1-1-1982

Fine structural studies of the uptake of horseradish peroxidase (HRP) in the hypothalamo-neurohypophysial neurosecretory system of the grass frog (Rana pipiens) following transection of the preoptico-hypophysial tract

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by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

Major: Veterinary Anatomy

Iowa State University
Ames, Iowa

1982

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GENERAL INTRODUCTION

It is well established that the hypothalamic neurosecretory neurons are essential for maintenance of the normal structure and function of the hypophysis (Harris, 1960; Martini et al., 1970). Studies on the hypothalamus have shown that it contains two systems for controlling pituitary secretion, the parvicellular neurons responsible for the synthesis and release of hypothalamic releasing and inhibiting factors (Blackwell and Guillemin, 1973; Schally et al., 1973; Hayward, 1974), and the magnocellular neurosecretory neurons which are responsible for the synthesis and release of neurohypophysial hormones (Bargmann and Scharrer, 1951; Sherlock et al., 1975; Sterba et al., 1980; Swanson and Kuypers, 1980). The peptidergic neurons of the preoptic nucleus of amphibians synthesize vasotocin and oxytocin and their associated neurophysins in their perikarya, transport them intraaxonally to the neural lobe and release them from perivascular axon terminals in the neural lobe (Matthews et al., 1973; Morris et al., 1978; Morris and Nordmann, 1980). Despite their endocrine function, these neurons possess all the morphological and functional characteristics of ordinary nerve cells; they receive nerve impulses from synapses and conduct them, and combine this activity with that of an endocrine cell which synthesizes and releases hormones.

Electron microscopic studies of neurosecretory neurons have provided much new information regarding the mode of synthesis of neurosecretory materials by these neurons and the contribution of various cell organelles to the elaboration of the secretory product (Morris et al., 1978). They have shown that the biosynthesis of neurosecretory materials is similar to that seen in other protein-secreting cells, and that their packaging into membrane-bounded granules is accomplished by the Golgi apparatus (Bern et al., 1961).

The neurosecretory granules that are synthesized in the hypothalamic neurons contain neurohypophysial hormones and specific proteins "neurophysins" (Ginsburg and Ireland, 1966; Dean and Hope, 1968; Brownstein and Gainer, 1977; Brownstein et al., 1980; Russell et al., 1981).

The first studies of neurosecretory systems with the electron microscope were confined to neurohaemal areas and to neurosecretory tracts (Gerschenfeld et al., 1960; Palay, 1957, 1960). The neurohypophysis was chosen as a model for studying the nature of these neurosecretory granules in several mammals, birds, reptiles, and amphibians (Palay, 1960; Gerschenfeld et al., 1960; Morris et al., 1978; Morris and Nordmann, 1980; Lescure and Nordmann, 1980). In all species, electron-dense granules were described by Palay (1955) who first reported the ultrastructural appearance of osmium-fixed neural lobes. He referred to the granules as elementary

neurosecretory granules. Many studies have revealed the presence of neurosecretory granulated vesicles (NGVs) in the course of the axons and their terminals, composed of dense granules surrounded by a definite membrane. Investigations have shown that the neurohypophysial hormone is localized within the NGVs of the neural lobe in the form of protein-hormone complex (Lederis and Heller, 1960; Heller and Lederis, 1961; Schapiro and Stjarne, 1961; LaBella et al., 1962; Barer et al., 1963). This was confirmed by Dreifuss et al. (1975) and Morris (1976b).

The neurosecretory axons in the neurohypophysis can be subdivided into three compartments: undilated axons, nerve endings, and axon swellings, all of which contain NGVs (Morris, 1976a). Some of the axon dilatations contain NGVs and microvesicles (Palay, 1957) and are commonly seen abutting the basement membrane of the capillaries. These dilatations are referred to as endings or terminals; undilated axons containing a few neurosecretory granules were also described in this location. Axon swellings containing NGVs and only a few or no microvesicles are only rarely observed in contact with the basement membrane (for review see Morris, 1976a).

Morris (1976a) found that stimulation of the neural lobe will cause release of only a small proportion of the total amount of hormone stored there. This observation has given rise to the concept that there exist within the neural lobe

a readily releasable pool of neurohypophysial hormones and a larger storage pool whose content is more difficult to mobilize (Sachs et al., 1967; Thorn, 1966; Sachs and Haller, 1968). It has been demonstrated that this readily releasable pool preferentially contains newly synthesized hormone (Sachs, 1971). The nerve endings contain the much larger greater proportion of the total number of granules stored in the neural lobe (Morris, 1976a), which act as the only site for the release of the neurohypophysial hormone (Nordmann and Morris, 1976; Morris and Nordmann, 1980).

Recently, Nordmann and Labouesse (1981) observed that neurosecretory granules undergo an aging process, during which the content of the granules mature while they are transported toward the neurosecretory nerve terminals. When the granules reach the neural lobe, almost all the precursor has been converted into protein neurophysins and peptides, which include the hormones oxytocin and vasopressin. The newly formed neurosecretory granules are located in the nerve endings, whereas most of the aged granules cannot immediately release their content and are thus likely to be found in the swellings. The newly formed granules and other granules located at the periphery of axon endings are preferentially released (Sachs and Haller, 1968). Those granules located in close proximity to the plasma membrane readily discharge their content following membrane depolarization.

The neurosecretory nerve fibers and nerve terminals in the neurohypophysis contain, besides elementary granules, other organelles such as electron-lucent vesicles first described by Palay (1957). He has shown two classes of small vesicles: The smaller ones are 43 nm in diameter and are found in densely packed clusters; the larger ones measure 61 nm and are scattered among the neurosecretory granulated vesicles (Theodosis et al., 1977). The nature and function of these microvesicles have been the subject of controversy for many years. Some authors have suggested that the small vesicles contain acetylcholine (Ach), and that Ach is intimately concerned in the release of neurohypophysial hormones from the neurosecretory nerve endings (DeRobertis and Bennett, 1954; DeRobertis, 1962). This concept is based on the similarity of these vesicles to the synaptic vesicles seen in the central and peripheral cholinergic synapses. However, the addition of acetylcholine to the incubation medium of neural lobe in vitro does not stimulate hormone release (Daniel and Lederis, 1966; Dicker, 1966; Douglas and Poisner, 1964; Nordmann et al., 1971). In contrast, acetylcholine stimulates release of vasopressin and oxytocin in vivo and in vitro when acting upon the supraoptic and paraventricular nuclei of the hypothalamus (Pickford, 1939; Pickford and Watt, 1951; Abrahams and Pickford, 1954, 1956). Others have suggested that the microvesicles represent the disintegration product

of neurosecretory granulated vesicles (NGVs) (Holmes and Knowles, 1960; Lederis and Heller, 1960; LaBella and Sanwal, 1965), or that the microvesicles originate by budding from the membranes of the elementary granules (Vollrath, 1970).

Gerschenfeld and his colleagues (1960) established that in the toad NGVs and microvesicles are two different components; the granules were found along the course of the preoptico-neurohypophysial tract, whereas microvesicles were confined to the nerve endings. However, Vollrath (1970) observed that the nerve fiber terminals are not the only sites in which microvesicles are present. They are also found in the preterminal regions of neurosecretory axons and are thought by Vollrath to be derived from sac-like dilatations of microtubules.

Experimental evidence strongly supports the idea that secretion occurs by exocytosis, resulting in the concommitant release of neurohypophysial hormone and neurophysin (Douglas, 1973; Dreifuss et al., 1975; Nordmann, 1976; Morris et al., 1978). During exocytosis, there is a calcium-dependent fusion of the granules with the plasma membrane, and an opening is formed at the site of coalescence which allows passage of the granule contents into the extracellular space (Douglas, 1974; Dreifuss et al., 1975; Thorn et al., 1978).

Matthews et al., (1973) have provided evidence that the granule membranes are not released during secretion, and that

microvesicles are a by-product of exocytosis, representing the form in which excess membrane is recaptured from the axon surface (Nordmann, 1969; Douglas et al., 1970; Nagasawa et al., 1971; Castel, 1973; Theodosis et al., 1976; Nordmann and Morris, 1976; Morris and Nordmann, 1978; Nordmann and Morris, 1980). However, vacuoles are the major route for membrane retrieval after hormone release (Lescure and Nordmann, 1980) and microvesicles are unlikely to arise from the division of vacuoles, recently, it has been shown that microvesicles can accumulate calcium in ATP-dependent manner (Chevallier and Nordmann, 1980).

In 1908, Herring described the presence of hyaline or granular masses in the neurohypophysis of the dog and cat. These masses subsequently have been referred to as Herring bodies. With a variety of techniques, Herring bodies have been found to be large axon dilatations within the hypothalamo-neurohypophysial tract. These dilatations represent sites of accumulation of neurosecretory materials, where degenerative and regenerative processes occur, which may be mechanisms for maintaining adequate hormone turnover in the nueral lobe (Dellmann and Rodriguez, 1970a; Dellmann et al., 1973).

The presence of lytic bodies at different stages of digestion in normal animals suggests that autophagy of secretory granules can take place at a slow rate under normal

conditions (Dellmann and Rodriguez, 1970a; Whitaker et al., 1970). Thus, autophagy of secretory granules may play an important role in the turnover of neurosecretory materials within Herring bodies, particularly for disposal of excess secretory products (Rufner, 1974).

Neurosecretory elements with numerous polymorphous inclusions have been revealed in different species (Dellmann, 1973; Polenov et al., 1974). Polenov et al., (1975) described three types of degenerating peptidergic neurosecretory fibers in the neural lobe of the long dehydrated rat: dark, light, and transitional forms that contain synaptic vesicles, single NGVs, lipid-like products, and lamellar bodies. Fibers that appear dark in light microscope seem to belong to degenerating pychnomorphous neurosecretory cells; their number increased immediately after low damage to the hypothalamo-neurohypophysial system (Zambrano and DeRobertis, 1968; Dellmann, 1973). They contain NGVs, microtubules, shrunken mitochondria, and diffusely distributed fine dense materials. The light neurosecretory fibers are the largest and may transform into large membrane bounded cavities containing flake-like materials and single membrane vacuoles.

Dellmann and Rodriguez (1970a) classified Herring bodies into three types, according to intraaxonal changes following interruption of hypothalamo-neurohypophysial axonal flow. According to their classification, type I Herring bodies

contain accumulations of neurosecretory granules. Type II Herring bodies are characterized by the presence of autophagic vacuoles and multilamellate bodies in addition to electron dense and empty vesicles; these authors suggest that this type of Herring body is in a degenerating phase. Type III Herring bodies are characterised by the presence of dense vesicles which contain materials of variable electron density and are connected to smooth axonal endoplasmic reticulum. They also contain microfilaments and long slender and very numerous mitochondria. Dellmann and Rodriguez believe it is likely that type III Herring bodies are undergoing a regenerative process.

The axonal transport mechanism has gained increased recognition since the first description by Weiss and Hiscoe (1948) of a slow axoplasmic flow directed from the nerve cell body to the periphery. By now, it is well-established that neurosecretory material which is synthesized in the perikarya is transported orthogradely within neurosecretory granules to the axon terminals (Pickering and Jones, 1978).

Recent studies have demonstrated axoplasmic transport in the peripheral nervous system (PNS) in retrograde (LaVail and LaVail, 1972) and anterograde (Grafstein, 1969; Lasek, 1970) directions. Various studies on axoplasmic transport have shown that microtubules are primarily involved in the process of fast transport (Paulson and McClure, 1974, 1975; Ellisman

and Porter, 1980). There is some evidence that the axonal smooth endoplasmic reticulum is also involved in transport (Droz and Giamberadino, 1974; Grafstein, 1978; Rambourg and Droz, 1980).

Recently, HRP has been used successfully in the nervous system as a protein tracer (Nauta and Britz, 1974). It can be demonstrated histo-chemically in cytoplasmic vesicles, multivesicular bodies, and in tubules of smooth endoplasmic reticulum (Kristensson and Olsson, 1976; Alonso and Assenmacher, 1978; Broadwell et al., 1980). Experiments on the hypothalamo-neurohypophysial system have shown accumulation of neurosecretory granules in both the proximal (Dellmann and Rodriguez, 1969; Rodriguez and Dellmann, 1970) and distal (Dellmann and Rodriguez, 1970 a,b) stumps of transected axons of the hypothalamo-neurohypophysial tract, attributed to a bidirectional axoplasmic flow. Application of HRP into the rat hypophysis results in its rapid uptake by the neurosecretory axon terminals (Price and Fisher, 1978). Peroxidase activity has been shown to be localized mainly in vacuoles (Castel, 1973; Theodosis et al., 1976; Theodosis, 1979) and the smooth endoplasmic reticulum (Price and Fisher, 1978; Tsukita and Ishikawa, 1980). LaVail et al., (1980), however, concluded that the smooth endoplasmic reticulum does not play a role in retrograde transport of HRP.

Pituicytes vary somewhat in their morphology, both within animals and between species. Fibrous (reticular) pituicytes, characterized by long branching processes, and adenopituicytes, which have an electron dense cytoplasm with signs of secretory activity, such as well-developed rER, Golgi and multivesicular bodies, have been observed (Dellmann and Owsley, 1969; Dellmann and Sikora, 1981). Kurosumi et al., (1964) believed there is only one type of pituicyte with a different appearance in different functional states. The pituicytes are arranged to form a network around the neuronal elements and are separated from one another by gap junctions (Dreifuss et al., 1975). Although they are not the source of neurohypophysial hormones, an increase in pituitary neural activity of the rat is associated with an increased content of lipid droplets in the pituicytes (Kurosumi et al., 1964; Sachs et al., 1971), and vacuoles with membranes of osmiophilic granules (Krsulovic et al., 1970; Whitaker et al., 1970).

The pituicytes in the neurohypophysis have been shown to include trophic and supportive functions (Hartmann, 1958; Fujita and Hartmann, 1961; Watson, 1974), maintenance of ionic composition of the extracellular space (Dellmann et al., 1974; Stoeckel et al., 1975), phagocytosis of axonal membrane or other neuronal components (Kurosumi et al., 1964; Knowles and Vollrath, 1966; Owsley and Dellmann, 1968; Dellmann, 1973), and feedback control on the hypothalamic centers

(Knowles and Vollrath, 1966; Watt, 1971; Norström, 1974).

Dellmann et al. (1974) have suggested that pituicytes might influence neurosecretion by regulating the ionic environment of the axons. Staining with potassium pyroantimonate gives extensive precipitates in pituicytes, which may indicate the presence of calcium. Therefore, pituicytes might have a role in the control of the availability of this important ion (Stoeckel et al., 1975). Phagocytosis of axons by pituicytes has been observed after transection of the hypothalamo-neurohypophysial tract (Dellmann and Owsley, 1969) and destruction of the paraventricular nucleus (Zambrano and DeRobertis, 1968), but, despite their close anatomical relationship, no physiological interaction between axons and pituicytes has yet been substantiated.

The purpose of this investigation was to study the uptake of HRP in an attempt to answer the following questions:

1- Following its uptake, in which organelles is HRP located?

2- At what rates is HRP retrogradely transported in intact and transected neurons?

3- Is there a quantitative difference in its uptake between intact and transected neurons?

4- What is the fate of HRP in the perikarya?

5- Are the distal portions of transected axons able to take up HRP, and if so, for how long after the transection?

6- Are there any changes in the uptake of HRP by pituicytes following transection of neurosecretory axons?

