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ULTRASTRUCTURE OF THE EPILEPTOGENIC FOCUS

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ALUMINA CREAM-INDUCED FOCAL MOTOR EPILEPSY IN CATS:
ULTRASTRUCTURE OF THE EPILEPTOGENIC FOCUS

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Velasco et al (1973 a, b) have shown that injection of a critical amount of alumina cream (AC) into the sensorimotor cortex of cats produced small and circumscribed lesions invariably accompanied by focal EEG and clinical seizures showing three consecutive sharply delineated stages: a latent, a convulsive and a remission stage.

The histopathological analysis of these epileptogenic lesions revealed in all stages a central deposit of alumina cream with macrophages, absence of fibrotic capsule at the edge and no gross inflammatory changes in the perilesional nervous tissue. In addition, a progressive and significant decrease in both thickness and cellularity through these various stages was observed in the perilesional motor cortex. In contrast, non-epileptogenic lesions produced by silicon (S) showed intense inflammatory reaction with fibrotic capsule, and a greater number of damaged cortical neurons of the perilesional nervous tissue. These results suggested that presence of clinical and EEG seizures in AC lesioned animals is independent of the scar formation and inflammatory changes of the perilesional tissue. Furthermore, spontaneous occurrence and remission of convulsive activity seems to depend upon a critical number of both intact and damaged cells of perilesional cortical tissue. Other possible morphological alterations which cannot be detected by light microscopy, however, should be also taken into account in the physiopathogenesis of this model of focal epilepsy.
The aim of the present study is to analyze the ultrastructural changes found in AC epileptogenic lesions of animals sacrificed in each stage of their spontaneous course. Data were compared with those observed both from animals with S non-epileptogenic lesions and from control animals operated but no lesion produced.

METHODS

Experiments were performed in 20 male cats weighing 3 kg. or more divided in three groups:

1. **Alumina cream.** In 15 animals a brain lesion was produced by a single intracortical injection of 0.02 - 0.04 ml. of a commercial product of alumina cream (Aldrox). All injections were into the same area of the right sensorimotor cortex (anterior sigmoid gyrus) at a point 4 mm lateral to the midline, 1 mm anterior to the cruciate sulcus and 3 mm in depth. The animals were divided into 3 subgroups according to the time when they were sacrificed: 5 animals were sacrificed during the latent stage (5 to 7 days after injection), 5 animals during the convulsive stage (40 days after injection) and 5 animals during the remission stage (80 days after injection).

2. **Silicon.** In two cats, a brain lesion was produced by injection of 0.04 ml of silicon*, at the same site as alumina cream. They

* Silicon - Silicacid Clay Adams Inc., York. 1% watersoluble silicon concentrate.
were sacrificed 40 days after operation.

3. Intact. In 3 cats, only a right frontal craniotomy and a dorsal incision were made. These animals were sacrificed 40 days after operation.

At the time of surgery, silver ball electrodes for cortical recordings were placed on the dura over the right anterior sigmoid gyri. After surgery, spontaneous behavior was observed and monopolar cortical recordings (referred to a common electrode attached to the frontal bone) were performed twice every decade*. Spike density at the right anterior sigmoid gyrus (number of spikes/20min/decade) of various groups of animals at the time they were sacrificed was as follows: Alumina cream: Latent ($1.3 \pm 0.3$), Convulsive ($920 \pm 120$), Remission ($1.5 \pm 0.8$), Silicon ($1.6 \pm 0.8$), Intacts ($0.5 \pm 0.1$). All AC animals sacrificed during the convulsive period showed at the day of autopsy clinical convulsions consisting in continuous rhythmic twitching of left facial, neck and forepaw muscles.

For electron microscopy studies, brains were perfused through both internal carotid arteries using the external jugular veins as outlets. Perfusions started with Ringer solution during 5 minutes, followed by a 3% glutaraldehyde-Ringer-water solution for 25 minutes. Physico-chemical composition of solutions was the same that those

* "Decade" is here employed to indicate 10-day period.
used by Feria and Karnovsky (1970) and Velasco et al (1973 b). After perfusion, 1 x 2 x 2 mm fragments were taken from a region located 1.0 - 2.2 mm posterior to the right frontal pole including lesions and perilesional cortices of AC and S lesioned brains and equivalent cortices of the intact brains. All tissue fragments were immersed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 for one hour at 4°C (Sabatini et al, 1963). After overnight washing in the same cacodylate buffer, samples were postfixed in cacodylate buffered-1% osmium tetroxide for 2 hours at 4°C (Palade, 1952) and dehydrated in graded ethanols to be embedded in Epon-812 for 24 hours at 60°C (Luft, 1961). The fragments were carefully handled and specially oriented in order to observe in the same section all the cortical layers. One micron thick sections were obtained in a Reichert OmU-2 ultramicrotome and stained with Paragon (Spurlock et al, 1963) to be examined in a light microscope to select the suitable areas for thin sectioning. With the same ultramicrotome thin sections in the silver color range of reflected light (Peachy, 1958) were obtained and collected in uncovered copper grids to be stained with lead citrate (Venable and Coggeshall, 1965) and uranyl acetate for observation in a Philips EM-200 and a Zeiss EM-9A electron microscopes.
RESULTS

