PERMEABILITY OF MEMBRANES OF CELL APPENDAGES IN THE BRAIN AFTER DEATH

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Preservation of the property of semipermeability of cell appendages in the brain (which is responsible for shrinking) during exposure to hypertonic buffer at various times after death was investigated in experiments on rats. Membranes of the appendages were found to retain the property of semipermeability even 48 h after death. Prefixation of cadaver material in glutaraldehyde has no effect on the sensitivity of the appendage membranes to the osmotic strength of the surrounding solution.

KEY WORDS: Semipermeability of membranes; hypertonic buffer solutions; glutaraldehyde; brain.

Tissue taken from the cadaver is being used increasingly at the present time for the investigation of human neurological and psychiatric diseases. However, the processes of autolysis that develop in cadaver material may distort the possible electron-cytochemical and morphological picture of the disease. This fact necessitates the study of certain aspects of postmortem autolysis of nerve tissue. After death destruction of the intracellular ultrastructures takes place in a liquid environment, i.e., under the influence of the high- and low-molecular-weight substances dissolved and assimulated in the cytoplasm. The brain has a wide spectrum of types of cells which undoubtedly differ in their complement of enzymes, substances dissolved in the cytoplasm, and the composition of the cell organelles. Meanwhile, these cells form large areas of contact with each other through the development of multiple appendages. In order to study the mechanisms of autolysis of nerve tissue cells, it is thus essential to discover whether the process is autonomous and specific for individual cells or whether the cytoplasmic membranes of the cells become permeable after death so that disintegration of the cell structure takes place under the influence of the dissolved substances in the cytoplasm of neighboring cells or even of neighboring regions of the brain.

Permeability of the cell membranes to low-molecular-weight substances at various times after death can be determined by studying changes in the osmotic properties of these membranes. Many investigators have shown that "mild" prefixation in solutions of aldehydes does not change the semipermeability of the membrane. During fixation of sea urchin eggs in solutions of formaldehyde [4] or glutaraldehyde [5], for instance, these fixatives did not disturb the sensitivity of the cells to the osmotic strength of the surrounding solution. The results of an investigation of membrane permeability of the eggs of Limnea stagnalis for ions showed that the membrane remained impermeable after fixation with glutaraldehyde and loses this property completely only after fixation with osmium tetroxide [2]. In analogous experiments with unmyelinated axons from crab legs, the same principles were discovered [1].

The essential object of this investigation was thus to study permeability of the membranes of cell appendages of the mammalian brain during short fixation in glutaraldehyde and the detection of its possible changes during postmortem autolysis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats. The animals were killed by acute ex-

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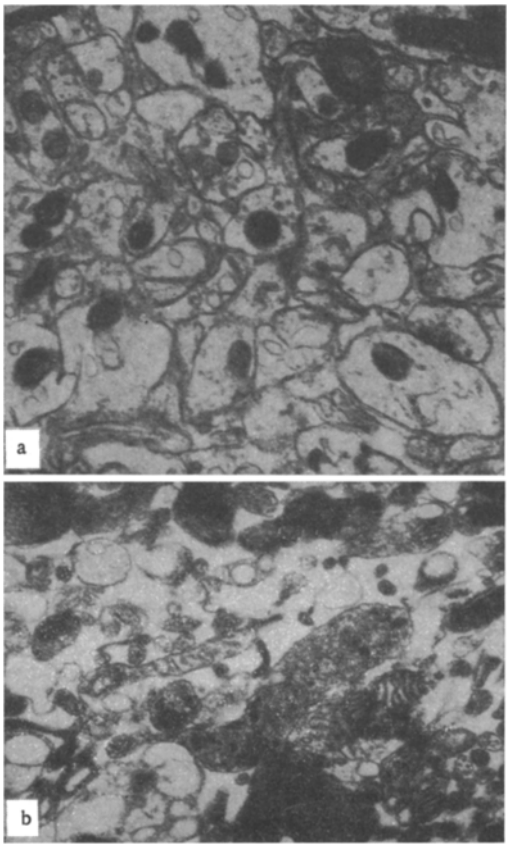

Fig. 1. Neuropil of rat hypothalamus. Material taken immediately after death: a) prefixation in glutaraldehyde diluted with 0.07 M buffer, 21,000 ×; b) prefixation in glutaraldehyde diluted with 0.28 M buffer, 30,000 ×.

sanguination. Small pieces (250-300 μ) of the parietal cortex and hypothalamus were treated by the rapid method [3] for electron-microscopic examination: fixation in 2% glutaraldehyde for 30 min at room temperature and postfixation in 1% OsO_4 solution for 15 min at 4° C. The solvent for the fixatives was NaH_2PO_4–NaOH buffer, pH 7.3 [6].

To determine the membrane permeability of the cell appendages, the fixatives in the experiment of series I were diluted with buffers of different concentrations: 0.07, 0.14, and 0.28 M.

In the subsequent experiments to study changes in membrane permeability of the cell appendages after death, a 0.28 M buffer was used. Two rats were investigated at each time, 0, 2, 6, 12, 24, and 48 h after death. The killed rats were kept at 24° C. To determine the possible action of glutaraldehyde as an agent inducing secondary development of semipermeability in the membranes of the cell appendages, which they may have lost after death, an experiment was carried out excluding glutaraldehyde: Pieces of cortex and hypothalamus were kept for 40 min in 0.28 M buffer at room temperature 48 h after death, and they were then fixed in 1% OsO_4 solution in 0.07 M buffer for 15 min at 4° C.

