ELECTRON MICROSCOPIC OBSERVATIONS OF THE EFFECTS OF DEXAMETHASONE AND MANNITOL IN THE HYPOXIC-ISCHEMIC RAT BRAIN (PART - I)

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SUMMARY :

In a modified LEVINE preparation, the effects of mannitol, dexamethasone alone and in combination in hypoxic-ischemic brain insults were studied by electron microscopy in which dexamethasone appeared to be more effective than mannitol, or its combination with mannitol, in reducing ischemic swelling and protecting the neural integrity.

KEY WORDS :

Rat, Electron microscopy, Ischemic edema, Dexamethasone, Mannitol.

INTRODUCTION

Several light and electron microscopic studies of the central nervous system have been reported by others in animals subjected to anoxia alone, simultaneous anoxia and ischemia, and simultaneous interruption of both arterial and venous circulation.

LEVINE reported that combination of anoxia and ischemia was a useful method for producing unilateral cerebral damage in rats; we, therefore in this experiment, employed hypoxia-ischemia combination with the addition of postischemic recirculation by re-opening the ligation on the carotid artery (16,18,19).

Hypoxic (or anoxic)-ischemic lesions of the rat cortex are usually characterized by some degree of cerebral edema, of which fluid accumulates intracellularly in the glial cells (CYTOTOXIC, ISCHEMIC EDEMA) with subsequent neuronal cell alterations. The earliest alteration described is microvacuolation of the cytoplasm, which is then usually succeeded by ischemic cell change and finally cell loss (5,12,25).

The results of medical therapy in acute cerebral ischemia are generally disappointing, although beneficial effects of a few agents have been demonstrated. Various hyperosmolar solutions, such as mannitol, glycerol and low-molecular-weight dextran have been reported to retard the development of edema and maintain microcirculatory patency when administered at an early stage (13,21,22,32).

Enthusiasm for the use of stereoids in the treatment of acute cerebral ischemia has developed recently with reports of the substantial beneficial effects of high doses of dexamethasone in severe head injury (8,9).

The object of this investigation was to study by electron microscopy (EM) the effects of mannitol, dexamethasone alone and in combination, upon the hypoxic-ischemic alternations in the rat brain. The ultrastructural examination was restricted to the hippocampus and cerebellum, because of their known vulnerability to hypoxia and/or hypoxia-ischemia (1). The results of study of the cerebellum are reported in the second part of the study (6).

MATERIALS AND METHODS

This experiment was carried out in Istanbul DETAM on the adult Wistar type male rats weighing 200-300g and divided into 3 and 6 hour (A and B respectively) experimental groups on the basis of duration of unilateral common caritod artery ligation for hemispheric ischemia prior to hypoxia to induce the effect of ischemia and re-opening the ligation on the carotid artery for postischemic reperfusion. Each experimental group (A,B) was also further subdivided to CONTROL (A1, B1) and TREATED groups. Treated groups were subdivided as MANNITOL TREATED (A2, B2), DEXAMETHASONE TREATED (A3, B3) and MANNITOL-DEXAMETHASONE TREATED (A4, B4) groups.

GROUP-A: In this experimental group, the left common carotid artery was ligated in 16 rats for 3 hour at the end of which all rats were placed in a low pressure camera, at the Department of Physiology Medical Faculty of Cerrahpaşa, to expose them to hypoxia at 320 torr for 48 hours. During the hypoxia 4 rats died, therefore 12 rats were to be employed for the experiment; 3 rats serving in the CONTROL, that is, HYPOXIC-ISCHEMIC-REPERFUSED GROUP (A1) and the rest, 9 rats, in the MANNITOL (A2), DEXAMETHASONE (A3) and MANNITOLDEXA METHASONE(A4) TREATED groups, being 3 rats in each respective treated group.

The rats in the CONTROL group received no treatment, following hypoxia, they were exposed to postischemic reperfusion for 60 minutes, by re-opening the ligation on the carotid artery, at the end of which all animals were perfused for fixation with 2 % paraformaldehyde and 2.5 % gluteraldehyde mixture solution in a 0.1M Na-phosphate buffer (buffer pH:7.35) given by the cardiac route in 20-30 minutes, prior to decapitation. Subsequent to perfusion fixation, rats were decapitated and their brains were removed as quickly as possible. Gross inspection was made on the cerebral hemispheres, to see the effect of hypoxia-ischemia, and on the cerebellum to see the effect of hypoxia. Both were swollen with no obvious evidence of herniation. Samples were taken from the left cortex of the hippocampus and immediately with 1 % osmium tetroxide. After the necessary follow up, samples were embedded in Vestopal-W and then cut into ultra thin sections which were stained with Uranyl acetate and Lead citrate (Reynol's) techniques. All samples were studied under JEOL-100C transmission electron microscopy.

The rats in the TREATED GROUP were subdivided to MANNITOL (A2), DEXAMETHASONE (A3) and MANNITOL-DEXAMETHASONE (A4) groups as mentioned earlier, with 3 rats in each group. Following the hypoxia procedure, the rats in A2 group were exposed to postischemic reperfusion for 60 minutes by re-opening the ligation on the carotid artery. In this group rats received mannitol % 20 2g/kg as an IV bolus through the penis vein immediately after the re-opening the ligation. At the end of reperfusion, that is, after 60 minutes, rats were perfused by the cardiac route with a fixative as described before. The rats in A3 group were treated with dexamethasone 0.14mg/kg as an IV bolus through the penis vein to with, and then it was continued 0.07mg/kg intraperitoneally q.i.d for 48 hours, at the end of which rats were exposed to postischemic reperfusion by reopening the ligation for 60 minutes, followed by cardiac perfusion fixation. The rats in A4 group were treated with dexamethasone as in group A3, but at the end they received mannitol as in group A2 immediately following the re-opening the ligation. Sixty minutes after the re-opening the ligation they were perfused intracardiacally for fixation as mentioned before. Decapitation and EM study procedures were exactly the same as in group Al.

