Electrical Anesthesia Produced by Combining Direct and Alternating Currents: Electronmicroscopy of the Dog Brain

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The brains of 3 dogs were studied by electronmicroscopy after electrical application. One was given ten hours of electrical anesthesia over a five-week period, another electroshock therapy ten times in five weeks, and the lungs ventilated during convulsions to avoid hypoxemia. The third animal acted as a control and no electrical current was applied. Sections from the brains of all three were examined by electronmicroscopy. There were no changes attributable to the passage of electrical current.

THIS STUDY is part of a continuing inquiry into the possibility of producing safe clinical anesthesia by the application of electrical current. Brain injury is a complication which must be considered and avoided if this form of anesthesia is to be accepted. Previous studies in this area have included (1) examination under the ordinary microscope of tissue slices from the brains of animals, to determine gross abnormalities, (2) observations of the effect of electrical anesthesia on learning and the performance of learned tricks, and (3) observation of behavior or "personality" pattern after repeated administration of electrical anesthesia.

The present study was designed to ascertain if there were any changes at the intracellular level in the brain consequent to the passage of electrical current.

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Method

Three female beagle litter mates six months old were studied. One, as the control, had no electrical anesthesia. The second had one hour of electrical anesthesia, ten times in five weeks. In each instance, the oral-vertex electrode placement was used. Four current patterns were employed: d.c. plus a square wave of a.c. origin; sine wave; square wave of a.c. origin; and, d.c. plus a square wave of d.c. origin. Table 1 shows the times and the patterns for electrical anesthesia. The techniques and pattern of current application were described in detail in previous articles on this subject.[1, 2, 3] The third dog was made to convulse (as in electroshock therapy) by the application of electrical current via the same placement of oral-vertex electrodes. Convulsions were produced ten times in five weeks by using 130 volts for 0.1 second. A standard EST unit was employed to produce the shocks. While awake, the trachea of this animal was intubated before each experiment and the lungs ventilated with room air throughout the convulsions to eliminate the possibility of hypoxic brain injury.*

The method of preparation and examination of brain sections was as follows: The dogs were given a lethal dose of pentobarbital sodium intravenously and the brains removed immediately. Two or 3 mm. square blocks of brain tissue were selected and fixed in Palade's[4] osmium tetroxide containing sucrose as suggested by Caulfield.[5] Tissues were placed in fixative within 10 minutes after the animals

* The animals were flown alive 24 hours after the last application of current to the Abbott Laboratories in North Chicago, Illinois.

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TABLE 1. Times and Patterns of Electrical Anesthesia Employed

Date	Type of Current	Milliamperes	Frequency	Weight	Remarks
2-28-64	Sine wave	12 to 15 a.c.	1200		Good anesthesia
3-2-64	Sine wave	14 a.c.	1000		Good anesthesia
3-5-64	Sine wave	12 a.c.	1,150-1,250	23.5	Good anesthesia
3-9-64	Square wave	10 d.c.-12 a.c.	300	22.5	Fair anesthesia
3-11-64	Square wave	11 d.c.-16 a.c.	300		Fair anesthesia
3-16-64	Square wave	16 a.c.	300	24	Fair anesthesia
3-19-64	Square wave	16 a.c.	300	23.5	Fair anesthesia
3-23-64	Sine wave	12 a.c.	700		Good anesthesia
3-26-64	Square wave	10 d.c.-6 a.c.	300		Fair anesthesia
4-1-64	Square wave	10 d.c.-6 d.c. pulse	750-1,200	26.5	Fair anesthesia

had been injected with pentobarbital sodium. The tissue was further trimmed and reduced in size while in cold fixative. Tissue was selected from 11 areas of the brain as follows: anterior cerebral cortex, caudate nucleus, internal capsule, thalamus, lateral cerebral cortex, hypothalamus, hippocampus, posterior colliculus, pons, vermis of cerebellum and the medulla. Following fixation, the tissue was dehydrated in a graded series of ethanol and embedded in Epon 812 according to the method of Luft.[6] Ultrathin sections were cut and examined on either a RCA EMU 3E or 3G electron microscope. A total of more than 150 ribbons of sections were examined. Each ribbon consisted of 3 to 4 sections, ½ by ½ mm. square. Sections were stained with uranyl acetate or lead citrate[7] to increase contrast.

