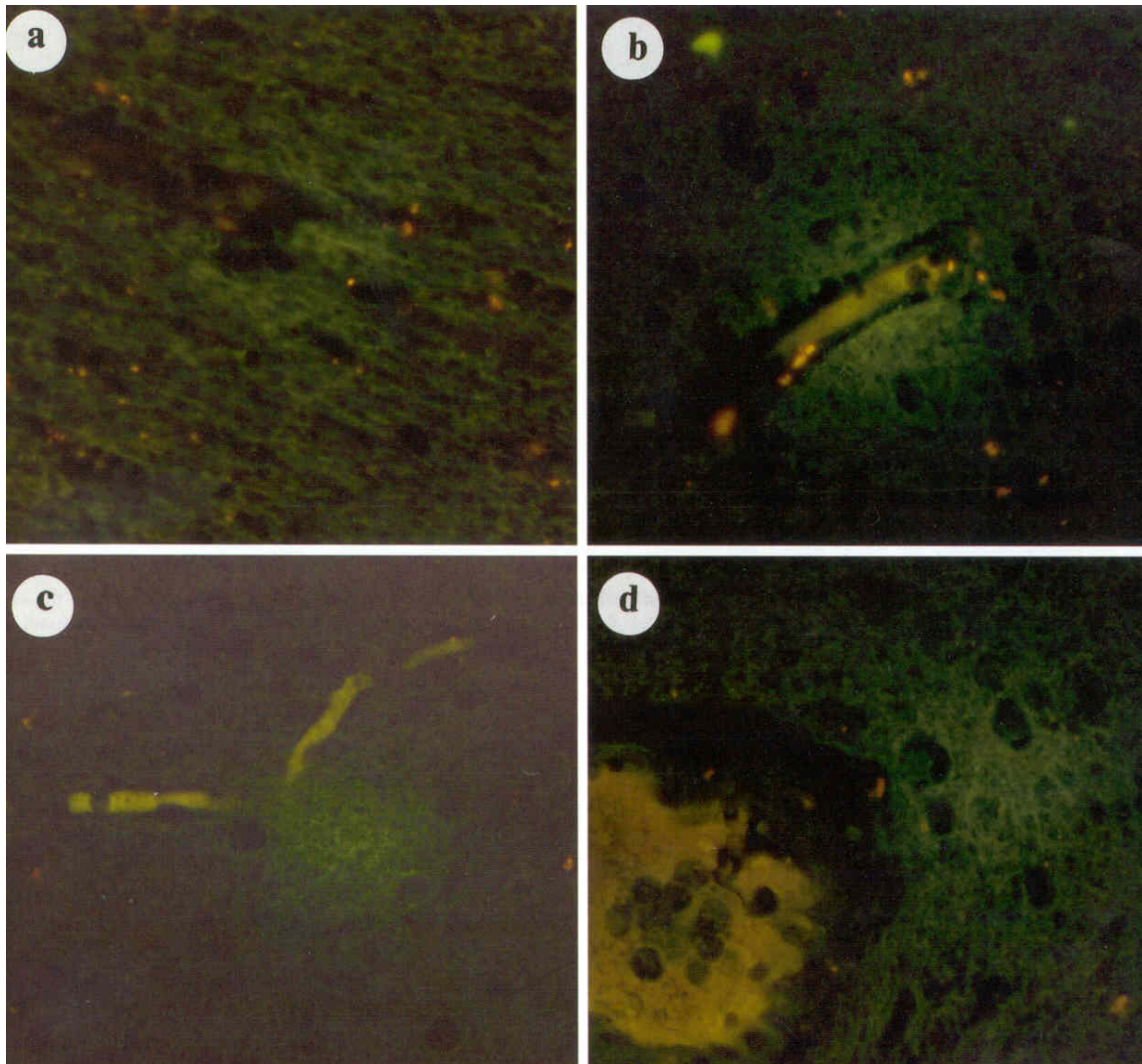
Initially tumour necrosis factor (TNF), which plays a pivotal role, interleukin (IL)-1, and gamma interferon **are produced** and these in turn **induce release** a cascade of other “pro-inflammatory” cytokines including IL-8, IL-12, IL-18.

These are **balanced** by production of the “anti-inflammatory” cytokines IL-6 and IL-10

Cytokines are responsible for many of the symptoms and signs of the infection, particularly fever and malaise.