7- Attempt to characterize the various vesicles and/or membrane-bounded compartments under normal conditions and in transected neurons.

PART I.

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

INTRODUCTION

It is now well-established that the hypothalamic neurosecretory neurons are essential for the maintenance of normal structure and function of the pituitary gland (Harris, 1960; Martini et al., 1970). The versatility of the hypothalamus in its regulation of endocrine, autonomic and other complex functions has been studied extensively (for review see Scharrer, 1974a,b).

In recent years, morphologic and physiologic studies have solved many problems and have established how this relatively small region mediates and organizes the diverse influences which play upon it to produce the appropriate final responses. These studies have shown that the basal hypothalamus contains two systems which control pituitary secretions. One system comprises the "parvicellular" neuroendocrine cells, which synthesize hypothalamic releasing and inhibiting hormones. These hormones control the secretion of the adenohypophysis; they are synthesized within the hypothalamic perikarya and transported via axoplasmic flow to the median eminence. At the median eminence, axon terminals make direct neurohaemal contact with the fenestrated portal capillaries into which the hormones are released to be conveyed by the portal veins to the pars distalis of the adenohypophysis (Blackwell and Guillemin, 1973; Schally et al., 1973; Hayward, 1974). The other system is located in the mediobasal hypothalamus and is composed of magnocellular neurosecretory neurons whose axons

course through the median eminence to terminate at the perivascular spaces of the pars nervosa. These neurosecretory axons store and release their hormones into the peripheral circulation (Bargmann and Scharrer, 1951).

It is now 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), transporting them to the neurohypophysis through the hypothalamo-neurohypophysial tract (Hild, 1951; Dierickx, 1962). These substances are stored in the neural lobe, waiting for an appropriate stimulus for release.

The peptidergic neurosecretory neurons of the preoptic nucleus in all lower vertebrates are highly heteromorphic (Polenov and Pavlovic, 1978), and their size, shape and staining affinity may vary within wide limits. This heteromorphism is thought to be the result of two asynchromous and independent simultaneous processes during development, i.e., the growth, differentiation and degeneration of neurosecretory neurons on the one hand, and the presence or absence of secretory granules in neurosecretory neurons on 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 cytoplasmic characteristics of neurosecretory neurons vary considerably both from species to species and in different stages of the secretory cycle in the same animal. All the elementary organelles have been identified by the classical cytologic methods and electron microscope examination. The cytoplasm, as seen light microscopically, has a granular or vacuolar appearance, the difference reflecting different metabolic activities. The shape of neurosecretory neurons cannot alone provide a criterion for identification of the neurosecretory neurons. The essential morphological feature of neurosecretory neurons that has actually been responsible for the discovery of these neurons are NGVs, which represent the major intracellular storage site for the peptide hormones, vasopressin and oxytocin (Silverman and Zimmerman, 1975) along with their respective neurophysins (Pickering and Jones, 1978).

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 take place at the level of ribosomes, and the product in a dilute macromolecular form is transferred into the cisternae of endoplasmic reticulum, and then condensed into granules within the Golgi complex. A

similar study in the toad (Zambrano and DeRobertis, 1968) showed that the perikarya of the preoptic nucleus are loaded with typical neurosecretory granules of peptidergic nature, and the distinctive feature of preoptic neurons in this species is the presence of large lipid droplets.

The magnocellular neurosecretory nuclei can be divided into different functional areas (Lederis, 1961; Stutinsky and Befort, 1962; Uemura and Kobayashi, 1963). It is known that part of the magnocellular neurosecretory preoptic nucleus is involved in the mechanism of osmoregulation (Bargmann, 1957; Dierickx, 1962; Dierickx et al., 1964), and could as well play a part in the mechanism of ovulation in amphibians (Dierickx, 1963, 1967; Dierickx and Vandesande, 1965).

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

It is generally accepted that the neurosecretory neurons of the magnocellular hypothalamic nuclei degenerate during their life cycle in different species and under normal and experimental conditions (for review see Polenov et al., 1975).

Physiological degeneration of neurosecretory neurons is widely spread in poikilothermic vertebrates and occurs most frequently during reproduction (Polenov, 1974). Two types of degenerative processes of the neurosecretory neurons have been observed 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.

Ultrastructurally, and after transection of neurosecretory axons of the rat supraoptic nucleus (SON), Raisman (1973) observed that the degenerative cells showed shrinkage with increased electron-density of their cytoplasm. Their organelles also showed degeneration. Eventually, these cells are 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 hypothalamo-neurohypophysial tract, in order to assess the uptake of HRP by the hypotalamo-hypophysial system in control animals and following transection of the hypothalamo-hypophysial tract.

MATERIALS AND METHODS

The hypothalamo-neurohypophysial tract of 36 frogs was transected and divided into four groups, following the transection of the hypothalamo-neurohypophysial tract three frogs were killed at each of the following times:

1. 1, 4, 24 hours (h.)
2. 13, 16, 36 h.
3. 25, 28, 48 h.
4. 97, 100 h., 5 days (d.)

In three frogs, the infundibulum was exposed as for transection, but transection was not performed and the animals were killed 24, 48 h., and 5 days. These sham-operated frogs were used as controls for the morphological study.

Surgical Procedure

Under anesthesia with 0.1% Finquel (Ayerst) solution, with the aid of a Bausch binocular technoscope, the base of the skull was approached paraorally 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 rostral edge of the pars distalis of the adenohypophysis.

Electron Microscopic Procedure

The brains were fixed by perfusion of the anesthetized frogs via the aorta with 5% phosphate buffered (0.1M, pH 7.4) glutaraldehyde. Whole brains were carefully removed and freed from meninges; then blocks of the hypothalamus containing the preoptic nucleus were excised and immersed in the fixative for an additional eight hours. Subsequently, the blocks were washed three times 15 min. each, with 0.2M phosphate buffer (pH 7.4) with 5% sucrose in phosphate buffer and stored eight hours at 4 C. The blocks were postfixed in 1% osmium tetroxide in 0.1M cacodylate buffer for 1½ hours, stained en bloc with 2% uranyl acetate in 0.2M veronal acetate buffer (pH 7.4) (Karnovsky, 1967) and dehydrated in graded ethanol and acetone 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[1] and lead citrate.[2]

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[1]Mallinckrodt Chemical Works, St. Louis, Missouri.

[2]Vaughn Inc., Memphis, Tennessee.

RESULTS

Light Microscopic Findings

PON of sham-operated frogs

The neurons of the preoptic nucleus 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 their oval or spindle shaped bodies. Their nuclei were large, spherical and centrally located with evenly disperse chromatin; they sometimes had a shallow indentation and contained more than two nucleoli.

PON of tract-transected frogs

Two to five days postoperatively (Fig. 1), 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. Moreover, a relatively large number of reactable neurons were degenerated. The degenerated neurons had intensely stained cytoplasm and pyknotic nuclei. Eventually, shrinkage of cell bodies and nuclei occurred toward the end of the experimental period.

Electron Microscopic Findings

The preoptic nucleus of sham-operated frogs

The magnocellular neurons of the preoptic nucleus 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 could be divided into peripheral and central zones. In the peripheral zone, the rough endoplasmic reticulum (rER) occurred in dilated and undilated varieties (Fig. 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).

Neurons having dilated cisternae of rER had electron dense cytoplasm due to the fact that they contained a large number of polysomes (Fig. 3). The undilated cisternae consisted of short parallel stacks of rER that were mostly seen located 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, which was located in a large area around the nucleus, was composed of elongated curved cisternae, and numerous vesicles of variable sizes some of the vesicles contained material of varying electron density and are referred to as neurosecretory granules (NGVs) (Fig. 5). The NGVs were located at the mature face of the Golgi complex. Sometimes

these were not detached yet from the Golgi membranes (Fig. 5). Furthermore, coated vesicles were also present in the vicinity of the Golgi complex. Dense bodies of different sizes and electron density, multivesicular bodies and autophagic vacuoles were also present in small numbers (Fig. 6). In addition, tubular profiles of smooth endoplasmic reticulum (sER) were associated with Golgi arrays. Some of sER had dense contents (Fig. 6).

The preoptic nucleus of tract-transected frogs

For ease of description, two periods were distinguished in the evolution of post-operative changes in the preoptic nucleus. 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 In this period, there were noticeable increases in cell bodies of reactive secretory neurons. These increases in size resulted mainly from an increase of the cell cytoplasmic volume due to an increase in the number of cytoplasmic autophagic vacuoles and pleomorphic dense bodies. The hypertrophy was confined to neurosecretory cells; it was not seen in any other cells in the neuropil.

As early as 28 h. after transection (Fig. 9), the cytoplasm of reactive secretory cells had scattered polysomes and 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 and 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 was located in the perinuclear region; it consisted of nondilated short parallel cisternae heavily studded with ribosomes (Fig. 8), and it very 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 reactive neurosecretory cells that abutted blood capillaries contained a large number of NGVs of variable sizes scattered among other organelles. The Golgi complex was widely dispersed and consisted of long cisternae in the vicinity of which a large number of vesicles occurred; the latter often contained electron dense material. A few but large pleomorphic dense bodies of varying electron density were observed (Fig. 12).

On the other hand, neurosecretory cells that were not located in direct contact with capillaries were seen to have few NGVs scattered in their cytoplasm (Fig. 13). The Golgi complex was surrounded by large pleomorphic dense bodies of different shapes and electron densities. It consisted of

approximately five to six stacks of cisternae, with small membrane-coated and smooth vesicles in the vicinity (Fig. 13).

Two to five days after transection : Four days (97 h.) after transection, the neurosecretory cells appeared hypertrophied, their hypertrophied nuclei were limited by an intensely stained unindented nuclear membrane (Fig. 16). The rER was located peripherally and consisted of long undilated parallel cisternae. The NGVs were confined to the central zone where 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 reactive cells was also seen (Fig. 18). These neurosecretory cells had widely dispersed fragmented forms of dilated cisternae of rER (Fig. 15). Their cytoplasm has scattered ribosomes and few NGVs (Fig. 18). The Golgi complexes were hypertrophied, and consisted of at least four layers of cisternae which were surrounded by a large number of pleomorphic vesicles. Occasionally these vesicles contained electron dense material (Fig. 14). In their cytoplasm, there were a few pleomorphic dense bodies, and multivesicular bodies which contained few vesicles of approximately uniform size and rather evenly distributed electron dense material. The cells contained few mitochondria.

The reactive cells, however, showed the same changes as in the previous period but well developed; there were massive

accumulations of NGVs in the central zone to an even greater extent than in the first period (Fig. 17). The nucleus was distorted and deeply indented. In some neurons, the rER consisted of dilated short cisternae, leaving small intracytoplasmic space between them, and therefore, the cytoplasm appeared dark. In other cells, the rER cisternae become displaced to the periphery of the cell where they formed an incomplete zone close to the plasma membrane (Fig. 16). The Golgi complex was widely dispersed in the perinuclear region and associated with it were a large number of NGVs as well as empty vesicles (Fig. 17). 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 cells were degenerated; others appeared to be recovered (Fig. 23). The recovered neurosecretory cells 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 (Fig. 20).

The rough endoplasmic reticulum, 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 appeared less active than in the earlier groups with fewer surrounding vesicles, and few flattened

agranular membranes.

The neurosecretory cells abutting the blood capillaries contained a large number of NGVs scattered in the cytoplasm (Fig. 19). The incidence of large dense bodies decreased, some of them contained electron-lucent vacuoles. A large number of rounded and elongated mitochondria were present in the cytoplasm. Reactive neurons were also seen five days after the transections. Such neurons had the same characteristics as at day four after transection; these neurons had nuclei with two to four nucleoli and intensely stained nuclear membranes. In addition to a large number of NGVs, mitochondria, as well as dense bodies appeared (Fig. 21).

Toward the end of day five after transection, there were some neurosecretory cells that had undergone degenerative processes; the cells were shrunken, the nuclei were dense, pyknotic (Fig. 24, 25) and shrunken. 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 (Fig. 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 between them, giving the appearance of electron-dense cytoplasm. Degenerated cells contained a hypertrophied Golgi complex and their cytoplasm was filled with NGVs as well as large dense bodies (Fig. 25).

Plate 1

Fig. 1. Preoptic nucleus of Rana pipiens, 5 days post-operative time. Hypertrophied neurons with large nuclei and prominent nucleoli. Dark neurons with pyknotic nuclei are degenerated X 862

Fig. 2. Preoptic neurons of a sham-operated frog. Smooth endoplasmic reticulum (small arrow) containing electron dense materials. Nucleus (N), Nucleolus (Nu), Golgi complex (GC) X10,400

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

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

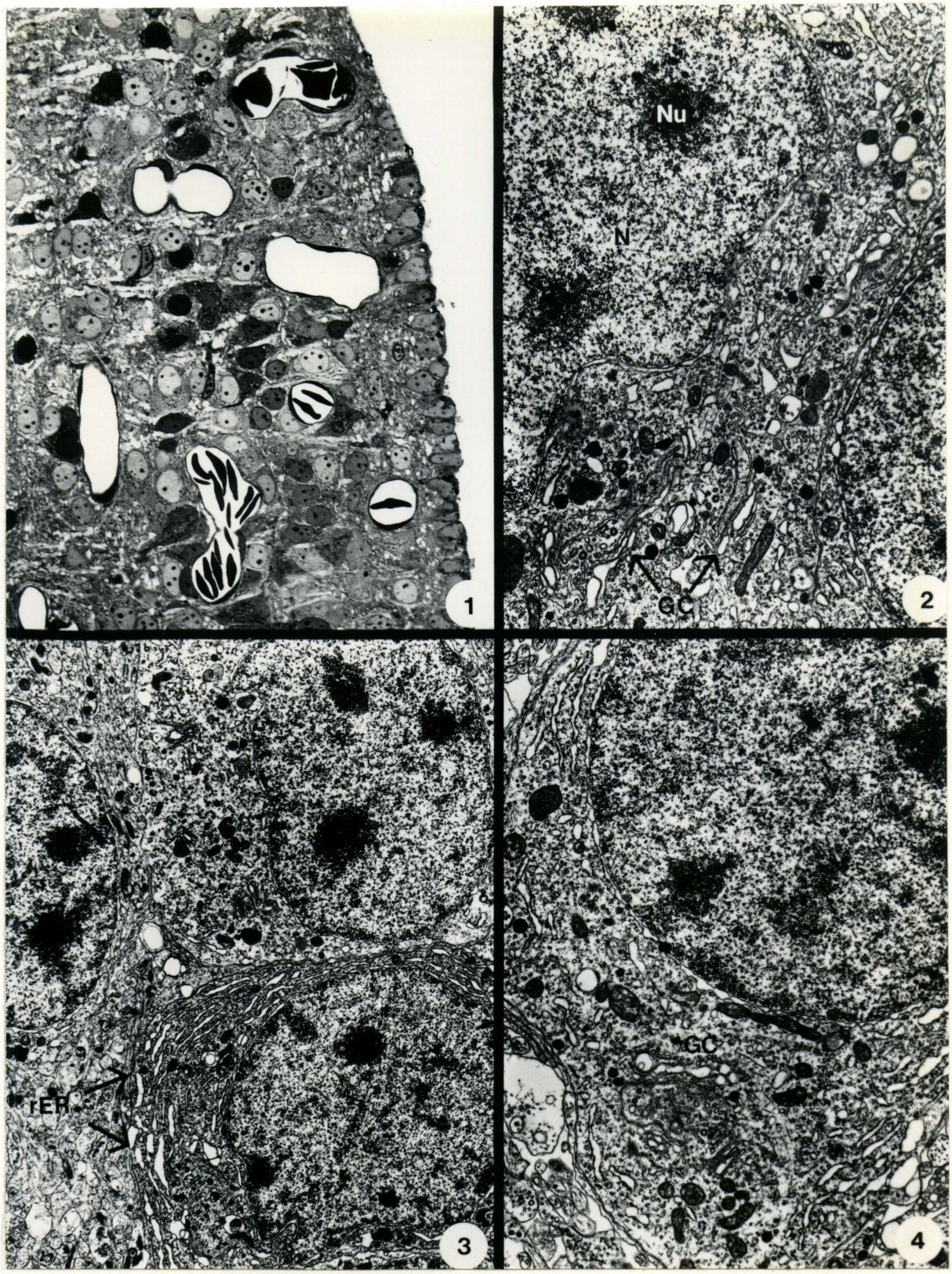

1
Nu
N
GC
2
rER
3
GC
4

Plate 2

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 in Fig. 5, coated vesicles (arrow). Dense bodies (DB). Smooth endoplasmic reticulum (sER) X 30,400