Sections 1-micron thick were stained with toluidine blue for study by light microscopy. Additional material from the same areas of the brain was fixed in 10 per cent buffered formalin for paraffin embedding and light microscopical evaluation. Sections were stained with hematoxylin and eosin, gallocyanin, Weil's stain, a modified Bodian silver stain, Holzer's stain for glial fibers, and Cajal's gold sublimate for astrocytes.

Specimens from the three animals were compared as unknowns. All tissues were studied by light microscopy of paraffin sections and Epon sections before examination by electron microscopy. The special stains listed above were utilized for study of nerve cells and fibers as well as glial cells and their processes. Careful study was made of the blood vessels.

Results

All structures were within normal limits. Electron microscopy did not reveal any significant difference between the control and experimental animals. All tissues were considered to be within normal limits with respect to neuron ultrastructure, myelinated and nonmyelinated fibers, supporting cells and capillaries, with the exception of isolated structures which may have been disturbed by fixation artifacts. In each instance the same type of change was found in all three animals.

Most capillaries and surrounding tissue were normal with no empty spaces, distorted membranes or unusual structures present. Occasional vascular channels were surrounded by one or more cross sections of neuronal processes with a light, almost structureless matrix (fig. 1). The lack of internal detail, except for a few mitochondria and vesicles, may be related to fixation. However, the cell membrane was normal and relationships to adjacent tissue did not suggest swelling or shrinkage. Basement membranes were distinct and

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FIG. 1. Capillary from cerebral cortex. A white blood cell (wbc) is present in the lumen. Several neuron processes (a) adjacent to the capillary are relatively empty indicating possible change related to fixation technique. Mitochondria (m) and vesicles are present in these processes. All structures are in close apposition and undistorted as seen in the endothelium, nucleus (n), basement membrane (bm) and surrounding neuropil. Magnification from 15,000 ×.

FIG. 2. Light micrograph of cerebral cortex. A neuron such as N is illustrated in figure 3. Hematoxylin and eosin stain, magnification from approximately 40 ×.

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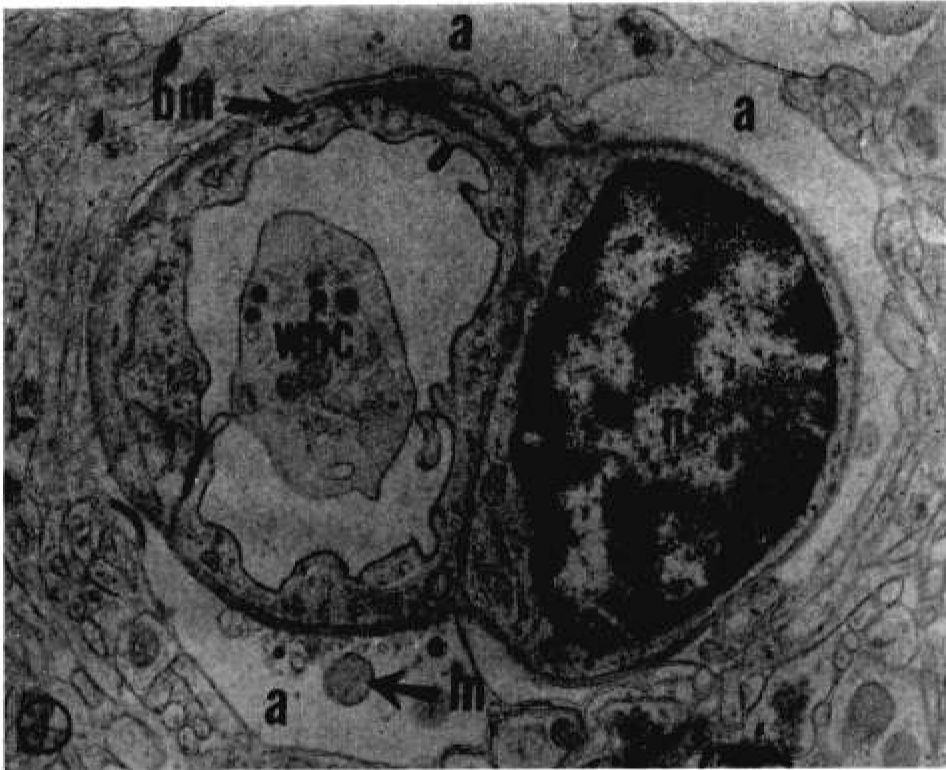

