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A light and electron microscopic study of the preoptic nucleus of the grass frog *(Rana pipiens)*, under normal conditions and following transection of the preoptico-neurohypophysial tract

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Summary. Degenerative and regenerative processes occur in the preoptic neurons following transection of the preoptico-neurohypophysial tract. Three types of responses after transection were observed: affected, recovered, and degenerated neurons. However, transection of the tract did not stop the synthesis of neurosecretory granulated vesicles. The affected neurosecretory neurons showed nuclear changes, increased number of Golgi complexes, and dilated cisternae of rER, as well as, an increased number of dense bodies. The recovered neurosecretory neurons contained long non-dilated cisternae of rER which were organized in a concentric manner. Also seen were large nuclei with evenly distributed chromatin, less active Golgi complexes, and vesicles. The degenerated neurosecretory neurons exhibit pyknotic nuclei, a net of dilated cisternae of rER, dense bodies, and electron dense cytoplasm.

Key words: Neurosecretory granules - Preoptic nucleus - Preoptico neurohypophysial tract - Frog - Transmission electron microscopy - Degeneration

Introduction

It is well established, that the hypothalamic magnocellular neurosecretory neurons synthesize and package into neurosecretory granulated vesicles (NGVs) the two neurohypophysial hormones, vasopressin and oxytocin, together with propressophysin and prooxiphysin (Brownstein et al., 1980) and transport them to the neurohypophysis through the hypothalamo-neurohypophysial tract (Hild, 1951). These substances are stored in the neural lobe, waiting for the appropriate stimulus for release.

The peptidergic neurosecretory neurons of the preoptic nucleus in all lower vertebrates are highly heteromorphic (Polenov and Pavlovic, 1978). Their size, shape, and staining affinity may vary within wide limits. This heteromorphism is thought to be the result of two asynchronous and independent simultaneous processes during development. These being growth, differentiation, and degeneration of neurosecretory neurons of the other (Polenov et al., 1972; Polenov, 1974). In most cases, their appearance is that of typical nerve cells. They have multipolar or bipolar perikarya, but the intracytoplasmic accumulation of secretory product may bring about considerable change in their shapes.

The hypothalamic neurohypophysial system of vertebrates has been the object of a number of electron microscope observations. Zambrano and DeRobertis (1966) suggested that the early stages of synthesis takes place at the ribosomal level, and the product in a dilute macromolecular form is transferred into the cisternae of endoplasmic reticulum. There it is condensed into granules within the Golgi complex. A similar study in the toad (Zambrano and De Robertis, 1968) showed that the perikaryon of the preoptic nucleus is filled with typical neurosecretory granules of peptidergic nature, and the distinctive feature of preoptic neurons in the toad is the presence of large lipid droplets.

Electron microscopic studies of the neurosecretory neurons have shown that their secretory product consisted of elementary granules of varying electron density, separated from the bounding membrane by a narrow electron-lucent ring. The ultrastructural characteristic and number of these NGVs and of other organelles are indications of the functional state of the cells (Zambrano and DeRobertis, 1966; Senchik and Polenov, 1967).

Under normal and experimental conditions, it is generally accepted that the neurosecretory neurons of the magnocellular hypothalamic nuclei degenerate during

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their life cycle in various species (Polenov et al., 1975). Physiological degeneration of neurosecretory neurons is wide spread in poikilothermic vertebrates and occurs most frequently during reproduction (Polenov, 1974). Degenerative processes of the neurosecretory neurons have been observed in two varieties with the light microscope. Cells may show shrinkage and pyknosis of the nucleus or swelling of the cell body and nucleus, followed by disintegration of the cells.

After transection of neurosecretory axons of the supraoptic nucleus in the rat, Raisman (1973) observed shrunken degenerating cells with increased electron-density of cytoplasm. Their organelles also showed degeneration. Eventually, these cells were removed by the phagocytic activity of the glial cells (Raisman, 1973).

The purpose of this investigation is to study the fine structure of neurosecretory neurons in normal conditions and after transection of the preoptico-neurohypophysial tract at different time intervals.

Materials and methods

Thirty-nine frogs *(Rana pipiens)* were used. Thirty-six were operated and had their preoptico-neurohypophysial tract transected and three were sham-operated.

Surgical Procedure

Under anaesthesia with 0.1% Finquel solution (Ayerst), with the aid of a Bausch binocular technoscope, the base of the skull was approached transorally and opened with a dental drill. After removal of the cartilage, the meninges were opened, and the infundibulum was exposed. With a microknife prepared from the edge of a razor blade, the tract was transected just rostral to the pars distalis of the adenohypophysis. In sham-operated frogs, the infundibulum was exposed as for transection, but was not transected.

The frogs were randomly divided into four groups and euthanatized at each of the following times:

Group 1: 1, 4, 24 hours (h.)
Group 2: 13, 16, 36 h.
Group 3: 25, 28, 48 h.
Group 4: 96, 100 h. 5 days

Sham-operated frogs were euthanatized 24 h., 48 h., and 5 days and used as controls for the morphological study.

Electron Microscopic Procedure

The brains were fixed by aortic perfusion of the anesthetized frogs with 5% phosphate buffered glutaraldehyde (0.1M, pH 7.4). Whole brains were carefully removed and their meninges removed. Sections of the hypothalamus containing the preoptic nucleus (PON) were excised and immersed in the fixative for eight hours. Subsequently the tissue was washed three times (15 min. each) with phosphate buffer (0.2M, pH 7.4) in 5% sucrose and stored eight hours at 4°C. The blocks were postfixed in 1% cacodylate buffered osmium tetroxide (0.1M, pH 7.4) for 90 minutes, stained en bloc with 2% veronal acetate buffered uranyl acetate (0.2M, pH 7.4) (Karnovsky, 1967), dehydrated in graded ethanol and acetone, series, and embedded in an Epon-Araldite mixture. Semithin sections, stained with 0.2% Azure II, were used to locate areas of interest. Thin sections were stained with 2% (w/v) aqueous uranyl acetate and lead citrate.