Fig. 7. Preoptic neuron shows an eccentric nucleus and concentric lamellae of dilated rough endoplasmic reticulum (rER) X 9,250

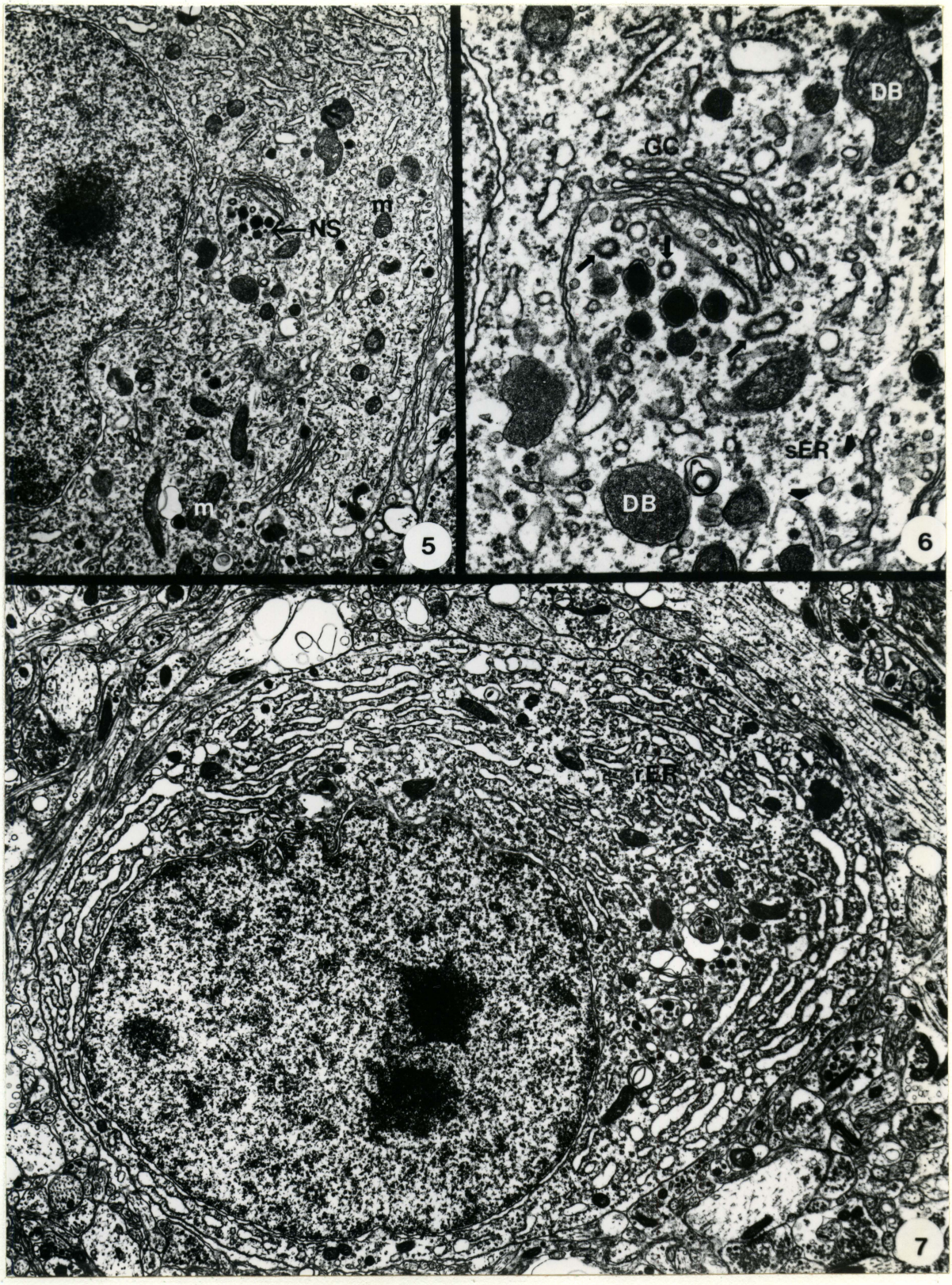

NS
m
m
5
DB
GC
sER
DB
6
rER
7

Plate 3

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

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

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

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

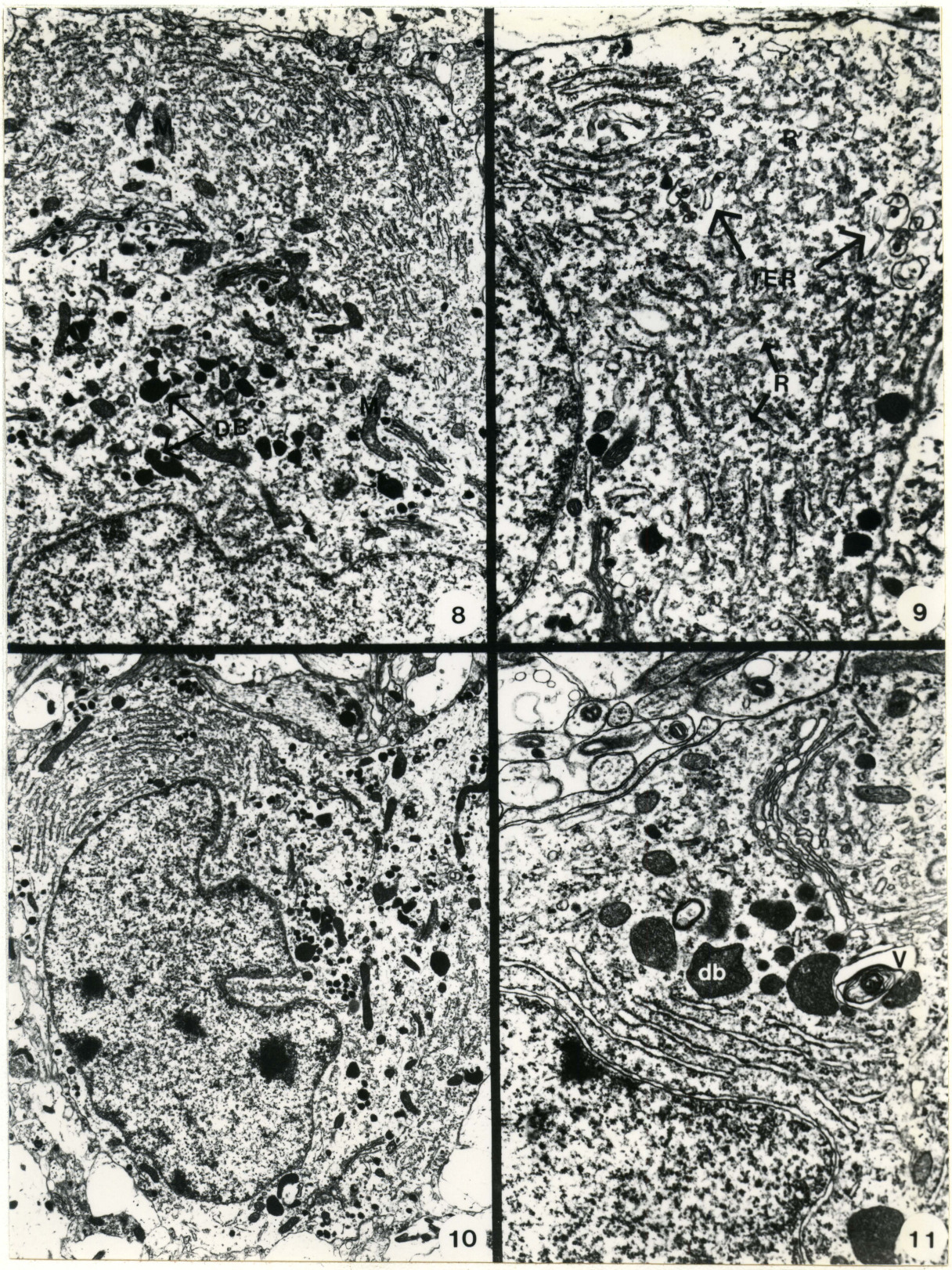

M
M
DB
8
R
TER
R
9
10
db
V
11

Plate 4

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

Fig. 13. A preoptic neuron, 36 h. after the transection. Hypertrophied Golgi complex (GC) surrounded by large dense bodies (DB). Mitochondria (M) X 21,000

Fig. 14. A preoptic neuron, 97 h. after the transection, several stacks of Golgi cisternae surrounded by vesicles of different size and electron density. Multivesicular body (MV) X 39,000

Fig. 15. A preoptic neuron, 97 h. after the transection, showing dilated cisternae of fragmented rough endoplasmic reticulum (rER), containing electron dense material X 22,100

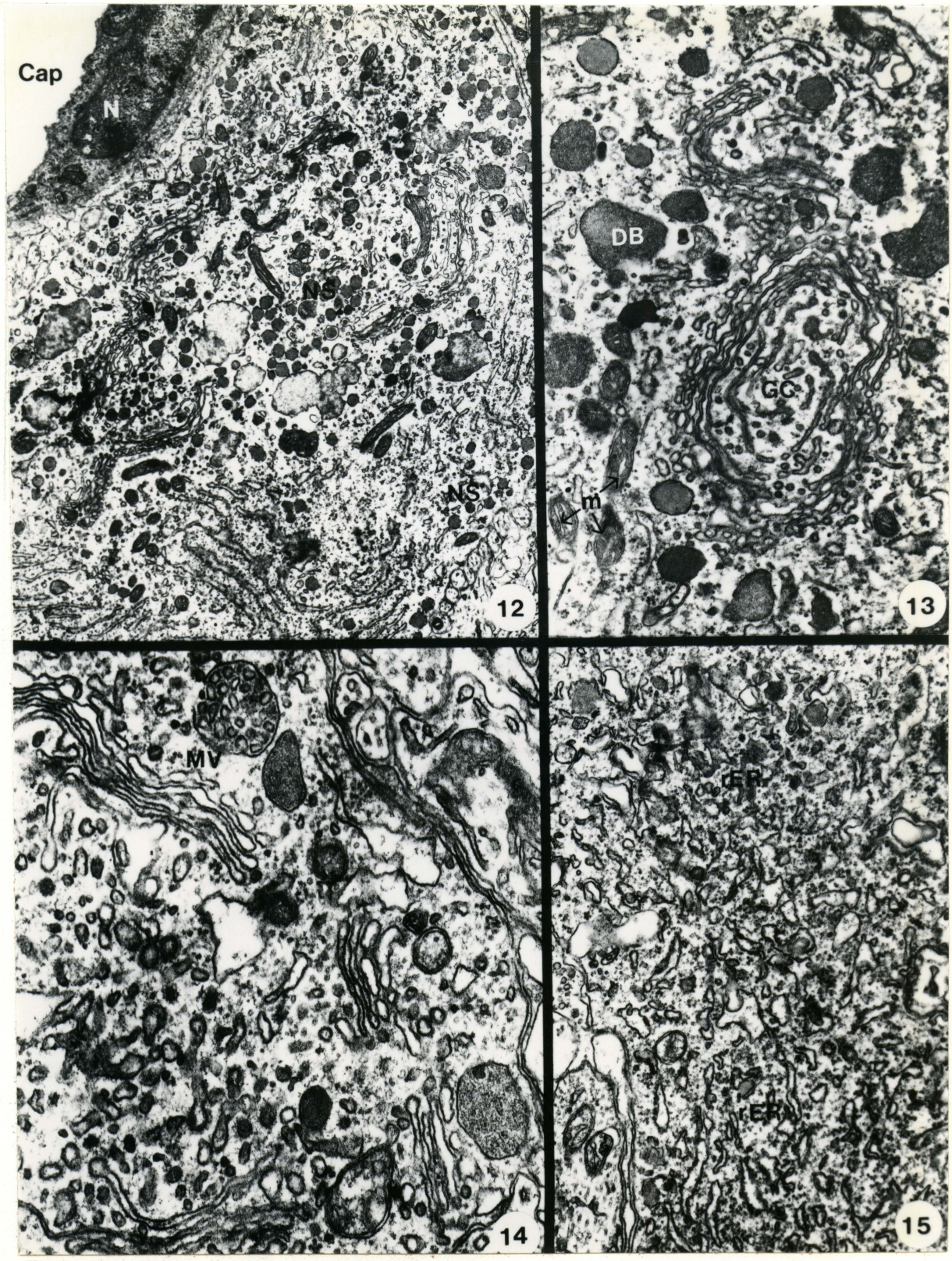

Cap
N
NS
NS
12
DB
B
GC
m
13
MV
14
ER
ER
15

Plate 5

Fig. 16. A recovered neuron abuts a blood capillary (Cap), seen with hypertrophied spheroid nucleus, 97 h. after the transection X 5,200

Fig. 17. A reactive neuron with a distorted and deeply indented nucleus, having massive accumulation of NGVs, 97 h. after the transection X 5,200

Fig. 18. A preoptic neuron at an intermediate stage, showing dilated and undilated cisternae of rER, 97 h. after the transection. Capillary (Cap) X 11,100