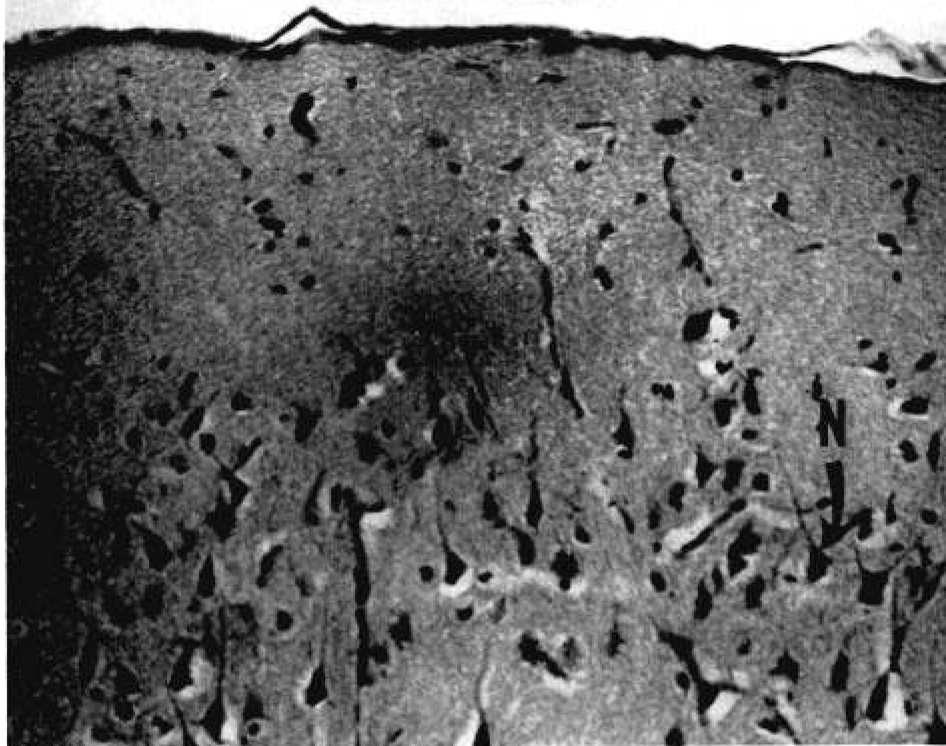

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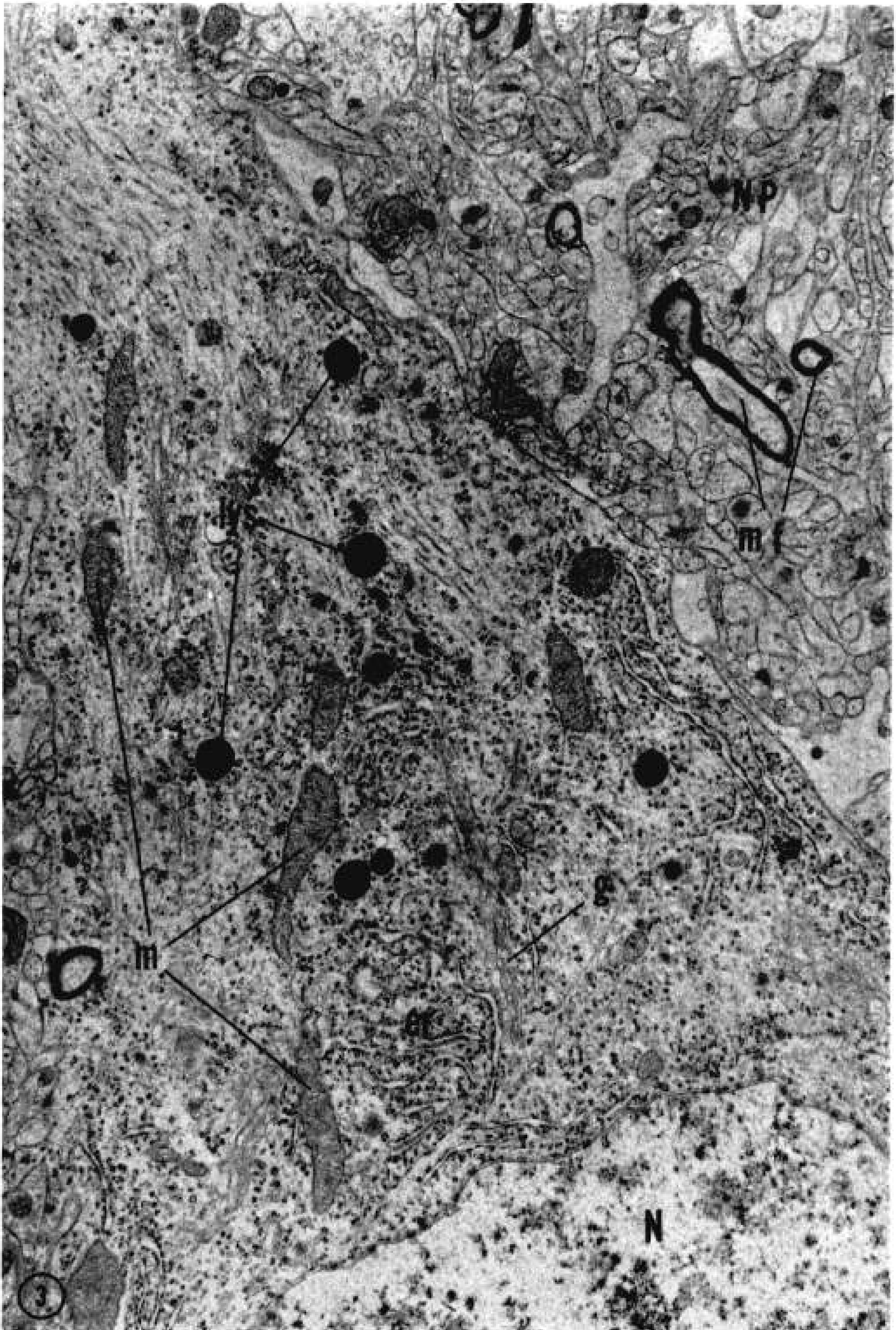

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all components of the capillary and surrounding neuropil were in normal apposition. Such processes of low density were seen near a few capillaries in all animals.

Neuron processes of low density were also seen near occasional isolated dark neurons in all animals. The occasional dark neurons were similar in structure to the more common lighter types, but all cytoplasmic components were densely packed. Dark cells have previously been related to variations in fixation.[8, 9, 10] A typical normal neuron as seen in these animals is illustrated in figure 3. It was located in the cerebral cortex and represents a cell similar to the one marked in figure 2, a photomicrograph of paraffin sectioned material.

Abnormalities of myelinated fibers were not observed (fig. 4). The nonmyelinated neuropil was also normal (fig. 5) with the exception of the processes of low density near a few capillaries and neurons as described above.

Unusual lamellar arrays were seen in neuronal processes of the molecular layer of the cerebellum and the posterior colliculus (figs. 6 and 7). Lamellar arrays have previously been reported from the cerebellum of the rat by Fernandez-Moran[11] and by Herndon[12] who attributed them to anoxia. They appear as stacks of 2 to 15 parallel membranes and each pair was separated by a dilated cisternum free of formed material. These neurons were normal in other respects.

Discussion

Previous reports dealing with electroshock as reviewed by Meyer,[13] indicate that lesions may occur following repeated electroshock but are not constant in all individuals and are of a minimal and isolated nature. Our electron microscopic studies did not establish any significant ultrastructural lesions associated with electroshock or electro-anesthesia. Such observations provide additional confidence that electro-anesthesia does not result in generalized permanent cellular lesions even though the many sections examined represent only a small portion of the brain tissue and isolated lesions may have been missed.

