The Response of Experimental Cerebral Edema to Glucosteroid Administration*

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The use of a variety of glucosteroids, notably dexamethasone, in the treatment of cerebral edema has been well substantiated on clinical grounds. Galicich and French\textsuperscript{10,11} presented the first account of the specific use of steroids for the treatment of cerebral edema. Rasmussen and Gulati\textsuperscript{24} substantiated these clinical impressions by detailing their experience with cortisone in the treatment of postoperative cerebral edema. Its use appeared to decrease the incidence of all of the postoperative complications which previously had been attributed to cerebral edema.

Little research has been done regarding the actual effects of steroid administration on either the functional or structural abnormalities in edema.\textsuperscript{3} Lippert \textit{et al.}\textsuperscript{16} reported that cortisone administration was followed by an improvement in the clinical status of dogs in which cerebral edema had been induced by the intracerebral implantation of psyllium seeds. Blinderman and his associates\textsuperscript{1} produced edema by the injection of vegetable oil into the carotid artery of dogs. Again a protective effect of cortisone was noted in these animals. Plum \textit{et al.}\textsuperscript{22} attempted to produce cerebral edema by experimental cerebral infarction. Dexamethasone was found neither to prevent the ischemic infarction nor to reduce the amount of cerebral swelling present in these anoxic-ischemic preparations. Corticoids did not improve the clinical course of these animals.

There has been no histological investigation in any of these studies. There still has been no definite proof that the beneficial effects of the steroids upon the signs and symptoms of cerebral edema have, in fact, been due to actual reduction in the amount of structural abnormality.\textsuperscript{23} Our study was undertaken in order to investigate the problem more thoroughly.

**Nature of Cerebral Edema**

The pathological changes we have used to define edema in this study are the same as those reported by a number of other observers of both human and laboratory animal material.\textsuperscript{27,29}

Grossly, there is an increase in brain volume, the gyri are flattened and there is narrowing of sulci. The subcortical white matter is hardly affected, but the deep white matter is markedly increased in volume; there is loss of clear demarcation of white and gray. Often there is a shift of midline structures.

The characteristics observed by light microscopy are distention of perivascular and pericellular spaces, venous congestion, degeneration of vascular endothelium, and pallor and swelling of axons and myelin. Sub-pial rarefaction is common as are anoxic nerve cell changes. Glial swelling is common, but the largest change in volume is caused by distension of the extracellular space of the white matter with a protein-rich fluid.

Electron microscopy defines the process further. In the cortex there is enlargement of astrocytes, particularly in the pericapillary area. Changes in oligodendroglia and neurons are less evident and basically consist of abnormalities of intracytoplasmic elements. The extracellular space at the cortical level is not enlarged, but there is an often remarkable increase in this space at the level of the deep white matter. Axonal and myelin changes occur, but fixation difficulties in non-perfused material make them difficult to interpret.

Originally ultrastructural studies indicated that edema was entirely intracellular.\textsuperscript{20,21} However, it has now been demonstrated that these studies which dealt for the

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most part with cortex and with alkyl-tin intoxication were incomplete, and that extracellular fluid does occur within the white matter in true edema. Thus, basically, cerebral edema is an extracellular phenomenon of the white matter accompanied by marked swelling of the astrocytes. None of the newer techniques has modified this interpretation.

Summary of Methods

In order to make this report clearer, we will give a brief summary first and follow it by more detailed description of certain parts.

The animals used in this study were normal adult male and female albino rabbits, weighing 2–4 kg., and normal adult male and female mongrel dogs, weighing 12–20 kg. Three normal rabbits and 2 normal dogs were used as controls. In addition, 2 rabbits and 2 dogs were pretreated with .06 mg./kg. dexamethasone intramuscularly every 6 hours for 48 hours. Cerebral edema was produced in 12 rabbits by the extradural or subdural placement of psyllium seed. These animals were treated with dexamethasone according to schedules which will be detailed later. Four dogs treated with steroids were also used in the study. Two of the dogs had edema produced by the intracerebral implantation of psyllium seed and 2 were treated after subdural balloon inflation.