EXPERIMENTAL RESULTS

On fixation of the fresh material in glutaraldehyde diluted in 0.07 M buffer, no shrinking of the cell appendages was observed either in the cortex or in the hypothalamus (Fig. 1a). This indicated that the osmotic strength of this buffer is near to that of the contents of the appendages. When 0.14 M buffer was used, shrinking of the cell appendages was observed, and the shrinking was greater still when a more hypertonic buffer (0.28 M) was used to dilute the fixatives (Fig. 1b).

Phenomena of postmortem autolysis in the cells of the parietal cortex and hypothalamus were clearly visible in material examined 24 h after death. A characteristic feature of these changes was the partial destruction of the intracellular membraneous structure: mitochondria, granular and agranular endoplasmic reticulum, nuclear membrane, and so on. The process of fragmentation of the ultrastructures became more

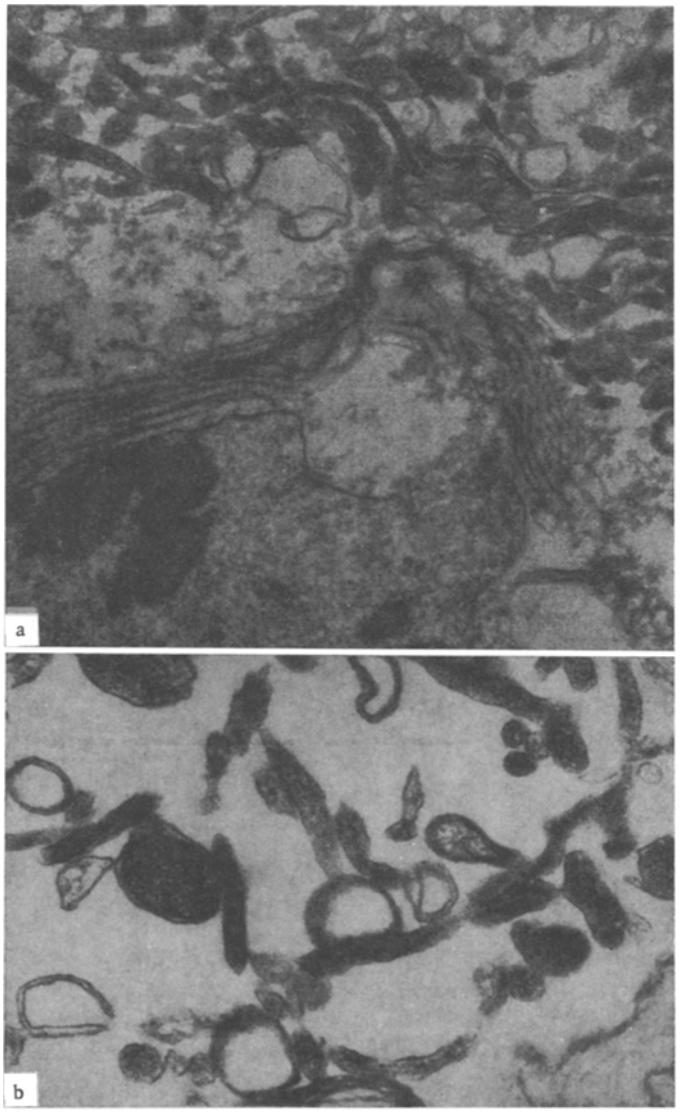

Fig. 2. Neuropil of rat hypothalamus 48 h after death: a) prefixation in glutaraldehyde diluted with 0.28 M buffer, 45,000 ×; b) incubation in 0.28 M buffer without glutaraldehyde, 42,000 ×.

marked 48 h after death, so that the contents of some cells by this time appeared as a homogeneous, finely granular matrix in which lay small fragments of the cytoplasmic ultrastructures. Separation and destruction of the myelin membranes were observed in the neuropil. The ultrastructural components of the axons were disintegrated and the axoplasm had become uniformly electron-dense. However, against the background of these clearly visible postmortem changes, the cell appendages even 48 h after death had not lost their power of shrinking during fixation of glutaraldehyde diluted with 0.28 M buffer, although here and there the membranes of the appendages were loose in texture and had lost their three-layered structure (Fig. 2a).

In the experiment excluding prefixation of the 48-h material in glutaraldehyde, shrinking of the cell appendages also was observed both in the cortex and in the hypothalamus (Fig. 2b). The brain was not examined at later times after death, for by 48 h visceral putrefaction of the cadaver was beginning to develop, although it did not yet affect the brain.

The membranes of the cell appendages of the brain thus retain the property of semipermeability at the time of the investigation until 48 h after death, and short prefixation in glutaraldehyde does not cause any change in this property. The membrane is probably impermeable to molecules or ions which are at least not smaller than the largest ion of the phosphate buffer, the PO_4^{-3} ion. Impermeability of the membrane of

the appendages to ions 48 h after death indicates that molecules and ions in the appendages and comparable in size with the PO_4^{-3} ion (or larger) do not diffuse from them and can play their role in postmortem autolysis only in situ.

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