Parallel lamellar arrays were seen and are reported for the first time in the dog and for the first time in tissue other than the cerebellum. Their significance and relationship to fixation is not critical to the interpretation of results of these experiments because they were found in control and experimental dogs. Herndon[12] believes that they do not occur when fixation is carried on by perfusion with warm fixative, but additional observations would be desirable to establish them as artifacts of fixation rather than specialized organelles of the neuron.

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Fig. 3. A typical neuron from the cerebral cortex similar to the one marked in figure 2. The location of nucleus (n), mitochondria (m), lysosomes (lys), Golgi complex (g) and granular endoplasmic reticulum (er) are marked. Note the many rosettes or ribonucleoprotein in addition to the particles attached to the endoplasmic reticulum. Fine neurofilaments can be seen in the upper left corner. The surrounding neuropil (np) contains many nonmyelinated fibers and a few myelinated tracts (mf). Magnification from 13,000 ×.

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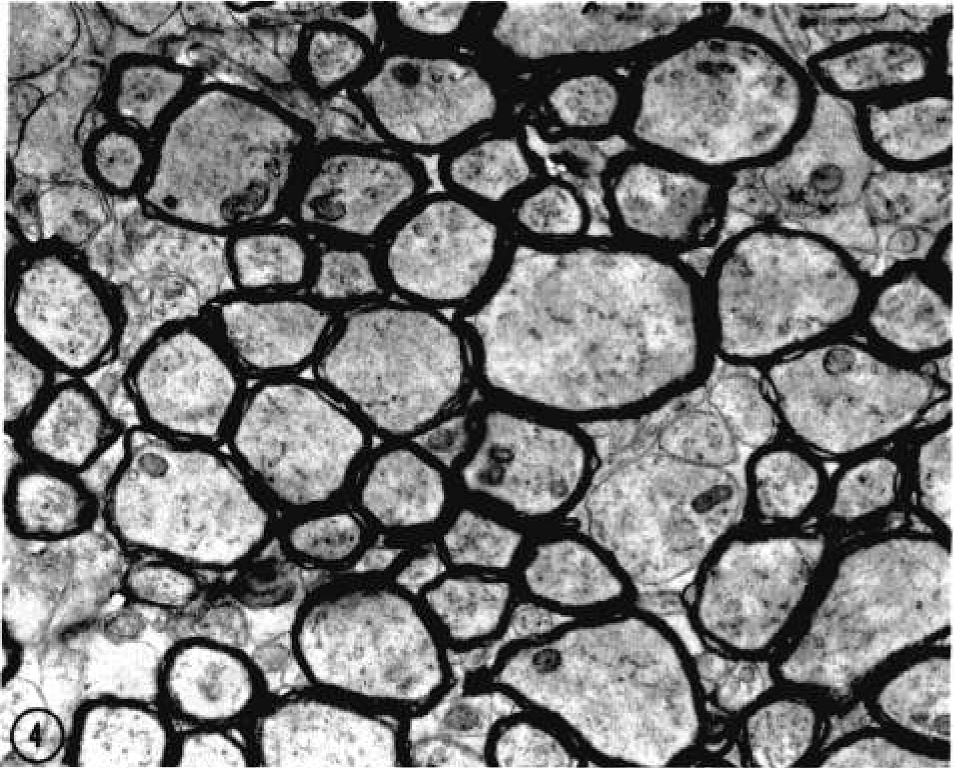