Summary of Results

The efficacy of these methods of producing cerebral edema has been previously established. All of the techniques employed give reproducible degrees of brain swelling. Moreover, in each method the ultrastructure of the resulting edema has been described so that material is available for comparison with the treated samples. The cerebral edema which follows these techniques appears in about 24 hours and is maximal at the end of 48 hours.

Untreated cerebral edema. Since the survey of large numbers of animals by electron microscopy is not feasible, it was important to determine whether dexamethasone would have a clinically discernible effect upon the animals in which edema had been produced. The clinical course of the untreated animals was quite striking. Twenty-four hours after the intracranial insertion of psyllium seed, the rabbits were irritable, lethargic and eating poorly. Animals that had undergone sham operations were normal at this point. The dogs which had undergone intracerebral seed implantation or subdural balloon inflation were usually hemiparetic or hemiplegic and semistuporous. At the end of 48 hours, the animals were all comatose. They lay in the cage breathing deeply, unresponsive to most forms of stimulation except pain. Many of these animals died 72 to 96 hours after surgery. Adequately supported survivors gradually began to recover after about 96 hours and eventually returned to what appeared to be a normal state in the case of the rabbits, although the dogs usually showed some definite neurological abnormality.

Treated cerebral edema. The course of the animals treated with dexamethasone was strikingly different. We used 3 methods of treatment. In all groups, .06 mg./kg. dexamethasone was given intramuscularly every 6 hours. This dose is comparable on a weight basis to that given clinically. In the first group of animals, the drug was given for 48 hours before the edema-producing procedure. The characteristic picture of lethargy proceeding to coma and possible death did not occur. The animals showed minor signs of irritability and reluctance to eat but rapidly recovered and were usually normal at the end of 48 hours. In the second group, dexamethasone was begun at the time of surgery and continued until sacrifice. These animals showed more symptoms but again rarely passed beyond the stage of lethargy; none of these animals died. At the end of 48 hours they were usually quite active and normal in appearance. In the third group, the animals were not treated for 48 hours. They actually were comatose at the time dexamethasone therapy was started. The response was dramatic. Twenty-four to 48 hours after the institution of therapy, the animals would be up, alert and eating well, although minor signs of irritation and focal neurological deficit persisted. An animal nearly dead at the time of the first drug injection often recovered to a normal state within 24 hours.

These procedures were carried out upon 30 animals equally divided in the 3 groups. Histologic studies generally bore out the impressions which were gained clinically. While the brains did not appear normal, there was a minimal amount of edema present. The appearance of this edema usually correlated well with the clinical status of the
animal. These results, using the light microscope, will not be described in detail since our primary purpose is to show the ultrastructural response of edema to steroid administration.

Experimental Procedure

Operation to create edema. All of the animals were lightly anesthetized with intravenous pentobarbital and allowed to breathe spontaneously. A linear midline incision along the sagittal suture was made and the skin retracted laterally. A hand trephine was used to remove a button of bone. In the rabbits it was removed in the midline while in the dogs it was placed 1 cm. to the right or left of the midline in order to avoid the sagittal sinus. After adequate hemostasis was obtained, the pyriform seeds were implanted in the subdural, extradural, or intracerebral position. In the rabbits 0.25 ml. of seeds were used in each case; in the dogs, 0.6 ml–1.2 ml. The dura was then closed (if it had been opened) and the bone button cemented in place. The skin was closed with skin clips and the animals returned to their cages. In the 2 dogs in which a subdural balloon was put in place the procedure was varied so that a small twist drill hole was made in the temporal region in these animals. A #8 Foley catheter was inserted through this hole and the hole then sealed with methyl methacrylate cement. The balloons were then inflated (1–4 minutes) to a pressure of 60 mm. of mercury and a volume of about 4 ml.

