The Effect of Proteolytic Enzyme Inhibitor on Experimental Brain Swelling

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INTRODUCTION

The importance of the brain swelling can be hardly overemphasized in neurosurgery and in neurology. A large number of studies have been reported on this theme[3)4)11)], whereas the problems which should be elucidated yet remain unsolved clinically and experimentally. It has been accepted that the brain edema means the condition of increased extracellular fluid which escape from the capillaries into the intercellular spaces and the brain swelling is the condition of increased intracellular fluid in glia and in nerve cells. While I have not attempted to distinguish between these two conditions, brain swelling and brain edema, therefore in this study it must include these two entities under the term brain swelling.

In experimental brain swelling there are included two types with regard to the different conditions of the blood-brain barrier, that is, the first is not destruction of it (water intoxication and triethyl tin intoxication); the second is the swelling due to barrier destruction (trauma, inflammation, tumor, irradiation, insulin intoxication, etc.). The brain swelling observing in clinical cases belongs almost to the latter, therefore, one of the important method in the treatment and prophylaxis of this condition is the maintenance of the barrier function. Modern medical management of the brain swelling consists of adequate oxygenation, controlled vascular hypotension, dehydration therapy by the hypertonic solutions, hypothermia, steroid therapy, controlled passive hyperventilation, tracheotomy, low sodium intake, administration of proteolytic enzymes, antihistamines and carbonic anhydrase inhibitors and drainage of cerebrospinal fluid[11)].

On the other hand, proteolytic enzymes were known to cause an increase in vascular permeability[6)], furthermore this effect was due to actions on the capillary wall. Anatomically, the blood brain barrier consists of capillary endothelium, basement membrane and pericapillary astroglial process. Therefore, the proteolytic enzymes may induce an increase in permeability of the blood-brain barrier. Beiler et al[5)]. reported that proteolytic enzymes, hyaluronidase and lecithinase were thought to increase permeability of the normal blood-brain barrier by the method of the

changes in latent period of barbiturates which was administered intravenously.

The author attempt to examine the effect of trasylol, the inhibitor of the many kind of proteolytic enzymes, in protecting the blood-brain barrier destruction in experimental brain swelling. This study is divided into two parts, the first is the check of the effects of trasylol by fluorescein sodium method. The second is the electron microscopic observation of the brain tissue including gray and white matters.

MATERIALS AND METHODS

Adult albino rabbits, both sexes, weighing 1.9 to 3.0 kg, were used for the study. The experimental brain swelling was produced by the extradural balloon method[18]. The experimental animals were anesthetized with 30mg/kg of intravenously administered pentobarbital sodium, thereafter, small trephine hole of 7 mm in diameter was made on the right parietal, through which a small empty condom balloon was attached to a polyethylene tube, was introduced into the extradural space of the right frontal area. After plugging the trephine hole with a small bone button, the wound was sutured. These surgical procedures were done under strictly sterile precautions. The polyethylene tube protruding from the incision was then connected to a syringe with saline. The balloon was slowly expanded with 0.022ml/min. increments of saline by an injector. Total amount of saline injected into the balloon was about 0.6 ml which required the interval of injection for 30 minutes. The polyethylene tube was sealed and the expanded balloon left in place for 24 hours. Then, the balloon was evacuated.

In trasylol-treated group, 50000 KIE (units) of trasylol was administered intravenously two times; the first, just before the filling of the extradurally introduced balloon with saline solution and the second, immediately after emptying this balloon 24 hours later. Experimental animals were sacrificed 24 hours after the evacuation of the balloon and the destruction of the blood-brain barrier was examined with fluorescein method[15]. That is, 5 ml/kg 20 % sodium fluorescein was injected via the right carotid artery, thereafter, bilateral jugular veins were cut and 300 ml of 10 % formalin solution was infused from the internal carotid arteries on both sides. Then, the whole brain was extirpated and examined under the ultraviolet light.

On the other hand, the animals for the electron microscopic study were sacrificed 24, 48, 72 and 168 hours after the evacuation of the balloon. In trasylol-treated group, trasylol was administered as same as in dye examination group. The specimens, 1 mm^3 in size, for electron microscopic examination taking from the right frontal gray and white matters were fixed in 2 % OsO_4 solution buffered with Caulfield's method[9] for 2 hours at 4°C. After dehydration in graded alcohol, the specimens were embedded in Epon 812 according to the method of Luft[23].

Thin sections were cut with glass knives on the Porter-Blum microtome and stained with uranyl acetate or lead citrate[42]. Then, the stained sections were observed under JEM 7 electron microscope JAPAN ELECTRON OPTICS LABORATORY.

RESULTS

I. Dye Examination

In all control animals, the right frontal area compressing with extradurally balloon was strongly stained with fluorescein (Fig. 2A). Ten rabbits were treated by trasylol in above mentioned manner. In 6 cases no fluorescein was found in whole brain, except for some areas which lack of the blood-brain barrier (Fig. 2B). In the other 2 cases slight fluorescein was seen in the compressed area, while the grade of contamination was fairly slight as compared with control animals. In the last 2 cases the brain was stained as same as in a control.

Therefore, it may be concluded that trasylol had the protective effect on the barrier function in this type of the brain swelling, since in 60 % of cases no stain by dye was observed and in further 20 % the grade of stain was inhibited. Then, in 80 % of cases the protective effect of the trasylol on the barrier function was observed.

II. Electron Microscopic Examination

(I) Normal Group

1. Nerve cell

Nerve cells contained large irregular ovoid nuclei with an evenly dispersed finely granular chromatin. Nucleoli were prominent and occasionally multiple. Associated with the nucleolus, there was frequently a clump of dense chromatin particles[24]. The nuclear membrane appeared double. The inner nuclear membrane was smooth, whereas the outer membrane presented fine undulations and tubular extensions into the cytoplasm. At irregular intervals the two nuclear membranes abruptly fused at a short distance. At some of these points the nuclear covering seemed to be discontinuous. Configurations of this type may actually have represented perforations or apertures in the nuclear membranes[13][17][32][37].

The cytoplasm, surrounding the nucleus and extending to the plasma membrane was abundant and contained numerous finely divided granules of varying sizes and of moderate densities. In the cytoplasm, the mitochondria, the Golgi components and the endoplasmic reticulum could be recognized. The mitochondria possessed an outer smooth membrane and an inner folded membrane that formed cristae projecting into the interior of the organelles. The cristae were usually of the conventional shelf-like type, extending more or less at right angles to the long axis of the mitochondrion[17][24][32].

The Golgi components consisted of vacuoles and tubules with walls made up of

smooth membranes.[16)17)24)] These Golgi aggregations were usually multiple and arranged perinuclearly. The endoplasmic reticulum consisted of membranous profiles associated with a dense, granular component. In addition to the structures enumerated above, the neurofilament and the lysosom were observed (Fig. 6, 7).

2. Astrocyte

The astrocyte contained an irregularly round to oval nucleus. The nucleoplasm was brighter than that of any other neuroglial cell and had a scant granular chromatin. The nucleolus could be recognized as a loosely organized condensation of nuclear granules.[12)13)17)24)27)]

The cytoplasm around the nucleus was abundant but contained relatively few electron-opaque components, so that the over-all effect was of a rather "watery" composition.[27)] In the soma, the organelles of these cells were easily visible in contrast of the pale backgound.[37)] There were a few mitochondria, vesicles, Golgi components and RNP particles through the perinuclear cytoplasm. The organelles of the membrane components in the cytoplasm were usually scanty. Dense bodies with granular substructure were occasionally encountered which conceivably were related to lysosomes.

The astrocytic processes were pale cytoplasm as that of the cell body and showed a highly important feature of astrocytes. They expanded directly upon the basement membrane overlying the capillary endothelial sheet.[37)] As specific view, unevenly scattered through the cytoplasm were dense granules 400 to 700 Å in diameter. These granules were named "specific fine granules" by K. Okada.[30)] He concluded that these granules were fixed well by potassium permanganate, stained distinctly by uranyl acetate, and not digested by saliva, thus being considered to be neither glycogen nor RNP granules but glycoprotein or glycolipid in nature (Fig. 8, 9).

3. Oligodendroglia

The nuclei were larger and less uniformly ovoid or round than those of the astrocyte, often brought slightly reniform. The nucleoplasm was slightly denser than that of the astrocyte, and the nuclear chromatin was finely and uniformly granular.[17)24)37)]

The cytoplasm amounted to little more than the content of the nucleus, and it was moderately dense. The organelles of these cells were relatively abundant. The cytoplasm contained moderate quantities of endoplasmic reticulum, a considerable density of RNA particles, mitochondria in moderate numbers and a few sparse groups of Golgi membranes (Fig. 10).

4. Microglia

The microglial cells were extreme over all density and of angular and irregular contour. Their nuclei were ovoid, large in relation to the size of the cells, and had a uniformly dense chromatin. The cytoplasm of microglial cells was scant, and was also stuffed with dense granular material. A moderate amount of endoplasmic reticulum could be found in microglial cells. Usually, more conspicuous

findings were clumps of Golgi membranes and vesicles. Mitochondria was not very numerous and seemed to be quite small. The processes of microglial cells kept their density terminally so that even small sections of microglial processes usually could be recognized without difficulty[17)24)37)] (Fig. 11).

5. Intercellular space

The neuropil was composed of a complicated arrangement of processes arising from the various cells of the nervous tissue, thus consisting of dendrites, axons, astrocytic, oligodendroglial and microglial projections. They were tightly packed against each other, and the intercellular space intervening between the cellular components was measured about 200 Å in width (Fig. 5).[24)28)30)37)39)43)]

6. Capillary

The capillary consisted of endothelium, pericytes and basement membrane.[7)17)28)] The endothelium, although considerably attenuate, presented a continuous sheet of cytoplasm without the fenestrations seen in some other capillaries. Endothelial cytoplasm was moderately dense. There were a few mitochondria, sparse endoplasmic reticulum and pinocytic vesicles in the cytoplasm. The margins of endothelial cells seemed overlap one another slightly. This area is called the "terminal bar" or "adhesion plates".[28)] Besides the endothelial cell, there was the pericyte surrounded by the basement membrane. Beyond the pericyte, the capillary wall was surrounded directly by glial processes. There was a basement membrane, which was interposed between the glial and endothelial elements and in places, was divided into two components. The two components of the basement membrane lined an endothelial and a glial side, one for each of the two.[19)] The total gap varied from 300 to 500 Å. The central zone was filled by relatively dense and osmiophilic material, and has been called the "lamina densa" or "basal lamina".[28)] On either side of the dense layer, between it and the adjacent cell layers, zones of low density, homogenous material were to be found. These layers have been called "lamina rara" and "cement layer".[28)] The pericapillary space, so-called the Virchow Robin space was not present (Fig. 3, 4).[17)19)25)30)]

(II) Non-Treated Control Group

1. Nerve cell

The cytoplasms of nerve cells appeared to be slightly expanded in volume as compared with normal. Mitochondria of nerve cells were vacuolized and their cristae deranged, and such intracytoplasmic membranous structures of these neurons as endoplasmic reticulum were distended. Nuclear membranes were partly distended and the outer membrane projected into the cytoplasm (Fig. 19).

2. Astrocyte

Astrocytic processes were swollen markedly. This finding was most prominent 24 to 48 hours after evacuation of the extradurally placed balloon and also was observed 72 hours later. However, 168 hours later the astrocytic processes was observed almost as the same as in normal ones. On the contrary, specific fine

granules in the astrocytic cytoplasm increased with the lapse of time. Mitochondria of astrocytes tended to be vacuolized and their cristae deranged (Fig. 20, 21, 22, 23).