Whereas high concentrations of cytokines appear to be harmful, lower levels probably benefit the host.

Involvement of cytokines in the histopathology of cerebral malaria



(Rachanee Udomsangpetch
et al., *Am J Trop Med Hyg*
1977, 57: 501-6)

Immunofluorescence
staining of the brain
of malaria autopsy
showing reactivity of
monoclonal antibody
with:

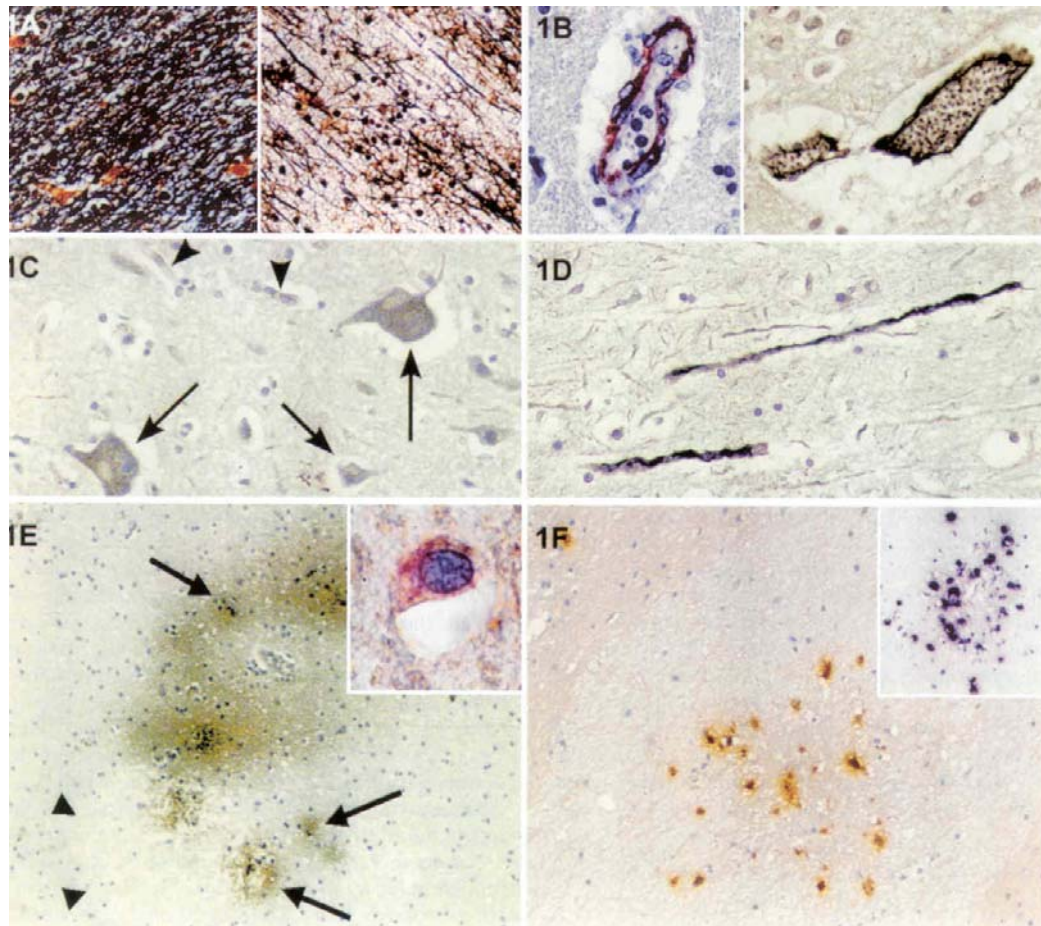
a. TNF or IFN

b. IL-4

c. and d. IL-10

Inducible nitric oxide synthase expression is increased in the brain in fatal cerebral malaria

(Yaowapa Maneerat *et al.*, *Histopathology* 2000, 37: 269-77)



This study indicates that an acute induction of iNOS expression occurs in the brain during CM. This occurs in a number of different cell types.

As NO may activate a number of secondary neuropathological mechanisms in the brain, including modulators of synaptic function, induction of iNOS expression in CM may contribute to coma, seizures and death.

Severe malaria, Pathogenesis :

- 1. Cytoadherence**
- 2. Sequestration**
- 3. Rosette formation, aggregation**
- 4. Red blood cell less deformability**
- 5. Soluble mediators of pathogenesis**