Treatment with dexamethasone. The rabbits were divided into 3 equal groups. In 6 rabbits the pyriform seeds were placed in the extradural space, in 6 in the subdural space and in the remaining 6 in an intracerebral position. Two rabbits in each group were pretreated with dexamethasone, 0.06 mg./kg. every 6 hours intramuscularly, for 48 hours before surgery and thereafter until sacrifice 48 hours postoperatively. In 2 animals, treatment with the same dose was begun at the time of surgery and continued until sacrifice 48 hours later. In the remaining 2 in each group, edema was allowed to develop for 48 hours and then treatment was instituted for another 48 hours before sacrifice.

One dog with intracerebral seed implantation and 1 with subdural balloon inflation were pre-treated with dexamethasone for 48 hours before surgery and for 48 hours thereafter. In the other 2 animals, the treatment was begun 48 hours after surgery when the animals were moribund and hemiplegic. Treatment was then continued for 48 hours before sampling.

Sampling the treated brain. To obtain the samples of treated material, all animals were reanesthetized with pentobarbital and a large craniectomy rapidly performed. Care was taken not to injure the underlying cortex. The dura was opened widely and 5×5 mm. blocks of tissue were removed from immediately beneath the seed mass or in close proximity to the offending lesion and at regular distances from the area of greatest abnormality. Cortex and subcortical white matter were removed in the first sample and, whenever possible, samples of deeper white matter were quickly removed by sharp dissection. The tissue was immediately immersed in 1 per cent buffered osmium tetroxide. After 1 to 2 minutes the tissue was removed from the osmium, cut into 1×1 mm. blocks and returned to the osmium for final fixation. The tissue was then embedded according to standard electron microscopic techniques in either methyl methacrylate (in the early part of the study) or epon 812. When the embedding process was complete, sections were obtained by use of a glass knife in the Porter-Blum-Serval MT2 ultramicrotome or the LKB ultramicrotome. Section thickness was judged to be about 600–1000 Å. The tissue was examined in the RCA Victor EMU 3F electron microscope or the Siemens Elmskop I.

Control material for light microscopy was obtained in each case. As soon as the tissue for electron microscopy had been removed, the animals were sacrificed by an intracarotid injection of 10 per cent formalin in saline. The brains were removed and immersed in the same solution for a minimum of 2 weeks. Appropriate tissue blocks were obtained and processed in the standard manner for light microscopy. All sections were stained with hematoxylin and eosin.

Electron Microscopic Study of Experimental Cerebral Edema Treated With Dexamethasone

Detailed Results

In the cortex, there was astrocytic swelling, similar in kind to that present in edematous areas but vastly different in amount. The astrocyte nucleus appeared normal. The cytoplasm of the cell body was often slightly increased but always less than in edematous specimens. The formed intracytoplasmic elements occasionally appeared to be increased in number, particularly the vacuoles, but this was not a consistent change (Figs. 1 and 2). In the neuropil, the majority of the astrocyte processes were of normal or near normal size. An occasional enlarged and clear astrocytic process was found but the general appearance of the neuropil was close to normal (Figs. 3 and 4).

The vascular processes of the astrocyte were usually normal in size or slightly enlarged. Increased vacuolization and increase
Fig. 1. A swollen astrocyte (AN) with clear cytoplasm (CY) in an untreated animal with edema produced by subdural psyllium seeds. Rabbit cortex. ×6510.

Fig. 2. In contrast this astrocyte from an animal receiving dexamethasone for forty-eight hours before an identical edema producing procedure is seen to be normal. Nucleus (AN). Cytoplasm (CY). Neuropil (NP). Rabbit cortex. ×10,200.
Fig. 3. Two swollen clear astrocyte processes (A) demonstrate the usual features of edema, increased volume and decreased electron density. Rabbit cortex. Subdural psyllium seed implantation. ×10,400.

Fig. 4. Administration of dexamethasone for forty-eight hours before operation prevents this astrocyte swelling. Astrocyte process (A). Neuronal element (N). Extracellular space (EC). Rabbit cortex. ×11,650.
in other formed elements was apparent but extremely difficult to estimate (Figs. 5 and 6). The oligodendroglial perivascular processes were normal. The capillary basement membrane was preserved. Endothelial cells appeared to be slightly enlarged and there was a definite increase in vacuolization in the cytoplasm.