3. Oligodendroglia and Microglia

No morphological change in oligodendroglial and microglial cells in these swollen brains was recognized.

4. Intercellular space

The cortical intercellular space maintained about 200 Å width similar to its normal one.

On the other hand, in the white matter, the intercellular space was distended, and presented the separated myelin sheath (Fig. 24).

5. Capillary

The endothelial membrane along the surface of the capillary lumen showed an irregular unevenness. The pinocytic vesicle in the cytoplasm of the endothelial cell was increased markedly. The basement membrane of the capillary wall became loose and low in density. The separation of the glial basement membrane from that of the endothelial cell was present partially (Fig. 12, 13, 14, 15, 16, 17, 18).

Table 1 showed the course of these main changes.

Table 1. The course of the ultrastructural change in the experimental cerebral swelling

Ultrastructural findings \ Time after evacuation of the balloon		24 hrs.	48 hrs.	72 hrs.	168 hrs.
Nerve cell	change of mitochondria	+	+	+	±
	distended vesicular structure	+	+	+	±
	extended nuclear membrane	+	+	+	±
Astrocyte	swelling of process	###	###	##	−
	increased specific fine granule	##	##	###	##
	change of mitochondria	±	±	±	−
Oligodendroglia		−	−	−	−
Microglia		−	−	−	−
Dilatation of intercellular space	gray matter	−	−	−	−
	white matter	+	+	+	±
Capillary endothelial cell	increased pinocytic vesicle	##	##	+	±
	unevenness of cytoplasma membrane	+	+	+	±
Capillary basement membrane separation		+	##	##	±

− : no change + : slight change ## : moderate change ### : marked change

(III) Trasylol Group

1. Nerve cell

Mitochondria of nerve cell in this group did not show the morphological changes as the vacuolation of its matrix and the derangement of its cristae. The nuclear membrane and the membranous structure of these nerve cells were not distended. Nerve cells in these brains seemed similar to those in normal ones (Fig. 32, 33, 34).

2. Astrocyte

Astrocytic processes in these brains were not swollen, but the specific fine granules in the astrocytic cytoplasm increased similarly in the non-treated swollen brain. Mitochondria of astrocytes in these brains did not show the morphological changes while tended to be slightly increased in number (Fig. 35. 36, 37).

3. Oligodendroglia and Microglia

No morphological change in oligodendroglial and microglial cells in these brains was recognized.

4. Intercellular space

No alteration of the intercellular space was present. This space in these brains maintained about 200 Å width similar to its normal one (Fig. 38).

5. Capillary

The pinocytic activity of the capillary endothelium was not so marked as in the case of non-treated swollen brain, and resembled that of normal brain. However, the basement membrane of the capillary wall in these brains seemed similar to those in non-treated one. The separation of the glial basement membrane from that of the endothelial cell was present (Fig. 25, 26, 27, 28, 29, 30, 31).

DISCUSSION

Dehydration therapy with hypertonic solutions is one on the most familiar method in the treatment of the brain swelling.[11)21)30)34)44)45)] This therapy is based on the principle that the swelling may be relieved by causing a shift of fluid from the central nervous system into the blood stream. Many drugs used in this manner have included glucose, sucrose, sorbitol, sodium chloride, dextran, urea, mannitol, concentrated human albumin, etc.. They have met with varying degrees of success, while none really proved entirely satisfactory, furthermore so-called "rebound phenomenon" appeared even if its grade varried. In present, 30% of ureal and 20 % of mannitol solutions are the most useful agents for the purpose of the treatment of the brain swelling, which is observed during the craniotomy or in a posttraumatic state since the dehydration effect of these agents is so strong and rapid, and further the rebound phenomenon appears moderately. However, we must pay attention to following opinion that these hypertonic substanses may

even do more harm than beneficial effects in the presence of a destructed blood-brain barrier, as they eventually could pass this barrier into the brain tissue, consequently fluid accumulation in these area would increase by drawing in water.

Recently, the large amount of glucocorticoid was used in clinics of neurosurgery as a treatment and prophylaxis of the brain swelling. Dramatic improvement of the neurological state and decrease of intracranial pressure had been reported by many authors.[8)11)14)22)30)33)40)] These large doses of steroids appear to restore the integrity of a blood-brain barrier that has become abnormally permeable in the presence of tumor or trauma, while the exact role played by corticosteroids is still unknown. Since it can be assumed that in every type of edema the excess of fluid derives its origin from the blood, the elucidation of vascular permeability and of a related blood-brain barrier phenomenon are of obvious importance. Scheinker[35)] considered the increased permeability of cerebral vessels to be an universal pathogenic mechanism in brain edema.

In regard to the anatomical localization of the blood-brain barrier two hypotheses have been presented, that is the pia-glia theory which was advocated by Tschirgi[41)] and capillary wall theory by Spatz.[38)] The latter had been almost forgotten since the appearance of the former, while it came to had a better opinion accompanied with the development of the electron microscopical studies. Bennett et al.[7)] were reported the morphological classification of the blood capillaries, in which they stated that capillaries may be classified by the difference of 1) basement membrane 2) endothelium and 3) pericapillary investment. The capillaries observing in the brain have a most compact wall, that is, capillary type A, capillaries with a complete continuous investment of basement membrane ; capillary type I, capillaries without fenestrations or perforations ; capillary type β, capillaries with a complete pericapillary cellular investment interposed between capillary and parenchymal cell. Therefore, at present, it has been accepted that the anatomical background of barrier function is situated both at the capillary wall and the pericapillary invested astroglial process.

The proteolytic enzyme inhibitor, trasylol, inhibits the activation of the kinin and fibrinolytic systems, further the functional increase of the coagulation system,[26)] these are mutual relation as Back[2)] showed in his triangular figure (Fig. 1). The functional increase of either apex causes mechanically the activation of the other apexes. Trasylol is able to act in three apexes of this triangular figure. Therefore, trasylol has been used in acute pancreatitis, increased fibrinolysis, some types of hemorrhagic diseases, severe inflammation, peritoneal adhesion, etc.. However, no report has been found that trasylol used as the treatment of brain swelling. We reported preliminarily trasylol had the protective effect on the blood-brain barrier in experimental brain swelling elsewhere.[1)]

The liberation of the proteolytic enzyme which isolates the pharmacodynamic active low molecular peptide, that is, the kinin from the kininogen, belongs to

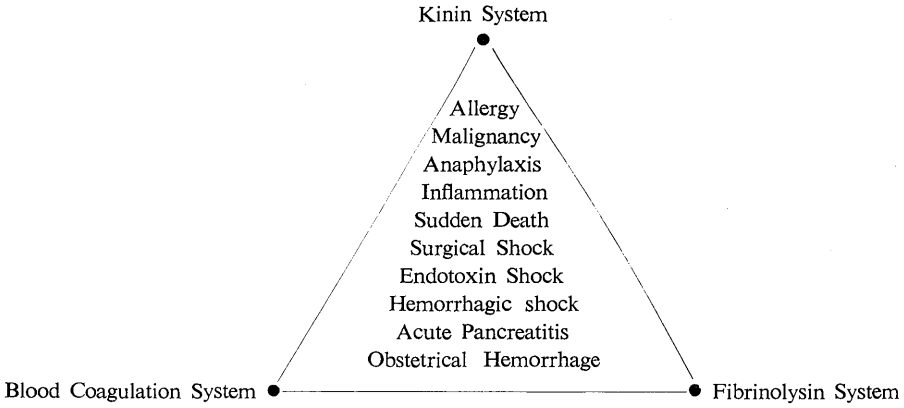

Fig. 1. Back's Triangle

the various active phenomenon to be caused by the tissue injury. The proteolytic enzyme inhibitor in the serum can inhibit the liberation of the kinin to some extent, but the action of the kinin is present when this inhibitor is spended. That affects the blood capacity, the blood component, the capillary circulation, the electrolyte and the acid-base balance caused to dilate the blood vessel, to down the blood pressure and to increase the permeability.

It may be thought that the liberation of kinin is a factor to be encouraged brain swelling in brain tumor, head injuries, cerebrovascular accidents and other brain lesions. In 1957, Beiler et al.[5] reported that the proteolytic enzyme increased the permeability of the blood-brain barrier.

In this study the effect of trasylol, proteolytic enzyme inhibitor, on the permeability of the blood-brain barrier was observed morphologically. Stain by the fluorescein sodium in the extradurally compressed region of the brain was strongly inhibited in trasylol-treated group. This fact suggests that the blood-brain barrier kept almost normal function.

In the observation of electron microscope, trasylol group differed obviously from the non-treated control group as shown in Table 2. As the pinocytosis of the capillary endothelium resemble that of normal brain, it was thought that the increase of the permeability of the wall was inhibited. The pinocytic vesicles of the capillary endothelium mentioned may represent the active and selective transmission through capillary wall and may account for the high permeability rate of the capillaries,[31] which was termed "cytopempsis" by Moore and Ruska.[29] It have been observed that the pinocytic vesicle in the alveolar capillary endothelium was increased in the edematous lung.[20][36] Therefore, it seemed that the increase of the permeability of the capillary wall was inhibited by the inactivation of the kinin system. However, the changes of the basement membrane of the capillary wall were present, then the beneficial effect of trasylol on the maintenance of the capillary wall was not complete.

Table 2. Electron microscopic findings of the compressed brain: Comparison of two groups (treated and non-treated groups)

Findings		Treated group	Non-treated group
Nerve cell	change of mitochondria	−	+
	distended vesicular structure	−	+
	extended nuclear membrane	−	+
Astrocyte	swelling of process	−	+
	increased specific fine granule	+	+
	change of mitochondria	−	+
Oligodendroglia		−	−
Microglia		−	−
Dilatation of intercellular space	Gray matter	−	−
	White matter	−	+
Capillary endothelial cell	increased pinocytic vesicle	−	+
	unevenness of cytoplasma membrane	−	+
Capillary basement membrane separation		+	+

In astrocytes, almost no swelling of the processes was observed and the mitochondria tended to be slightly increased, which showed a few of the vacuolation of the matrix and the derangement of their cristae. The increase of mitochondria suggested that the cell had the high breathing enzyme activity and the active energy production. This fact suggested the functional increase of astrocytes which form a link in the chain of the blood-brain barrier considering the active transport system. Since the astrocytes were not swollen, it was thought that the function of the blood-brain barrier was maintained.

Cohen[10] reported that the ATP synthetic activity of the cell decreased in the brain anoxia. On such occasion the energy supported the function of the active transport system was reduced. Then, the brain swelling developed due to the functional impairment of the blood-brain barrier. Biochemical process in the central nervous system following administration of the trasylol remained obscure, further it is unknown that whether or no ATP synthetic activity is effected by trasylol. Whereas, according to this study trasylol was related to the permeability of blood-brain barrier and the nerve cells remained entirely normal. Intercellular space showed normal width, about 200 Å, and no separation of the myelin sheath was observed in white matter. Therefore, protective effect of trasylol on the blood-brain barrier was observed by both fluorescein method and electron microscopic study, in experimental brain swelling which was induced by extradural compression. However, chemical process induced by the trasylol was remained as future problem.

SUMMARY AND CONCLUSION

The results of the fluorescein study and the electron microscopic observations concerning the effect of the proteolytic enzyme inhibitor, trasylol, on the experimental brain swelling, which was induced by extradurally compression, were as follows.

(1) In dye experiment using fluorescein, function of the blood-brain barrier was protected completely in 60 % of cases and in further 20% it was maintained partially.

(2) In electron microscopic study, the pinocytosis of the capillary endothelium did not show remarkable change and similar to that of normal brain.