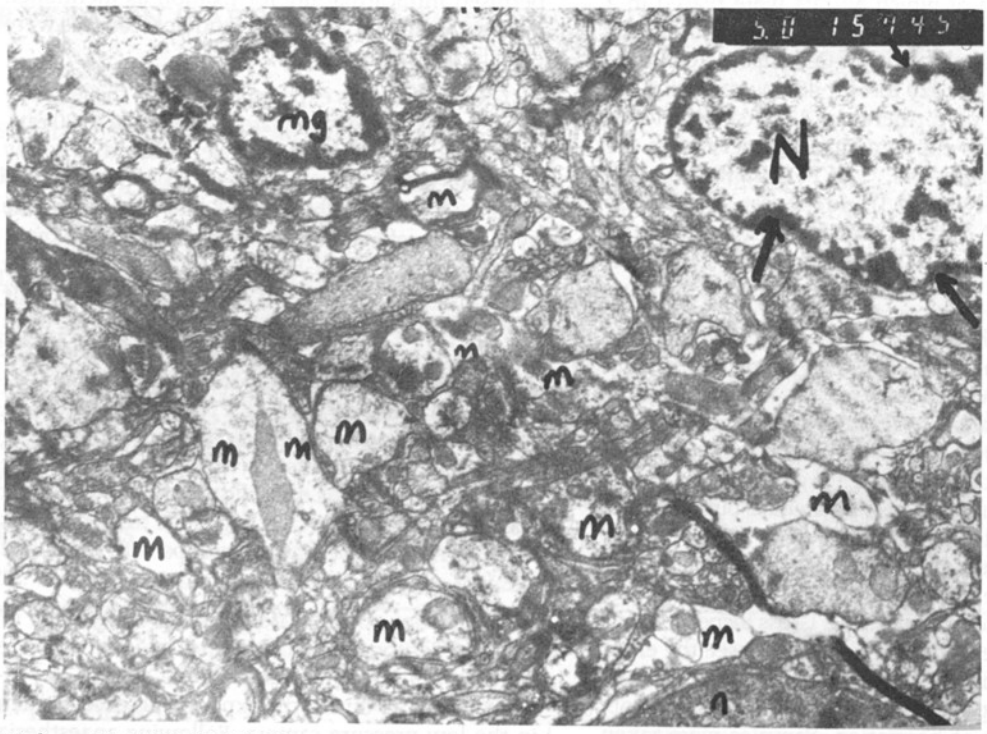
GROUP-B: In this experimental group the left common carotid artery was ligated for 6 hours in 20 rats, at the end of which all rats were placed in a low pressure camera to expose them to hypoxia at 320 torr for 48 hours. In this group 8 rats died during the hypoxia, therefore again 12 rats were to be employed, 3 in the CONTROL GROUP (B1), 3 in each of the TREATED GROUPS (B2, B3 and B4). The rest of the procedure applied in this group was the same as in GROUP (A) for both CONTROL (B1) and MANNITOL (B2), DEXAMETHASONE (B3) and MANNITOL-DEXAMETHASONE (B4) TREATED groups. Gross inspection of the cerebral tissue again revealed marked swelling on the left side, when compared to the other parts, but it was difficult to make any comment about the lessening of swelling in the treated groups of either A or B experimental groups with the naked eye.

All ligations of the carotid artery, re-opening, IV and intraperitoneal treatment and decapitation were carried out under ether anaesthesia and all rats inspired room air during the experiment, except when in the low pressure camera. Following anaesthesia and ligation of the carotid artery, the neurological abnormalities observed in all rats were permanent left sided Horner-syndrome and right sided weakness for a short duration, which persisted longer in the rats which survived hypoxia, being about 60-120 minutes. Following hypoxia, the rats were also lethargic for some time and became more sensitive to ether anaesthesia, especially in the longer treated groups, which was put down to the developing ischemic oedema.