Results

Light Microscopic Findings

The neurons of the preoptic nucleus (PON) of sham-operated frogs were located in the mediobasal hypothalamus in contact with a dense capillary bed. These neurons were easily distinguished from other nerve cells in the neuropil by their large size and oval to spindle shaped bodies. Their nuclei were large, spherical, and centrally located with evenly dispersed chromatin; they sometimes had a shallow indentation and contained at least two nucleoli.

Light Microscopic Findings

In PON of tract-transected frogs two to five days postoperatively Chromatolysis of Nissl substance was accompanied by central aggregation of dense bodies, displacement and deep indentation of nuclei, and increased staining intensity of the nuclear membrane (Fig. 1). A large number of neurons responded to transection by degeneration. The degenerated neurosecretory neurons had intensely stained cytoplasm and pyknotic nuclei. Toward the end of the experimental period, shrinkage of cell bodies and nuclei was noted.

Electron Microscopic Findings

The magnocellular neurons of the PON of sham-operated frogs were large cells containing large spherical, eccentrically located nuclei that sometimes had a shallow indentation. The nuclei were limited by a perinuclear membrane and contained chromatin of even electron density, as well as, two to three prominent nucleoli (Fig. 7).

The cytoplasm was divided into peripheral and central zones. In the peripheral zone, the rough endoplasmic reticulum (rER) occurred in dilated and undilated varieties (Figs. 4, 5, 7). The dilated rER consisted of long, parallel, densely packed stacks of cisternae that were preferentially located in the central zone in the vicinity of nuclear indentations (Fig. 2). The neurosecretory neurons had electron dense cytoplasm (Fig. 3). The undilated cisternae consisted of short parallel stacks of rER. These were located mainly in the central zone and in the vicinity of the nucleus (Fig. 5).

The central zone of the neurosecretory neurons contained numerous spherical or elongated, multilocular

mitochondria. The Golgi complex, located near the nucleus, was composed of elongated curved cisternae and numerous vesicles of variable sizes. Some of the vesicles contained material of varying electron density which is referred to as neurosecretory granulated vesicles (NGVs). The NGVs were located at the mature face of the Golgi complex. Sometimes these were not yet detached from the Golgi membranes (Fig. 5). Furthermore, coated vesicles were also present in the vicinity of the Golgi complex. Dense bodies of diffferent sizes and electron density, multivesicular bodies, and autophagic vacuoles were also present in small number. In addition, tubular profiles of smooth endoplasmic reticulum (sER) were associated with Golgi arrays. Some of sER had dense contents (Fig. 6).

The PON of tract-transected frogs: For ease of description, two periods (28-36 h. and 2-5 days) were distinguished in the evolution of post-operative changes in the PON. During the period 1 to 16 h., cells were not different from those of the sham-operated frogs.

Twenty-eight to thirty-six hours after transection there were noticeable increases in cell bodies of affected neurosecretory neurons. The hypertrophy was confined to neurosecretory cells; hypertrophy was not seen in any other cells in the neuropil.

As early as 28 h. after transection (Fig. 9), the cytoplasm of affected neurosecretory neurons had scattered polysomes. The nuclei were hypertrophied, deeply indented, and contained two to five hypertrophied nucleoli. The perinuclear membrane was intensely stained (Fig. 10). Large and small pleomorphic dense bodies were scattered in the perinuclear region and in the vicinity of the Golgi complex (Figs. 8, 11). The Golgi complex was widely dispersed in the cytoplasm, hypertrophied, and consisted of more than four layers of dilated and undilated cisternae. In the vicinity of its forming face, there were few NGVs (Fig. 11). The rER, located in the perinuclear region, consisted of nondilated short parallel cisternae, heavily studded with ribosomes (Fig. 8). The rEr often contained electron dense material. The NGVs were aggregated mainly in the central zone, facing the indented side of the nucleus (Fig. 10).

Thirty-six hours after transection, the affected neurosecretory neurons that abutted blood capillaries contained a large number of NGVs of variable sizes. These NGVs, were scattered among other organelles. The Golgi complex was widely dispersed and consisted of long cisternae that often contained electron dense material. Near the Golgi were large number of vesicles. A small number of large pleomorphic dense bodies of varying electron density were observed (Fig. 12).

On the other hand, neurosecretory neurons that were not located in direct contact with capillaries had few NGVs in their cytoplasm (Fig. 13). The Golgi complex had five to six stacks of cisternae. It was surrrounded by large pleomorphic dense bodies of different shapes and electron densities. Small membrane-coated and smooth vesicles were scattered near the cisternae (Fig. 13).

Two to five days after transection: Two days after transection, the affected neurosecretory neurons showed changes similar to those described at thirty six hours. Four days (96 h.) after transection, the affected neurosecretory neurons appeared hypertrophied. Their hypertrophied nuclei were limited by a intensely stained unindented nuclear membrane. The rEr was located peripherally and consisted of long undilated parallel cisternae. The NGVs were confined to the central zone; and most were associated with the Golgi complex. Neurons with such characteristics were usually seen abutting the blood capillaries (Fig. 16).

An intermediate stage, between recovered and affected neurosecretory neurons was also seen (Fig. 18). These neurosecretory neurons had widely dispersed fragmented profiles of dilated rER cisternae (Fig. 15). Their cytoplasm contained scattered ribosomes and few NGVs (Fig. 18). The Golgi complexes were hypertrophied and consisted of at least four layers of cisternae. The cisternae were surrounded by numerous pleomorphic vesicles. Occasionally these vesicles contained electron dense material (Fig. 14). Occasional pleomorphic dense bodies and multivesicular bodies which contained occasional vesicles were observed. These vesicles were uniform in size and had evenly distributed electron dense material. The cells contained few mitochondria.

The affected neurosecretory neurons showed similar but more advanced changes as in the previous period. There were greater accumulations of NGVs in the central zone (Fig. 17). The nucleus was distorted and deeply indented. In some neurons, the rER consisted of dilated short cisternae, leaving a narrowed cytoplasmic space between the cisternae. In other cells, the rER cisternae became displaced to the periphery of the cell forming an incomplete zone close to the plasma membrane (Fig. 16). The Golgi complex was widely dispersed in the perinuclear region. Associated with it were a large number of NGVs, as well as empty vesicles. There were also a few large dense bodies, autophagic vacuoles, and mitochondria scattered in the cytoplasm (Fig. 17).