(3) The basement membrane of the capillary wall in the treated brain seemed similar to that in the non-treated swollen brain. The separation of the glial basement membrane from that of the capillary endothelial cell was present.

(4) Astrocytic processes in the treated brain were not swollen. Mitochondria of astrocytes did not show the morphological changes and tended to be slightly increased in number.

(5) Nerve cells and its organelles in treated brain were seen similar to those in normal brain.

(6) No alteration of the intercellular space was present. The intercellular space in treated brain maintained about 200 Å width similar to the normal one. No separated myelin sheath was found.

(7) In conclusion, it was recognized by the dye method and electron microscopic examination that the brain swelling due to the extradurally compression was protected by the administration of trasylol. No report has been seen in regard to such usage of trasylol. However, some important obscure problems were remained, that is, the action points of intravenously administered trasylol was unknown, whichever in blood, in capillary wall and in central nervous tissue. Further, the chemical changes in the barrier function induced by trasylol was not clarified. These problems should be elucidated in future.

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Fig. 2A. Strong fluorescein stain is seen right frontal. (Non-treated swollen brain, 24 hours after evacuation of the balloon)

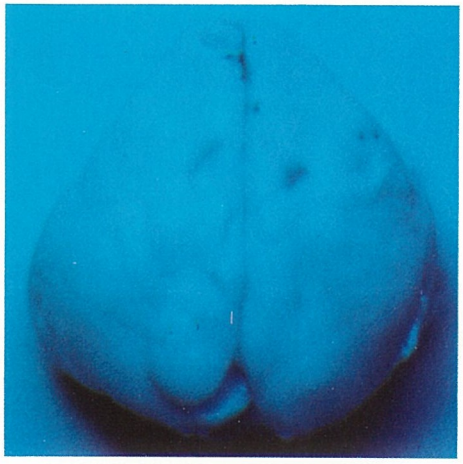

Fig. 2B. No fluorescein stain is seen in whole brain (Trasylol treated brain, 24 hours after evacuation of the balloon)

Legends for Figures

Figures 3 to 38 are electron micrographs.

ap : astrocytic process
ebm : endothelial basement membrane
ery : erythrocyte
gr : specific fine granules
ni : nucleolus
bm : basement membrane
end : endothelial cell
g : Golgi apparatus
m : mitochondria
pv : pinocytic vesicles
cl : capillary lumen
er : endoplasmic reticulum
gbm : glial basement membrane
n : nucleus
x : extracellular space

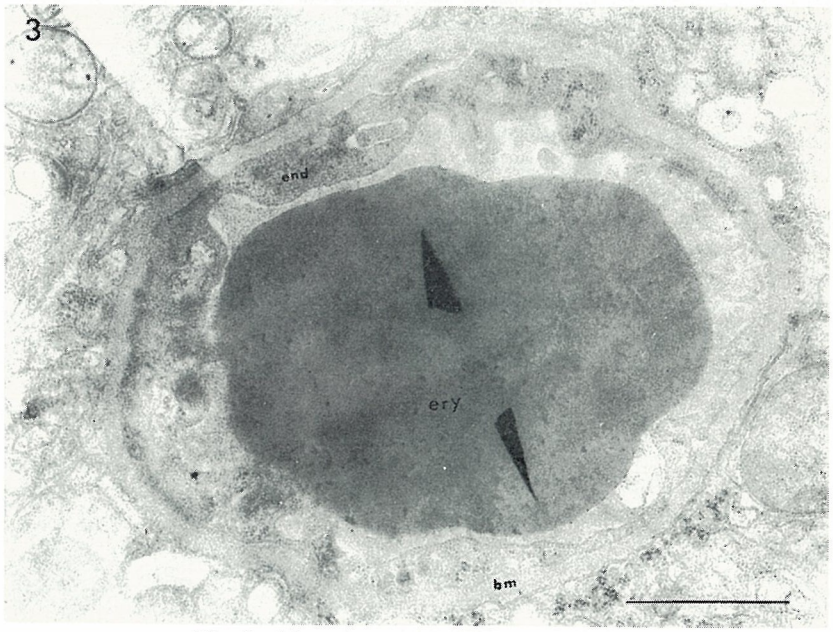

Fig. 3. Normal capillary in the white matter.

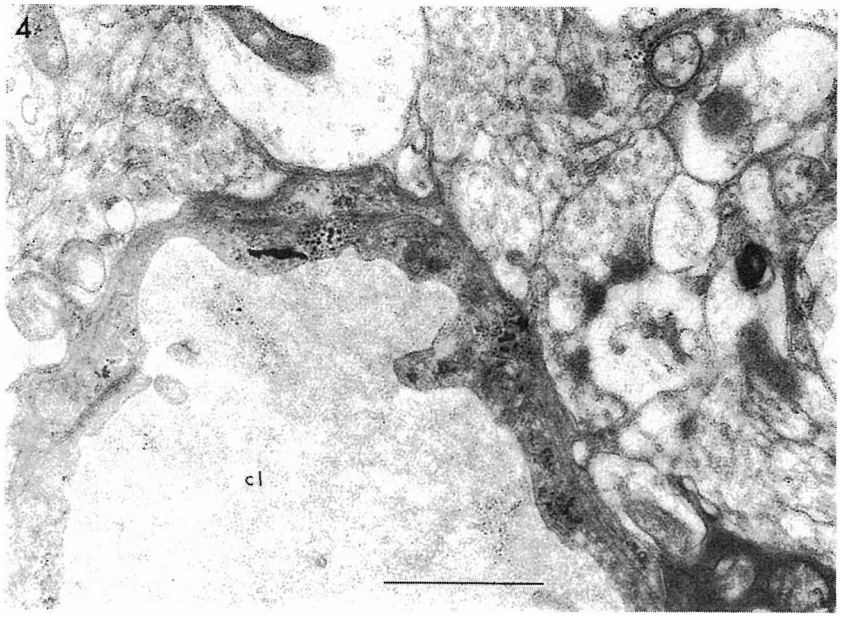

Fig. 4. Normal cortical capillary. The capillary wall is surrounded directly by glial processes. There is a basement mambrane, which is interposed between the glial and endothelial elements.

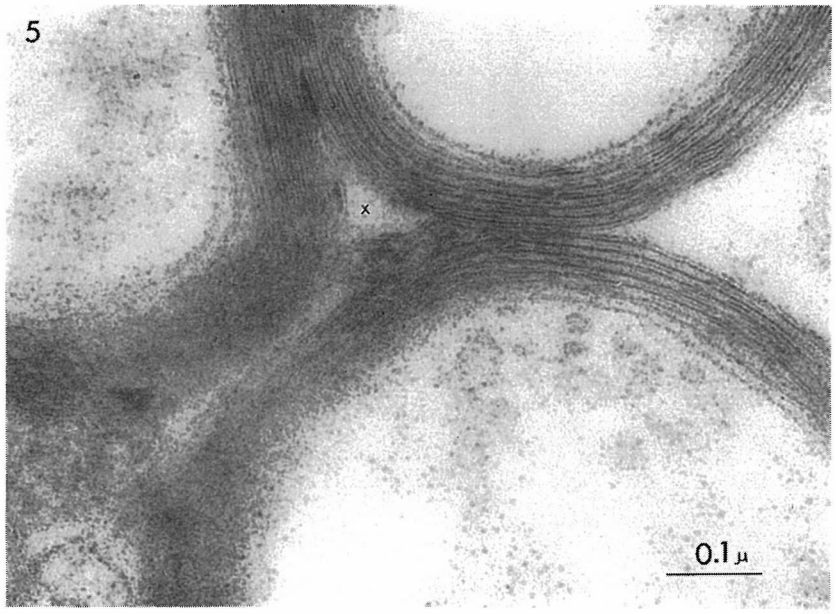

Fig. 5. Normal myelin sheath in the white matter. The myelin sheath shows no separation. The triangular extracellular space formed by three ovoid-shaped myelinated fibers is about 200 Å wide.

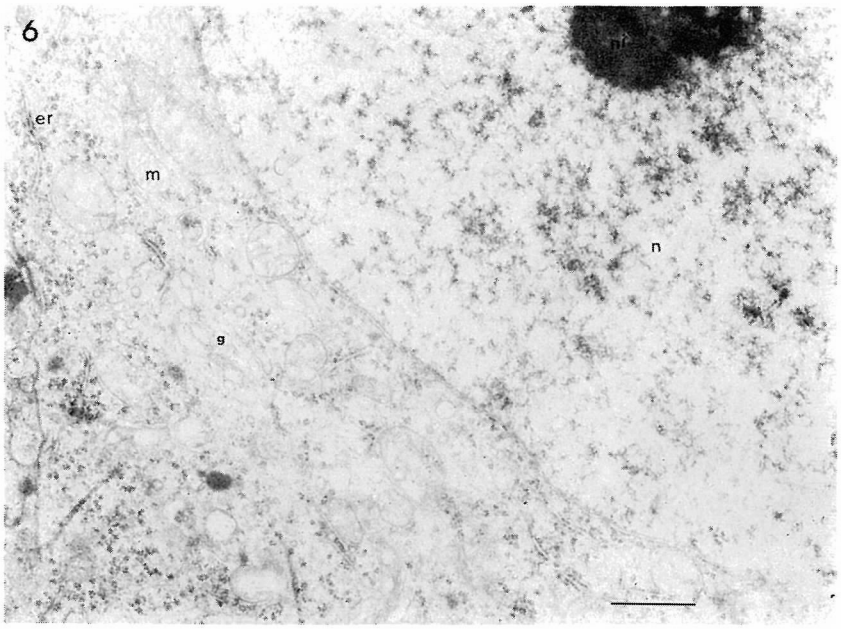

Fig. 6. Normal cortical nerve cell.

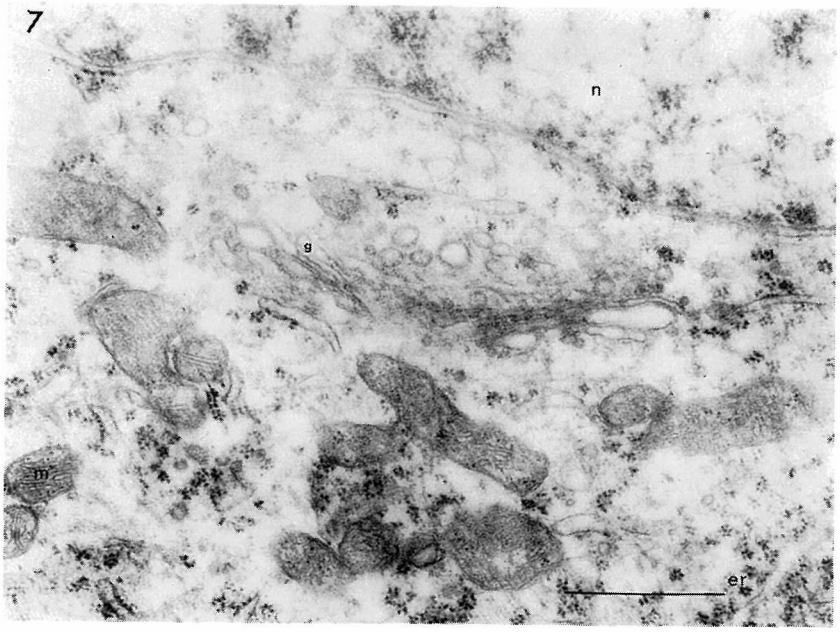

Fig. 7. Normal nerve cell in the white matter. The nuclear membrane appears double. In the cytoplasm, the mitochondria, the Golgi components and the endoplasmic reticulum can be recognized.

