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# HUMAN CEREBRAL MALARIA: A PATHOLOGICAL STUDY

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Cerebral malaria is one of the most serious complications of falciparum malaria and 20-50% of deaths in falciparum malaria is due to the involvement of the central nervous system (MacPherson et al., 1985). Although pathogenesis of cerebral malaria is still unknown, the blockage of cerebral vessels by parasitized cells, endotoxin, immune complexes and reduced humoral or cell-mediated immune responses have been suggested as possible factors contributing to the development of cerebral malaria (Tharavanij, 1983). Recently, several authors (MacPherson et al., 1985) reported, by electron microscopy, pathological aspects of cerebral malaria in seven authopsied patients from eastern Thailand. They demonstrated that cerebral vessels were packed with *P. falciparum* infected erythrocytes. Therefore, they suggested that the blockage of cerebral vessels by parasitized cells was the most important factor responsible for damage to the central nervous system.

In Burma, there were 800,000 cases of malaria reported in 1982 (Anon, 1985) and *P. falciparum* is the major agent. We have examined brain tissue specimens from 19 Burmese patients who died of cerebral malaria using light and electron microscopy and an immunoperoxidase technique (Oo et al., 1986). Our study showed that blockage of cerebral capillaries by *P. falciparum* infected erythrocytes was the major pathological change in cerebral malaria as previously reported (MacPherson et al., 1985). The junction formation between capillary endothelium and knobs on the infected erythrocytes was responsible for capillary blockage. Contrary to previous reports (MacPherson et al., 1985), we were able to demonstrate, for the first time, the presence of *P. falciparum* antigens and IgG in capillary basement membranes. This findings indicates that immunological events also play a role in the pathogenesis of cerebral malaria.

## MATERIALS AND METHODS

Postmortem brain tissues were collected from three geographically widely separated Burmese hospitals, i.e. Rangoon General Hospital, Defense Services General Hospital and Tharrawaddy Hospital. All of the patients selected for our study were infected only with *P. falciparum*. Patients with mixed infections (i.e. *P. vivax* and *P. falciparum*) were excluded as far as possible by clinical and laboratory evalucation. These patients had varying parasitemia levels, a variety of neurological signs, and all eventually became unconscious (Table I). Other causes of unconsciousness in these patients, e.g. tuberculosis, syphilis, leprosy and other encephalitides were excluded by clinical examination and laboratory tests. This left 19 patients remaining in our study. A full autopsy was performed on these patients with a routine pathological examination. Electron microscopy of brain tissue was done on seven patients (Table II).

The brain tissue collected was fixed in 10% buffered formalin for light microscopy. It was dehydrated in a routine manner, embedded in paraffin and stained with H&E. Brain tissue for electron microscopy was fixed in 2.5% glutaraldehyde with 0.05M phosphate buffer for one hour (hr.) (pH 7.4) followed by routine embedding and cutting procedures. The sections were then examined with a JEOL 100CX electron microscope.

A peroxidase anti-peroxidase (PAP) method was applied to the paraffin embedded material from seven patients which was also examined by electron microscopy (Burns, 1982). The antibody used in this study was obtained from Cappel Worthington Biochemicals, Malvern, PA. Controls were performed by substituting the antibody with normal rabbit serum.

## RESULTS AND DISCUSSION

As shown in Table I, the age of these patients ranged from five to 50 years, with most falling between 20 and 40 years old. Four patients were females and 15 were males. The parasitemia varied from 1% to 30% in peripheral blood. High or low levels of parasitemia did not cor-

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TABLE I

Human Cerebral Malaria – Demographic and Clinical Features

| Case Number | Age (years) | Sex | Parasitemia | Duration of Illness (days) |
|---|---|---|---|---|
| 1. | 26 | F | 24% | 9 |
| 2. | 40 | M | 1% | 8 |
| 3. | 25 | F | > 3% | 7 |
| 4. | 25 | F | 30% | 13 |
| 5. | 43 | F | 30% | 7 |
| 6. | 40 | M | 30% | 9 |
| 7. | 24 | M | > 3% | 10 |
| 8. | 13 | M | 2% | 4 |
| 9. | 12 | M | 1% | 4 |
| 10. | 40 | M | > 3% | 26 |
| 11. | 21 | M | > 3% | 2 |
| 12. | 22 | M | 2% | 17 |
| 13. | 19 | M | 1% | 4 |
| 14. | 43 | M | ? | 3 |
| 15. | 39 | M | 20% | 7 |
| 16. | 20 | M | > 3% | 33 |
| 17. | 50 | M | 1% | 9 |
| 18. | 28 | M | > 3% | 10 |
| 19. | 5 | M | > 3% | 5 |

F = Female; M = Male; (Oo, M.M. et al., 1986. J. Neuropath. Exptl. Neuro., In press.).