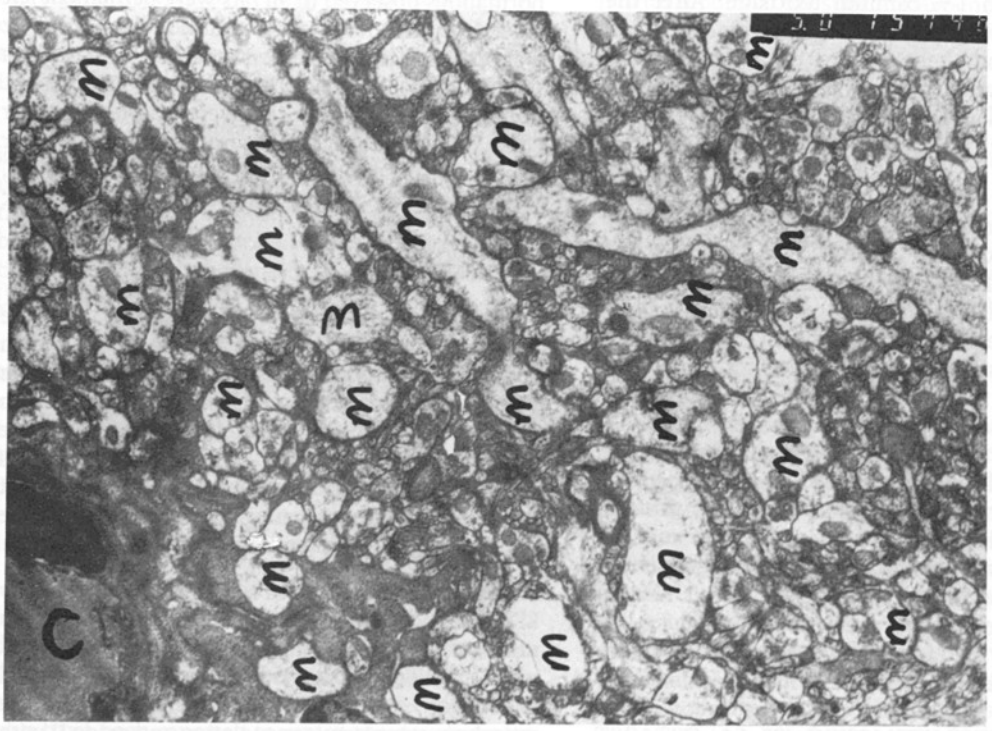
DISCUSSION

The object of this experiment was to study the effects of mannitol, dexamethasone alone and in combination upon the neuronal alterations in hypoxic-ischemia. In cerebral ischemia, it has been experimentally shown that neuronal alterations are usually diphasic (5,19,20). The primary phase is thought to be reversible with tissue preservation but the secondary phase is characterised by tissue necrosis and is therefore irreversible (20).

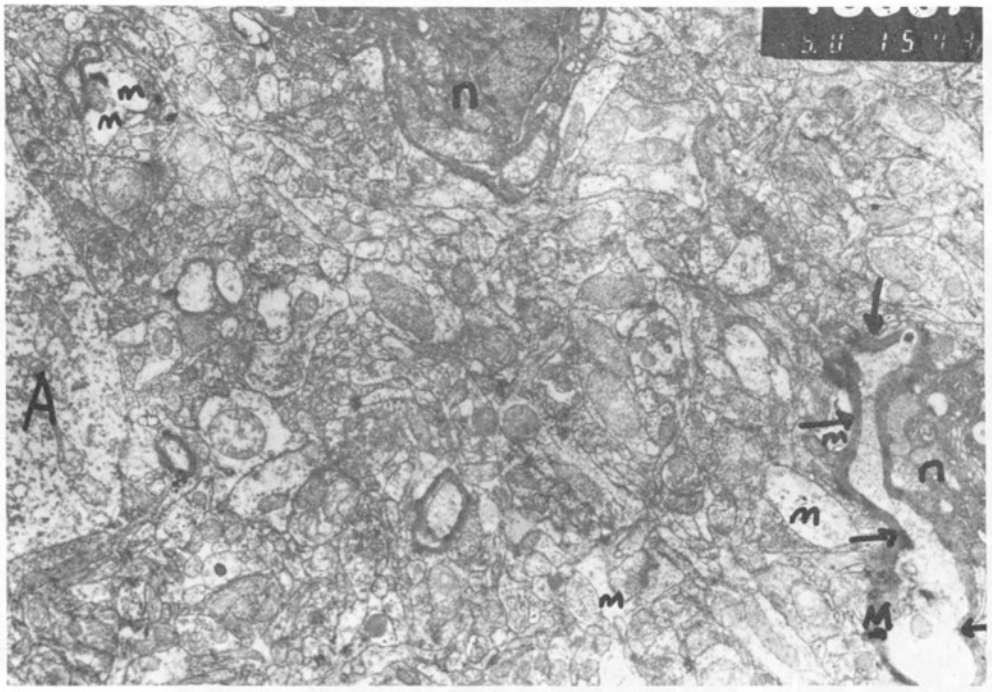
Neuronal alterations in ischemia were graded by LITTLE and et al, with their light and electron microscopic observations, in which, grade-I presented with swollen mitochondria, dispersion of nissl substance, distension of rough endoplasmic reticulum, cytoplasmic vacuolation and grade-II with moderate shrinkage, increased or decreased density of cytoplasm; grade-III with severe shrinkage,



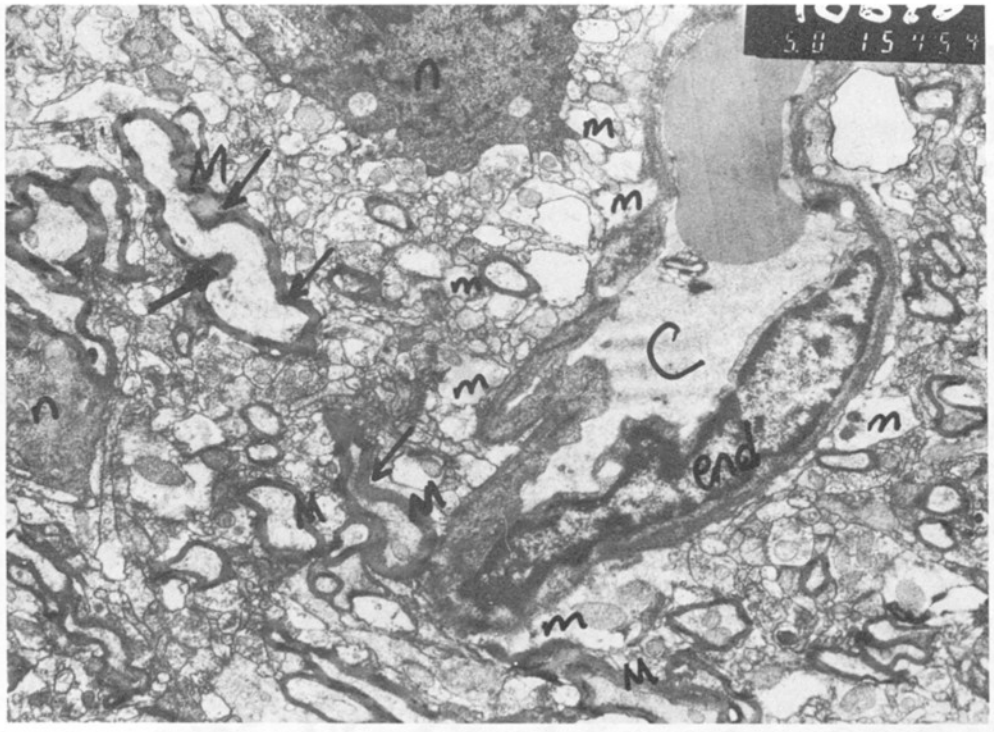
EM figure-Al/1 (x8500) (CONTROL) :