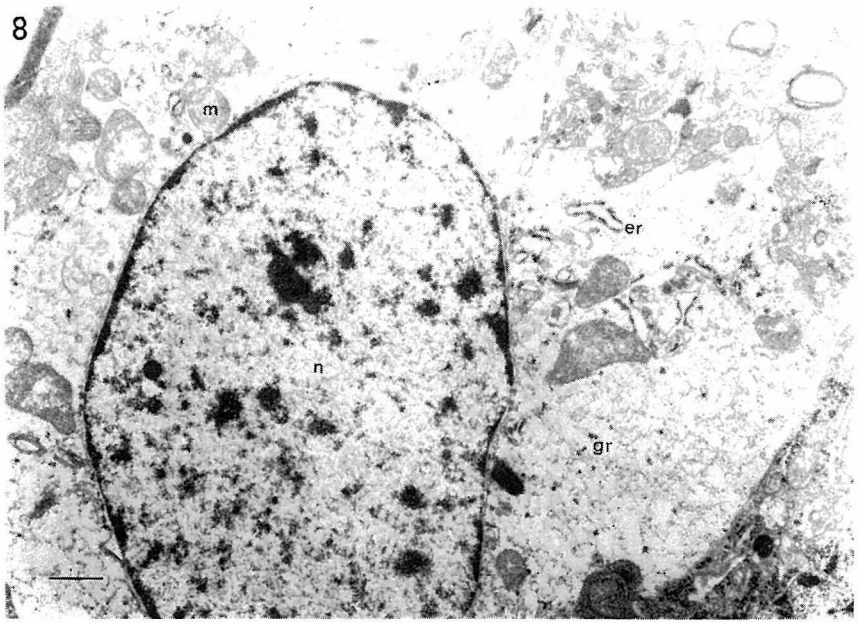

Fig. 8. Normal astrocyte in the white matter.

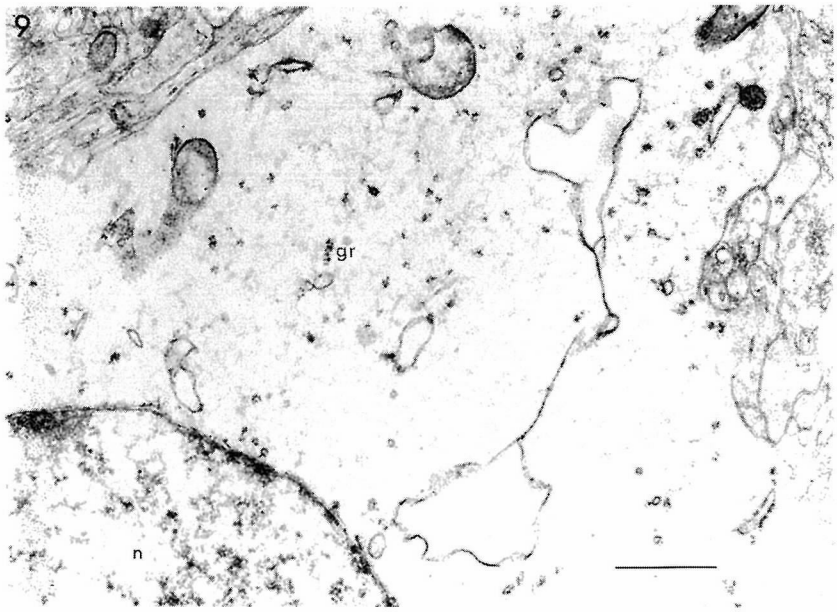

Fig. 9. Normal cortical astrocyte. The cytoplasma is abundant but contains relatively few electron-opaque component, therefore the over-all effect is of a rather watery composition. There are a few specific fine granules in the cytoplasm.

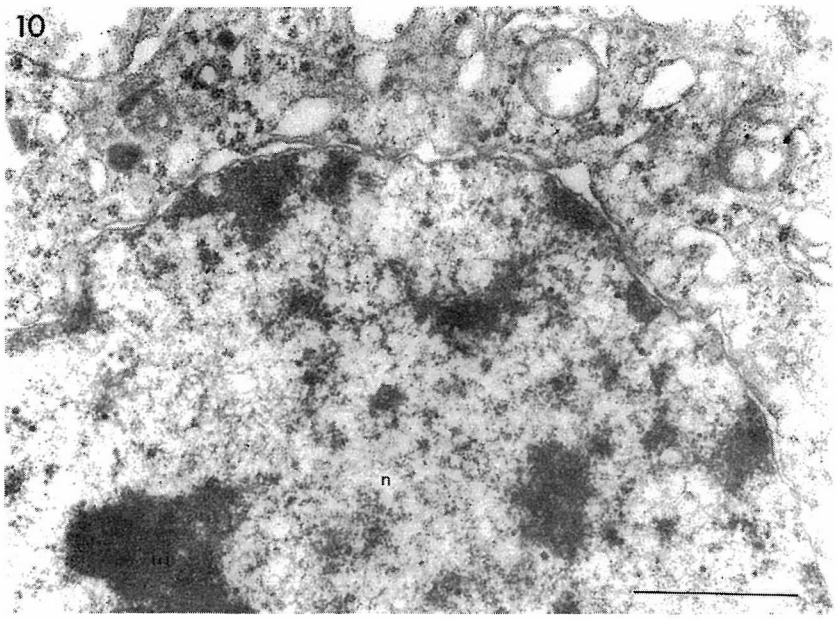

Fig. 10. Normal oligodendroglia in the white matter. The cytoplasm amounts to little and it is moderately dense. The organelles of these cells are relatively abundant.

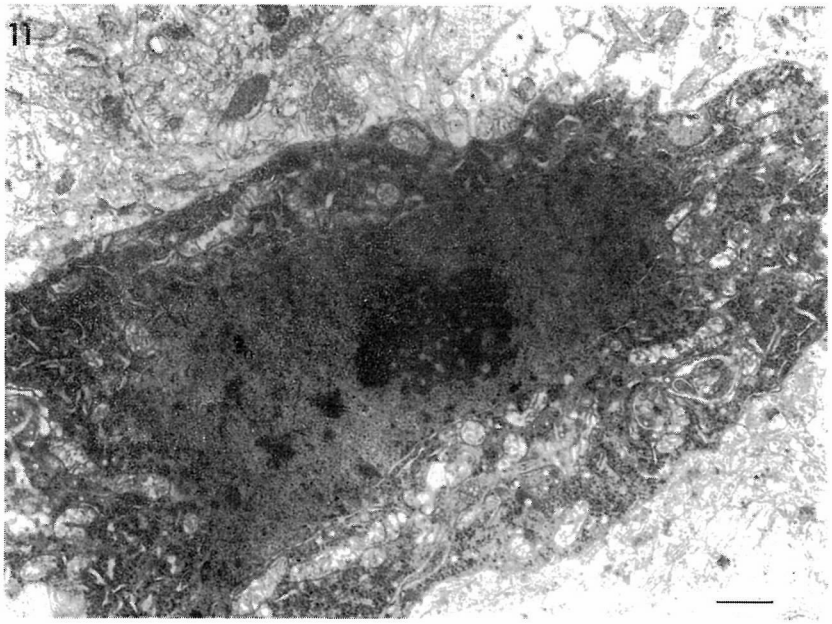

Fig. 11. Normal microglia in the white matter. The cytoplasm is scant, and is also stuffed with dense granular material.

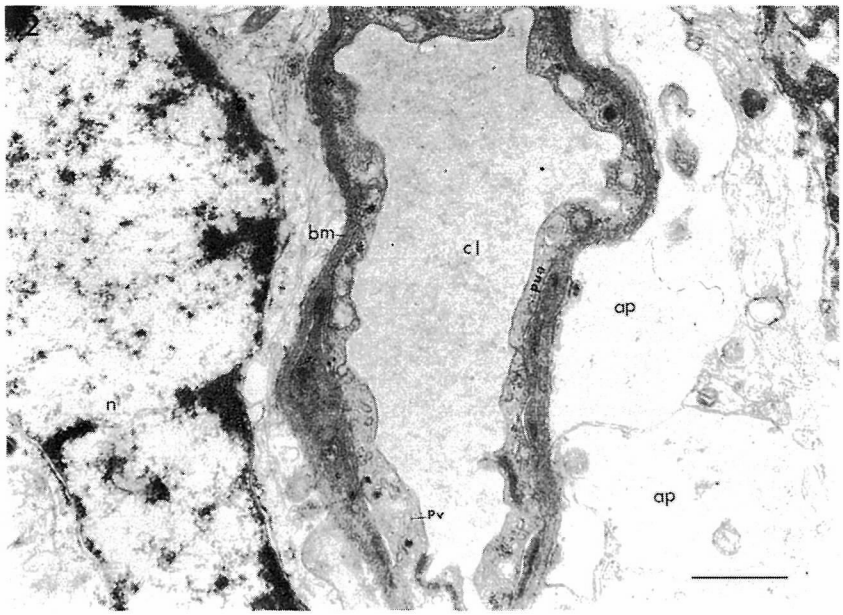

Fig. 12. Cortical capillary, 24 hours after evacuation of the balloon (Non-treated swollen brain). The astrocyte becomes enlarged along the capillary wall. The pinocytic vesicles in the capillary endothelial cell are increased markedly. Swelling of the glial process is seen.

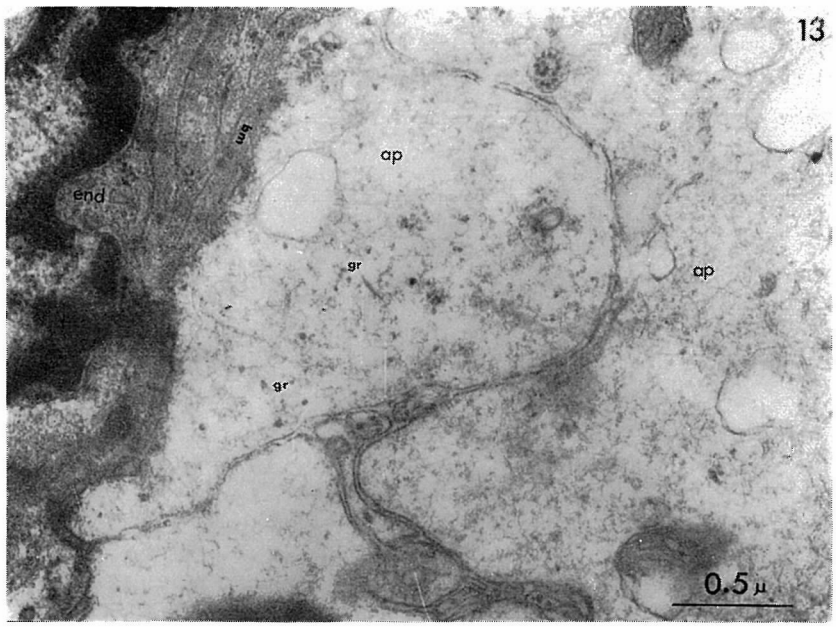

Fig. 13. Cortical capillary in swollen brain. 24 hours after evacuation of the balloon. Astrocytic processes are swollen markedly and the increase of the specific fine granules in the astrocytic cytoplasm are seen.