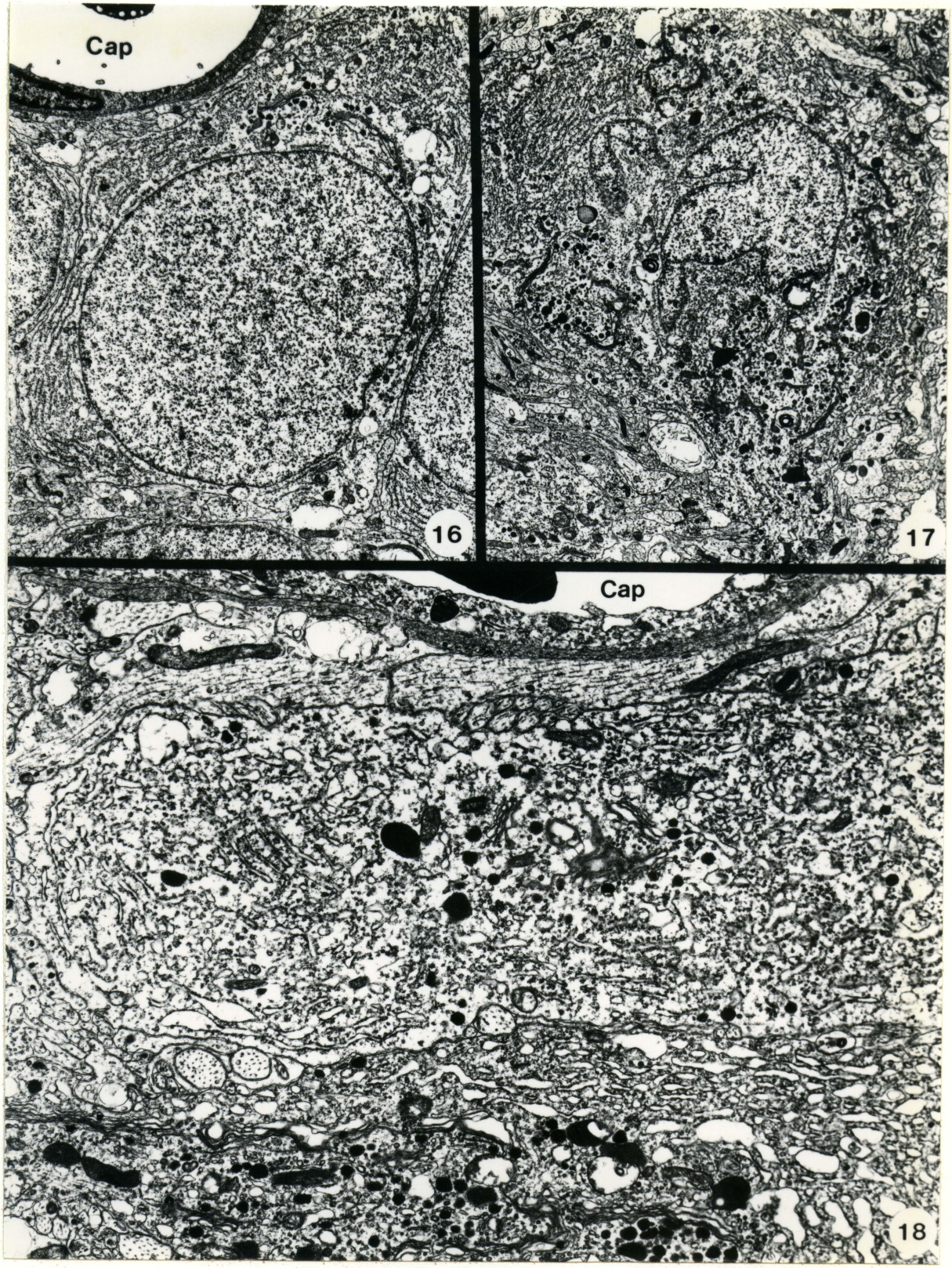

Cap
16
17
Cap
18

Plate 6

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

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

Fig. 21. Reactive neurons, 5 days after the transection, have intensely stained perinuclear membranes and their central zones are filled with mitochondria, dense bodies and NGVs X 5,600

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

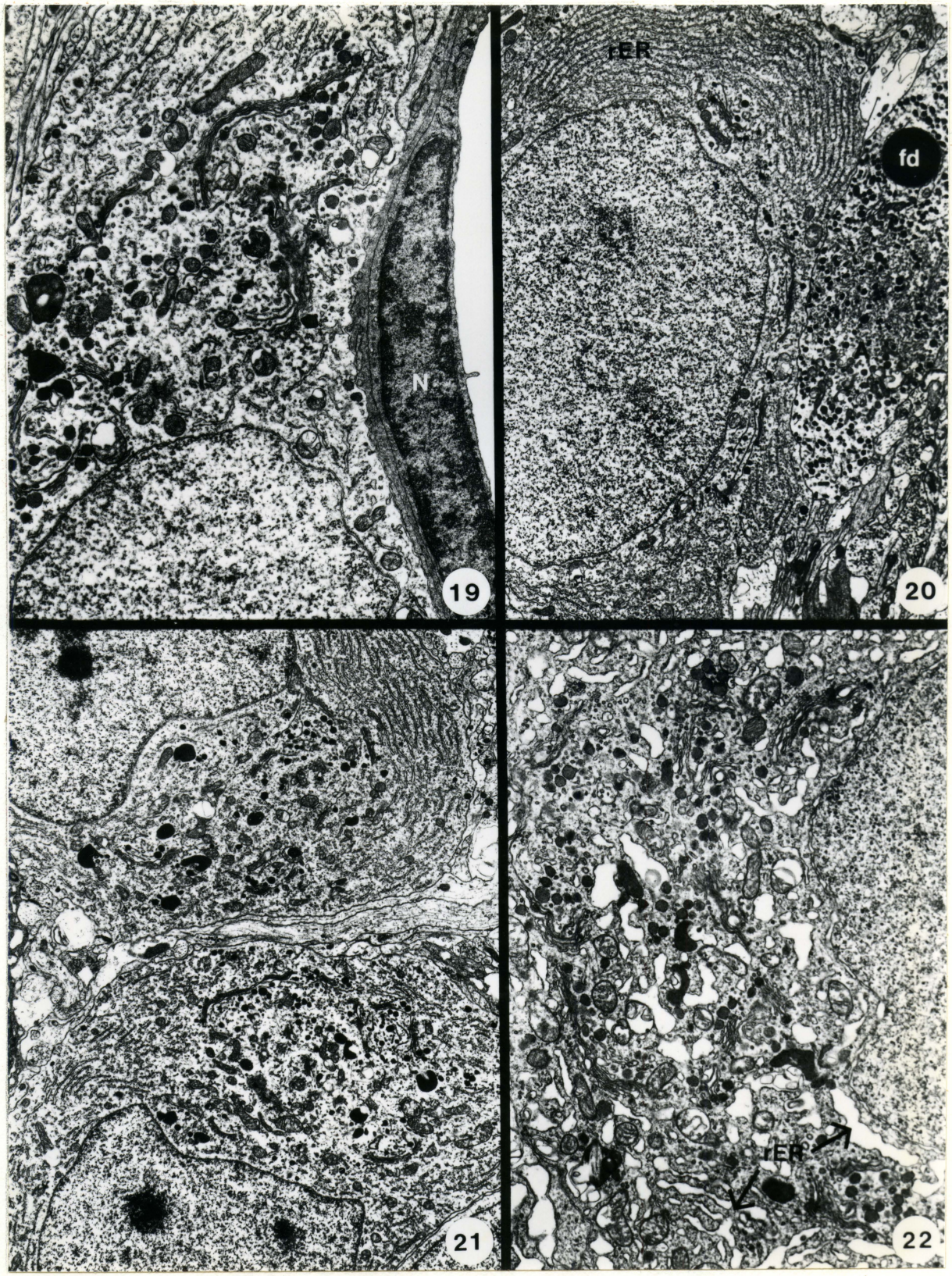
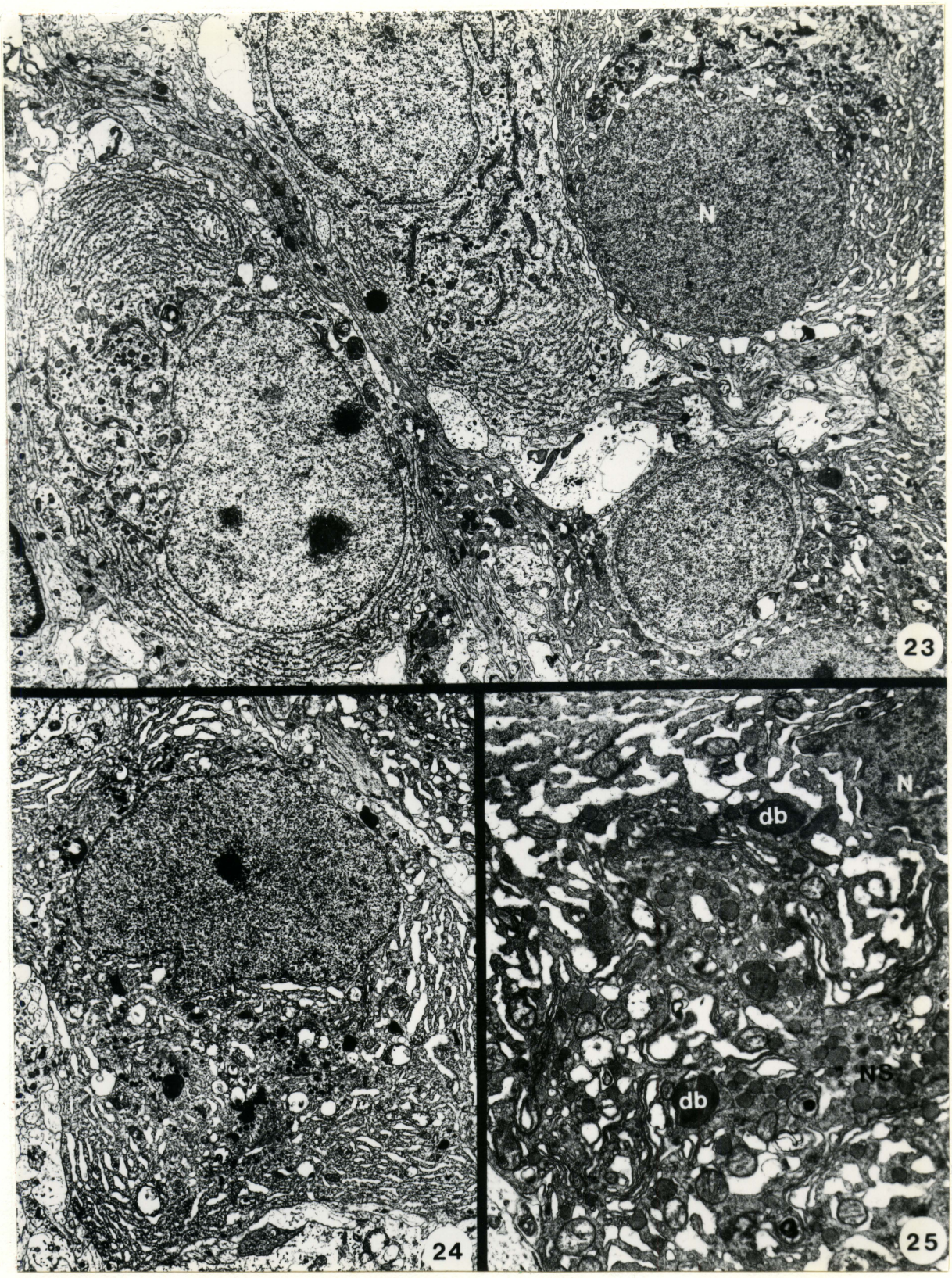

N
19
rER
fd
20
21
rER
22

Plate 7

Fig. 23. Five days after transection, compare the degenerated neuron at the upper right corner which has a spherical pyknotic nucleus (N) with the recovered one at the lower left corner X 4,625

Fig. 24. This degenerative neuron, 5 days after the transection, shows displacement of the pyknotic nucleus and a dark cytoplasm X 5,200

Fig. 25. This degenerated neuron 5 days after the transection, has communicating channels of dilated cisternae of rER, and a pyknotic shrunken nucleus (N). Large dense bodies (db), neurosecretory granules (NS) X 15,600


N
23
24
N
db
db
NS
25

DISCUSSION

It is likely that the ultrastructural characteristics of neurons of the preoptic nucleus 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 perikarya of the PON, the peripheral Nissl substance of light microscopy was seen to consist of rough elements of endoplasmic reticulum in concentric parallel lamellae. The rER can exist in two principal forms, dilated and undilated, with a spectrum of appearances between these two extremes.

In control animals, the majority of neurons have short undilated cisternae of rER. A few dilated cisternae of rER were also observed located at the periphery. In addition, neurons with dilated cisternae have a large number of ribosomes on and between the cisternae of endoplasmic reticulum. Furthermore, coated vesicles were also present in the vicinity of the Golgi complex. Dense bodies of variable sizes and electron density, multivesicular bodies, and autophagic vacuoles were also present in small numbers and were considered normal cytoplasmic inclusions. This observation agrees with those by Zambrano and DeRobertis (1966, 1968).

The reason for dilatation of the rER is not known. Possibly the cisternal dilatation could represent either disturbance of synthesis of secretory material formed on the ribosomal level of the rER, or an imbalance between synthesis of secretory materials in the rER and packaging by the Golgi complex.

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 (Rodriguez and Dellmann, 1970). NGVs accumulate in the perinuclear region of the perikarya, which is similar to their location in normal animals, but many more are found peripherally. Only the neurosecretory neurons of transected animals appeared hypertrophied in agreement with Morris et al. (1978), due to the fact that their cytoplasmic organelles have been increased.

During the observation period, which lasted 5 days, some of the neurosecretory neurons assumed an appearance characteristic of a recovery period. It is not clear why these neurons adjusted to the transection. It can perhaps be attributed to the following reasons. There may be regeneration of some injured nerve fibers of the hypothalamo-neurohypophysial tract (Dellmann, 1973), may be the one whose collateral projections are spared (Morris et al., 1978); they are reacted in order to compensate the degenerated neurons. Also, it could be possible that the surviving neurons may

have an axon dilatation somewhere along their course before the axons end in the neural lobe, and these dilatations could act as a temporary reservoir until neurohaemal contact is established (refer to Fig. 20).

The dark cells of light microscopy are the cells that underwent degeneration; these had a large number of ribosomes and dilated rER.

In our experiment, degeneration can occur at all times during the survival period, but it was more pronounced five days after the transection. The degenerated neurons had dark nuclei, their cytoplasm became increasingly dense and packed with ribosomes while the cellular structures became unrecognizable. Finally, the affected preoptic neurons were very shrunken, became round and showed evidence of cellular degeneration; the cytoplasm contained autophagic vacuoles and cytoplasmic dense bodies. These observations correspond to those described by Novikoff (1967), Holtzman et al. (1967), Holtzman (1969), Raisman (1973), Picard et al. (1977) and Morris et al. (1978). The electron-dense bodies have been shown to contain lytic enzymes, such as acid phosphatase, and to be involved in a process of cytoplasmic involution (Matthews and Raisman, 1972).

The cytoplasmic changes due to transection of the hypothalamo-neurohypophysial tract indicate that there is 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 represent the indigestible remnant resulting from the activity of lysosomes.

In conclusion, degenerative and regenerative processes occur in the preoptic neurons following transection of hypothalamo-neurohypophysial tract. Transection of the tract did not stop the synthesis of NGVs. The reactive neurons show nuclear changes, increased Golgi complexes and dilated cisternae of rER as well as an increased number of dense bodies. The degenerated neurons exhibit pyknotic nuclei, a net of dilated cisternae of rER, few dense bodies and electron dense cytoplasm. It is presumed that dense bodies are secondary lysosomes involved in intracytoplasmic resorption of cell constituents.

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PART II.

A FINE STRUCTURAL INVESTIGATION OF THE UPTAKE OF HORSERADISH PEROXIDASE (HRP) BY THE MAGNOCELLULAR HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM OF THE GRASS FROG (Rana pipiens) UNDER NORMAL CONDITIONS AND FOLLOWING TRANSECTION OF THE PREOPTICO-NEUROHYPOPHYSIAL TRACT

INTRODUCTION

The mechanisms of axonal transport have been extensively studied in neurosecretory neurons, and particularly in the hypothalamo-neurohypophysial system. In these neurons, neurosecretory material, consisting of neurophysin and peptide hormones, is present in membrane-bounded granules, which are the most numerous of the axonal organelles. Following their biosynthesis within the hypothalmic perikaryon, they are transported orthogradely to the axon terminals (Palay, 1955; Heller and Lederis, 1961).