Five days after the transection, some of the neurosecretory neurons were degenerated; others appeared to have recovered (Fig. 23). The recovered neurosecretory neurons were hypertrophied with eccentric nuclei and hypertrophied nucleoli. The nuclei were generally circular in profile but became flattened on the side facing the main mass of the cytoplasm. The rER, which was disorganized by the accumulation of NGVs in the previous period, was again organized into long non-dilated concentric cisternae (Fig. 20). The Golgi complex, which appeared less active than in the earlier groups, had fewer vesicles associated with it and had a few flattened agranular membranes. The affected neurosecretory neurons, abutting the blood capillaries, contained a large number of NGVs scattered in the cytoplasm (Fig. 19). The incidence of large dense bodies decreased; some contained electron-lucent vacuoles. A large number of rounded and elongated mitochondria were present in the cytoplasm. Affected neurosecretory neurons, five days after transection, had similar characteristics to neurons four days after transection.

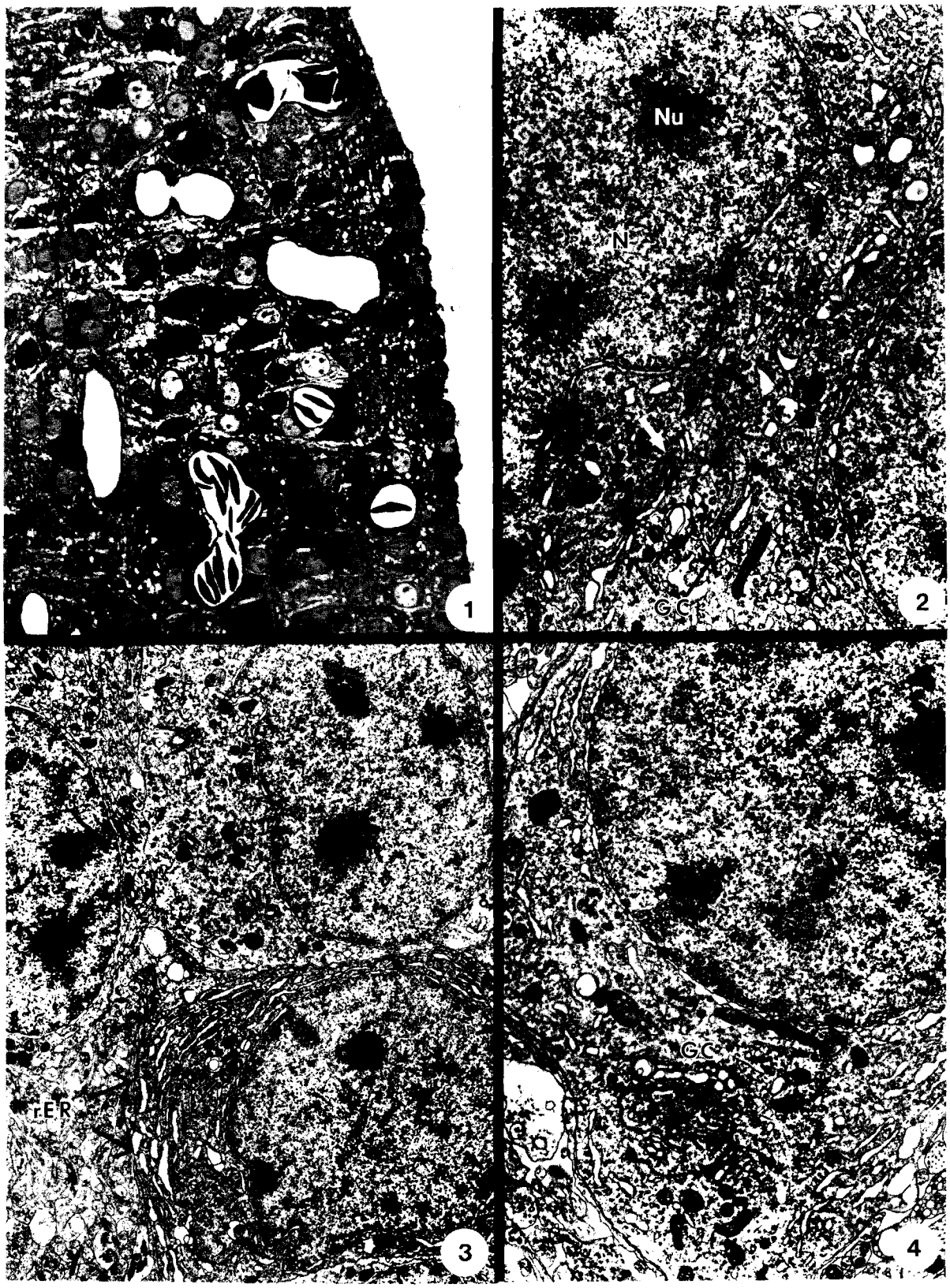

Fig. 1. Preoptic nucleus of *Rana pipiens*, 5 days post-operative. Hypertrophied neurons with large nuclei and prominent nucleoli are seen, along with degenerating dark neurons with pyknotic nuclei × 862

Fig. 2. Preoptic neurons of sham-operated frog. Smooth endoplasmic reticulum (arrow) containing electron dense materials. Nucleus (N), Nucleolus (Nu), Golgi complex (GC) x 10,400

Fig. 3. Preoptic neurons of sham-operated frog. Rough endoplasmic reticulum (rER) greatly dilated, and the cytoplasm packed with ribosomes × 6,500

Fig. 4. Preoptic neurons of sham-operated frog. An unindented nucleus with dilated Golgi cisternae (GC) × 10,400