TABLE II

Human Cerebral Malaria – Pathological Features

| Case Number | Time of Autopsy After Death | Ring Hemorrhages | Capillary Blockage | Gross Cerebral Edema | Knobs | Adhesion |
|---|---|---|---|---|---|---|
| 1. | 5 min | – | + | – | + | + |
| 2. | 25 min | – | + | - | + | + |
| 3. | 25 min | - | + | – | + | + |
| 4. | 15 min | – | + | – | + | + |
| 5. | 10 hrs. | – | + | . | + | + |
| 6. | 10 hrs. | + | + | -- | + | + |
| 7. | 11 hrs. | – | + | - | + | + |
| 8. | 13 hrs. | – | + | + | NA | NA |
| 9. | 11 hrs. | + | + | + | NA | NA |
| 10. | 5 hrs. | – | + | – | NA | NA |
| 11. | 9 hrs. | + | + | + | NA | NA |
| 12. | 10 hrs. | + | + | + | NA | NA |
| 13. | 12 hrs. | + | + | – | NA | NA |
| 14. | 12 hrs. | + | + | – | NA | NA |
| 15. | 16 hrs. | + | + | + | NA | NA |
| 16. | 2 hrs. | + | + | + | NA | NA |
| 17. | 2 hrs. | + | + | – | NA | NA |
| 18. | 30 hrs. | – | + | – | NA | NA |
| 19. | 22 hrs. | – | + | + | NA | NA |

NA = not available for electron microscopy. (Oo, M.M. 1986. J. Neuropath. Exptl. Neuro., In press.)

relate with brain involvement. The duration of illness varied from three days to 33 days with an average duration between three to ten days. Specific clinical symptoms did not occur prior to unconsciousness. Clinical signs and symptoms such as jaundice were not useful in predicting the development of cerebral malaria with *P. falciparum* infections.

The cut sections of the brain showed swelling in seven out of 19 patients and in some cases, petechial hemorrhages. Light microscopy revealed cerebral capillaries filled with *P. falciparum*-infected erythrocytes admixed with non-infected erythrocytes (Figs. 1-2). Erythrocytes which were infected with *P. falciparum* contained all stages of maturation. Electron microscopy demonstrated multiple electron dense knobs, 40x80nm in size, protruding from the membrane of the infected erythrocyte (Figs. 4-5). The number of knobs increased as the parasites became more mature in these cells. These electron dense knobs formed focal junctions with the endothelial cells and adjacent erythrocytes (Figs. 4-5).