The cortical extracellular space was not enlarged. Nerve cells were consistently found to be normal. The oligodendroglial cells were normal and microglial cells were unchanged unless evidences of tissue damage appeared. The general impression was one of normal cortical tissue. Occasionally there were minimal resolving changes similar to those characteristic of cerebral edema. There was definite astrocytic enlargement with increased clarity of processcyttoplasm, although this was never so great as in edematous preparations (Figs. 1 and 2). The perivascular processes having been the first to react in cerebral edema appeared to lag in the resolution and were usually still abnormal (Figs. 5 and 6). Rupture of the plasma membrane, where present, had not healed and these dying cells might be found in a small pseudoeextracellular pocket, interpreted as a residual of the more severe edematous process.

The astrocytes within the white matter were mildly swollen in a manner similar to that in the cortex. Rarely, quite large processes could be seen compressing surrounding tissue. Again the pericapillary astrocyte processes were larger in some instances. These processes were frequently filled with vesicles and intracellular formed elements. No abnormalities of the capillary basement membranes were observed. There was moderately increased vacuolization of endothelial cytoplasm in the capillary.

The white matter extracellular space was occasionally slightly enlarged but no such enlargement as that present in the edematous areas was ever seen (Figs. 7 and 8). Rarely, the myelin sheath showed spaces between the myelin lamellae. Axonal compression was also seen although both changes were less apparent than in the edema preparations. There are recognized difficulties in determining whether these changes are real or artificial. It is likely that some represent artifact from immersion fixation. No changes in the oligodendroglia or microglia were evident.

The ultra-microscopic changes correlated well with the clinical status of the animal, the degree of swelling estimated at the time of sampling, and the histologic picture obtained by light microscopy.

Findings in 3 Groups of Treated Animals

The differences between the various groups of animals are quantitative rather than qualitative.

Group 1. In those animals receiving dexamethasone for 48 hours prior to surgery and continuously after surgery until sacrifice, there were few signs or symptoms of cerebral edema. No evidence of brain swelling was found at that time of sampling, except in the area of surgical manipulation. Little or no ultra-microscopic variation from the normal was present in the brains of these animals. Only rarely could large and clear astrocytic processes be discovered in the cortex. Occasionally the capillary processes were enlarged. There was sometimes very minimal increase in white matter extracellular space. These brains were otherwise normal.

Group 2. In those animals receiving treatment only after edema-producing procedure, the variation from normal was more marked. Astrocytic enlargement was more constant and a greater increase in the extracellular space of the white matter was seen. However, neither of these 2 groups demonstrated changes as great as those in the untreated edema preparations.

Group 3. In those animals allowed to develop edema before therapy was begun, changes were more severe. There was slight astrocytic enlargement together with minimal increase in white matter extracellular space. However, the abnormality was not great and correlated in degree with the clinical recovery of the animal.

The general impression gained from all 3 groups of treated edema was one of minimal-to-moderate abnormality of the central nervous system. In other words, the typical findings of cerebral edema were present in these preparations although the degree of structural abnormality was remarkably less than that observed in similar untreated experimental preparations.

Examination of the brain substantiated this opinion for little gross evidence of cere-
Fig. 5. Capillary-glial detail from edema produced by intracerebral psyllium seed implantation reveals huge, clear astrocyte processes (A) abutting a cortical capillary (CAP). Dog. X10,000.

Fig. 6. Pretreatment for forty-eight hours in a similar preparation has prevented the astrocyte swelling. This cortical capillary (CAP) is normal in appearance. Basement membrane (BM). Endothelium (EN). Lumen (L). Dog. X14,000.
Fig. 7. Edematous white matter is characterized by enlargement of the extracellular space (EC) with swelling or deformation of axons (AX) and disruption of myelin lamination (MY). Dog. Subdural balloon inflation. ×18,750.