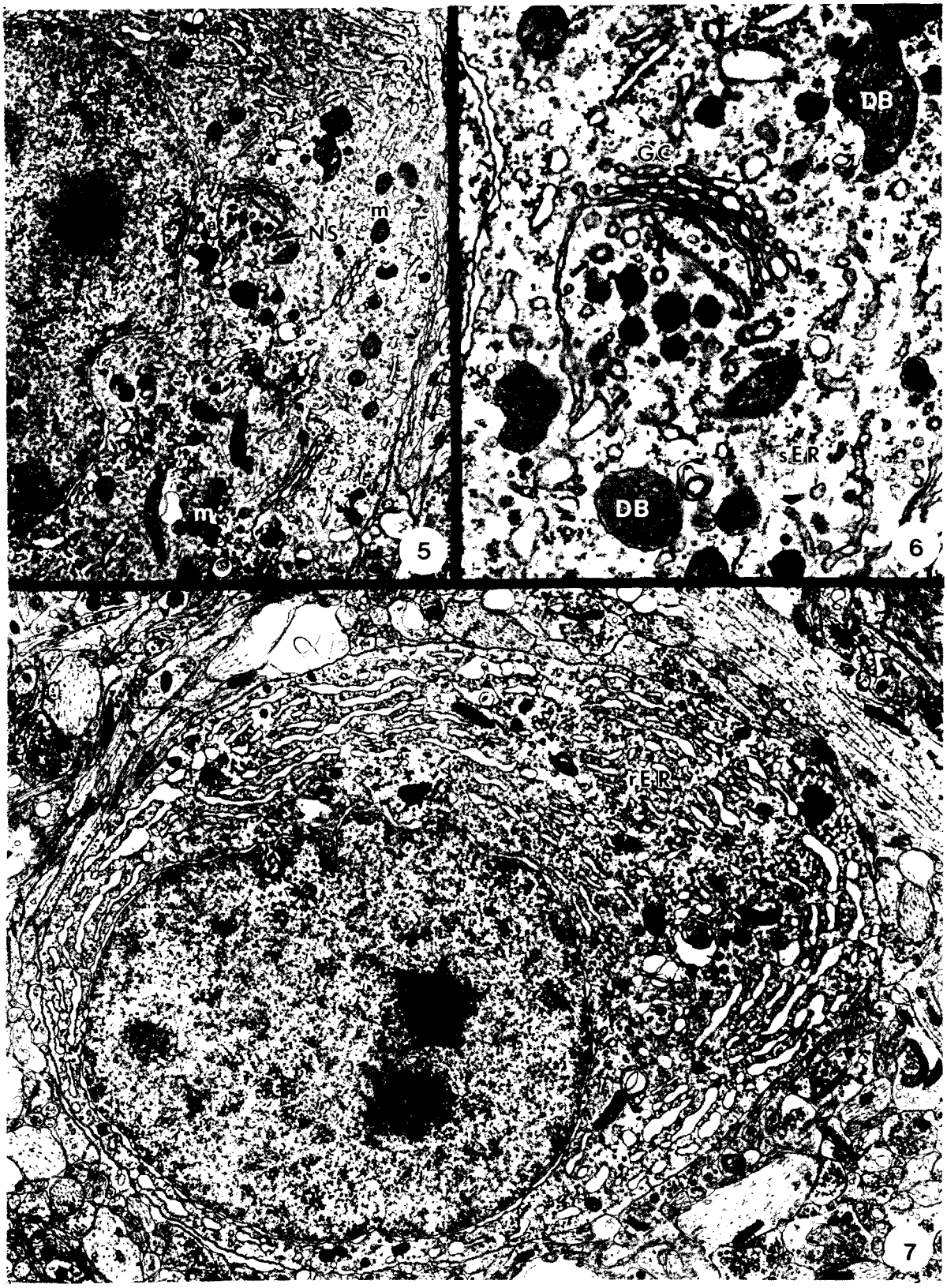

Fig. 5. Preoptic neuron of sham-operated frog. Neurosecretory granules (NS) in the vicinity of Golgi complex. Mitochondria (m) x 10,400

Fig. 6. High magnification of Golgi region (GC) in Fig. 5, coated vesicles (arrow). Dense bodies (DB). Smooth endoplasmic reticulum (sER) × 30,400

Fig. 7. Preoptic neuron of sham-operated frog shows an eccentric nucleus and concentric lamellae of dilated rough endoplasmic reticulum (rER) × 9,250