Since the first description by Weiss and Hiscoe (1948) of a slow axoplasmic flow directed from the nerve cell body to the periphery, the axonal transport mechanism, which involves the active movement of materials within axons, has gained increased recognition. The bidirectional movements described by Lubinska (1975) within axons gave direct support to the hypothesis that axonal transport is also in the somatopetal or retrograde direction. It has been demonstrated in a variety of neurons (Lasek, 1970; Dahlström, 1971), and shown to exist at least two distinct rates of movement of materials.

Slow transport has been observed to occur at 1-5 mm/day (Lasek, 1970; Heslop, 1975; Schubert, 1976), while the fast rate of transport is 200-400 mm/day, depending upon the tissue studied (Lasek, 1970; Ochs, 1972). Transport of materials from axon terminals toward the cell bodies has been

observed in mammals at rates of about half of the maximal orthograde value (Kristensson et al., 1971; Edström and Hanson, 1973). However, LaVail and LaVail (1974a) indicated that the retrograde rate of movement is about 84 mm/day.

The axon transport of rapidly migrating proteins has been extensively studied in various systems and its dependence on temperature (Ochs and Smith, 1971a; Gross, 1973), age (Hendrickson and Cowan, 1971; Marchisio et al., 1973), oxidative metabolism (Ochs, 1971; Ochs and Smith, 1971b; Banks et al., 1973), and microtubules (Banks et al., 1971; Byers et al., 1973) are well-documented.

Axoplasmic transport is shown experimentally in a variety of ways, including morphological changes that occur in nerve fibers above a constriction, which indicates a flow of all the nerve content down inside nerve membrane (for review see Grafstein, 1978). These experiments present the best example of what might occur after interruption of axonal flow and the involvement of retrogradely transported materials needed to maintain normal function of the fiber, which also includes the components that enter the membrane to maintain its excitability.

Anterograde and retrograde axonal transport have been widely used during the past decade in the field of neuroanatomy. The methods used are varied: ligature of sciatic nerve trunk, which showed the accumulation of materials at the site of the ligature (Lubinska, 1964; Dahlström, 1971);

cutting of the vagus nerve and tracing the HRP marker up to the perikaryon (DeVito et al., 1974); transection of the hypothalamo-neurohypophysial tract and the formation of tubules in the proximal stump (Dellmann and Rodriguez, 1971). Finally, injections of plant alkaloids such as colchicine and vinblastine, also are shown to block fast (Sjöstrand et al., 1970) as well as slow axonal transport (Fernandez et al., 1970) by binding to tubulin, the protein subunit of the microtubules. Most of these methods have shown evidence for the involvement of microtubules in the process of fast axoplasmic transport (Porter, 1966; Paulson and McClure, 1974; 1975).

It has been suspected that the smooth endoplasmic reticulum convey materials from their sites of synthesis in the perikaryon to the plasma membrane of axons and nerve endings and to the synaptic vesicles (Palay, 1958; Byers, 1974; Droz and Giamberadino, 1974; Grafstein, 1978; Rambourg and Droz, 1980). Droz et al. (1975) observed that sER appears as a continuous intraaxonal pathway bridging the perikaryon and the axon terminals, conveying macromolecular components with the fast axonal transport.

Orthograde and retrograde active transport of macromolecules along the axons has now become one of the most important methods in neuroanatomy. A retrograde transport of material was first shown for the nerve by Lubinska (1964),

who found an accumulation of acetylcholine esterase (AchE) below ligations made in the sciatic nerve as well as above such ligations.

Horseradish peroxidase, a relatively small protein (molecular weight = 40,000), has been used successfully as a protein marker both in vivo (Lasek, 1970; Kristensson and Olsson, 1971b, LaVail and LaVail, 1972) and in vitro (Litchy, 1973). Sensitive localization of HRP is obtained by virtue of the amplification of visible marker through enzymatic activity. Thus, a few molecules of enzyme yield a large number of molecules of electron opaque reaction product (Graham and Karnovsky, 1966).

It has been shown (Nauta and Britz, 1974) that HRP can be used successfully in retrograde axonal transport for identifying the origin of neuron connections which terminate outside or within the central nervous system (CNS), without inducing pathological changes in the labeled cell bodies (LaVail and LaVail, 1972; Kristensson and Olsson, 1973). Initially the technique was thought to depend upon the vesicular uptake of the protein by intact axon terminals and transport of these vesicles back to the neuron cell body (Kristensson and Olsson, 1971a; LaVail et al., 1973). However, in both peripheral nervous system (PNS) and CNS, severed axons also transport HRP-positive granules back to their cell bodies (DeVito et al., 1974; Adams and Warr, 1976). Kristensson and Olsson

(1976) observed that HRP diffused through the damaged axons of the sciatic nerve forming columns of reaction product; at a more proximal level, axons contained HRP in vesicular and tubular organelles, and later nerve cell bodies of the corresponding spinal ganglia showed HRP accumulations in cytoplasmic vesicles, cup-shaped bodies, multivesicular bodies, and tubules of smooth endoplasmic reticulum. Supporting these findings, Alonso and Assenmacher (1978) observed HRP mainly in tubules of sER in the more proximal part of the neurosecretory axons, and in granules and microvesicles of the axon terminals. In contrast, LaVail et al., (1980) observed that HRP was only localized in granules and microvesicles of the axons of PNS.

The phenomenon of retrograde axoplasmic flow may be of significant neurobiological importance, since it could provide a mechanism by which information reaches the nerve cell body from the axons.

There are several possible mechanisms by which a nerve cell body can respond to an axonal injury. The effects on the neuronal cell body of axonal injury to the PNS have been studied extensively by both light and electron microscopy (Cragg, 1970; Matthews and Raisman, 1972). One effect is that alterations in retrograde axonal transport of macromolecules provide the signal for chromatolysis. Materials normally transported in the retrograde direction are hypothesized

to include trophic substances from neighboring cells and target organs (Paravicini et al., 1975; Stoeckel and Thoenen, 1975; Stoeckel et al., 1976), and materials from axons and nerve terminals, some of which may be playing a regulatory role for retrogradely transported trophic substances, nerve growth factor (NGF), or chromatolytic response and nerve regeneration (Stoeckel et al., 1976; Purves and Nja, 1976; Tsukita and Ishikawa, 1979, 1980).

Retrograde transport appears to carry enzymes or signal-substances back to the cell body, which regulates the overall level and perhaps the type of materials synthesized in the soma (for review see Ochs, 1974). This retrograde transport has been examined with light microscopy (Hammond and Smith, 1977; Forman et al., 1977a,b), with the movement of label in damage nerves (Bisby, 1976, 1977; Bisby and Bulger, 1977), and after the uptake of exogenous substance (Rosenbluth and Wissig, 1964; LaVail and LaVail, 1974b).

The retrograde transport of HRP has been found to label different cell components (Kristensson and Olsson, 1971a). Tsukita and Ishikawa (1980) classified membrane-bounded components into two systems from the view point of axonal transport: axonal sER and vesiculo-tubular structures in the anterograde direction. The vesiculo-membranous structures increased in amount inside both myelinated and non-myelinated axons. These structures were composed mainly of

multivesicular bodies and lamellated membranous structures, and some of them seemed to be continuous with the axonal sER (Tsukita and Ishikawa, 1980).

The hypothalamo-neurohypophysial system has been studied extensively by using HRP under normal and experimental conditions to demonstrate the origin of microvesicles that are present in the axon terminals and to study the neuroanatomical configuration of that system (Sherlock et al., 1975; Theodosis et al., 1976; Price and Fisher, 1977; 1978; Mikami et al., 1978; Alonso and Assenmacher, 1978; Theodosis, 1979; Broadwell and Brightman, 1979; Broadwell et al., 1980).

Peroxidase reaction product within the secretory axons was found mainly in vacuolar and C-shaped structures of a size comparable to or larger than the NGVs (Castel and Hockman, 1976; Theodosis et al., 1976). Pituicytes were also shown to take up HRP within numerous membrane-bounded vesicles of various size and morphology (Theodosis, 1979). Price and Fisher (1978) found that axon terminals in the pars nervosa of the rat took up HRP rapidly by pinocytosis into small vesicles. The HRP was subsequently transferred to angular reticulum, in which it was retrogradely transported to the perikarya of the supraoptic nucleus (SON).

The purpose of this investigation was to study the uptake of HRP in an attempt to answer the following questions:

1- Following its uptake, in which organelles is HRP located?

2- At what rates is HRP retrogradely transported in intact and transected neurons?

3- Is there a quantitative difference in its uptake between intact and transected neurons?

4- What is the fate of HRP in the perikarya?

5- Are the distal portions of transected axons able to take up HRP, and if so, for how long after the transection?

6- Are there any changes in the uptake of HRP by pituicytes following transection of neurosecretory axons?

7- Following transection of neurosecretory neurons, where can HRP first be detected in the proximal stump?

MATERIALS AND METHODS

Five groups of 48 grass frogs (Rana pipiens) were subjected to the following treatments.

1. Group One: Injection of HRP followed immediately by transection of the infundibulum and killing of three frogs each after 1 h., 4 h. and 24 h.
2. Group Two: Transection of the infundibulum; 12 h. later injection of HRP, then killing of three frogs each after 1 h., 4 h. and 24 h.
3. Group Three: Transection of the infundibulum, 24 h. later injection of HRP, then killing of three frogs each after 1 h., 4 h. and 24 h.
4. Group Four: Transection of the infundibulum, four days later injection of HRP, then killing of three frogs each after 1 h., 4 h. and 24 h.
5. Group Five: Non-transected and non-injected frogs, then killing of three frogs each after 1 h., 4 h. and 24 h.

Fifteen mg. Horseradish peroxidase (HRP, Type II Sigma, MW 40,000) dissolved in 0.2 ml amphibian saline solution were injected into the dorsal lymph sac. For each time interval in each group one frog was injected with 0.2 ml amphibian saline solution only, and three frogs were sham-operated.

Surgical Procedure

Under anesthesia with 0.1% Finquel (Ayerst) solution, with the aid of Bausch binocular technoscope the base of the skull was approached paraorally and opened with a dental drill. After removal of the cartilage, the meninges were opened and the infundibulum was exposed. The tract was transected just rostral to the rostral edge of the pars distalis of the adenohypophysis with a microknife prepared from the edge of a razor (Fig. 26).

Fixation and Thick Sectioning

The brains were fixed by perfusion of the anesthetized frogs via the aorta with 5% phosphate buffered glutaraldehyde (0.1 M, pH 7.4). The whole brains were carefully removed, freed from meninges and immersed in fixative for an additional eight hours. Subsequently, they were washed repeatedly with 0.2M phosphate buffer containing 5% sucrose and stored over night at 4 C. Blocks of the hypothalamus containing the preoptic nucleus, infundibulum and hypophysis were serially sectioned at 60µm on a vibratome in a bath of the same buffer. They were placed in order in compartments of a staining tray filled with the same buffer.

Horseradish Peroxidase Histochemistry and Postfixation

After collection of all 60µm thick sections, in addition to 0.5 to 1mm thick slices of the blocks which may contain

HRP stained neurons and axons, both were rinsed in fresh buffer and reacted for peroxidase in a solution that contained 20mg of 3-3 diaminobenzidine tetrahydrochloride (DAB) in 10ml 0.1M cacodylate buffer at pH 5.1 for 30 min. at room temperature, then they were transferred into the same solution with an addition of 0.1 ml hydrogen peroxide and incubated for 60 min. at room temperature in the dark. Subsequently, the sections were washed twice for 5 min. with cacodylate buffer at pH 7.2, then postfixed in 1% osmium tetroxide in cacodylate buffer at pH 7.2 for 90 min. Finally, the sections were washed and stored in cacodylate buffer at pH 7.2.

Dehydration, Infiltration and Embedding

The 60µm sections were dehydrated in graded ethanol and acetone and embedded in Epon-Araldite mixture by placing the sections between two slides previously coated with 1% Dimethyldichlorosilane in benzene together with a few drops of embedding medium. Plastic squares containing selected sections were mounted on plastic stubs with a drop of resin. The thick slices were embedded in weighing dishes.

Preparation for Electron Microscopy

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[1] and 0.4% (w/v) aqueous lead citrate.[2]

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[1]Mallinckrodt Chemical Works, St. Louis, Missouri.

[2]Vaughn Inc., Memphis, Tennessee.

RESULTS

Light Microscopic Findings

Neural lobe of sham-operated frogs

The neural lobe contained axons and axon terminals of the preoptico-neurohypophysial tract. The nerve terminals ended in intimate relationship with the rich capillary plexus of the pars nervosa (Fig. 28). Pituicytes were scattered among these fibers with their short processes extending between them.

Neural lobe of the tract-transected frogs

The degenerative changes that occurred after transection of the hypothalamo-neurohypophysial tract depended on the time intervals between the transection and the killing of the animals. These changes became visible 48 h. after transection, when a pronounced accumulation of the neurosecretory granules appeared in the axon terminals. This accumulation caused rounding up and an increase in the size of the axon terminals. Degenerative changes became very conspicuous between 24 and 48 h. after the transection. The extracellular spaces were clearly seen at this period. The staining intensity of the NGVs decreased, especially at the axon terminals, and an increase was seen in the size of pituicytes.

Striking changes were observed 4 to 5 days after the transection. There were more degenerated axons and axon terminals and a remarkable increase in the width of extracellular spaces (Fig. 29). The pituicytes became actively phagocytic and engulfed degenerating axons (Fig. 30). These processes were reflected microscopically by atrophy of the neural lobe.

Hypothalamo-neurohypophysial tract of sham-operated frogs

The neurosecretory fibers in the subependymal region were grouped in a bundle, passing through the infundibulum before entering the median eminence. These fibers were partly filled with neurosecretory materials, which sometimes accumulated in small Herring bodies.

Hypothalamo-neurohypophysial tract of tract-transected frogs

After transection of the hypothalamo-neurohypophysial tract, a significant increase in the amount of neurosecretory material was observed. The amount depended on the time interval between the transection and the sacrifice of the animal, and on the number of axons involved. Accumulation of neurosecretory materials was observed over a distance proximal to the site of transection (Fig. 27).

At the cut end the axons had cone- and club-shaped outgrowths filled with neurosecretory material; these protruded beyond the original site of the transection (Fig. 27).

Electron Microscopic Findings

Neural lobe of sham-operated frogs

The neural lobe consisted of non-myelinated axons that terminated on the blood capillaries by means of nerve endings, and pituicytes (Fig. 32 and 33). The nerve fibers contained neurofilaments and mitochondria. The mitochondria varied in size, shape and distribution, occuring in nerve fiber swellings. In other swellings, the mitochondria occupied the greater part of the neurosecretory axons (Fig. 31).

The axon terminals adjacent to the capillaries were characterized by the presence of two types of neurosecretory elements (Fig. 33). One is represented by dense NGVs, and the other by small electron-lucent microvesicles. The microvesicles were aggregated in closely packed arrays in different sizes. The neurosecretory materials were separated from the neuroplasm by a limiting membrane around their surface, and comprised a homogeneous material of varying electron density (Fig. 31). In the terminals, the electron density of NGVs varied in different regions of the neural lobe. Those near the capillary showed a decreased density compared to others (Fig. 33).