Lesion

Light microscopy abnormalities associated to AC and S lesions by examining one micron thick sections of Epon-embedded material were confirmatory to those reported in a previous study (Velasco et al, 1978 b).

Under the electron microscope, AC lesions showed similar characteristics in all three stages. They consisted in a central necrotic region formed by numerous macrophages with irregular nucleus disclosing coarse clumps of chromatine and clearly discernible nucleolus. The cytoplasm of these cells contained a large number of ovoid vacuoles filled with moderately electron dense amorphous material and dense spherical bodies limited by single membranes which most likely corresponded to lysosomes (figure 1). Rests of destroyed cells and electron dense amorphous granular material were observed within the interstitial space of this central region. Neither fibrotic capsule nor other inflammatory elements were found at the edge of AC lesions. 

S lesions also showed a central area with macrophages, rests of destroyed cells and electron dense amorphous material. However, at their edge these lesions showed morphological signs of a non-specific chronic granulomatous inflammatory reaction, i.e. fibrotic capsule formed by fibroblasts and bundles of collagen fibers, presence of polymorphonuclear leukocytes, lymphocytes and few epithelioid and multinucleated giant cells. Unusual features of these inflammatory
elements were observed under the electron microscope.

Perilesional tissue

Changes in number of glial processes and morphological signs of progressive degeneration of neuronal elements were the most remarkable findings at the cortical tissue adjacent to AC lesions. The apparent number of glial processes was small during the latent stage, reached its maximum during the convulsive stage and decreased during the remission stage (figure 2). At the convulsive stage glial processes were better delineated showing a clear cytoplasmic matrix containing densely packed rows of thin filaments, with individual diameters from 7 to 10 mm, frequently arranged in bundles along with other cytoplasmatic organelles (figure 2c). These glial processes morphologically corresponded to fibrous astrocytes, based upon descriptions of normal human brains and fibrous astrocytomas of the CNS (Luse 1960, 60 and Duffel et al, 1963). Whether the apparent increase in number of the astrocytic processes is due to hypertrophy or hyperplasia of normal glial elements cannot be asserted from the present work.

Degeneration signs were incipient during latent stage consisting of few swollen dendrites and dilacerated myelin fibers (Figs. 2L and 3L). During the convulsive stage degenerative changes of dendrites and myelin fibers were more pronounced and other degeneration signs appeared, i.e. residual and multivesicular bodies at dendritic cyto-
plasm and increased density of cytoplasmic matrix, multivesicular bodies and distortion of myocondria at the synaptic terminals (figures 2C and 3C). During the remission stage a complete distortion of neuronal somas, dendrites, synaptic buttons and myelinated fibers was observed (figures 2R and 3R).

Cortical tissue adjacent to S lesions showed no proliferation of astrocytic processes. Degeneration signs in neuronal elements were similar, although less intense to those found in the convulsive stage of AC lesions. No ultrastructural alterations were found at the cortical tissue obtained from intact brains.

DISCUSSION

Lesion

At the central core, all AC epileptogenic and S non epileptogenic lesions showed histopathological signs of a non-progressive coagulation necrosis. By contrast, at the edge, all AC epileptogenic lesions showed neither fibrotic capsule nor other inflammatory changes while S non-epileptogenic lesions showed elements of a chronic granulomatus reaction. These results were confirmatory to those reported by Velasco et al 1973 b, and suggest that presence of clinical and EEG seizures in AC lesioned animals is independent to the scar formation and other chronic inflammatory elements. Present findings at the lesion edge are in contradiction to those of other electron mi-
Electroencephalography investigations showing a chronic granulomatous reaction in both epileptogenic lesions produced by AC in monkeys (Harris, 1973) and by cobalt gelatine rods in rats (Fischer, 1968). These differences may be explained on the basis of the type and quantity of the convulsant agent used and the scrupulous aseptic methods employed in implantation and maintenance of the animals.