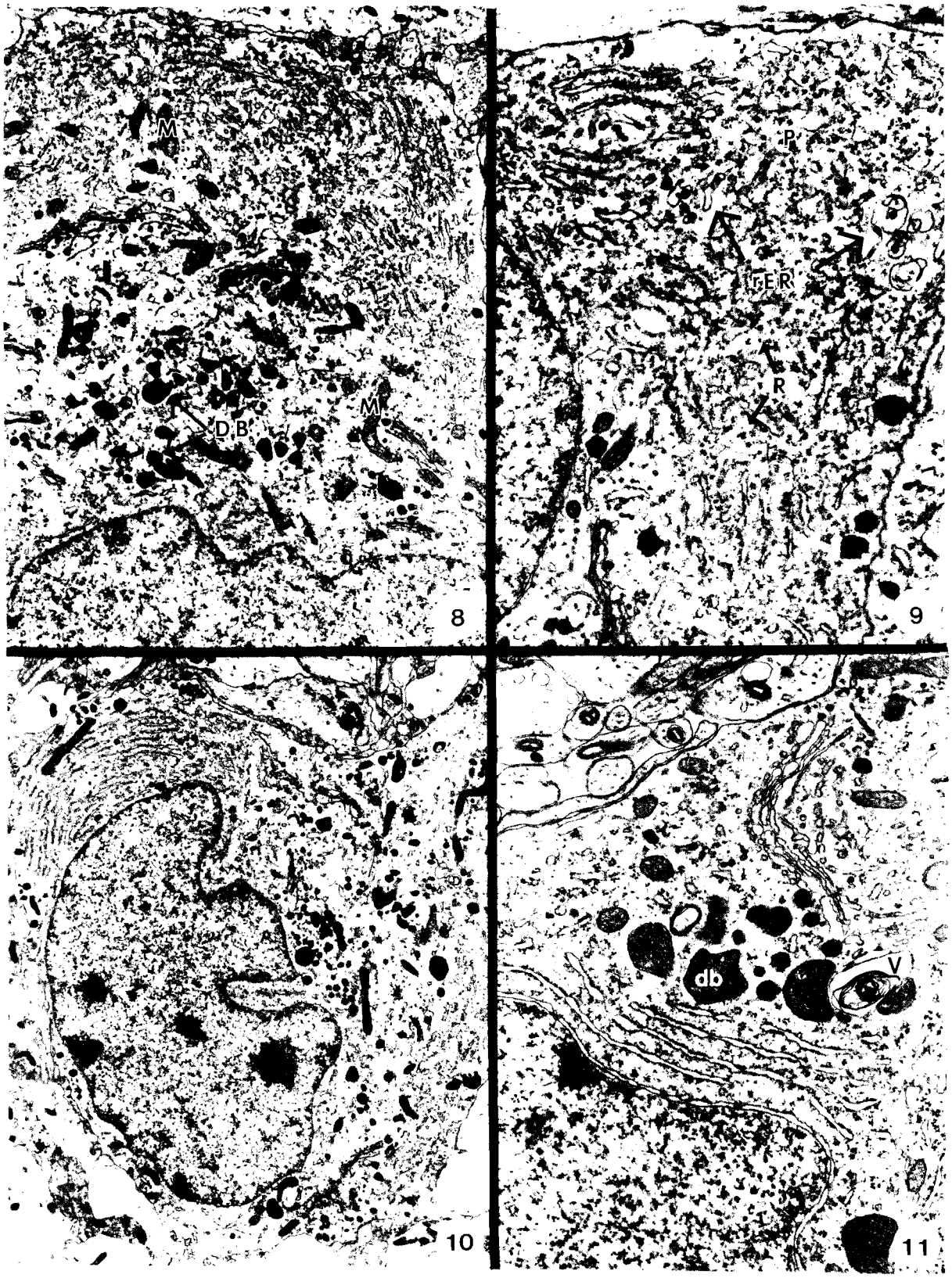

Fig. 8. Preoptic neuron, 28 h. after transection, showing dense bodies (DB) of different size and shape. Mitochondria (M) × 7,800

Fig. 9. Rough endoplasmic reticulum (rER) of PON, 28 h. after transection, is heavily studded with polyribosomes (R) and contains electron dense material × 15,600

Fig. 10. Preoptic neuron, 28 h. after transection, contains very few ribosomes and has a deeply indented nucleus with an intensely stained perinuclear membrane × 6,500

Fig. 11. Few stacks of rough endoplasmic reticulum are seen in the central zone, 28 h. after transection. Large dense bodies (db) and an autophagic vacuole (V) in the vicinity of Golgi complex are seen × 19,500

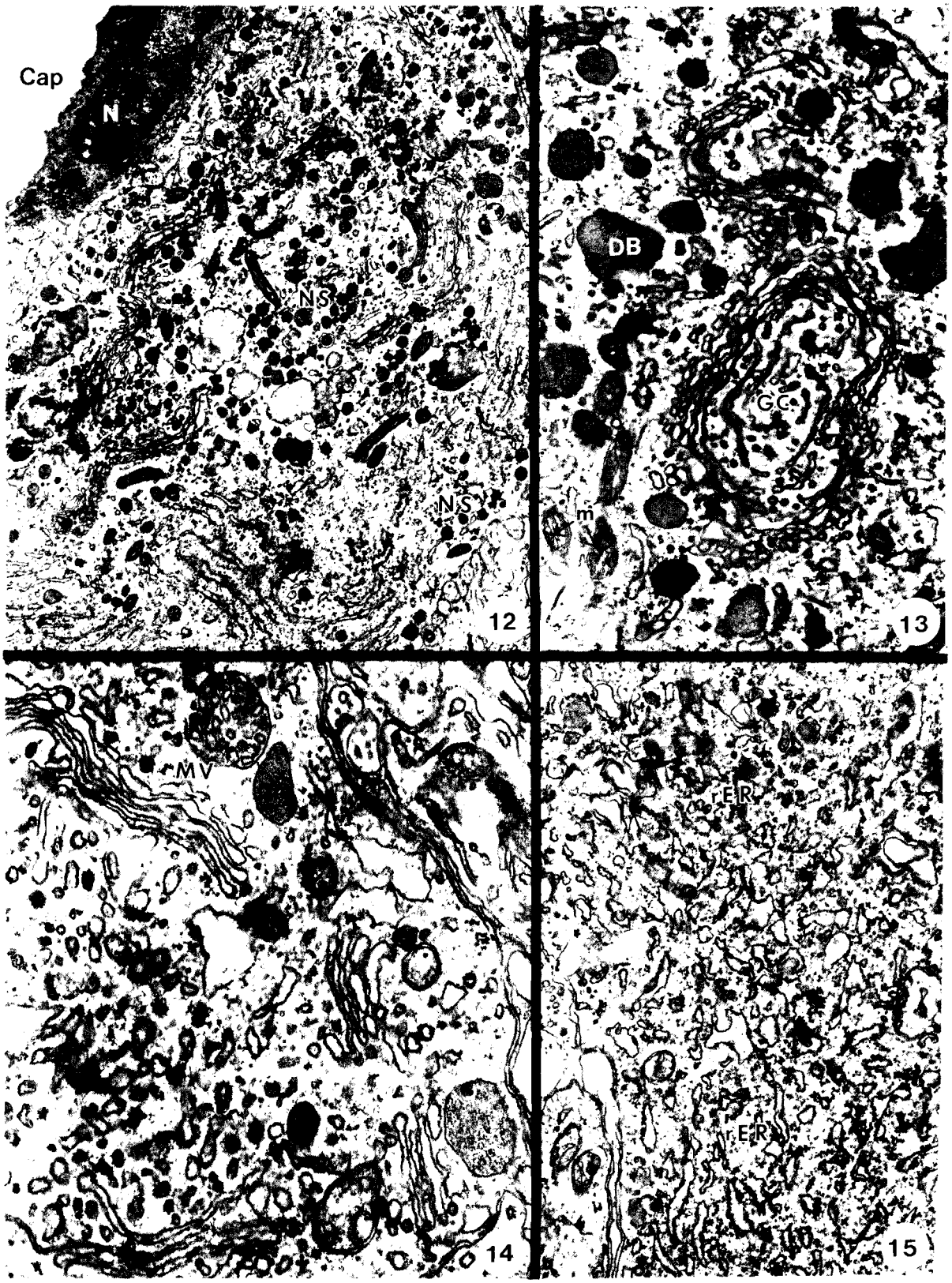

Fig. 12. Preoptic neuron, 36 h. after transection, abutting a blood capillary (Cap), containing massive accumulation of neurosecretory granules (NS). Nucleus of the endothelial cell (N) × 10,400