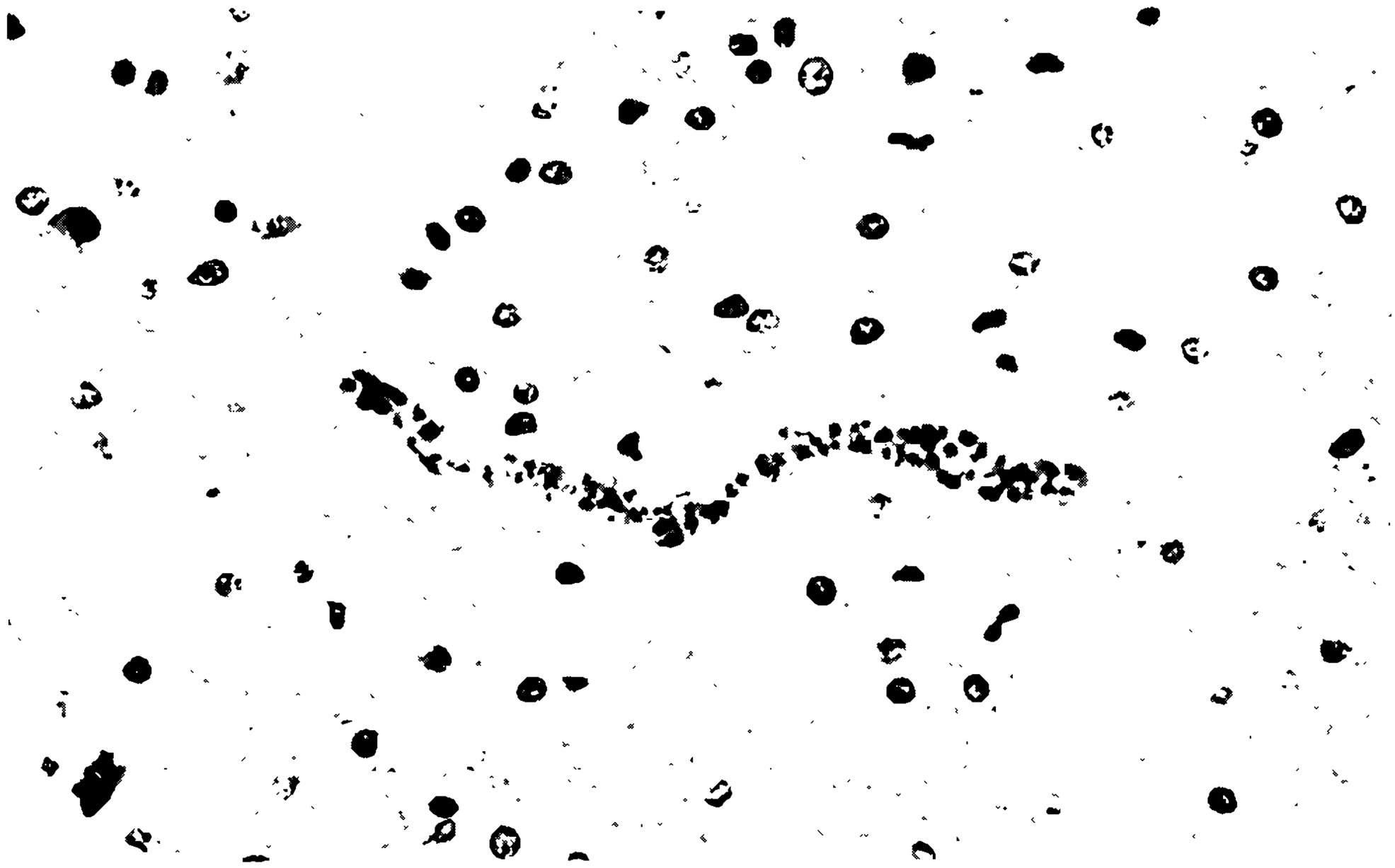

Fig. 1: A brain section from a cerebral malaria patient. Cerebral capillaries are blocked with infected and non-infected erythrocytes. H & E. X180.

Fig. 2: A high power micrograph showing capillary blockage due to infected and non-infected erythrocytes. Occasional inflammatory cells (arrow) are adjacent to the capillary. H & E. X840.

Knob protrusions and associated submembrane electron dense material (EDM) have been described on the membrane of erythrocytes infected with *P. falciparum* (Aikawa, Rabbege & Wellde, 1972; Luse & Miller, 1971). These knobs formed focal junctions with the endothelial cells of capillaries of various organs, resulting in the sequestration of infected erythrocytes along the vascular endothelium (Aikawa, Rabbebe & Wellde, 1972; Aikawa, Suzuki & Gutierrez, 1980; Gutierrez et al., 1976; Luse & Miller, 1971). Parasites that do not produce knobs (K– phenotype)