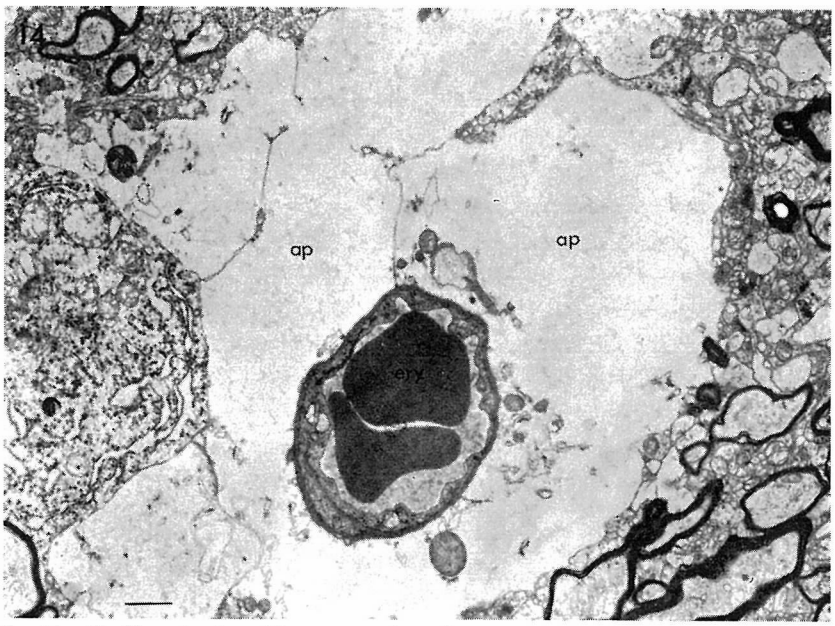

Fig. 14. Capillary in the white matter of swollen brain, 24 hours after evacuation of the balloon. Astrocytic processes are swollen markedly.

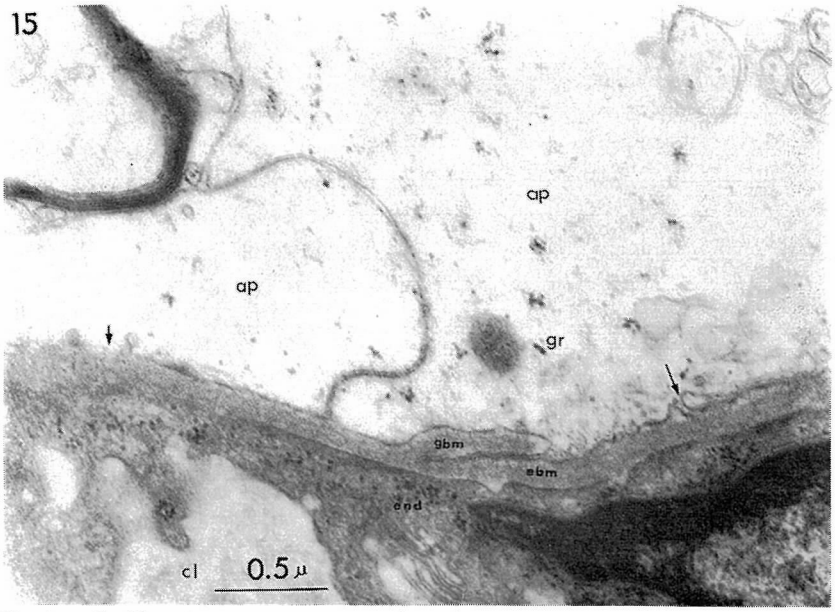

Fig. 15. Capillary in the white matter of swollen brain, 24 hours after evacuation of the balloon. The endothelial cytoplasmic membrane along the surface of the capillary lumen shows irregular unevenness. The basement membrane of the capillary wall becomes loose (arrow). Astrocytic processes are swollen markedly and the increase of the specific fine granules in the astrocytic cytoplasm are seen.

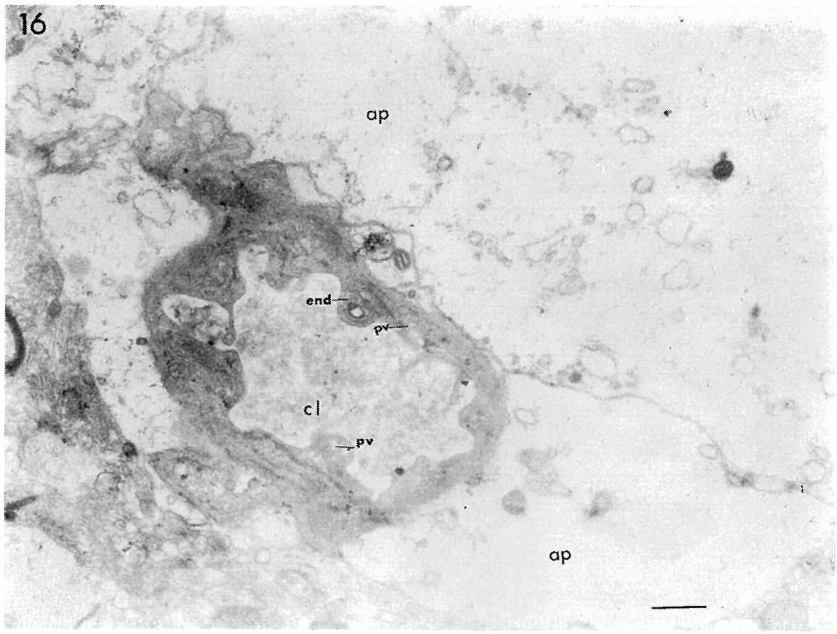

Fig. 16. Capillary in the white matter of swollen brain, 48 hours after evacuation of the balloon. Astrocytic processes are swollen markedly. The endothelial cytoplasmic membrane along the surface of the capillary lumen shows irregular unevenness and increased the pinocytic vesicles.

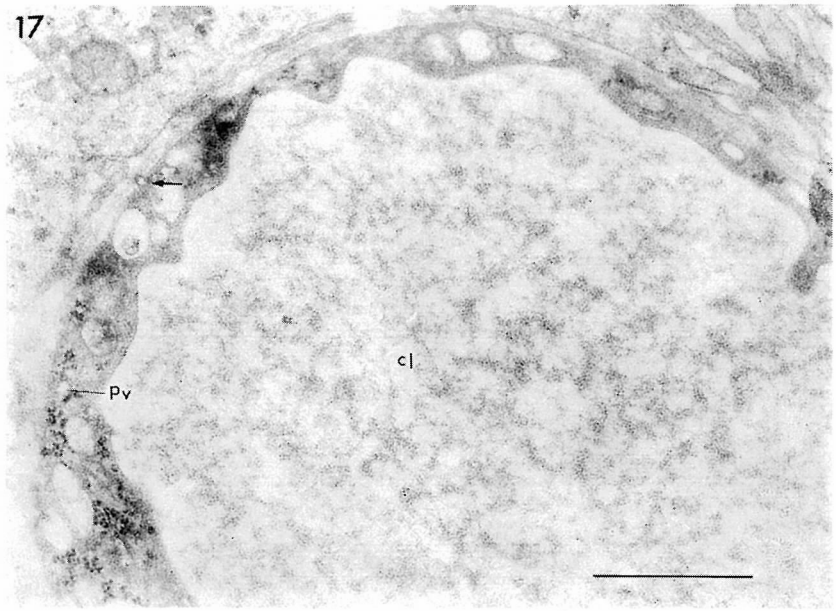

Fig. 17. Capillary in the white matter of swollen brain, 48 hours after evacuation of the balloon. The pinocytic vesicles in the cytoplasm of the endothelial cell is increased markedly (arrow).

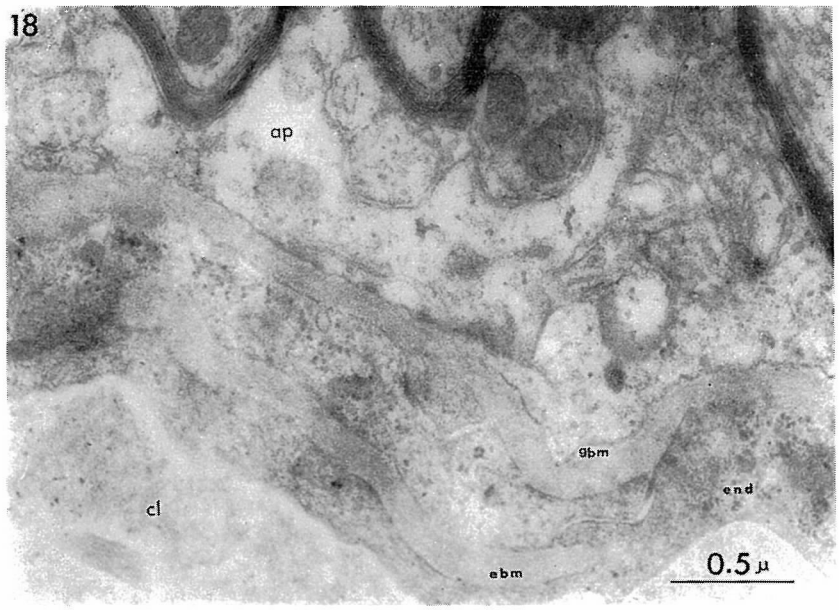

Fig. 18. Capillary in the white matter of non-treated brain, 168 hours after evacuation of the balloon. Astrocytic process is not swollen. The basement membrane of the capillary wall becomes loose. The separation of the glial basement membrane from that of the endothelial cell is present.

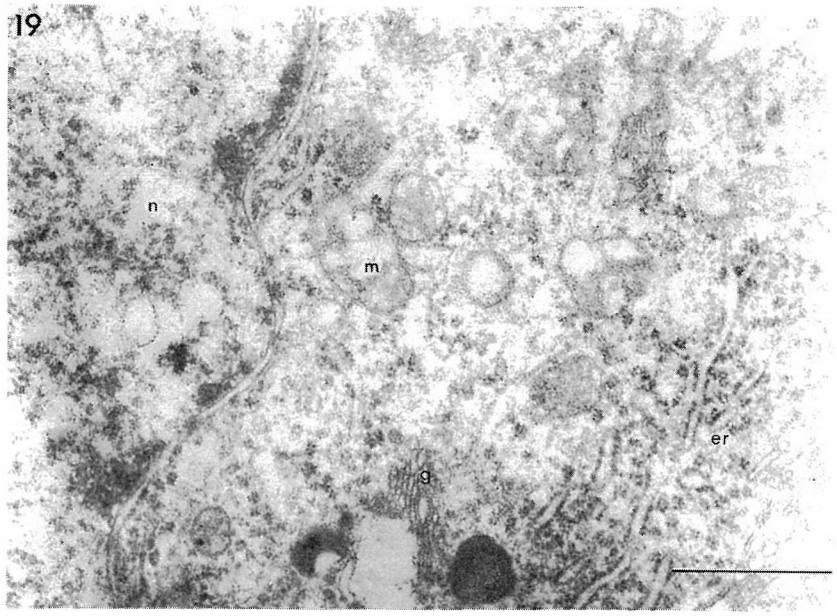

Fig. 19. Cortical nerve cell in swollen brain. 72 hours after evacuation of the balloon. Mitochondria are vacuolized and their cristae deranged.

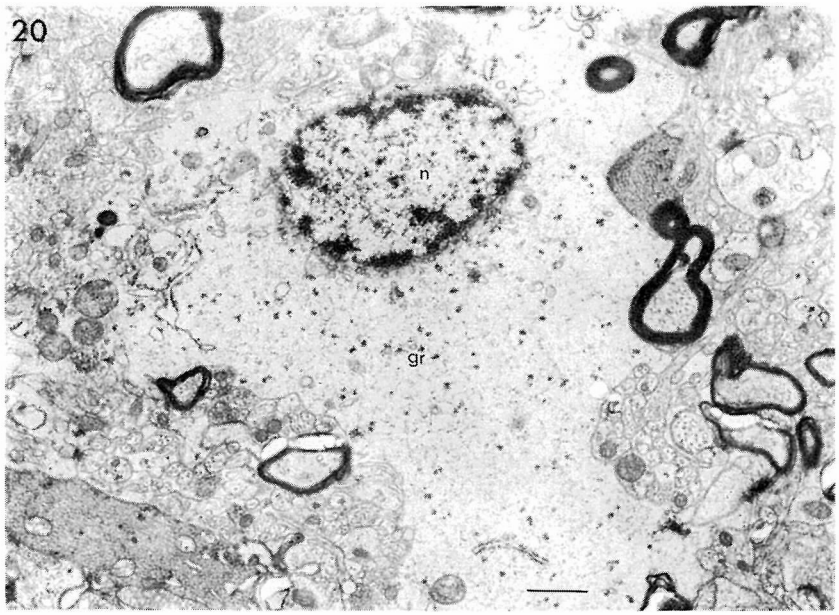

Fig 20. Astrocyte in the white matter of swollen brain. 24 hours after evacuation of the balloon. Specific fine granules in the cytoplasm are increased markedly.