Fig. 13. Preoptic neuron, 36 h. after transection. Hypertrophied Golgi complex (GC) surrounded by large dense bodies (DB). Mitochondria (m). × 21,000

Fig. 14. Preoptic neuron, 96 h. after transection, several stacks of Golgi cisternae surrounded by vesicles of different size and electron density. Multivesicular body (MV) × 39,000

Fig. 15. Preoptic neuron, 96 h after transection, showing diilated cisternae of fragmented rough endoplasmic reticulum (rER), containing electron dense material × 22,100

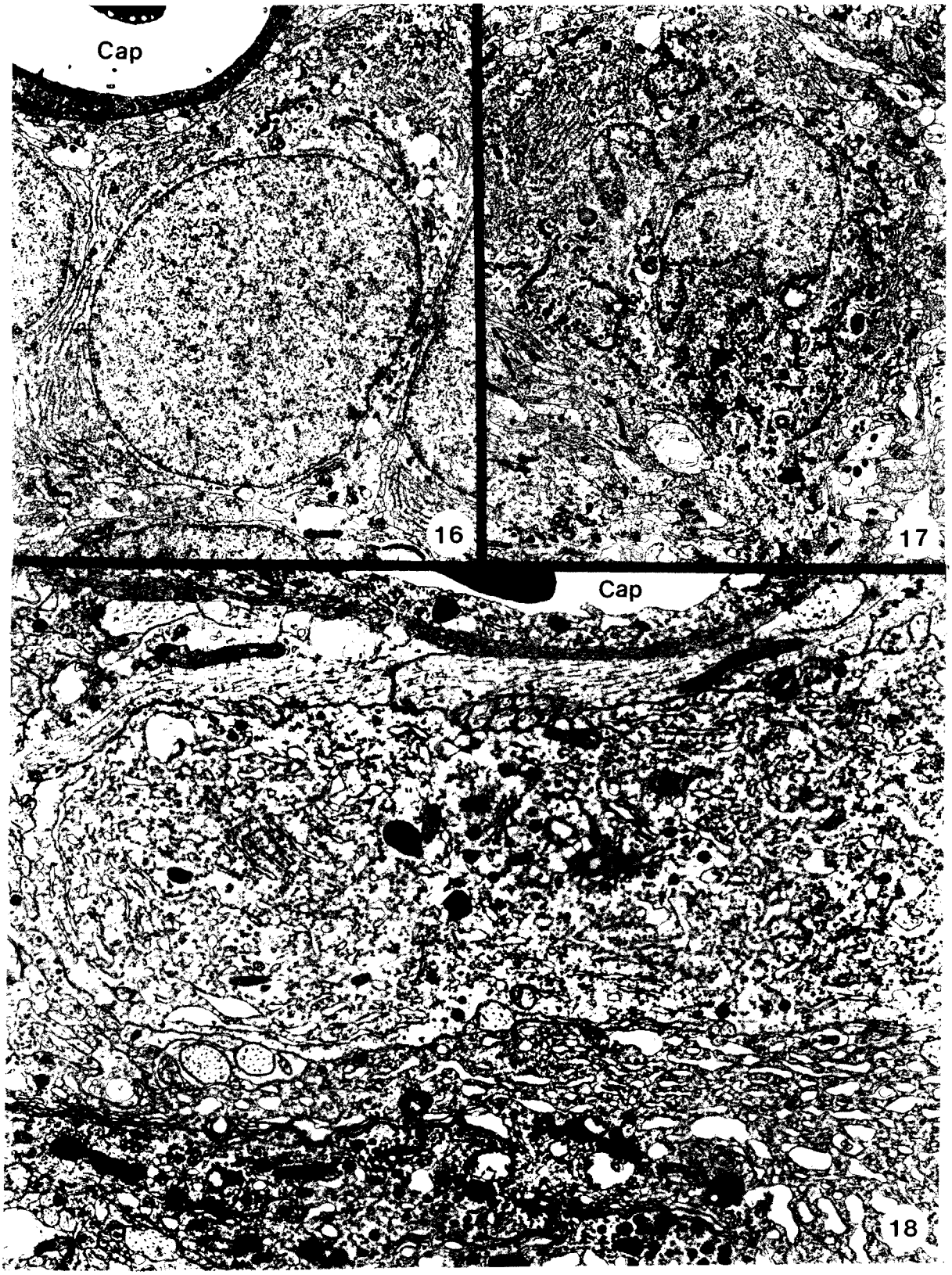

Fig. 16. Recovered neuron abuts blood capillary (Cap), seen with hypertrophied spheroid nucleus, 96 h. after transection × 5,200
Fig. 17. Affected neurosecretory neuron with distorted and deeply indented nucleus, having massive accumulation of NGVs, 96h. after transection × 5,200
Fig. 18. Preoptic neuron in an intermediate stage, showing dilated and undilated cisternae of rER, 96 h. after transection. Capillary (Cap) × 11,000