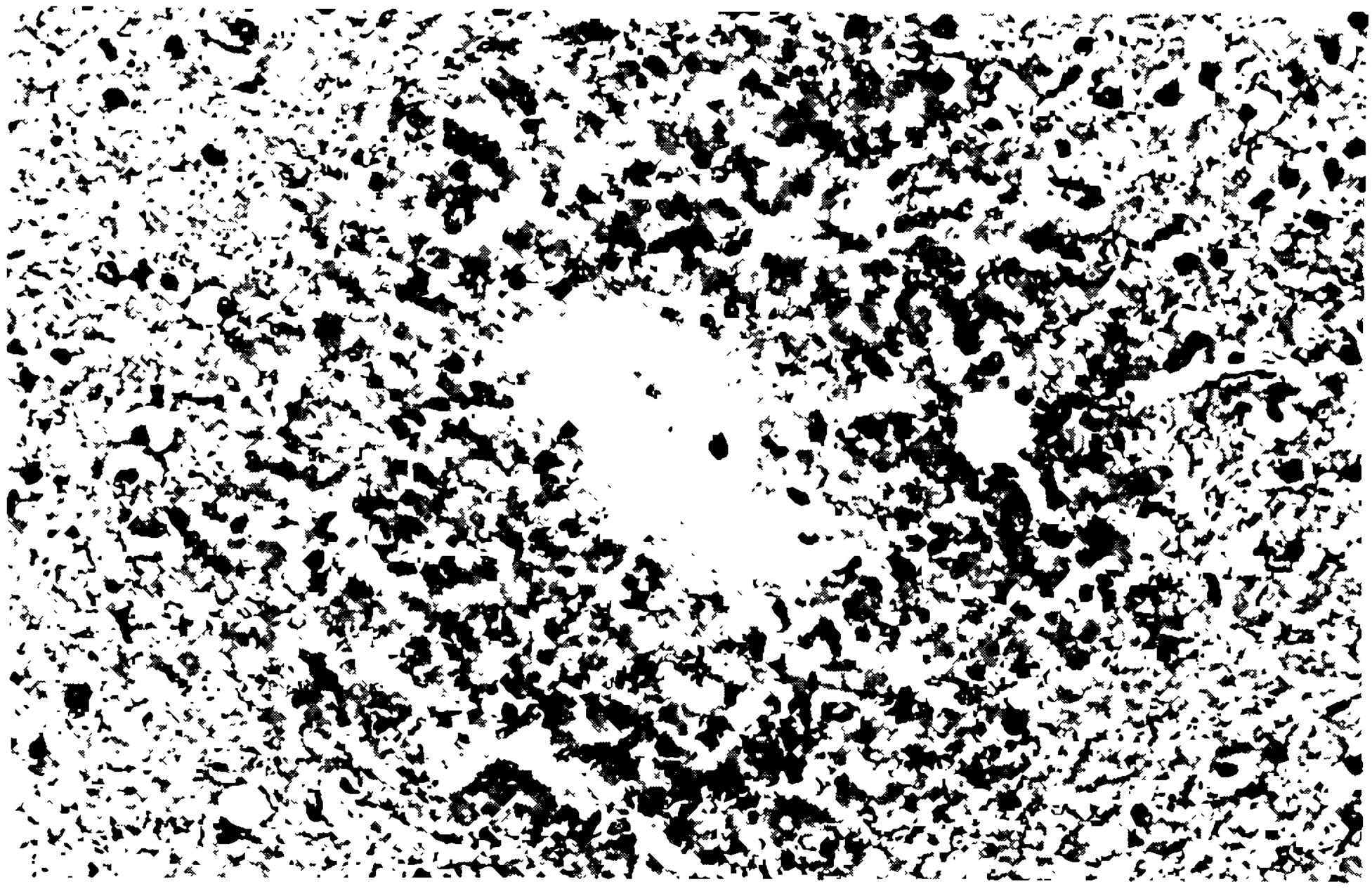

Fig. 3: A ring hemorrhage with a centrally located necrotic capillary containing infected erythrocytes. H & E. X350.