Fig. 8. Administration of glucosteroid for forty-eight hours in a similar preparation has returned the extracellular space (EC) to normal volume. Axon (AX). Myelin (MY). ×15,490.
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bral edema was discovered. The light microscopic picture also correlated well with the ultrastructure. Most of the brains appeared normal although minimal evidence of cerebral edema was seen in some cases. In all instances, the clinical status of the animal, the gross estimate of edema at the time of sampling, and the light microscopic histology were compatible with the degree of abnormality observed ultrastructurally.

Tissue from animals with untreated cerebral edema showed such marked structural abnormalities that these samples were easily separated from those animals which had received glucocorticoid therapy. The histopathologic findings in untreated cerebral edema have been reported in another communication.17

The characteristics of cerebral edema as viewed with the electron microscope are as follows:

1. Pericapillary enlargement of astrocyte processes with decreased process electron density (Fig. 5).
2. Generalized swelling and increased clarity of astrocyte processes throughout the neuropil (Fig. 3).
3. Great increase in the size of the astrocyte cell body without nuclear change (Fig. 1).
4. Remarkable increase in white matter extracellular space (Fig. 7).
5. Disruption of myelin lamination and axonal compression (a finding of questionable significance).
6. Rupture of plasma membranes and creation of a "pseudoextracellular space," especially within the cortex.
7. Signs of tissue damage and ischemic cell changes.
8. Tissue necrosis.

The cortical extracellular space does not increase, and neurons show ischemic changes only. Microglia undergo reactive change only if tissue necrosis has occurred. The oligodendroglia are rarely involved. Capillary endothelium is occasionally increased in volume and increased vacuolization may occur. Intracytoplasmic inclusions do not appear to be changed. However, further study is required to clarify the possibility of endothelial cell vacuolization and the status of the formed elements of cell cytoplasm.17-19

Cerebral edema, treated with dexamethasone, is strikingly different. The pericapillary astrocyte processes remain slightly enlarged and increased vacuolization is more common than in untreated edema (Fig. 6). The astrocytic processes scattered throughout the neuropil are generally normal although an enlarged process may occasionally be found (Fig. 4). Astrocytic cell bodies generally appear normal or slightly enlarged (Fig. 2). The most common abnormality is a minimal-to-moderate increase in the white matter extracellular space. Myelin disruption and axonal compression are much less marked than in untreated edema preparations. The possibility that these findings may be artifacts has been mentioned. Areas of pseudoextracellular space may persist but these are small. There is no apparent effect in areas of frank tissue necrosis, and where damage existed previously the administration of the steroids does not appear to change the abnormality. The neurons, microglia, oligodendroglia, and capillaries are not remarkably changed. Capillary endothelium volume may be increased, and increased vacuolization may occur. Intracytoplasmic inclusions do not vary appreciably from the normal. It is of course possible that future methods for studying small structures may reveal abnormalities in these inclusions and substantiate or refute the impression of increased endothelial cell volume and vacuolization.

It cannot of course be proved that similar degrees of edema existed in the treated and untreated animals prior to therapy or that similar degrees of swelling would have developed without steroid administration. However, the clinical course of the animals certainly suggests comparable degrees of edema as well as the subsidence of edema following glucocorticoid administration.

Discussion

It seems likely that the study of experimental cerebral edema can at least partially applied to the human situation. The remarkable effect of high doses of glucocorticoids on patients suffering from cerebral edema complicating numerous types of intracerebral lesions has been well documented.8 A recent ultrastructural review of this type of brain tissue has indicated that one of the actions of the glucocorticoids is an actual re-
duction in the amount of the structural abnormality in edema. However, clinical material of this sort is difficult to control. The present study was undertaken in order to evaluate the effects of glucosteroids upon experimental edema and to compare these effects with the human material already available.