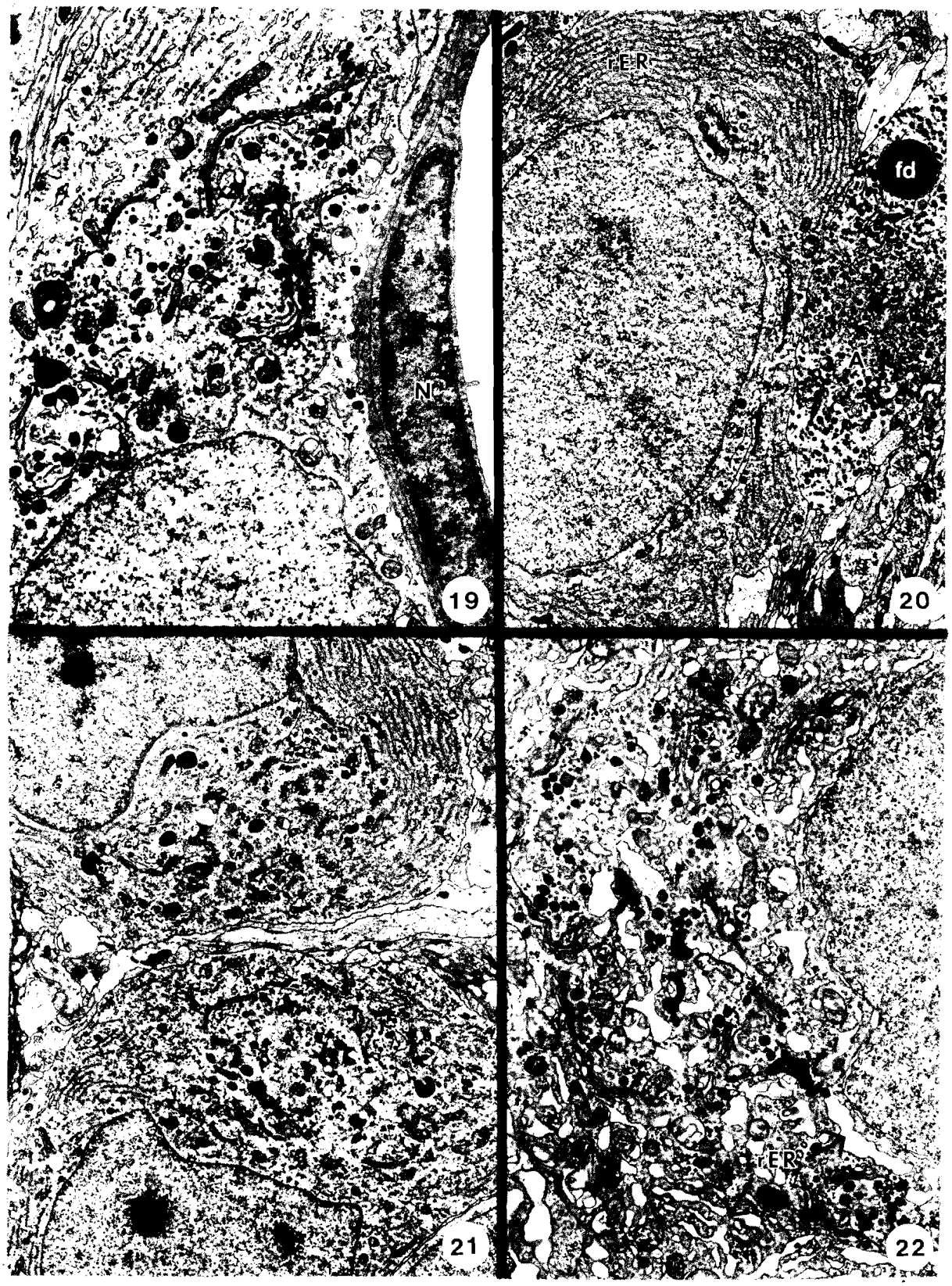

Fig. 19. Preoptic neuron, 5 days after transection, abutting the blood capillary has normal characteristics. Nucleus of endothelium (n) × 10,000

Fig. 20. Recovered preoptic neuron, 5 days after transection, has a large central nucleus and concentric lamellae of rough endoplasmic reticulum (rER). Axon (A) is filled with NGVs and contains a fat droplet (fd) × 6,250

Fig. 21. Affected neurosecretory neurons, 5 days after transection, have intensely stained perinuclear membranes and central zones are filled with mitochondria, dense bodies, and NGVs ×5,600

Fig. 22. Preoptic neuron, 5 days after transection. Large number of dilated fragments of rough endoplasmic reticulum (rER) × 10,000