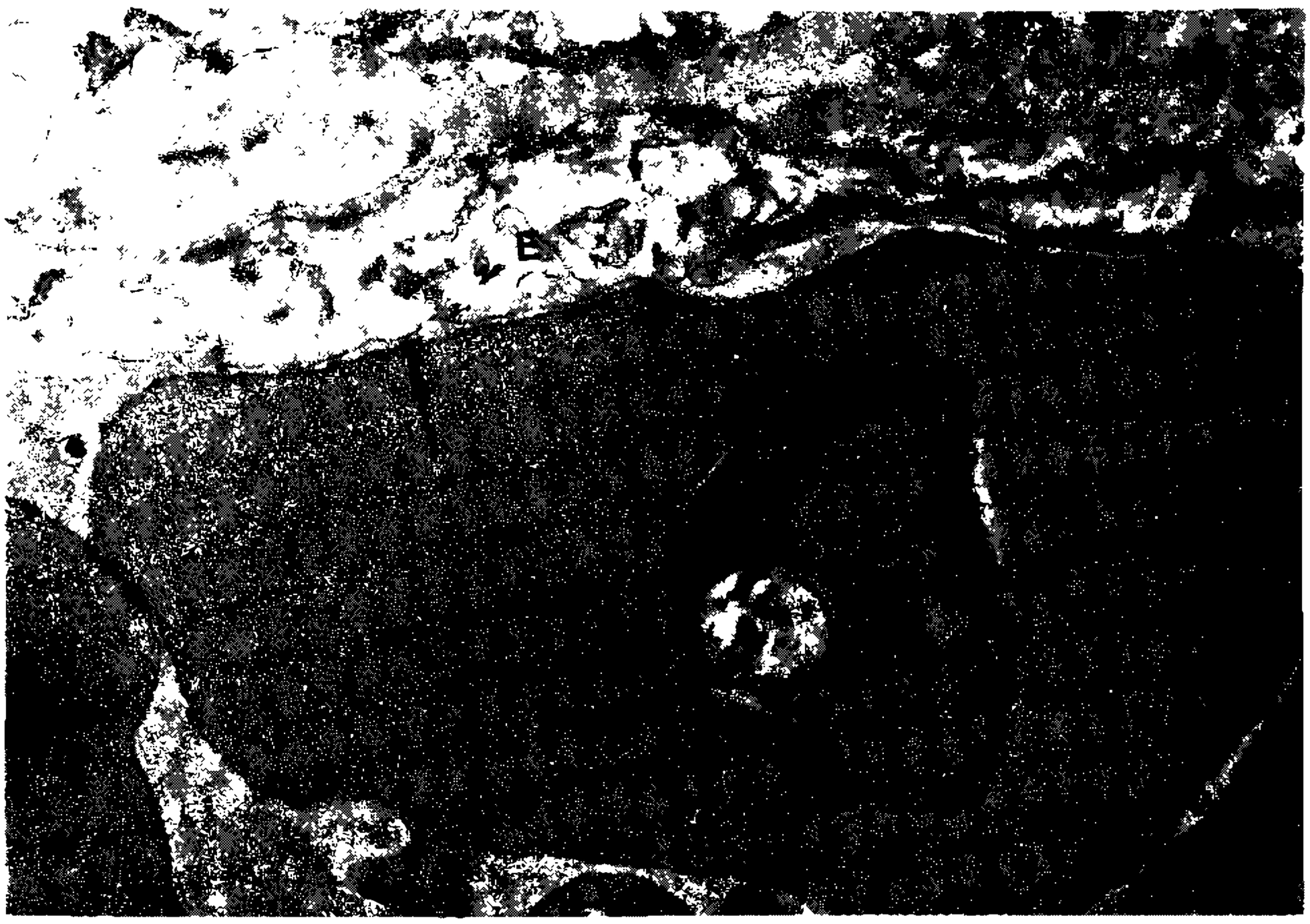

Fig. 4: A cerebral capillary. An *P. falciparum* infected erythrocyte is adhered to the endothelial cells (E) by electron dense knobs (arrows). Note that knobs are also present on the free surface of infected erythrocytes. X18,000. (Oo, M.M. et al., 1986. J. Neuropath. Exptl. Neuro., In Press.)

Fig. 5: Focal junctions (arrows) are formed between the endothelial cells and knobs on the membrane of the infected erythrocyte. X40,000. (Oo, M.M. et al., 1986. J. Neuropath. Exptl. Neuro., In press.)