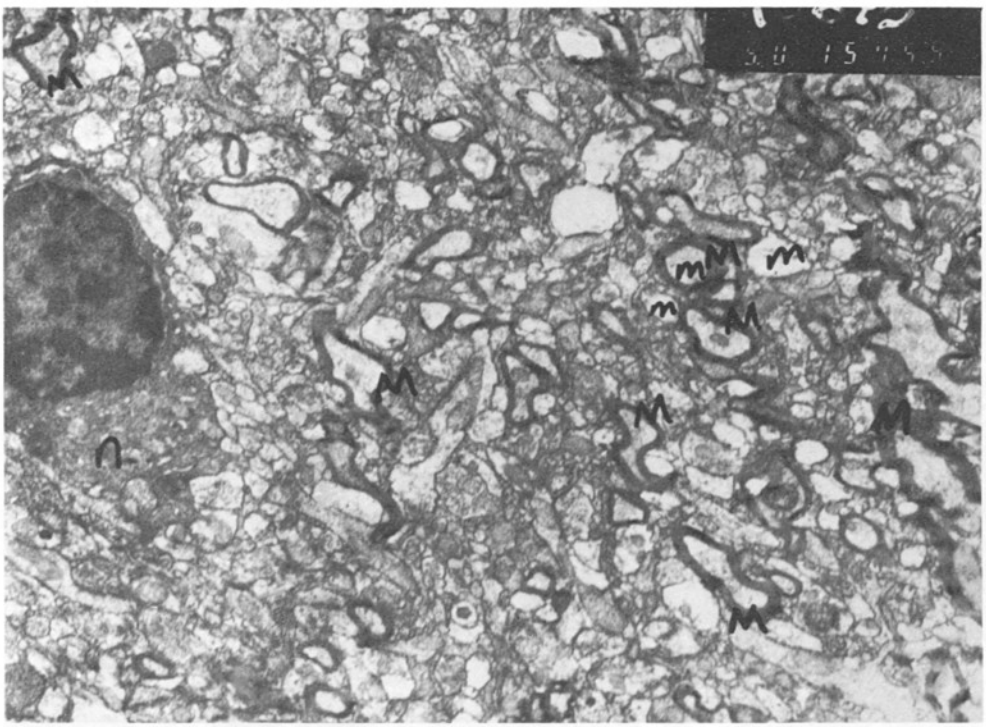
EM figure-Al/2 (x8500) (CONTROL): Widespread microvacuolation (m), disconfiguration of the astrocyte nucleus (N. arrow) are seen. (C: capillary, mg: microglia, n: neurone).



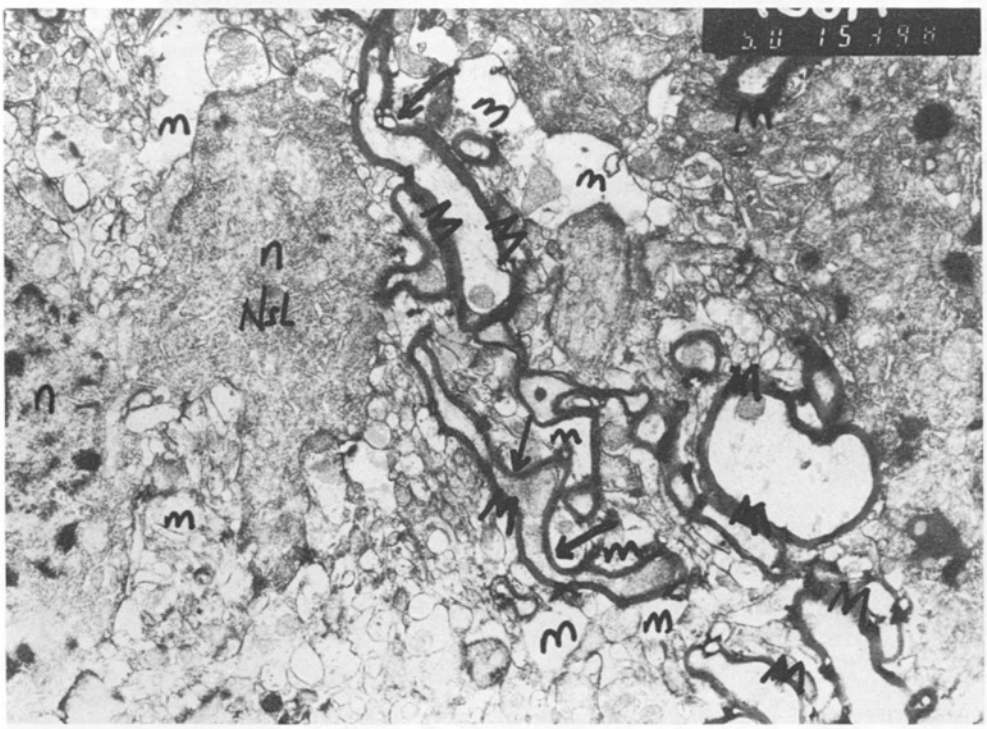
EM figure-A2/1 (x8500) (MANNITOL TREATED:) Although disconfiguration (arrows) of the myelin tissue (M) is pre- sent, less microvacuolation (m) and more or less normal neronal structure are seen. (A:astrocyte, n:neurone).