The blood capillaries were lined by a fenestrated endothelium. The most distinct feature of the neural lobe blood capillaries were wide perivascular spaces onto which abut the neurosecretory nerve endings (Fig. 33). The perivascular

spaces had ramifications between the axon terminals into the tissue of the neural lobe and hence, a large number of neurosecretory nerve endings came into contact with this perivascular space. The wide perivascular connective tissue spaces contained fibroblasts and collagen fibrils. They were connected by processes of pituicytes. In some places, the perivascular spaces formed channels which were lined with the transection (Fig. 34 insert).

Pituicytes were stellate or elongated cells with pleomorphic nuclei. Their cytoplasm contained ribosomes associated with the endoplasmic reticulum. Lipid droplets, usually roughly circular in outline, and well-developed Golgi complex, in addition to spherical and elongated mitochondria were also observed. The pituicytes were very often in intimate contact with the axons and axon terminals (Fig. 32).

Neural lobe of tract-transected frogs

The evolution of the ultrastructural changes in the neural lobe following transection of the hypothalamo-neurohypophysial tract can roughly be divided into three periods, with frequent overlapping, especially in the last two periods, due to differences in the development changes among axons.

Four to sixteen hours post-operative time In this period, the neurosecretory axon endings were packed with NGVs due to anterograde passive accumulation of NGVs, reducing

the space between the granules. This condensation of NGVs at the terminal caused the terminal to become spherical (Fig. 34). A few NGVs lost their staining affinity and sometimes merging of two or three occurred especially 16 h. after the transection (Fig. 34 insert).

Twenty-eight to forty-eight hours post-operative time

A considerable enlargement of the extracellular spaces was seen in the hilar region 28 h. after transection (Fig. 36). The NGVs appeared empty and only the bounding membranes persisted (Fig. 38). Fusion of two or three granules was frequently observed (Fig. 37). Moreover, axons in close proximity to pituicytes became partially or totally surrounded by pituicytes (Fig. 38). Pituicytes increased in size and became closely associated with the degenerating axons. Their cytoplasmic processes extended between and around the degenerating axons (Fig. 39). Toward the end of this period, some axons that had been totally engulfed by pituicytic cytoplasm were almost completely depleted of NGVs (Fig. 39).

Two to five days post-operative time Free NGVs were observed in the intercellular as well as perivascular spaces, due to the fact some of the neurosecretory axons had lost their axolemma (Fig. 42 and 43). More axons were engulfed by pituicytes (Fig. 41), and the intercellular spaces extended to the axon terminals. Axons contained few NGVs and these had lost their staining affinity. Some of these NGVs, during

this time, possessed electron-lucent small tails (Fig. 42). Engulfed axons contained empty NGVs; their surrounding membranes fused with pituicytic cytoplasm and often disappeared. The fusion of membranes giving a different appearance of the degenerated axons appeared (Fig. 42).

Herring bodies of the neural lobe were observed to contain NGVs located at the periphery, leaving the center filled with microtubules, neurofilaments, and mitochondria (Fig. 40). Toward the end of this period, free NGVs were still found in the perivascular connective tissue spaces (Fig. 43), and axons that had started degeneration were engulfed by the hypertrophied pituicytes (Fig. 41).

Hypothalamo-neurohypophysial tract of sham-operated frogs

Various sizes of neurosecretory axons with few axonal dilatations were usually grouped in a bundle. The axoplasm was occupied mainly by numerous neurofilaments, some mitochondria and occasional NGVs. Tubules resembling the smooth endoplasmic reticulum were also observed (Fig. 45).

Hypothalamo-neurohypophysial tract of tract-transected frogs

The evolution of the ultrastructural changes in the hypothalamo-neurohypophysial tract after transection was divided into two phases; the first phase included changes that occurred 4 to 48 hours after transection, while the second phase occurred between 2 to 5 days after transection. Two

different sites of the infundibulum were observed: the terminal region of the proximal stump and the preterminal region which was approximately 2 mm proximal to the terminal region.

Phase I

As early as 4 h. after the transection a massive accumulation of NGVs was observed mainly at the terminal portion of the neurosecretory fibers (Fig. 44). This accumulation resulted in the formation of small axon dilatations, within which a considerable number of microvesicles of varying sizes were observed. The microvesicles become pronounced toward the end of this phase (Fig. 48c). Many of them were spheroid or ovoid, and some were irregular in shape, but not all shapes were seen in the same axon.

The NGVs had varying sizes and elctron densities and were located mainly at the periphery, leaving the center of the axons filled with neurofilaments, mitochondria and tubules (Fig. 47). The tubules appeared as early as 4 h. after transection in both the preterminal and terminal regions of the tract (Fig. 44). They consisted of short elongated membrane-bounded cisternae intermingled with neurofilaments. The tubules increased considerably in quantity and width at approximately 36 h. after transection, and occasionally contained electron dense material (Fig. 48c). In the preterminal region, the accumulation of NGVs together with tubules caused

local dilatation of the axons. A few fibers underwent intra-axonal changes with increasing time after the operation. Thirty-six hours after the transection the NGVs lost their electron staining affinity. Two or three NGVs merged, and occasionally a connection between them and the tubules was observed.

Pituicytes were seen concentrated around the degenerating axons mainly at the terminal region (Fig. 46). Later in this phase, they extend to include the preterminal region (Fig. 48a). Phagocytic activity represented by changes occurred in axons or axon fragments within the cytoplasm of the pituicytes was obvious during this phase, especially approximately 16 h. after transection (Figs. 48b and 49).

Phase II

The changes during this phase were mainly limited to the axon dilatation in the preterminal and terminal regions. In addition to accumulation of NGVs, there was a considerable increase in the number of mitochondria and tubules. Tubules with connections to NGVs were frequently seen (Fig. 51). Compared to the previous phase, the tubules were dilated and their contents became more electron dense (Fig. 51).

The presence of dense bodies at the preterminal and terminal regions was the most striking feature of this phase (Figs. 50 and 53). The dense bodies were irregular in shape, and sometimes contained a homogeneous material. Pituicytes

in the terminal region surrounded the degenerated axons (Fig. 49). In the preterminal region, the neurosecretory axons were filled with NGVs and few tubules. The tubules consisted of long non-dilated tubes that were filled with material of varying electron density (Fig. 54). Toward the end of this phase, some of the affected axons in the terminal region contained a few widely separated NGVs which had lost their staining affinity. The tubules were very few and not dilated; in addition, a few axons were filled with microvesicles which contained moderately electron-dense material (Fig. 52).

Non-transected HRP-reacted frogs

In this group, sections from the preoptic nucleus, infundibulum, and neural lobe were incubated in DAB to reveal the presence of endogenous peroxidase. Peroxidase reaction products were confined to red blood cells.

Non-transected HRP-injected frogs

Preoptic nucleus HRP reaction product consisted of discrete granules similar in size and shape to the NGVs in all the neurons regardless of the time interval between the injection and the sacrifice of the animals (Fig. 55a, b). HRP reaction products appeared as homogeneous bodies with an increased electron density as compared to the pleomorphic dense bodies of the cytoplasm (Fig. 55a). HRP reaction

products were present neither in discrete smooth cisternae nor in the small pinocytotic microvesicles, but infrequently were found in large labeled vesicles.

HRP reaction products were associated with cytoplasmic dense bodies which were capable of degrading the amount of peroxidase delivered to the cells (Fig. 55c, d, e).

Infundibulum HRP reaction product was present mainly in the extracellular spaces between the hypothalamo-neurohypophysial axons (Fig. 67a). Little reaction product was found within the axons and was similar in shape to that present in the perikarya. In addition, there was some peroxidase reaction product in the form of rings as well as in the form of a small aggregation associated with the NGVs (Fig. 67b).

Neural lobe A variable amount of HRP reaction product was present in the extracellular spaces in the form of irregular spherical bodies within axons and axon terminals (Fig. 61). In the pituicytes, peroxidase reaction product was found in small pinocytotic vesicles, coated vacuoles, and multivesicular bodies (Figs. 62 and 64). Peroxidase reaction product was not seen in the cisternae of the smooth axonal endoplasmic reticulum. HRP reaction product was also taken up by the capillary endothelium in the form of macromolecules (Fig. 63).

Tract-transected HRP-injected frogs

Preoptic nucleus Neither transection of the hypothalamo-neurohypophysial tract nor the surviving time after the transection has an effect on the quantity of HRP taken up by the neurons.

Peroxidase activity within the perikaryon was localized in a variety of membrane-delimited organelles. It was demonstrated in vacuoles, ring-shaped bodies and in microvesicles (Figs. 56e, d, 59). The microvesicles were scattered throughout the cytoplasm, but mainly were in the vicinity of the Golgi complex (Fig. 56a, b). Few labeled vacuoles (Fig. 56d), labeled short and long smooth cisternae as well as varicosed cisternae were observed in the perikarya (Fig. 56a, b, c, e). These cisternae were mainly found in close proximity to the Golgi complex (Fig. 56b, c). Small and large peroxidase labeled dense bodies were morphologically identical to the dense bodies presumed to be secondary lysosomes or residual bodies (Fig. 56b, c).

Infundibulum HRP reaction product diffusely filled some of the axons as early as 1 h. after transection of the hypothalamo-neurohypophysial tract (Fig. 68a, b). HRP was also observed in short smooth cisterns of the axons in the preterminal region as well as axons in the preoptic region (Fig. 71a, b). These cisternae were similar to those found in the perikarya. Occasionally, HRP reaction product was

seen in some axons in close association with the NGVs. There was no uptake of HRP by the proximal stump of the transected hypothalamo-neurohypophysial tract at any time (Fig. 70).

Neural lobe HRP reaction product has been recognized in four portions of the neural lobe: hilar region, axon terminals, extracellular space and in the pituicytes. In the hilar region, peroxidase reaction product was observed diffusely filling some of the axons as early as four hours after HRP injection (Fig. 69). Sixteen hours after the injection, HRP reaction product was also observed labeling short cisternae (Fig. 71c). At the axon terminals, HRP reaction product was in the form of aggregates of different size and shape 24 h. after the injection. It was also seen labeling the electron-lucent tails of the NGVs. In one instance, the HRP reaction product was within the membrane of the NGVs (Fig. 66a). HRP reaction product was observed in the extracellular space in the form of large dense molecules (Fig. 66b). Pituicytes also took up HRP in the form of labeled vacuoles and multivesicular bodies approximately 36 h. after the injection (Fig. 65).

Plate 8

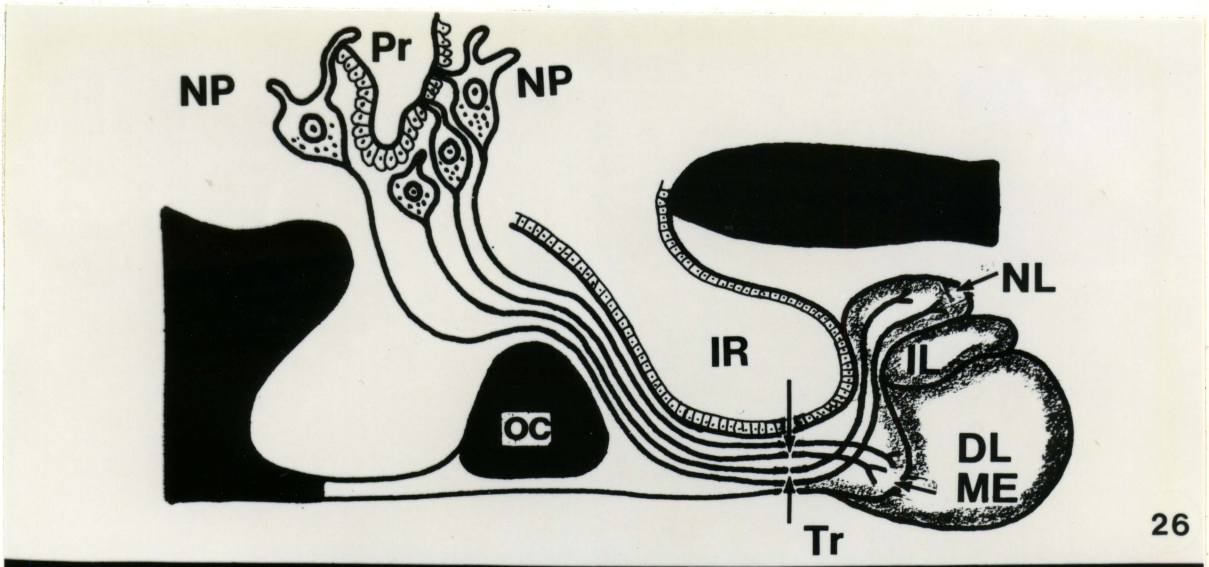
Fig. 26. Schematic drawing of a longitudinal section of the frog hypothalamus. Nucleus preopticus (NP), Preoptic recess (PR), Infundibular recess (IR), Optic chaisma (OC), Neural lobe (NL), Intermediate lobe (IL), Distal lobe (DL), Median eminence (ME), area of transection (Tr)

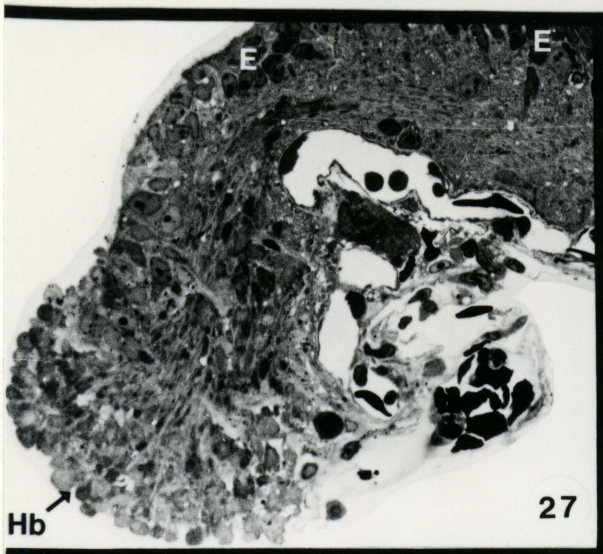
Fig. 27. Proximal stump of the infundibulum, 97 h. after the transection. Herring bodies (Hb). Ependyma (E) X 325

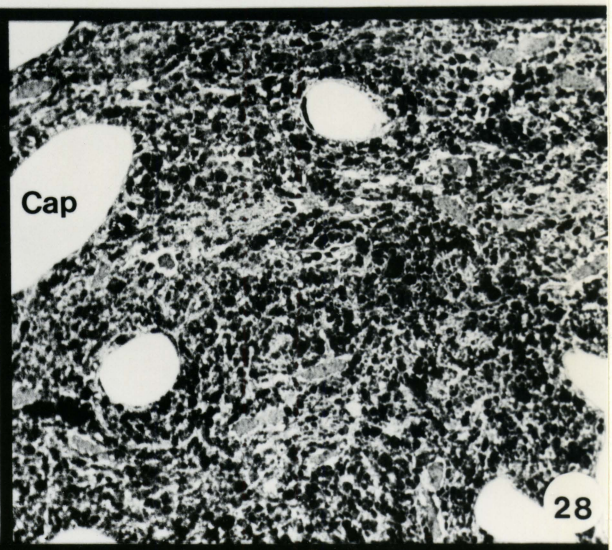
Fig. 28. The neural lobe of sham-operated frog. Blood capillary (Cap) X 350