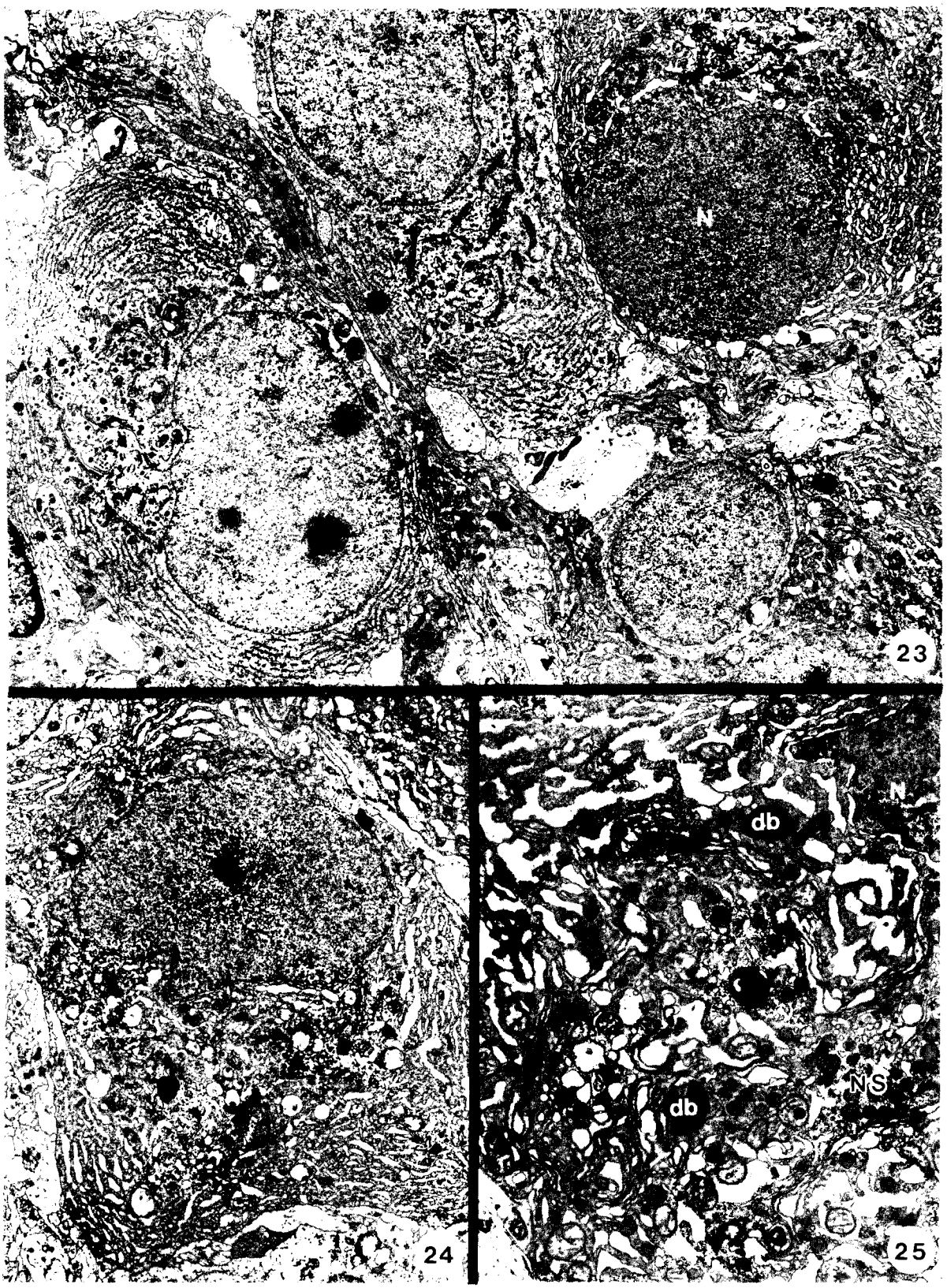

Fig. 23. Five days after transection, compare the degenerated neuron (upper right corner) which has a spherical pyknotic nucleus (N) with the recovered one, lower left corner × 4,625
Fig. 24. Degenerated neuron, 5 days after transection, shows displacement of the pyknotic nucleus and dark cytoplasm × 5,200
Fig. 25. Degenerated neuron, 5 days after transection, has communicating channels of dilated cisternae of rER, and pyknotic shrunken nucleus (N). Large dense bodies (db), neurosecretory granules (NS) × 15,600

These neurons had nuclei with two to four nucleoli, intensely stained nuclear membranes, a large number of NGVs, mitochondria, and dense bodies (Fig. 21).

Five days after transection, there were some neurosecretory neurons showing degenerative changes. The cells were shrunken. The nuclei were dense, pyknotic, and shrunken (Fig. 24, 25). The cytoplasm was packed with ribosomes and contained autophagic vacuoles and dense bodies. The rEr appeared as fragmented, dilated cisternae in the central zone and peripheral cytoplasm. The dilated cisternae fused to form continuous channels that occupied most of the cytoplasm and caused displacement of the nucleus toward the cell membrane (Figs.22, 23). Finally, it became difficult to identify the cellular organelles of the degenerated cells with certainty. The intricate anastomoses of dilated cisternae of rEr reduced the intracytoplasmic space, giving the appearance of electron-dense cytoplasm. Degenerated cells contained hypertrophied Golgi complex, and their cytoplasm was filled with NGVs as well as large dense bodies (Fig. 25).

Discussion

The ultrastructural characteristics of neurons of the preoptic nucleus (PON) are similar to those described by Palay (1960) in the fish. Sometimes they abut on the wall of the blood capillaries, and sometimes the neurons are closely apposed to each other. Within the perikaryon of the PON, the peripheral Nissl substance (viewed by light microscopy) consisted of rough endoplasmic reticulum (rER) in concentric parallel lamellae.

Transmission electron microscopy of controls showed that the rER neurosecretory neurons had shortened cisternae. The rER was observed in two principal forms, dilated and undilated, with a variety of forms in between these two. A few dilated cisternae of rER were observed near the periphery of cell membrane. In addition, neurons with dilated cisternae had a large number of ribosomes on and between the cisternae. The reason for dilatation of the rER is not known. Possibly the cisternal dilatation represents either a disturbance in the synthesis of secretory material formed at the ribosomal level of the rER, or an imbalance between synthesis of secretory materials in the rER and packaging by the Golgi complex. Furthermore, coated vesicles were present in the vicinity of the Golgi complex. Dense bodies of variable sizes and electron density, multivesicular bodies, and autophagic vacuoles were present in small numbers and were considered normal cytoplasmic inclusions. This observation is similar to those of Zambrano and De Robertis (1966, 1968).

Transection of the hypothalamo-neurohypophysial tract does not stop the synthesis of neurosecretory material. Therefore, neurosecretory material will accumulate distally in the axons, close to the site of transection (Rodríguez and Dellmann, 1970). NGVs accumulate in the perinuclear region of the perikarya, which is similar to their location in normal animals. However, there are increased numbers found peripherally. Only the neurosecretory neurons of transected animals appeared hypertrophied due to an increase in the number of cytoplasmic autophagic vacuoles and pleomorphic dense bodies, in agreement with Morris et al. (1978).

Five days after transection, affected neurosecretory neurons assumed the appearance of normal neurons. It is not clear why these neurons recovered after transection. It perhaps can be attributed to regeneration of some injured nerve fibers of the hypothalamo-neurohypophysial tract (Dellman, 1973), or possibly the ones whose collateral projections are spared (Morris et al., 1978). They have responded in order to compensate for the degenerated neurons. Also, recovered neurons may have an axon dilatation somewhere along their course, before the axons end in the neural lobe. These dilatations may act as a temporary reservoir until neurohaemal contact i established (refer to Fig. 20).

In our experiment, degenerative changes were observed in all time periods postoperatively, but it was most pronounced five days after the transection. The dark cells of light microscopy are the cells that underwent degeneration; these had a large number of ribosomes and dilated rER. The degenerated neurons had dark nuclei, their cytoplasm had become increasingly dense and packed with ribosomes, while the other cellular structures became unrecognizable. Finally, the affected preoptic neurons were shrunken, rounded, and showed evidence of cellular degeneration. The cytoplasm contained autophagic vacuoles and cytoplasmic dense bodies. These observations correspond to those described by other investigators (Novikoff, 1967; Holtzman et al., 1967; Holtzman, 1969; Raisman, 1973; Picard et al., 1977; Morris et al., 1978). They have been shown to contain lytic enzymes, such as acid phosphatase, and involved the process of cytoplasmic involution (Matthews and Raisman, 1972).

The cytoplasmic changes due to transection of the preoptico-neurohypophysial tract indicate a phase of intracytoplasmic digestion or resorption of cell constituents. Small and large electron-dense bodies, multivesicular bodies, and autophagic vacuoles are electron-dense structures which respresent the indigestible remnant resulting from the activity of lysosomes.

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