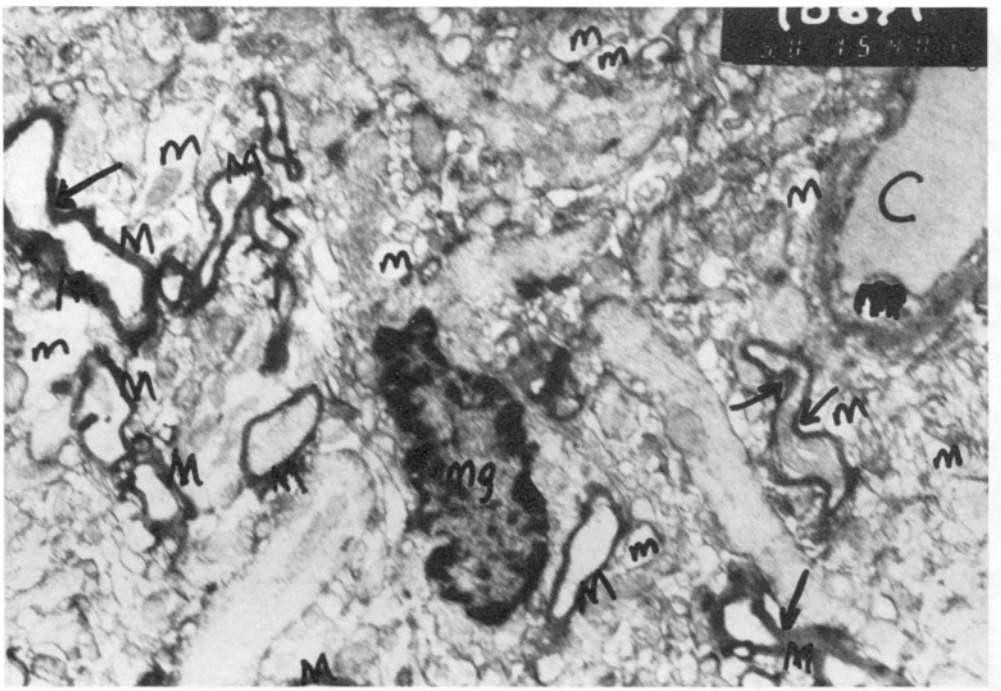
EM figure-A3/1 (8500) (DEXAMETHASONE TREATED):



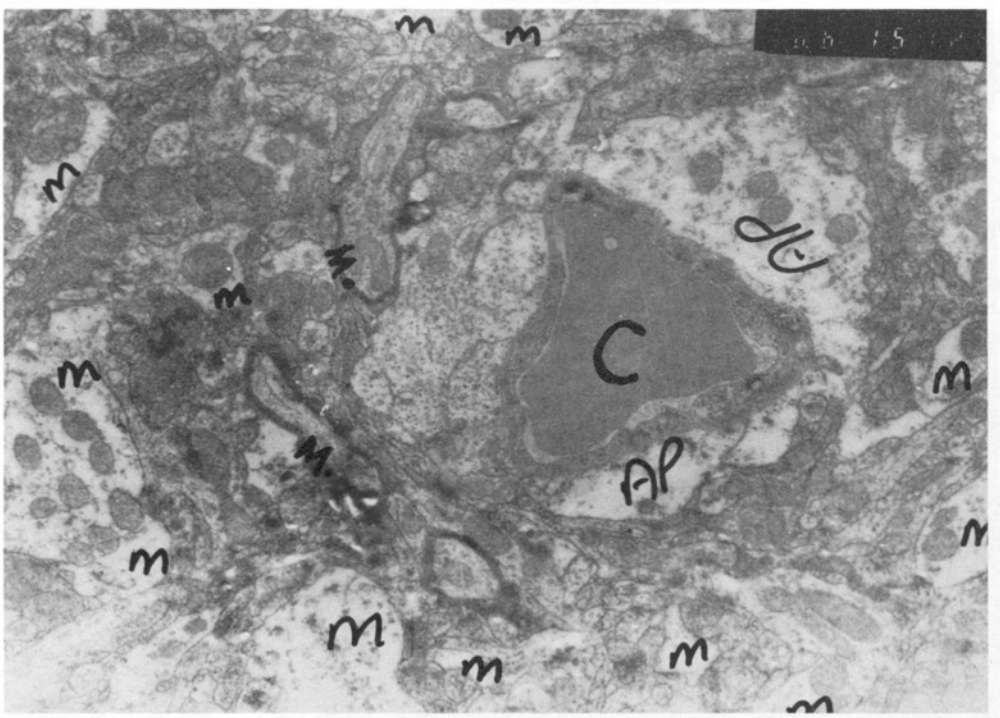
EM figure-A3/2 (x8500) (DEXAMETHASONE TREATED): It appears that myelin tissue (M), capilary (C) and neurones (n) are better protected than in the MANNITOL TREATED group. (arrows:distortion, end:endothel, m:microvacuolation).



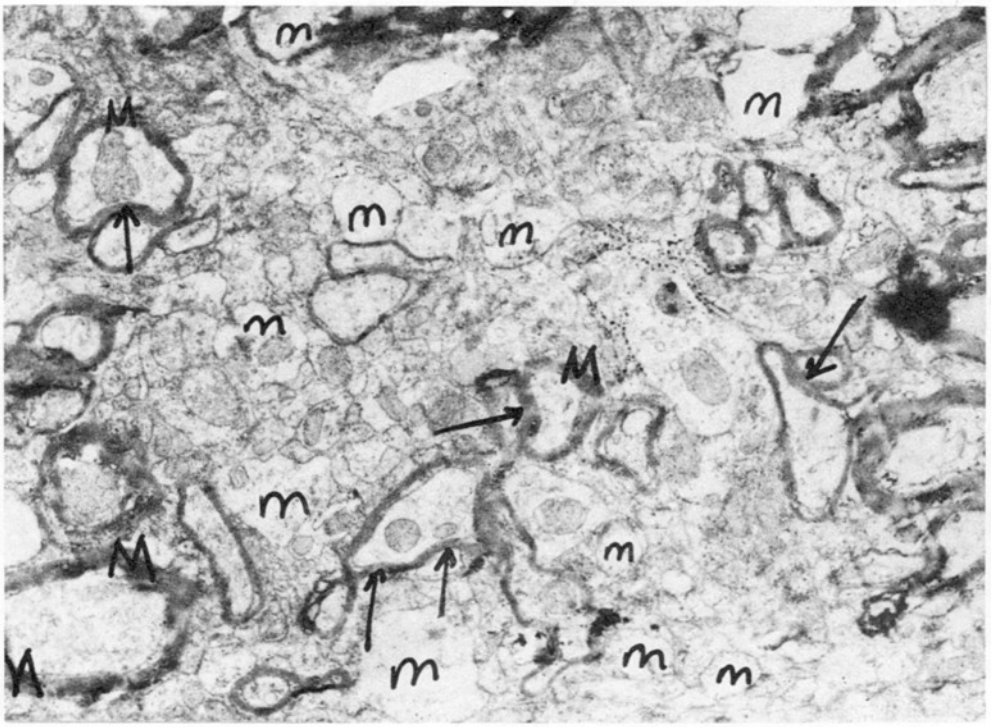
EM figure-A4/1 (x8500) (MANNITOL-DEXAMETHASONE TREATED):