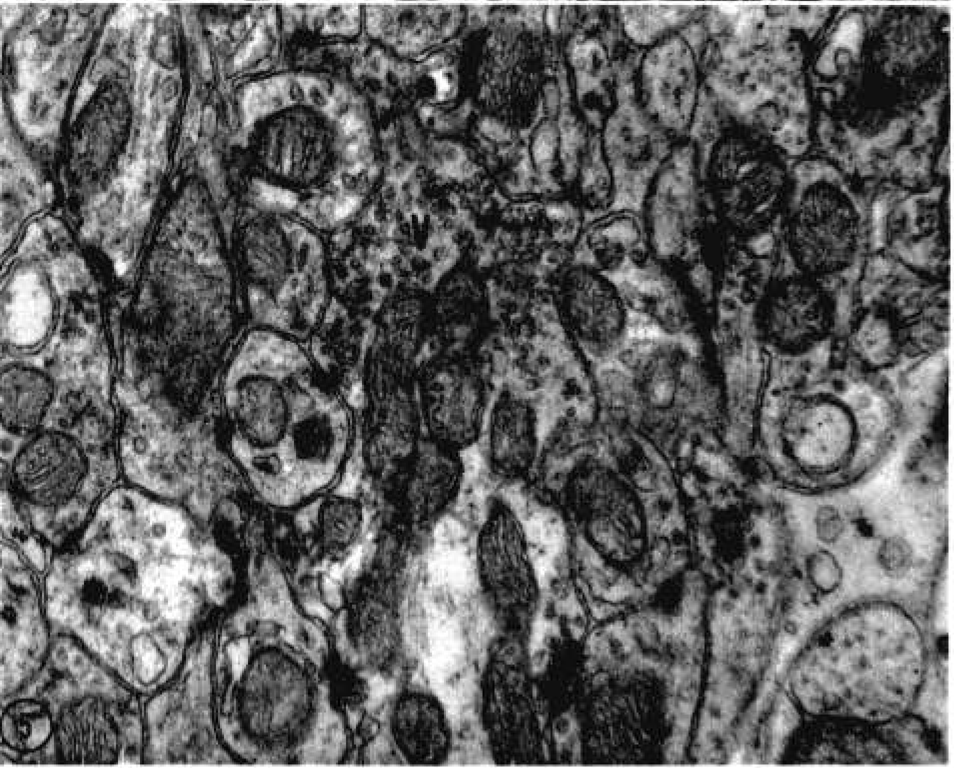

FIG. 4. Cross section of myelinated fibers from the internal capsule. Magnification from 14,500 ×.

FIG. 5. Nonmyelinated portion of the neuropil. Vesicles (v) are typical of presynaptic sites. Numerous mitochondria are present. Cerebral cortex. Magnification from 30,000 ×.

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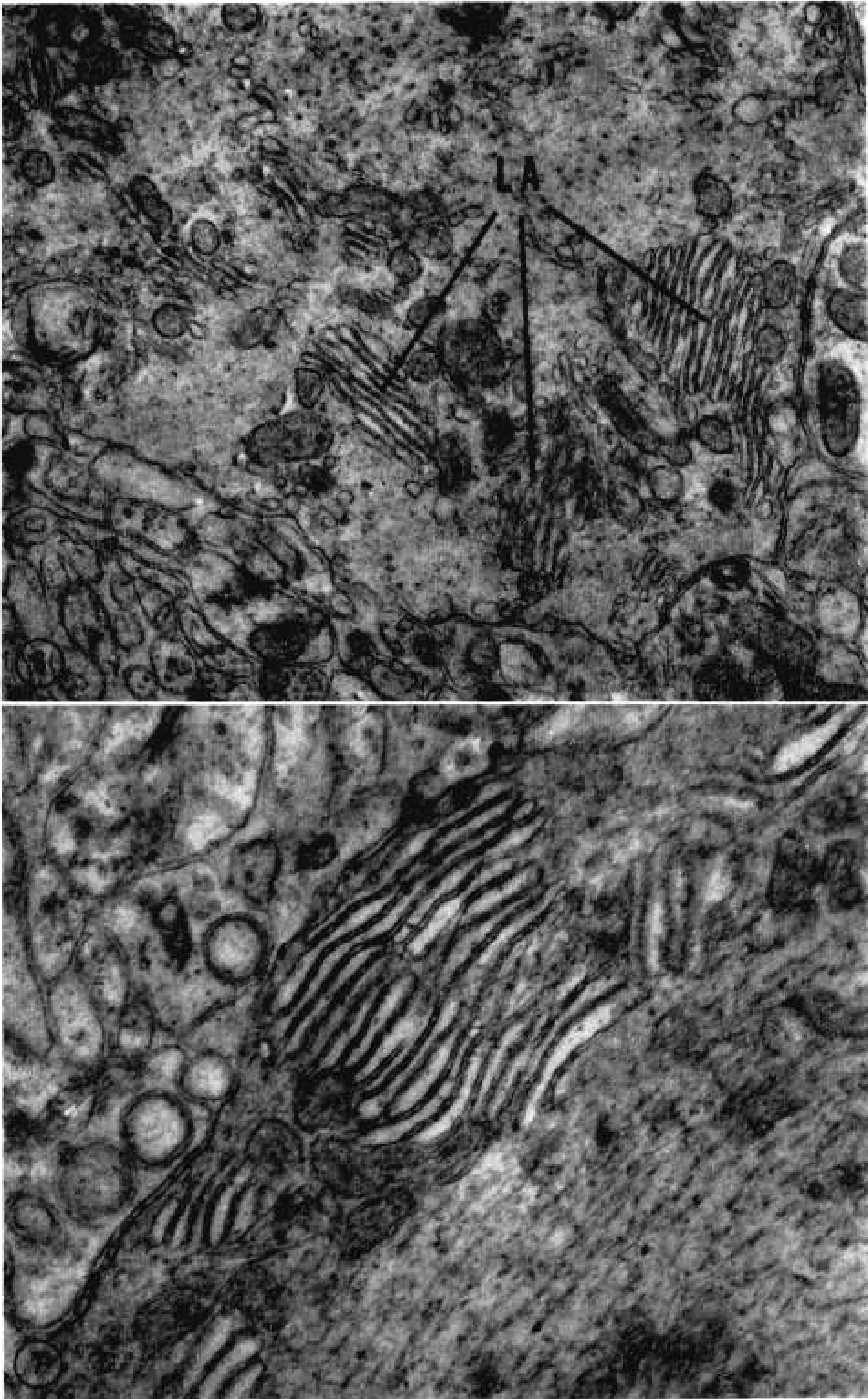