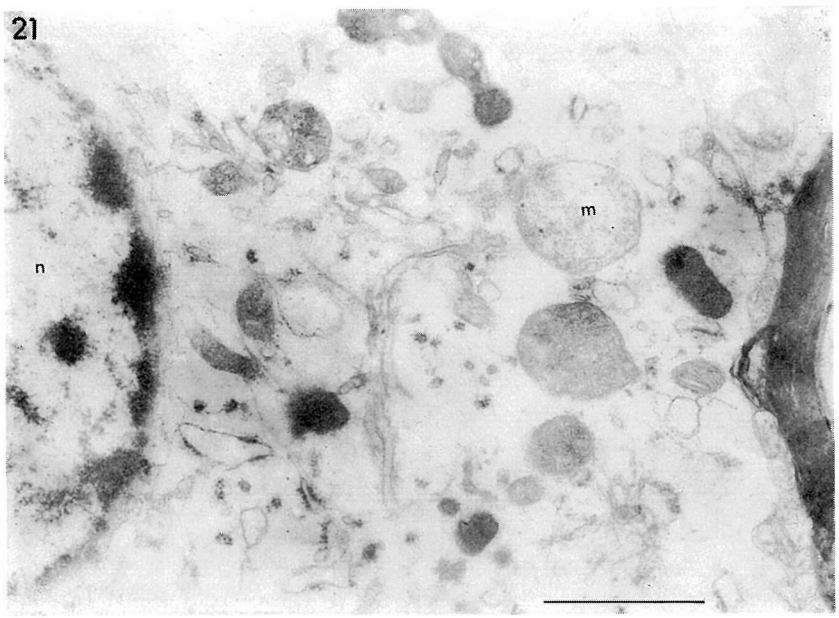

Fig. 21. Astrocyte in the white matter of swollen brain, 24 hours after evacuation of the balloon. Mitochondria tend to be vacuolized and organelles are somewhat swollen.

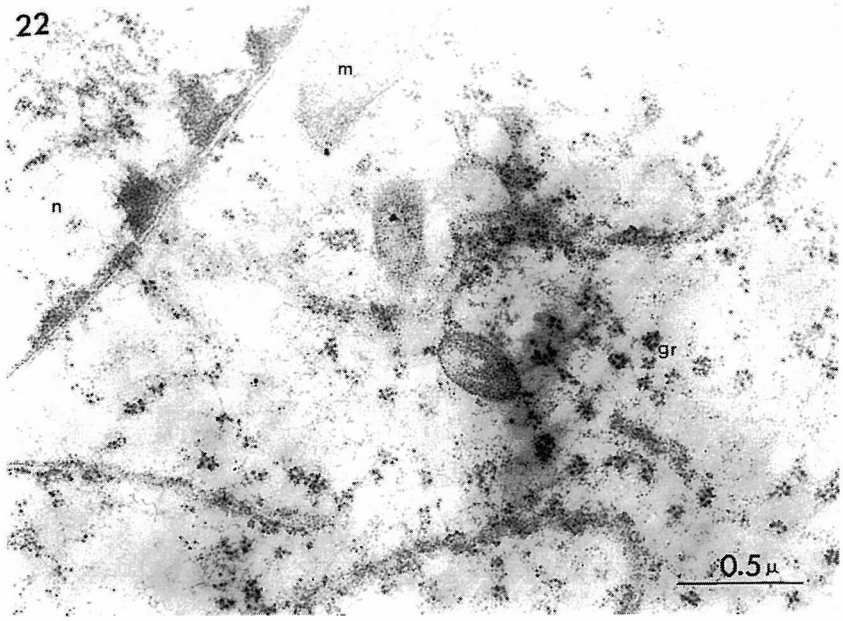

Fig. 22. Cortical astrocyte in swollen brain, 24 hours after evacuation of the balloon. Specific fine granules in the cytoplasm are increased markedly.

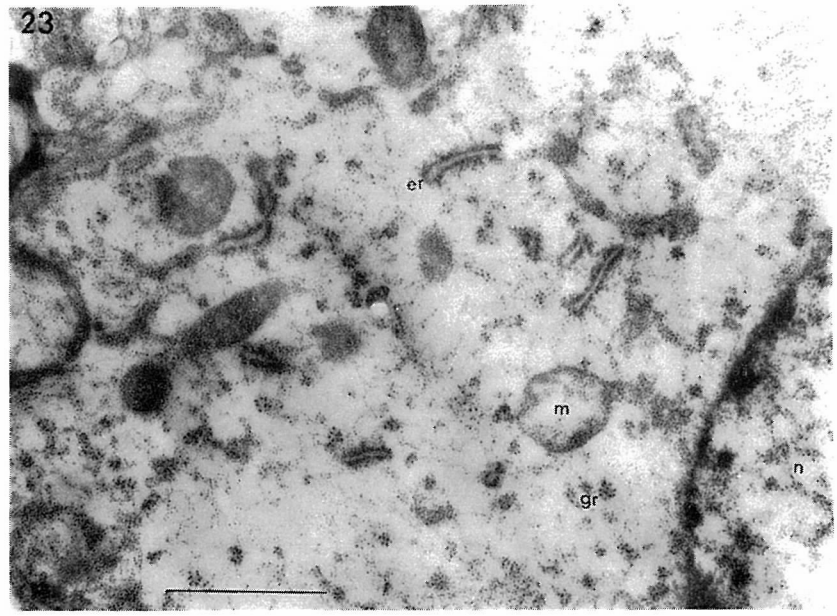

Fig. 23. Astrocyte in the white matter of swollen brain, 72 hours after evacuation of the balloon. Specific fine granules in the cytoplasm are increased. Mitochondria tend to be vacuolized and organelles are somewhat swollen.

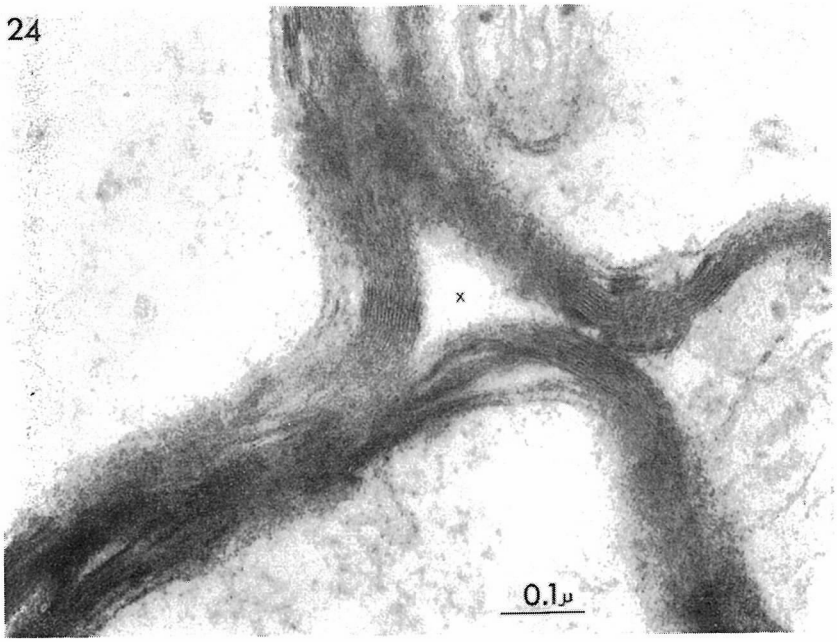

Fig. 24. The white matter in swollen brain, 24 hours after evacuation of the balloon. Myelin sheaths are separated. The triangular extracellular space formed by three ovoid-shaped myelinated fibers in somewhat wider than the normal one.

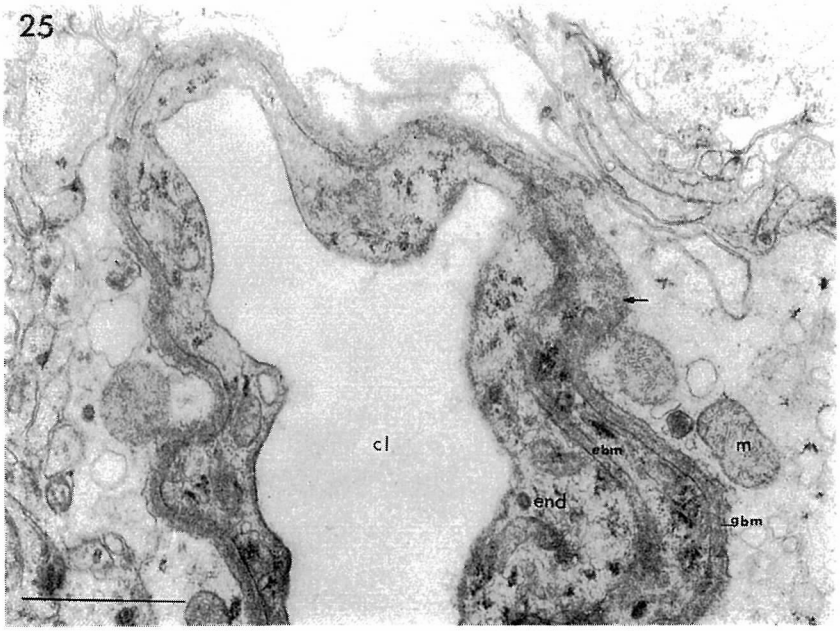

Fig. 25. Capillary in the white matter, 24 hours after evacuation of the balloon (Trasylol group). The pinocytic vesicles of the capillary endothelium are not so marked. The basement membrane of the capillary wall becomes loose. The separation of the glial basement membrane from that of the endothelial cell is present partially (arrow).

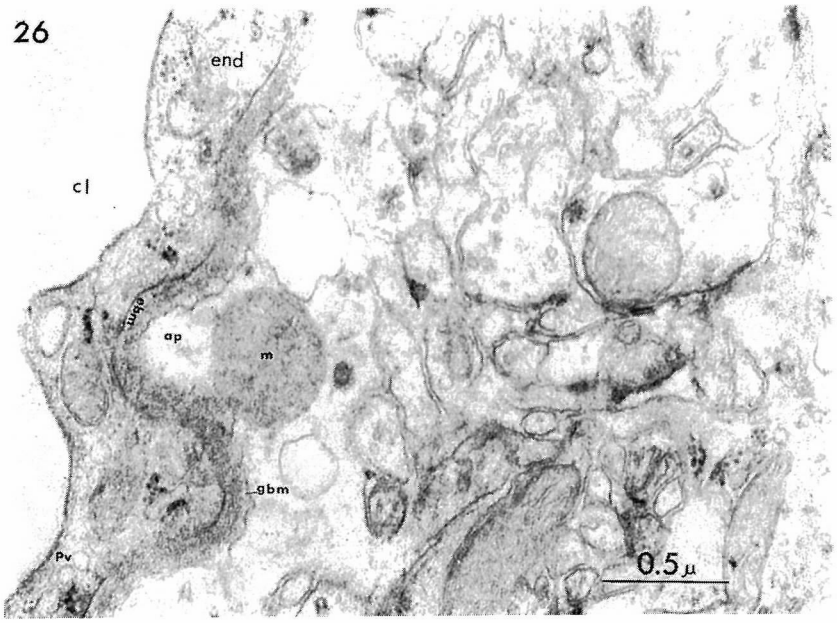

Fig. 26. Capillary in the white matter, 24 hours after evacuation of the balloon (Trasylol group). Astrocytic processes are not swollen.