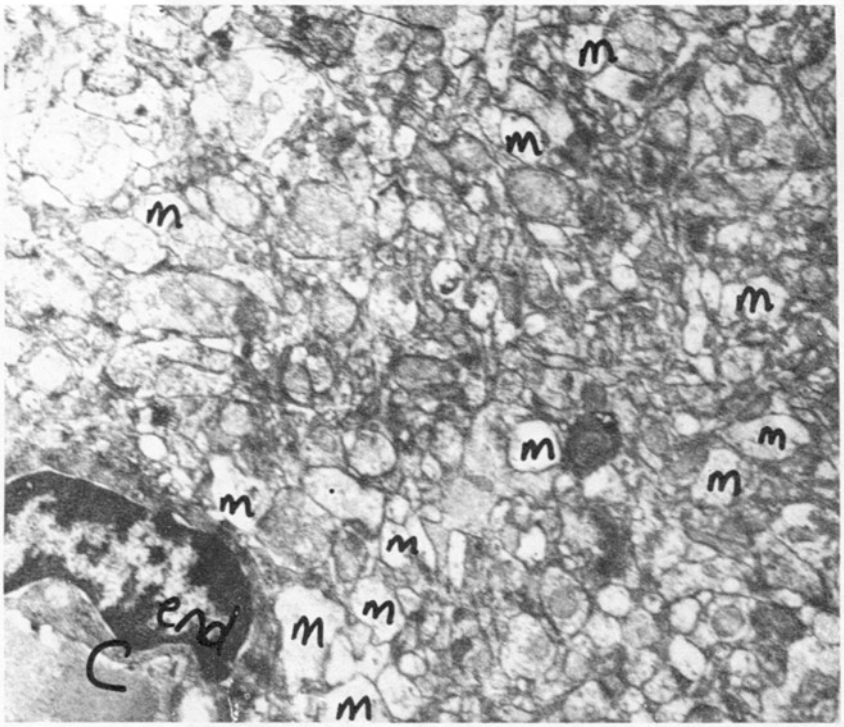
EM figure-A4/2 (x8500) (MANNITOL-DEXAMETHASONE TREATED): Disconfigurations (arrows) of the myelin tissue (M) around axons and microvacuolations (m) appear to be more numerous than in the DEXAMETHASONE TREATED group. (mg:microglia. C:capillary. n:neurones. Nsl:nissl substance).



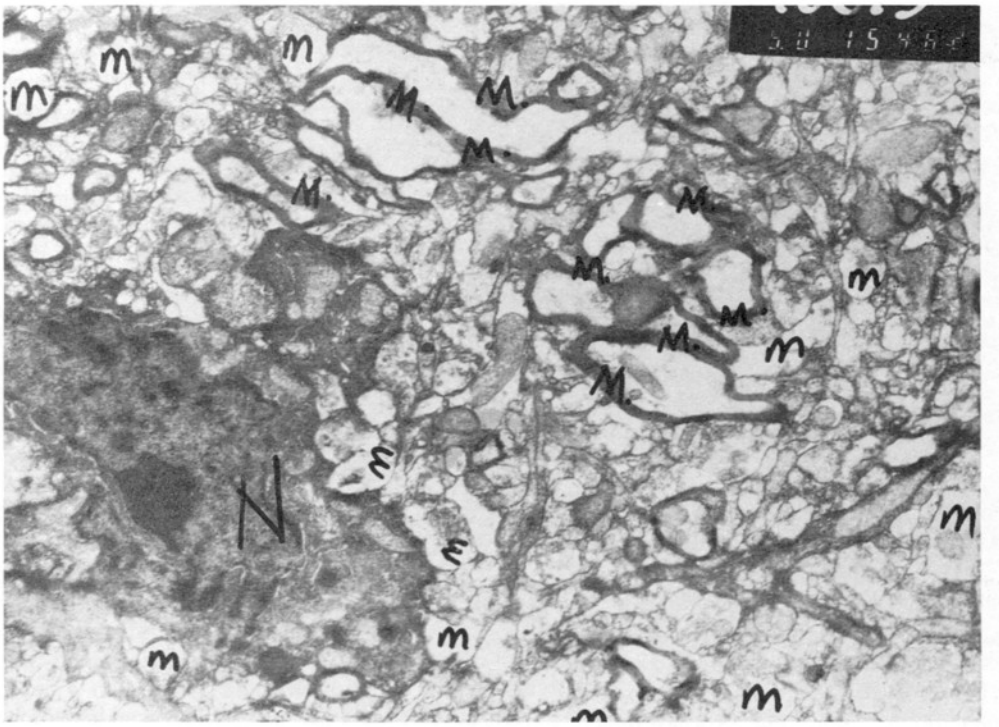
EM figure-B1/1 (x8500) (CONTROL): Widespread perivascular edema in the astrocytic processes (AP) and microvaculations (m) are seen.