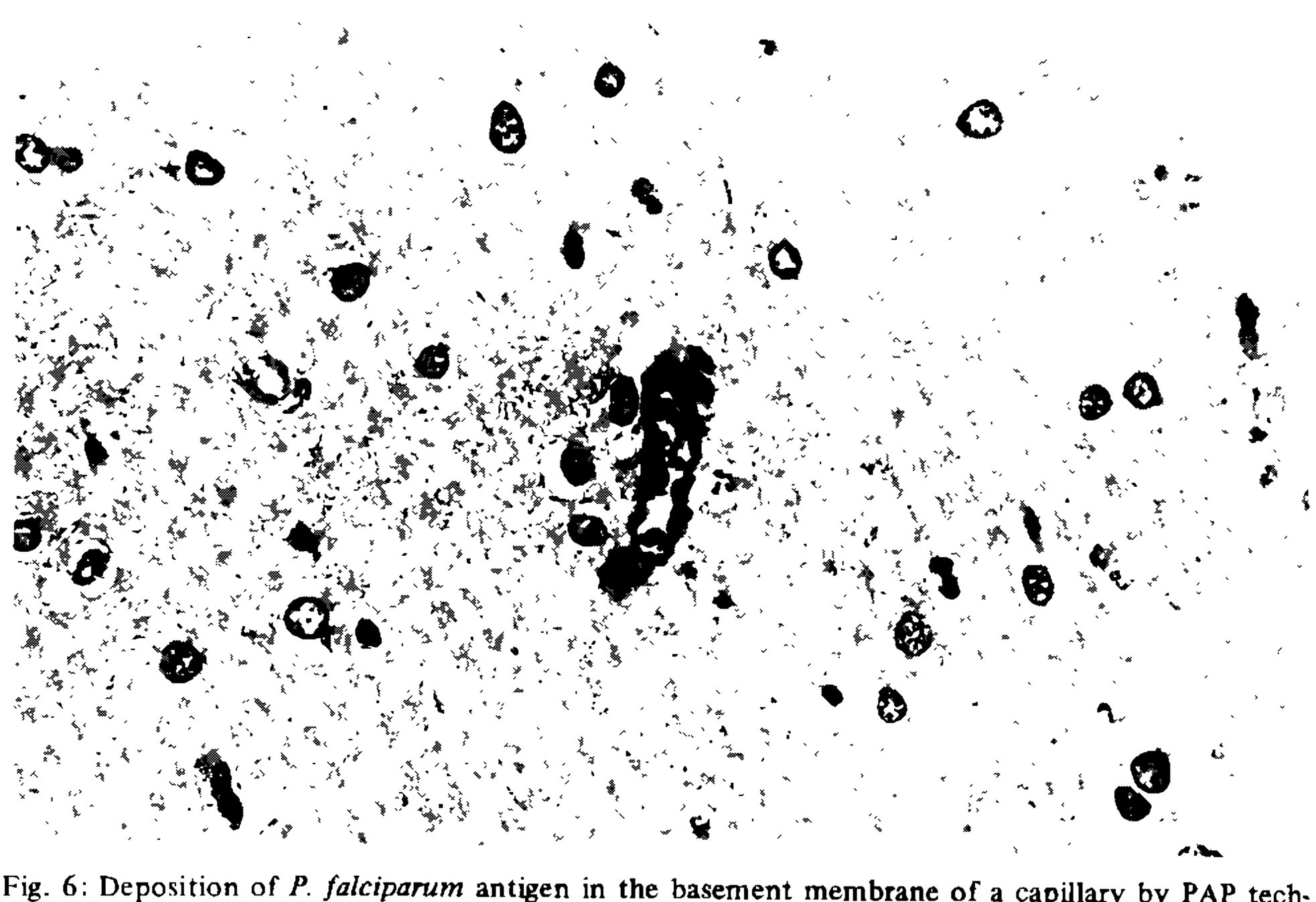

Fig. 6: Deposition of *P. falciparum* antigen in the basement membrane of a capillary by PAP technique. X780.

neither attach to endothelial cells *in vitro*, nor do they sequester *in vivo*. These findings together with our present data demonstrate the presence of cytoadherence properties at knobs (Howard & Barnswell, 1983).

Macrophages containing malarial pigment particles were present between packed erythrocytes. Infected erythrocytes were seen in small and medium sized arteries attached to the endothelial cells, however, they rarely blocked the lumen of these vessels. Endothelial cells were well preserved in the samples taken within a few hours after death. Severe autolysis such as, vacuolization and swelling of the endothelial cells, was found in tissue removed five hrs. or later after death.

A group of authors (MacPherson et al., 1985) described changes in the cerebral capillary endothelial cells e.g. vacuolization which they thought were due to infection. We found similar changes in our material, however, we could not differentiate these changes from autolysis. These same authors (MacPherson et al., 1985) described numerous pseudopods from the endothelial cells. Another paper (Pongponratn et al., 1985) reported vesicular membranes which were suggested by the authors to be the extension of the endothelial cells attached to parasitized erythrocytes. Whether these vesicular membranes are the same as the pseudopods of endothelial cells by MacPherson is difficult to interpret, since their illustration of these vesicular membranes did not show direct extensions from the endothelial cells. Endothelial cells pseudopods and vesicular membranes were not definitely identified in our material. Similarly, no pseudopods were found in *in vitro* study by Udeinya et al. (1981).

Perivascular or ring hemorrhages were present in nine out of 19 patients (Fig. 3). Necrotic arterioles and capillaires were present in the center of some of these hemorrhages, which contained parasited erythrocytes. The ultrastructural study of the ring hemorrhage revealed extravasated red cells in the cerebral tissue admixed with fibrin strands. Ring hemorrhages commonly have been found in the central nervous system of patients with cerebral malaria (Rest & Wright, 1979; Tharavanij, 1983). The mechanism of their formation and necrosis in some capillary walls is unknown, however, it might be secondary to vasoactive peptides and circulating antigen-antibody complexes (June et al., 1979). Rest & Wright (1979) postulated from experimental models that immune complexes could be taken up in cerebral vessel endothelium, resulting in their damage.

Subtle gliosis and slight sprinkling of red cells were seen in the white matter of some sections especially in the vicinity of packed vessels. Occasional inflammatory cells are adjacent to some of the capillaries engorged with infected erythrocytes (Fig. 2). The localization of *P. falciparum* antigens, IgG, and C3 was examined in brain tissues from seven patients with cerebral malaria. Linear staining of *P. falciparum* antigens was found along the basement membrane of the capillaries (Fig. 6). IgG was also seen along these same membranes of capillaries (Fig. 7). No staining of C3 was observed. Normal human brain, used as control, showed negative staining for *P. falciparum* antigens, IgG and C3 in the capillaries (Fig. 8). Other controls were also negative. The presence of *P. falciparum* antigen and IgG in capillary basement membrane might imply that capillary damages, such as necrotic arterioles and capillaries were in some way secondary to immune mechanisms. On the other hand, we should also consider the possibility that *P. falciparum* antigens and IgG were deposited in the capillary membrane as a result of tissue damage.