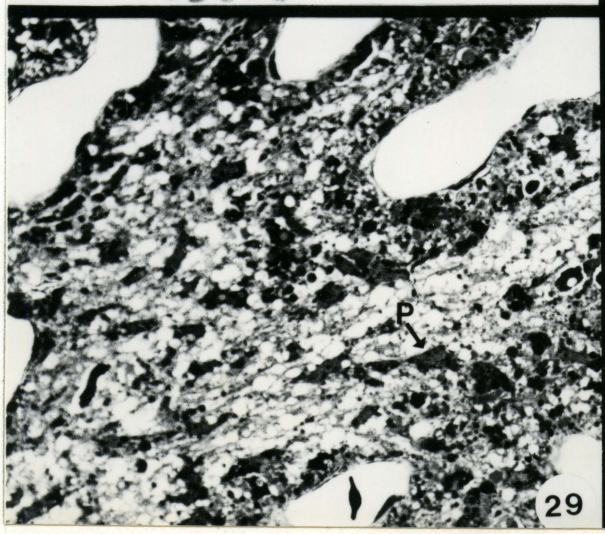
Fig. 29. The neural lobe, 97 h. after the transection. Hypertrophied pituicytes (P) and large extracellular spaces X 350

Fig. 30. The neural lobe, 5 days after the transection, degenerated axon remnants (F) surrounded by pituicytes. Intermediate lobe (IL) X 350


NP
Pr
NP
NL
IR
IL
OC
DL
ME
Tr
26


E
E
Hb
27


Cap
28


P
29

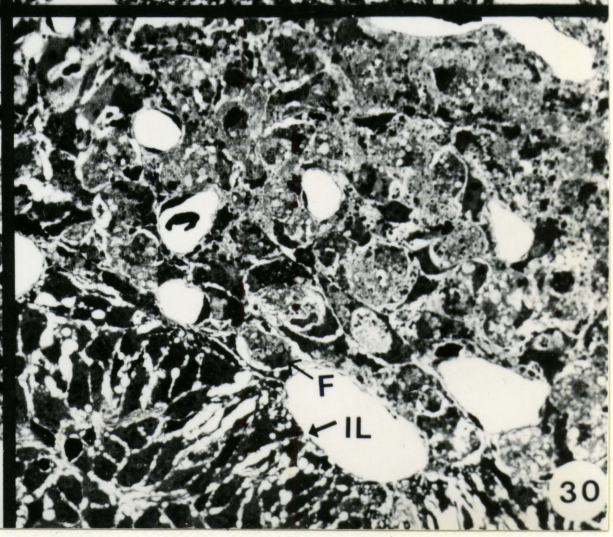

F
IL
30

Plate 9

Fig. 31. Neurohypophysis of sham-operated frog. Neurosecretory axons filled with NGVs. Microvesicles (MV) X 12,000. Insert showing NGVs (NS) X 22,800

Fig. 32. Neurohypophysis of sham-operated frog. A pituicyte (PIT) in direct contact with neurosecretory axons X 13,000

Fig. 33. Neurohypophysis of sham-operated frog. Axon terminals abutting a blood capillary (CAP) with a wide perivascular space (PV) X 15,600

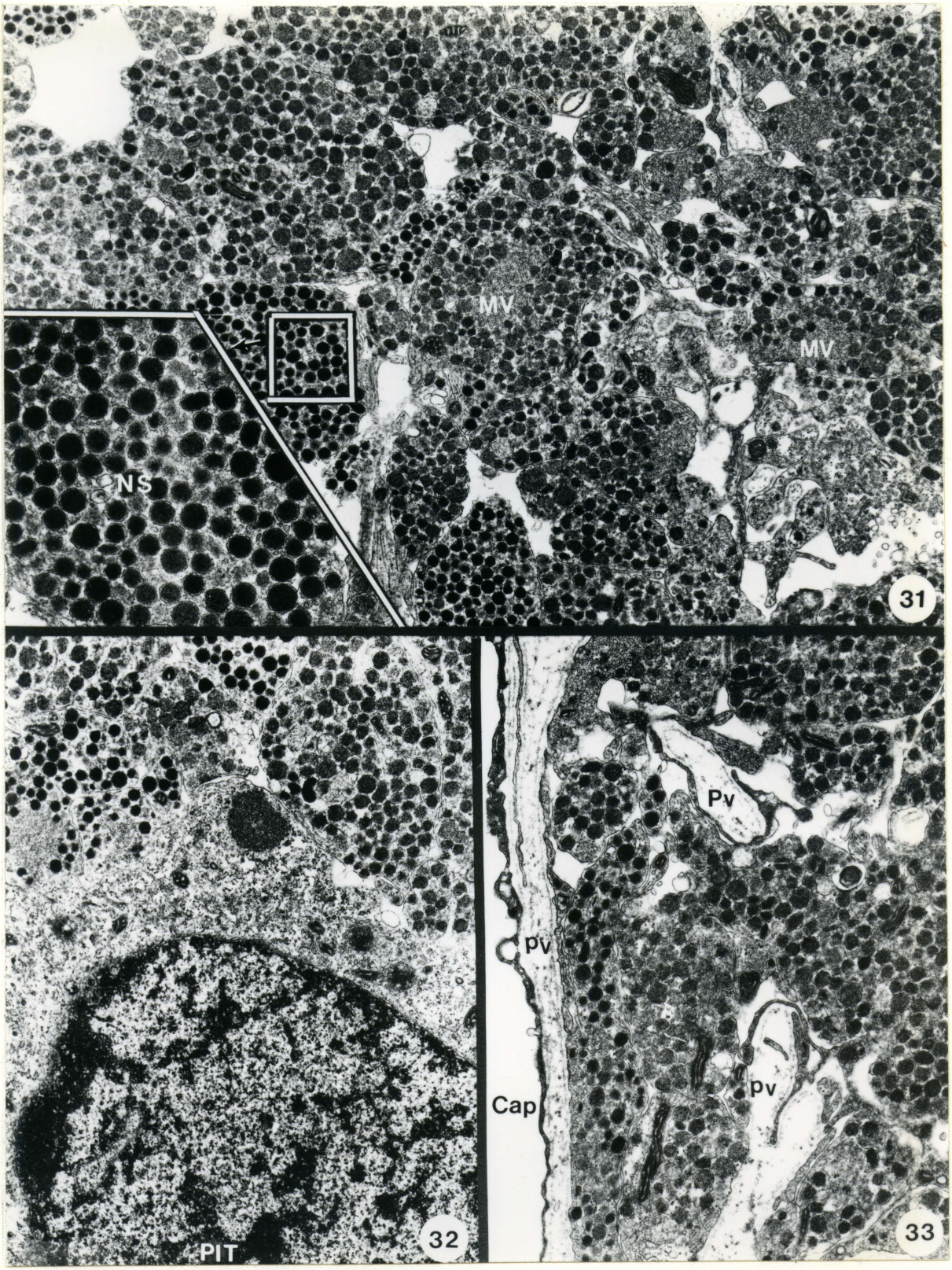

MV
MV
NS
31
PIT
32
Pv
pv
Cap
pv
33

Plate 10

Fig. 34. Four hours after the transection, neurosecretory axon terminals filled with NGVs. The blood capillary (Cap) is fenestrated (arrows). Perivascular space (PV) X 12,000. Insert shows merging of two or more NGVs X 30,400

Fig. 35. The neural lobe, 16 h. after the transection, shows dense lamellar body (DLB) in the neurosecretory axon X 30,600

Fig. 36. Twenty-eight hours after the transection, the neural lobe shows a wide extracellular space in the hilar region X 15,000

Fig. 37. The neural lobe, 36 h. after the transection. Degenerated axons contain small dense lamellar bodies (arrows) X 37,500

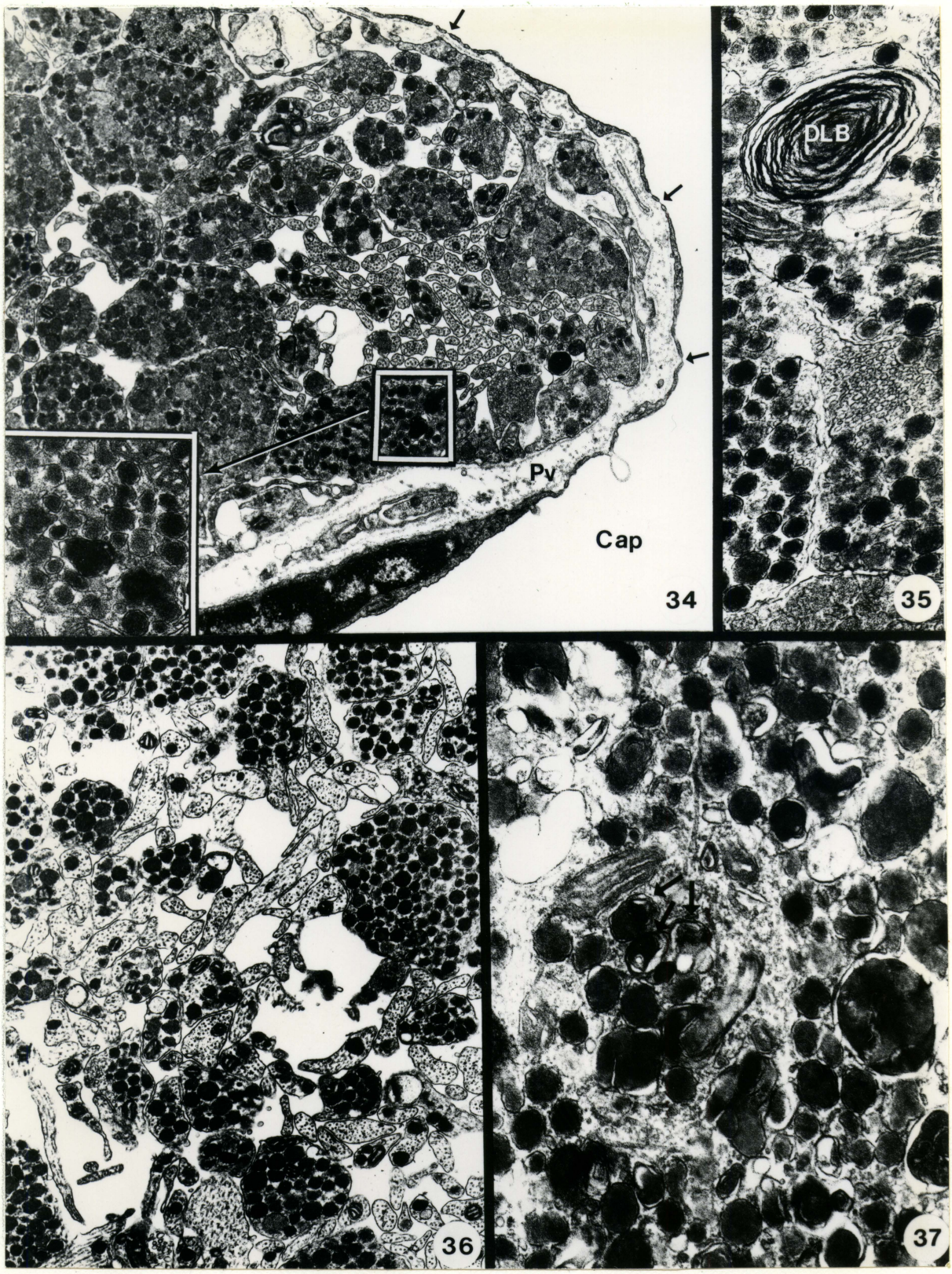

Pv
Cap
34
PLB
35
36
37

Plate 11

Fig. 38. The neural lobe of transected frog. Degenerated neurosecretory axons (A) completely surrounded by a pituicyte (PIT), 48 h. after the transection X 10,400

Fig. 39. In the neural lobe. Intracytoplasmic digestion of the axons, 48 h. after the transection. Note some axons appeared empty of NGVs (A) X 15,000

Fig. 40. A Herring body of the neural lobe contains large numbers of mitochondria (M), large dense bodies (d) and tubular formations (TF), 97 h. after the transection X 26,000

Fig. 41. The neural lobe, 5 days after the transection. Intrapituicytic axons have been transformed into multilamellate bodies. Pituicyte (PIT) X 10,400

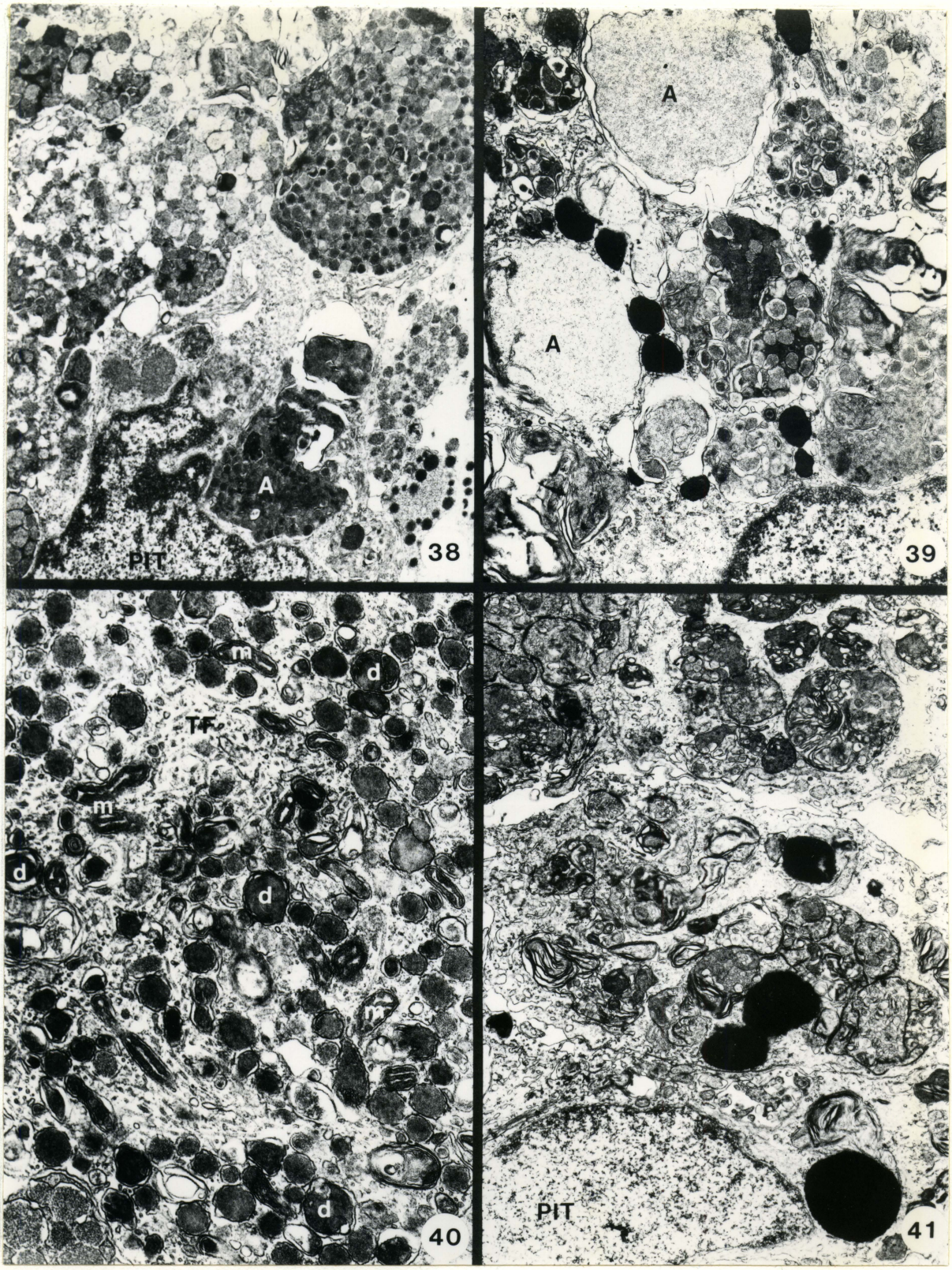

A
PIT
38
A
A
39
m
d
TF
m
d
d
m
d
40
PIT
41

Plate 12

Fig. 42. Neural lobe, 97 h. after the transection. Intrapituicytic axons are transformed into multilamellate bodies (MLB). Note in the upper left corner the neurosecretory axons have lost their axolemma. Pituicytic cytoplasm (P) X 15,000. Insert exhibits NGVs with electron-lucent tails (arrows) X 76,000