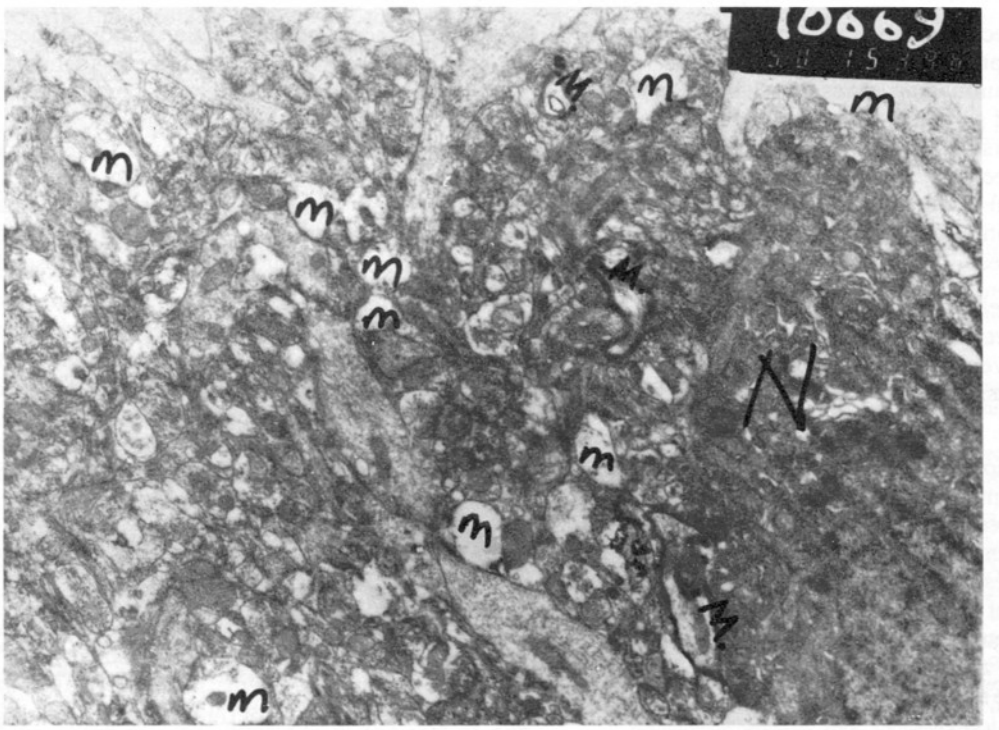
EM figure- B2/1 (x8500) MANNITOL TREATED): Microvaculations (m) and disconfigurations (arrows) of the myelin tissue (M) are still obvious.



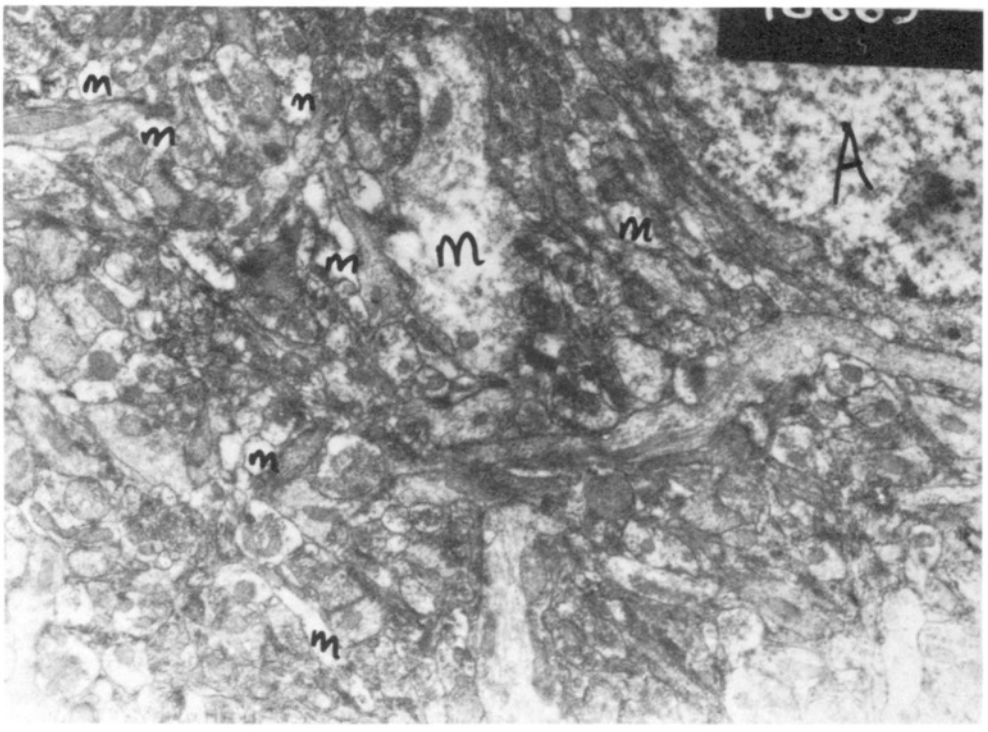
EM figure-B3/1 (x8500) (DEXAMETHASONE TREATED):



EM figure-B3/2 (x8500) DEXAMETHASONE TREATED It is again obvious than myelin tissue (M), capillary (C) and neuronal (N) structures appear to have been protected better than in the MANNITOL TREATED group. (m:microvaculations, end:endothel).