Significance of cerebral edema in cerebral malaria is difficult to interpret. Gross swelling of brain was present in seven out of 19 patients. Variability of edema may be due to the stage of the disease, result of different treatment, or the difficulty of interpretating clinical and radiological findings (Looaresuwan et al., 1983).

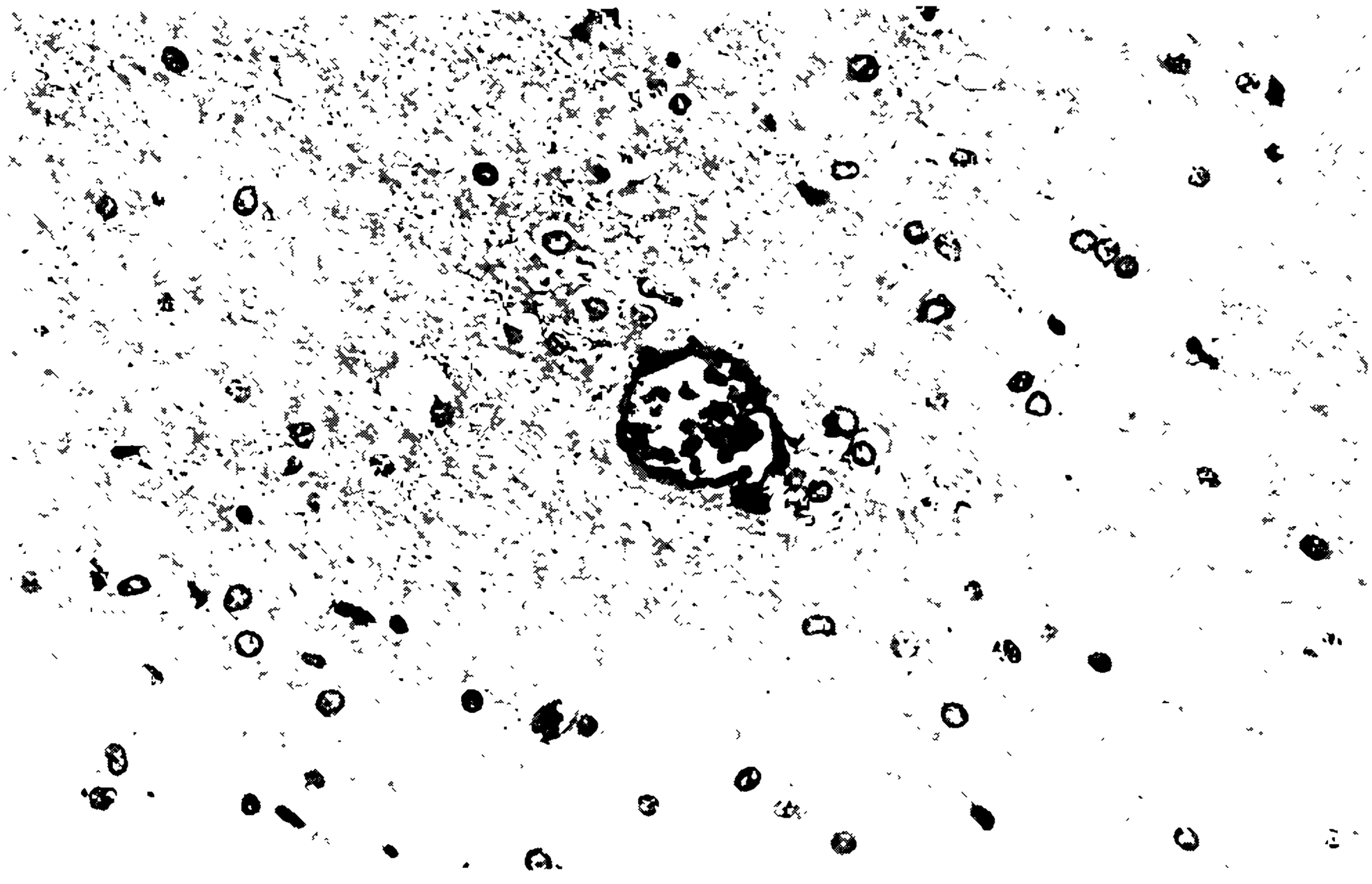

Fig. 7: Deposition of IgG in the basement membrane of a capillary by PAP technique. X350.