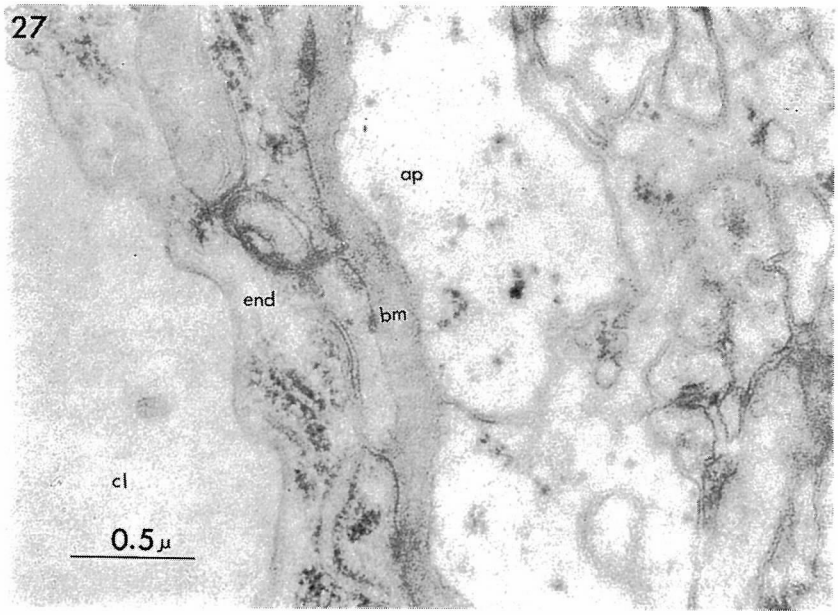

Fig. 27. Cortical capillary, 24 hours after evacuation of the balloon (Trasylol group). The pinocytic vesicles of the capillary endothelium are not so marked. Astrocytic processes are not swollen.

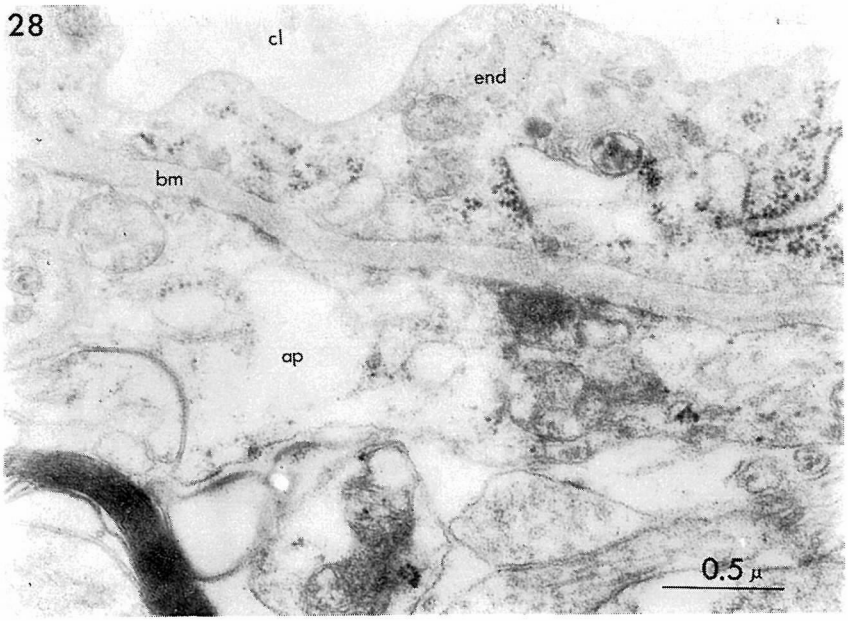

Fig. 28. Capillary in the white matter, 72 hours after evacuation of the balloon (Trasylol group). Astrocytic processes are not swollen.

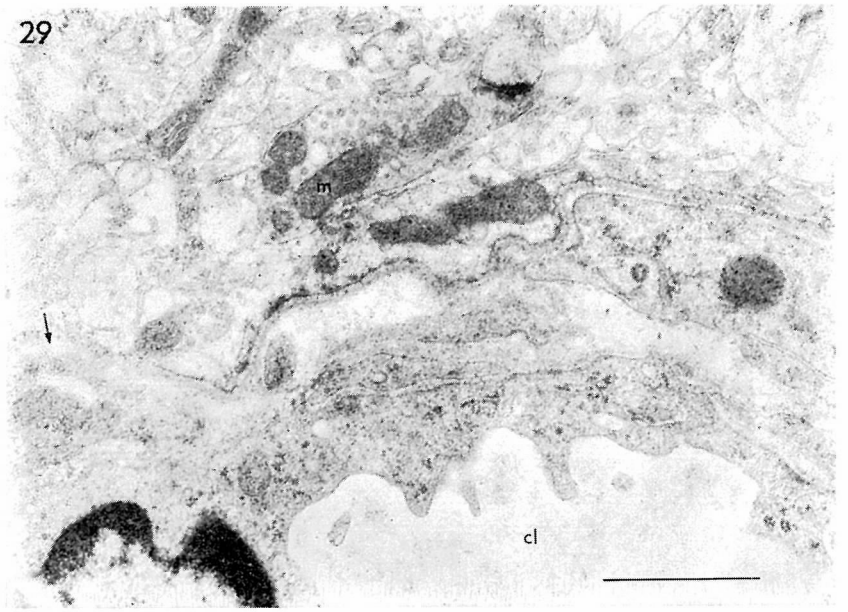

Fig. 29. Cortical capillary. 72 hours after evacuation of the balloon (Trasylol group). The basement membrane of the capillary wall becomes loose. The separation of the glial basement membrane from that of the endothelial cell is present (arrow). Astrocytic processes are not swollen.

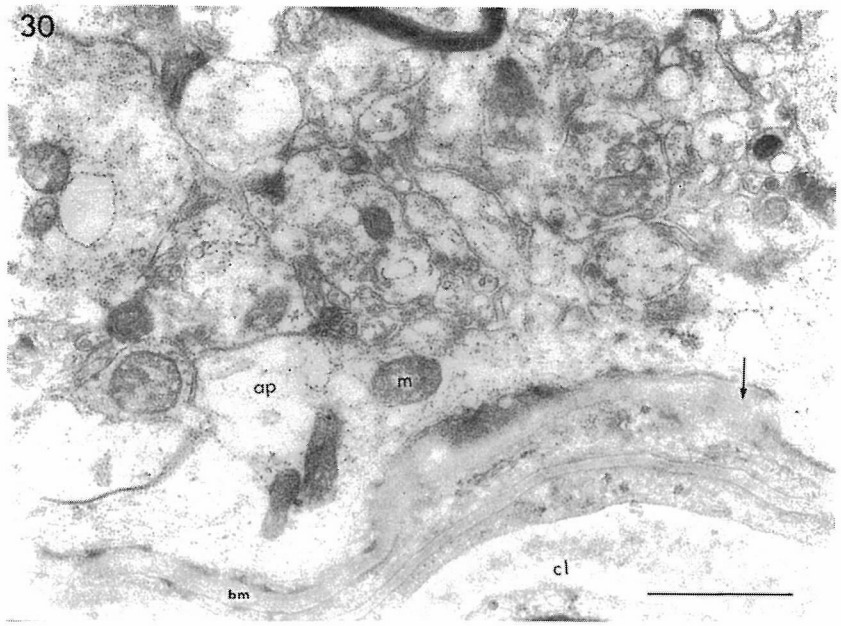

Fig. 30. Capillary in the white matter, 72 hours after evacuation of the balloon (Trasylol group). The separation of the glial basement membrane from the endothelial cell is present (arrow).

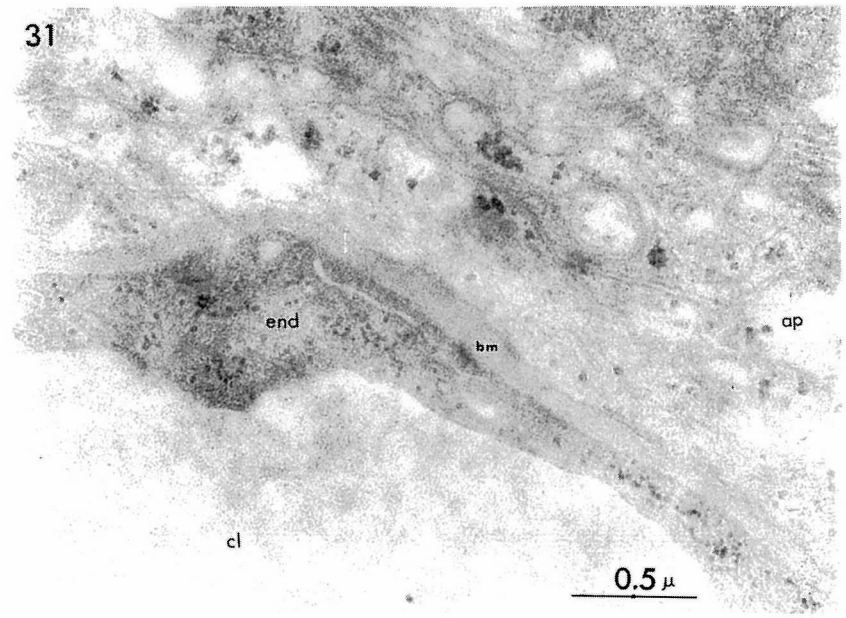

Fig. 31. Capillary in the white matter, 168 hours after evacuation of the balloon (Trasylol group). Astrocytic processes are not swollen. The pinocytic vesicles of the capillary endothelium are not so marked.

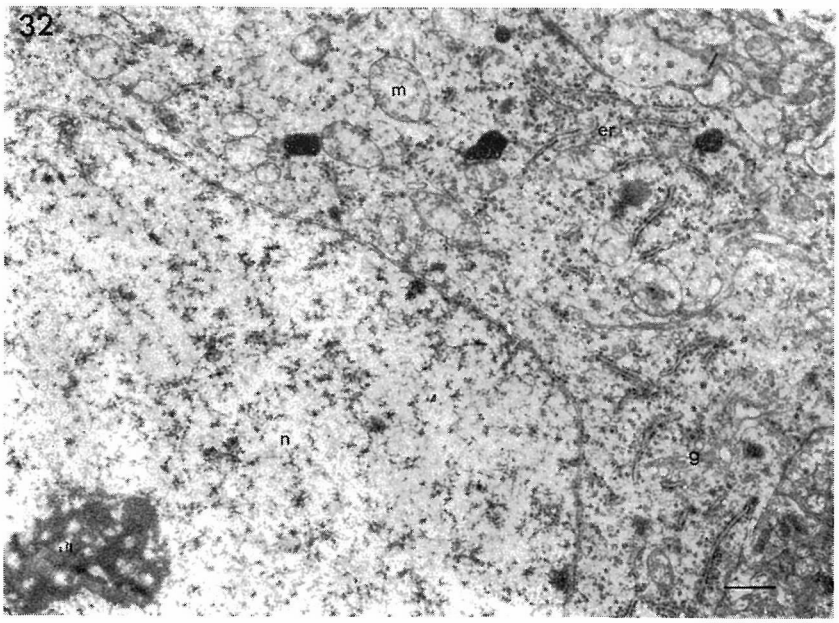

Fig. 32. Cortical nerve cell, 24 hours after evacuation of the balloon (Trasylol group). Mitochondria shows no morphological change. The nuclear membrane and the membranous structure are not distended.