**Perilesional cortical tissue**

Changes in number of fibrotic astrocytes and morphological signs of progressive degeneration of neuronal elements were the most remarkable findings at the cortical tissue adjacent to AC lesions. In normal brains, fibrous astrocytes seem to participate in regulating the microenvironment of central nervous tissue (Palay, 1969). The significance of increase number of these elements during the convulsive stage of AC lesions, however, remains unknown.

Progressive degenerative signs associated to AC lesions morphologically correspond to those observed in both distal and proximal axonal degenerations (Colonnier 1964, Guillery 1970). They can be interpreted as a slow, progressive, non-specific toxic effect of alumina cream on all cortical neurons. However, current experiments in our laboratories show that this non-specific, toxic effect is preferentially exerted upon presumably inhibitory neurons located at layers II and VI of the cerebral cortex adjacent to AC lesions during convulsive stage and therefore convulsions may result from a focal excitatory-inhibitory cortical imbalance (Ferr. Velasco et al, 1974).
SUMMARY

Present study analyzes the ultrastructural changes of AC epileptogenic lesions in animals sacrificed during latent, convulsive and remission stages. Analysis include the histopathological elements found at the lesion core and edge as well as the perilesional cortical tissue. Data were compared with those observed both from animals with S non-epileptogenic lesions and from control animals operated but no lesions produced.

1- At the central core all AC epileptogenic lesions and S non epileptogenic lesions were similar, showing macrophages with dense spherical bodies and vacuoles filled with electron dense amorphous material. Rest of destroyed cells and amorphous material were also seen at the interstitial space.

2- At the edge all AC epileptogenic lesions showed neither fibrotic capsule nor other inflammatory changes while S non epileptogenic lesions showed morphological signs of a chronic granulomatous inflammatory reaction. No peculiar features of these inflammatory elements were observed under the electron microscope.

3- Changes in number of glial processes (fibrous astrocytes) and morphological signs of progressive degeneration of neuronal elements at the perilesional cortical tissue were found through the latent, convulsive and remission stages of AC lesions. By contrast, cortical tissue adjacent epileptogenic lesions showed no astrocytic proliferation whereas degeneration signs of neuronal elements were similar al-
though less pronounced than those found in the convulsive stage of AC lesions. No ultrastructural alterations were found at the cortical tissue obtained from intact brain.

Present findings at the lesion edge support the idea that presence of clinical and EEG seizures in AC lesioned animals is independent of the scar formation and other inflammatory chronic elements. On the other hand, further studies should be done to clarify the significance of increased astrocytic processes and progressive degeneration signs of the perilesional cortical tissue in the physiopathogenesis of convulsive activity.
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REFERENCES


LEGENDS FOR FIGURES

Fig. 1:
Portion of a macrophage at the edge of the central region of AC lesion, showing dense cytoplasmic bodies, which most likely correspond to lysosomes (L) and fine granular material within phagocytic vacuoles (V). This particular macrophage was located at the vicinity of a blood capillary, showing the endothelium (E) and vascular lumen (VL) (22,000X).

Fig. 2:
Neuropil at deep layers of the motor cortex adjacent to AC lesions. 2l. Intact animal showing the normal appearance of tissue elements (16, 600X). 2L Latent stage showing minor structural changes, i.e. slight swelling of mitochondria (sm), dilaceration of myelin (dm) and a multivesicular body (mb) in a nerve ending (20,000 X). 2C. Convulsive stage showing numerous astrocytic processes (A) (16,600 X). Inset: Detail of glial filaments (f) within an astrocytic process (41,500 X). 2R. Remission stage showing complete distortion of the architecture with marked degenerative changes in the neuropil elements (22,000 X). Inset: a multivesicular body (mb) is observed within a dendritic process (50,000 X).

Fig. 3:
Synaptic elements at the motor cortex adjacent to AC lesions. 3l. In intact animals no alterations were observed at synaptic vesicles (v) within synaptic
endings. D = dendritic spine; Arrow = synaptic cleft (48,000 X).

3L. Latent stage showing slight swelling of the synaptic endings (S) and accumulation of vesicles at the presynaptic membrane (v). Some glial processes (G) were also noticed (42,000 X). 3C. Convulsive stage showing marked changes in a synaptic ending with a degenerated mitochondria (m) and dilated vesicles (v) (43,000 X). Inset: A membranous residual body (m) is depicted within a postsynaptic nerve process (ps) (16,600 X). 3R. Remission stage showing complete distortion of both, presynaptic (S) and postsynaptic (ps) elements (32,000 X). Inset: A membranous residual body (m) is depicted in a nerve terminal among the synaptic vesicles (v) (39,000 X).
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