The ultrastructural characteristics of cerebral edema have been established. It is not difficult to add the response of edema to glucosteroid therapy to this schema. There are 2 basic changes in the resolution of the abnormality. The astrocyte swelling is greatly reduced. No other space is seen to enlarge concomitantly and so the most likely place for the discharge of this "fluid" is into the vascular system. The persisting enlargement of the pericapillary astrocyte processes, when all other evidences of brain swelling have disappeared, substantiates this observation. The abnormalities in the white matter extracellular space also return toward normal. It seems most likely that this fluid is discharged into the vascular system but the route is less evident in the white matter. The remarkable difference in the treated and untreated groups of animals seems to establish that at least one of the actions of the glucosteroids in cerebral edema is actual reduction in the amount of structural abnormality present.

The pathophysiological understanding of cerebral edema is still not clear in spite of voluminous literature on the subject. Vascular stasis and mechanical factors were most often used to explain the appearance of edema. It now seems likely that such simple explanations are not enough and several investigators have suspected abnormalities of water and electrolyte transport systems within edematous brain tissue. Some of the arguments supporting this concept have been reported elsewhere.

In cerebral edema something allows the influx of "water," sodium, chloride, and probably other materials as yet undefined into the astrocyte in the cortex and into the white matter extracellular space. Perhaps this "something" is a derangement of the water-electrolyte transport mechanism across the capillary-glial interface and possibly elsewhere. Similar transport systems have been studied in mammalian kidney, and in amphibian bladder and skin. Enzymes responsible for sodium and potassium transfer within the brain have been isolated and there seems little doubt that such systems exist. If the transport system in an amphibian bladder preparation is disrupted, a great influx of sodium and water occurs. This abnormal sodium and water movement across the damaged bladder correlates to some extent with the observed electrolyte disturbances in cerebral edema. The addition of large quantities of glucosteroids to the in vitro amphibian bladder preparation will reconstitute the transport function of the bladder. The water and electrolyte influx is halted and excess is rapidly excreted. Dexamethasone, a potent glucosteroid, may well have the same effect upon the transport system within the brain.

Certain situations which exist in the human also support this theory. Erythrocytes from hypoadrenal patients swell appreciably when placed in isotonic saline. This swelling can be prevented and reversed by the addition of glucosteroid to the preparation or by adequate replacement therapy for the animal or patient involved. Slices of brain and liver removed from hypoadrenal animals exhibit the same swelling. In the brain this abnormality is particularly noticeable in the astrocytes. The swelling can be prevented or reversed by the preoperative use of steroid, or by the addition of glucosteroids to the fluid medium used for incubation.

These speculations can certainly not be regarded as proofs but the analogies are attractive. To summarize, cerebral edema may be considered to be the result of a deranged water and electrolyte transport mechanism within the brain. The disruption probably occurs at the capillary-glial interface but may also be present elsewhere at levels as yet unknown. Abnormal accumulations of fluid, electrolytes, and probably other substances occur primarily within the astroglia and in the white matter extracellular space. The continued accumulation of this fluid may result in tissue disruption, compression, ischemia, and may well account for certain of the later features present in severe brain swelling. The administration of large quantities of glucosteroids is capable of reversing these abnormalities and reducing
the amount of structural change present in the edematous brain. It is quite possible that this reduction is effected by reconstitution of the disrupted water ion transport mechanism and a reversal of the abnormal fluid and electrolyte transfer. While no more than a speculation, the concept of such a mechanism is supported by analogy with similar transport systems available for study in the human and in the experimental animal. It is also in agreement with the observed ultrastructural morphology of human and laboratory animal cerebral edema and the temporal and structural response of this edema to glucocorticoid administration.

Summary

Dexamethasone and other glucocorticoids are known to be of value in the treatment of patients suffering from cerebral edema. The histological changes which accompany the resolution of the signs and symptoms of cerebral edema following therapy have not been previously studied. We have shown that dexamethasone will prevent the ultrastructural characteristics of experimental cerebral edema from developing. The administration of the drug after the appearance of the signs and symptoms of brain swelling is effective in reducing the amount of structural abnormality present. We have reviewed the ultrastructural characteristics of cerebral edema and have given a possible explanation for the action of dexamethasone.

References