FIG. 6. Lamellar arrays (LA) from the cerebellar molecular layer may be associated with technique of fixation. Magnification from 20,000 ×.

FIG. 7. A higher magnification of a lamellar array. Note the empty cisternae and dense material between the parallel membranes. Magnification from 40,000 ×.

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STEROID THERAPY Topical applications of steroids may suppress the pituitary-adrenal axis. Concentration of steroid is a significant factor in the suppression of adrenal function. Extent of the body area exposed and the status of the skin barrier also are important variables in influencing the presence or absence of percutaneous absorption sufficient to produce adrenal suppression. (*March, C., and Rea, T. H.: Adrenal Function and Topical Steroid Therapy, Clin. Pharmacol. Ther. 6: 43 (Jan.–Feb.) 1965.*)

NEWBORN PNEUMOTHORAX Spontaneous pneumothorax can occur in the newborn infant, and if not recognized may have fatal consequences. It should be suspected in any infant in respiratory distress. It may be caused by over-enthusiastic attempts at resuscitation with pressure breathing apparatus, but more often it occurs spontaneously. In the latter instance it is probably due to occlusion of some of the smaller air passages by aspirated meconium. The patient with a small pneumothorax and only moderate distress may recover completely with no treatment other than observation in an oxygen-enriched atmosphere. If considerable distress persists, continuous drainage of the pneumothorax should be provided by means of an intercostal drain and an underwater seal. (*Ashmore, P. G.: Spontaneous Pneumothorax in the Newborn, Canad. Med. Ass. J. 92: 309 (Feb. 13) 1965.*)

CONSTANT DEPTH ANESTHESIA Anesthesia may introduce an undefined variable in experimental situations, particularly in respiratory studies. More dependable control of anesthetic depression should be achieved by constancy of the agent rather than in the responses it produces. In dogs, each increment of halothane diminished respiratory response to carbon dioxide. In deep halothane anesthesia respiratory response to carbon dioxide was flat or negative. Respiratory response to carbon dioxide was essentially constant during an eight hour period of halothane anesthesia with a constant alveolar concentration near the minimum anesthetic concentration. A single injection of 150 mg. of thiopental diminished the response to carbon dioxide in dogs for a minimum of two hours. (*Brandstater, B., Eger, E. I., II, and Edelist, G.: Constant Depth Halothane Anesthesia in Respiratory Studies, J. Appl. Physiol. 20: 171 (Mar.) 1965.*)

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