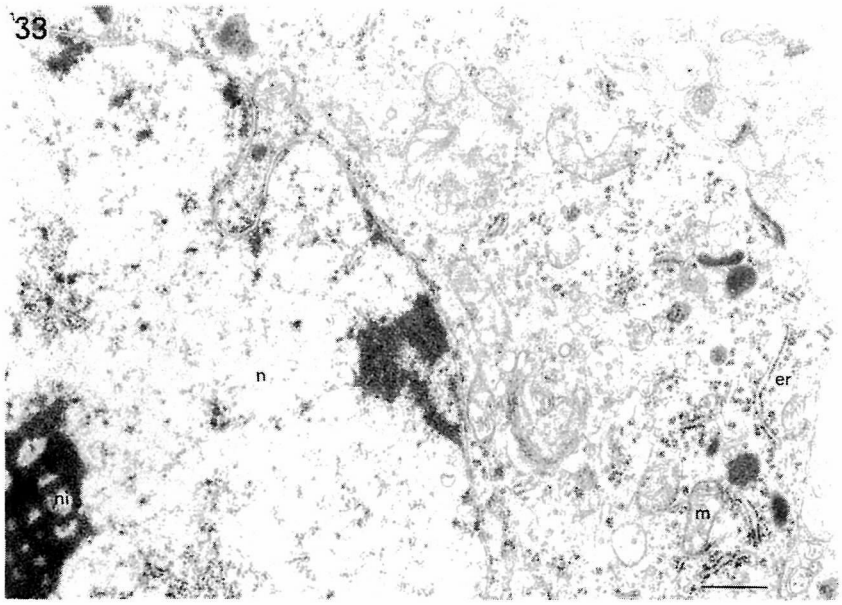

Fig. 33. Nerve cell in the white matter, 24 hours after evacuation of the balloon (Trasylol group). No particular change is seen as compared with normal one.

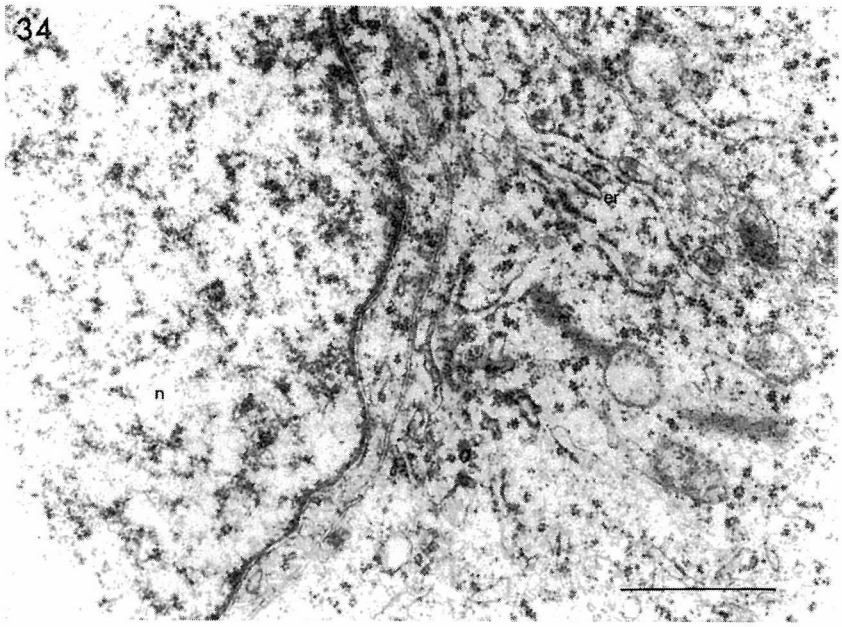

Fig. 34. Cortical nerve cell, 72 hours after evacuation of the balloon (Trasylol group). No particular change is seen as compared with normal one.

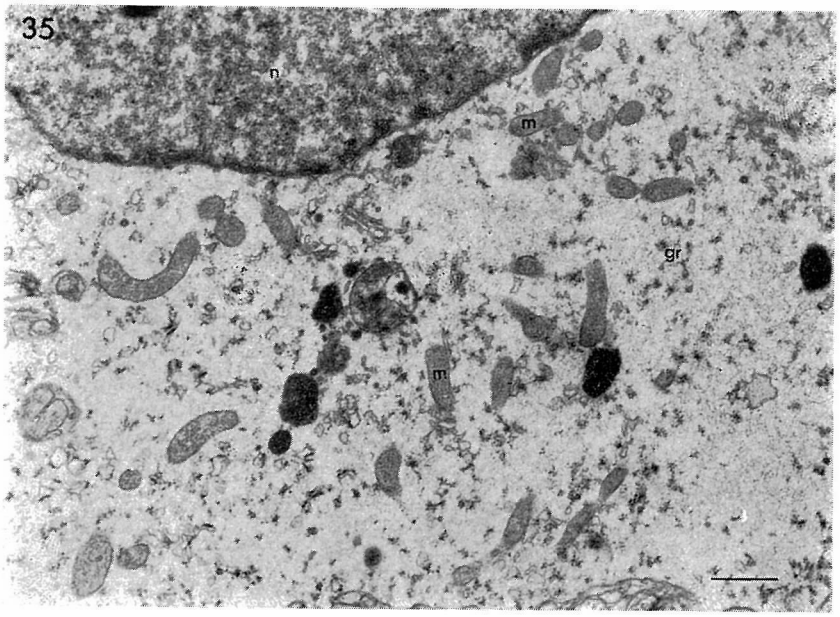

Fig. 35. Cortical astrocyte, 24 hours after evacuation of the balloon (Trasylol group). Specific fine granules are not reduced. Mitochondria tend to be increased, and are not vacuolized.

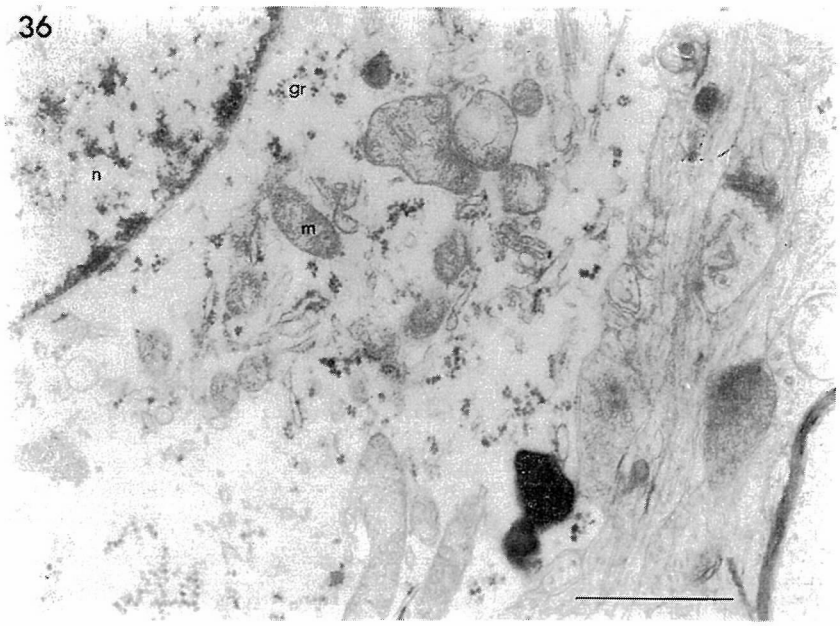

Fig. 36. Astrocyte in the white matter, 24 hours after evacuation of the balloon (Trasylol group). Almost same change is seen, as shown in Fig. 35.

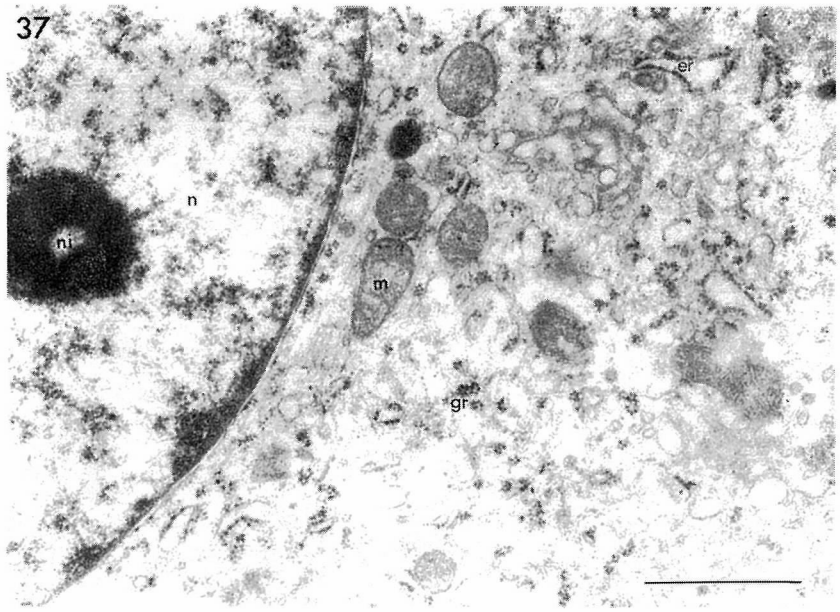

Fig. 37. Astrocyte in the white matter, 72 hours after evacuation of the balloon (Trasylol group). Almost same change is seen, as shown in Fig. 35.

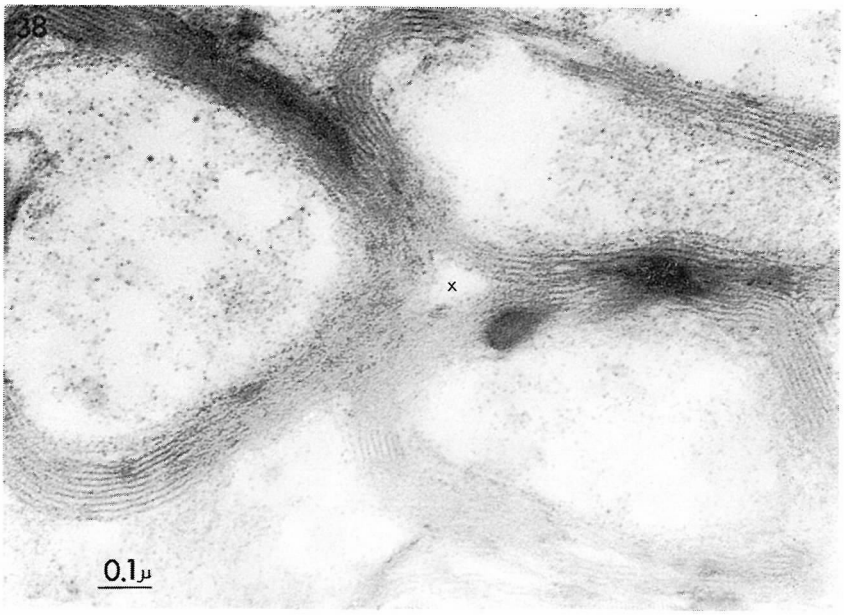

Fig. 38. The white matter in Trasylol group, 24 hours after evacuation of the balloon. The myelin sheath show no separation. The triangular extracellular space formed by three ovoid-shaped myelinated fibers is about 200 Å wide, similar to its normal width.