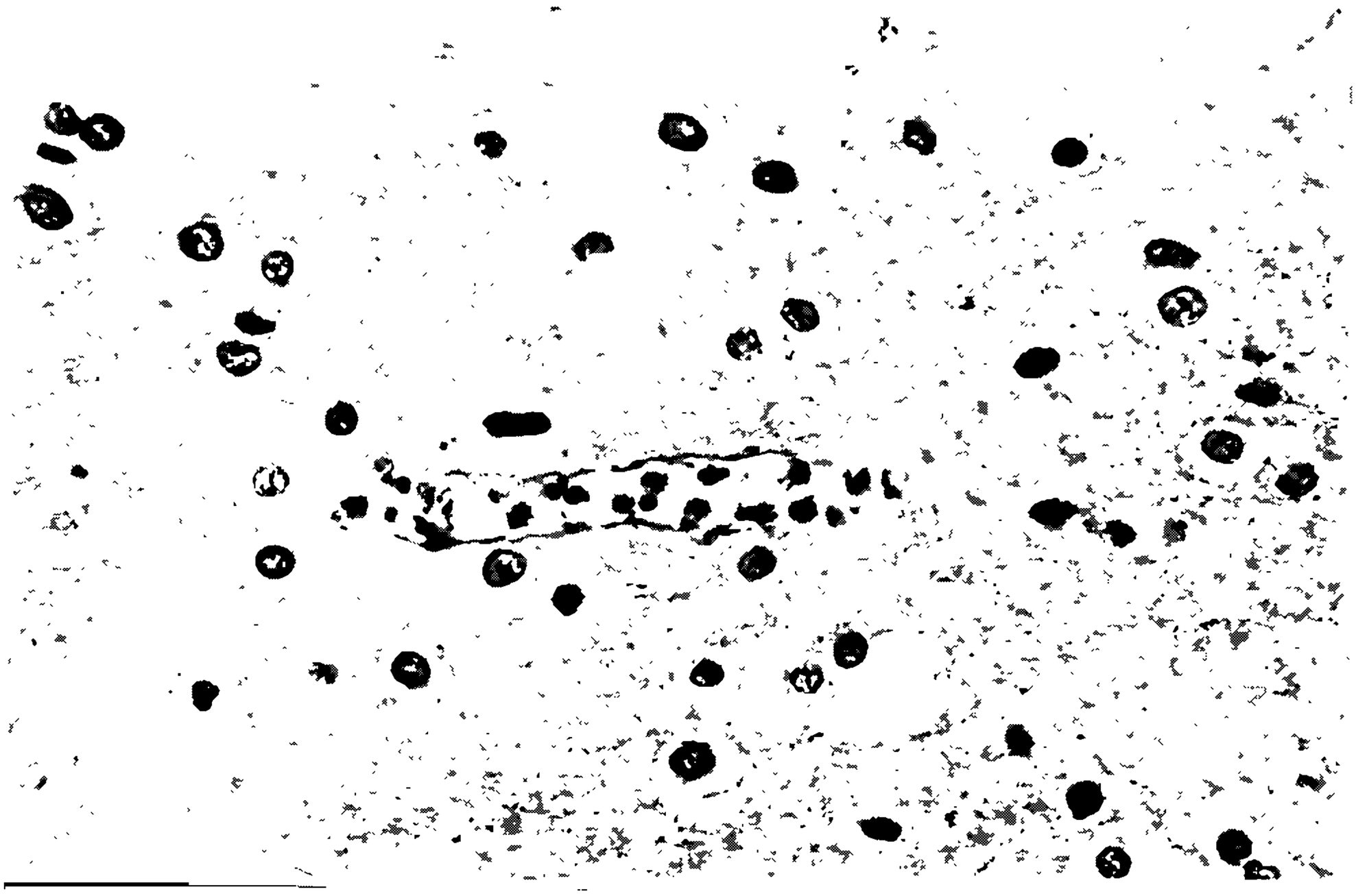

Fig. 8: Negative staining sections for IgG in the basement membrane of a capillary taken from a normal brain (control). X780.

## CONCLUSIONS

A series of 19 Burmese patients who died of cerebral malaria was studied by light and electron microscopic and immunological techniques. The major pathological changes are blockage of cerebral capillaries by *Plasmodium falciparum* infected erythrocytes, ring hemorrhages and segmental necrosis of cerebral capillaries. Cerebral edema is variable in our cases. Electron dense knobs which protrude from the membrane of infected erythrocytes and formed focal junctions between endothelial cells and erythrocytes. These junctions result in the sequestration of erythrocytes and cause blockage in capillary lumen. This finding encourages further work in the development of a vaccine to knob proteins which could retard the development of capillary blockage, thereby preventing cerebral malaria. Our immunoperoxidase study revealed for the first time *P. falciparum* antigens and IgG deposits in the capillary basement membrane. This might imply that damage to the cerebral capillary could be also related to immune mechanisms.

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