EM figure-B4/1 (x8500) (MANNITOL-DEXAMETHASONE TREATED):



EM figure-B4/1 (x8500) (MANNITOL-DEXAMETHASONE TREATED): Structures appear to be rather protected from the effect of ischemic edema and the edema reducing effect of the combined treatment is not so obvious as in the DEXAMETHASONE TREATED group. (N:neurone, m:microvacuolations, A:astrocyte, M:mylein tissue).

cytoplasmic eosinophilia, pyknotic nucleus, membrane disruption, cellular fragmentation (19). Therefore, neuronal responses to ischemia could be in the from of shrinkage and swelling which represent the reversible phase or in the ischemic phase (cytoplasmic eosinophilia, nuclear pyknosis, cellular fragmentation). In our experiment, Em findings were usually compatible with neronal changes in grade-I/II. (see EM figures).

Ideally, treatment of acute cerebral ischemia should be directed toward protecting cerebral tissue during the reversible phase by preventing or retarding the transition into the irreversible ischemic phase (13,32).

The development of brain edema following cerebral ischemia is a matter of great concern, and the effectiveness of mannitol is still unresolved in both experimental animals and humans. Part of the difficulty arises from differences in the time of administration of the drug in relation to onset (pre-or post-insult treatment), duration of the ischemic insult, differences in the dose of mannitol used and differences in the mode of administration (bolus or continuous) infusion (17).

MARSHALL and et al. demonstrated in brain injured patients and LITTLE experimentally in cats that if chronic mannitol therapy is necessary, smaller doses permit more frequent administration without any obvious side effects such as systemic dehydration, electrolyte balance disturbances, and have the same effect as larger doses (23,24). On the other hand, it was also experimentally shown by LITTLE that a single standard (1-2g/kg) dose of mannitol was effective in retarding development of ischemic edema and thereby preventing capillary compression (21,22). In this experiment, we used a single dose of mannitol (2g/kg as an IV bolus).

Focal cerebral ischemia increases brain water content and tissue pressure locally; therefore the gradient of hydrostatic pressure develops between ischemic and normal brain, which may be important in subsequent events in and around the ischemic focus (i.e:increased tissue pressure further reduces CBF in the ischemic zone by capillary compression) (14).

As mentioned earlier, LITTLE and also WATANABE et al. have shown by their ultrastructural studies that mannitol has beneficial effects on cerebral ischemia (21,33), this was supported by PENA (30) who related its effect on cerebral blood flow (CBF) enhancement, improvement in microcirculatory dynamics or amelioration of cerebral edema. In this study, as we have not studied CBF or microcirculation, no comment can be made. HANAMURA and et al. showed that mannitol withdraws water from the normal and ischemic brain tissue, the effect be-

ing more marked within the moderately ischemic area (penumbra) than the severely ischemic area (core)(10). They proposed that for the mannitol to be effective in reducing ischemic edema water from the penumbra, functioning brain capillary would be essential. As BBB is still partially preserved in the penumbra, mannitol could open and free edema water could more easily be extracted together with sodium, but as within the ischemic core where most edema water is present tissue pressure increased and BBB is severely damaged, mannitol would not be expected to be effective in extracting edema water(10). It is therefore particularly important, if the brain is to be protected by mannitol effectively, it should be administed as early as possible before BBB damage ,which occurs in the secondary phase of ischemia. These important remarks are also supported by LITTLE and MEYER et al. (21,22,26).

In our experiment, EM findings on the rats exposed to hypoxic-ischemia were compatible with the neronal changes of the primary phase of ischemia as mentioned previously, and EM observations proved that mannitol appeared to have affected these primary neuronal changes in both A and B experimental treated groups (see the related EM figures).

On the other hand, we also treated the rats with dexamethasone the effect of which is also unresolved, both experimentally and clinically, in the hypoxic-ischemic brain insults. Various mechanisms (including membrane stabilization, inhibition of lysosomal enzyme release and free radical scavengering) have been proposed for steroid action(3). In ischemic cerebral edema, the peak water increment precedes the significant alteration of BBB permeability. Therefore, membrane stabilization of the BBB by dexamethasone is probably not relevant in ischemic edema (3). FAUPEL et al. and GOBIET reported substantial beneficial effects of high doses of dexamethasone in severe head injury in 1976(8,9), but the latest study carried out by BRAAKMAN et al. on 161 head injury cases showed that steroids were ineffective(4). In PATTEN et al's report on 31 patients with acute stroke (excluding aneurysm or AVM rupture) in 1972, it was concluded that dexamethasone could have a beneficial effect on patients with severe stroke due to its ability to decrease brain edema secondary to brain infarction(29). NORRIS and NORRIS et al. on 53 and 113 patients with acute stroke treated by dexamethasone (or placebo) in 1976 and 1986 respectively reported that they found steroids were ineffective in acute stroke(27,28). As a result of clinical studies, experimental studies were also conflicting. In PLUM and POSNER's experimental study carried out on rats in 1963, there was no difference in the rats of non-treated, pre and/or post-insult treated with dexamethasone from the ischemic infarction and reduction of cerebral swelling points of view(31). In an experiment carried out on gerbils by HARRISON and RUSSEL, dexamethasone was found to be effective in reducing mortality due to experimental infarction from % 65 to % 19(11). In experiments carried out on monkeys, gerbils and neonatal rats by DONLEY et al., ITO et al., ALTMAN et al., respectively neither low nor high doses of dexamethasone were found to be effective, in ameliorating ischemic brain edema or on histological appearances(2,7,15).

In both our experimental groups, as seen in the related EM figures, dexamethasone appeared to be much more effective than mannitol in reducing ischemic swelling and thereby protecting neural integrity.

We also compared the effects of mannitol-dexamethasone combination and on the basis of EM observations the combined treatment did not seem to be as effective as dexamethasone alone in reducing ischemic edema and protecting neural integrity.

In conclusion, our EM observations showed that in hypoxic-ischemic brain insults treatment with dexamethasone alone was more effective than mannitol or its combination with mannitol in reducing ischemic edema and protecting neural integrity. Our conclusion was also supported by another study, part-II(6).

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