Fig. 43. The neural lobe, five days after the transection, shows axon terminals abutting a blood capillary (CAP) are completely degenerated and engulfed by a pituicyte (PIT). Note the NGVs (NS) are free in the extracellular spaces X 9,600

Fig. 44. The terminal region, 4 h. after the transection. The terminal portions of the neurosecretory fibers forming small Herring bodies X 9,000

Fig. 45. Hypothalamo-neurohypophysial tract of sham-operated frog. Microtubules (MT), neurofilaments (arrows), tubular formations (TF) and NGVs (NS) X 82,500

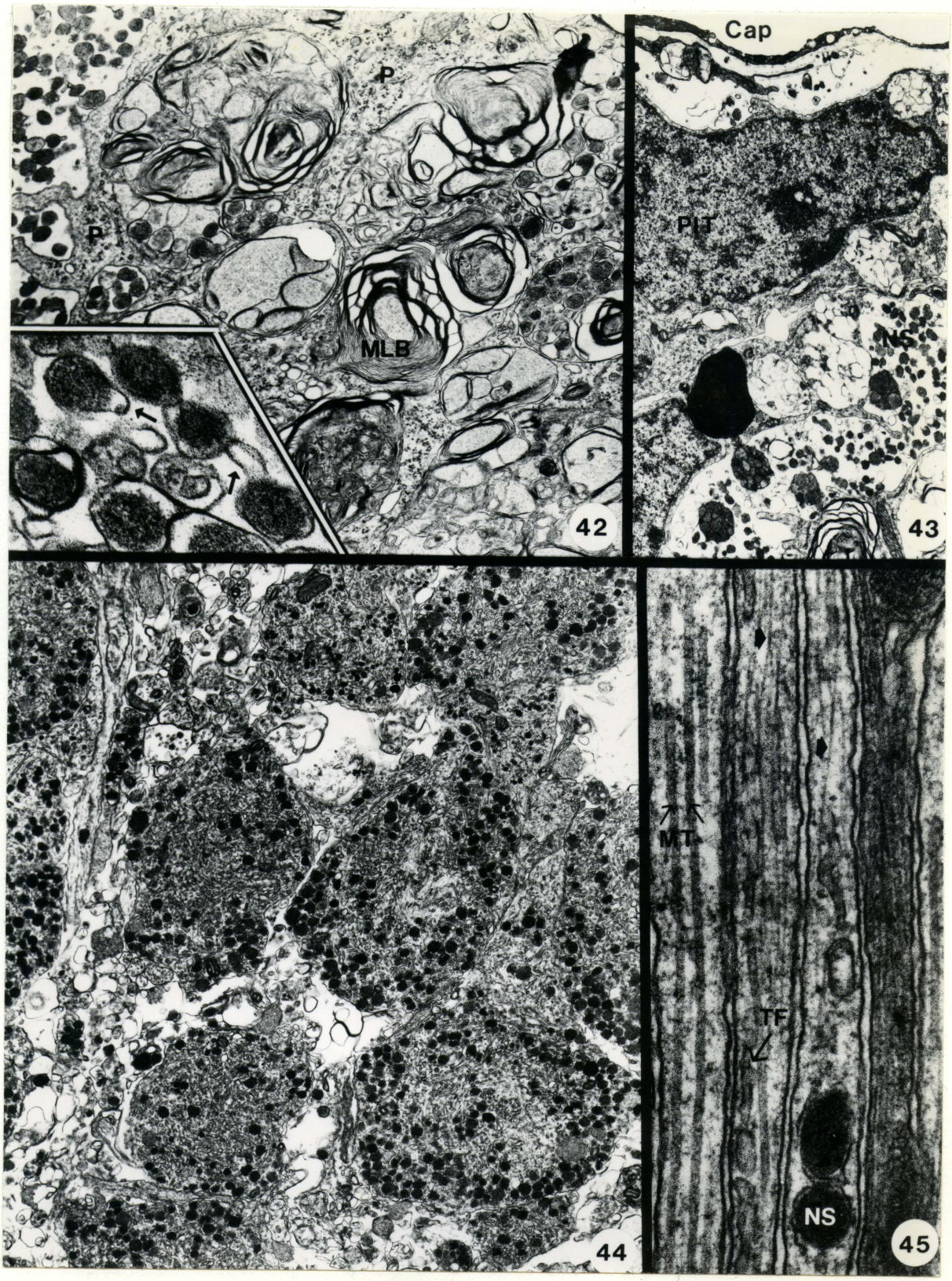

P
P
MLB
42
Cap
PLT
NS
43
44
MT
TF
NS
45

Plate 13

Fig. 46. The terminal region, 16 h. after the transection. Pituicyte (PIT) surrounding the degenerating axons (A) at the terminal end. Herring bodies (Hb), infundibular recess (IR) X 9,000

Fig. 47. The terminal region, 24 h. after the transection. Tubular formations (TF) are a very prominent feature of the axon terminals. Infundibular recess (IR) X 15,600

Fig. 48. Thirty-six hours after the transection of the hypothalamo-neurohypophysial tract

a. Preterminal region; pituicyte (PIT) in contact with large Herring bodies filled with NGVs X 3,600
b. A pituicyte shows intracytoplasmic digestion of axon. Note the NGVs lose their electron intensity X 6,500
c. At the terminal end, axonal dilatation contains empty vesicles (VS) of different sizes. Multivesicular body (MV). Infundibular recess (IR) X 30,600

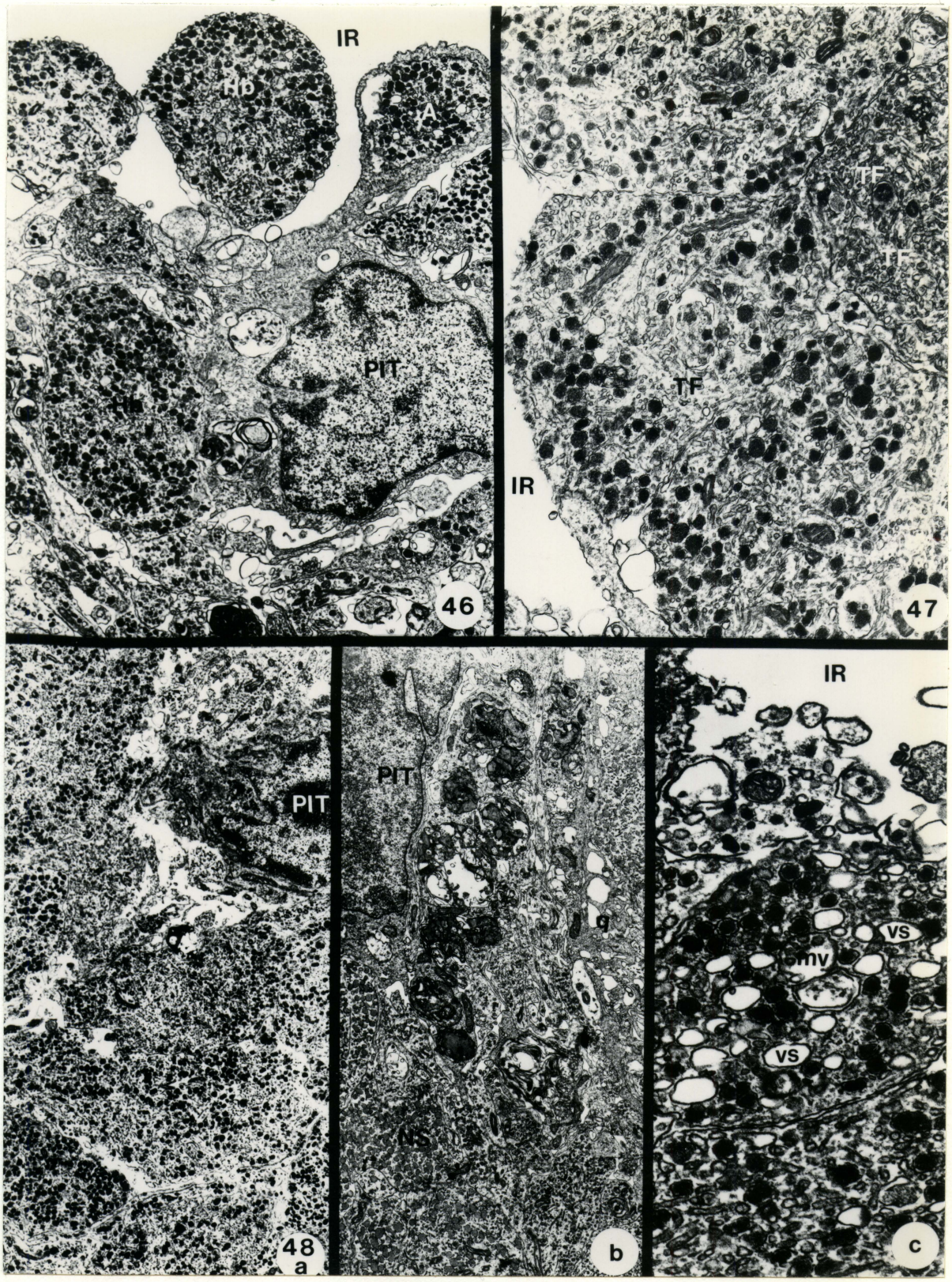

IR
A
PIT
46
TF
TF
TF
IR
47
PIT
48
a
PIT
NS
b
IR
VS
mv
VS
c

Plate 14

Fig. 49. A pituicyte in the preterminal region phagocytizing neurosecretory axons; 48 h. after the transection X 19,500

Fig. 50. Axonal dilatations are adjacent to the subependymal pituicyte (EP), 97 h. after the transection. Dense bodies (d) X 12,000

Fig. 51. The preterminal region, 97 h. after the transection. Dilated tubular formations (TF), and NGVs connected to an electron dense tubular formation (arrow). Mitochondria (M), dense body (d) X 39,000

Fig. 52. Axons in the infundibulum are filled with tubular formations (TF), and electron dense vesicles (V), 5 days after the transection. Note the scarcity of NGVs and partial loss of their electron intensity X 26,000

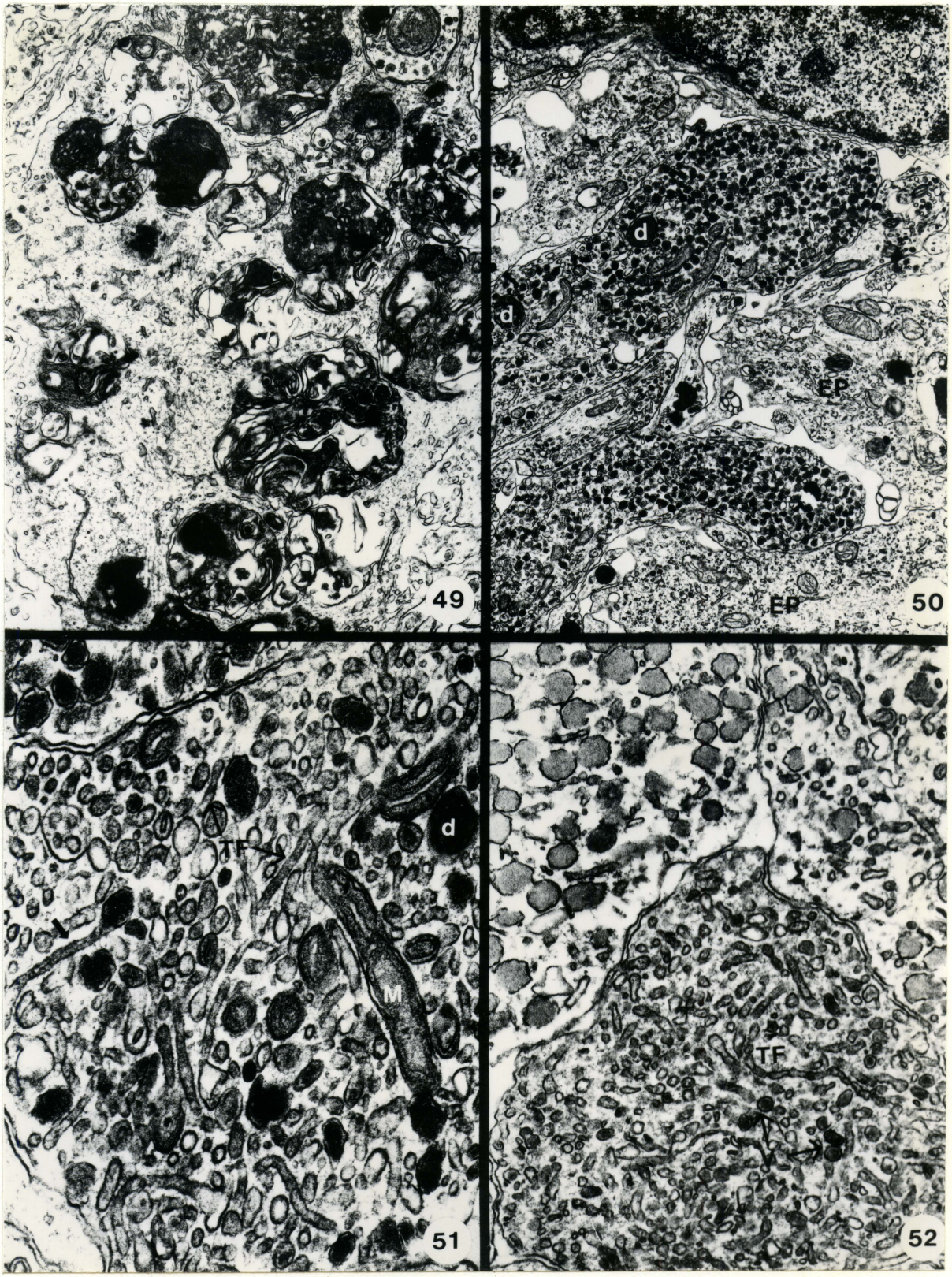

49
d
d
EP
EP
50
d
TF
M
51
TF
52

Plate 15

Fig. 53. Formation of large Herring bodies (Hb) at the terminal ends (TE). These are filled with a large number of dense bodies. Note the axonal dilatations in the preterminal region (PT), four days after the transection X 7,200. Insert shows pituicyte (PIT) at the terminal region X 4,800

Fig. 54. Ninety-seven hours after the transection, the preterminal region has axons filled with NGVs. Axon dilatation (AD) X 16,000. Insert shows long tubular formations (TF) in the preterminal region filled with electron dense materials X 24,000

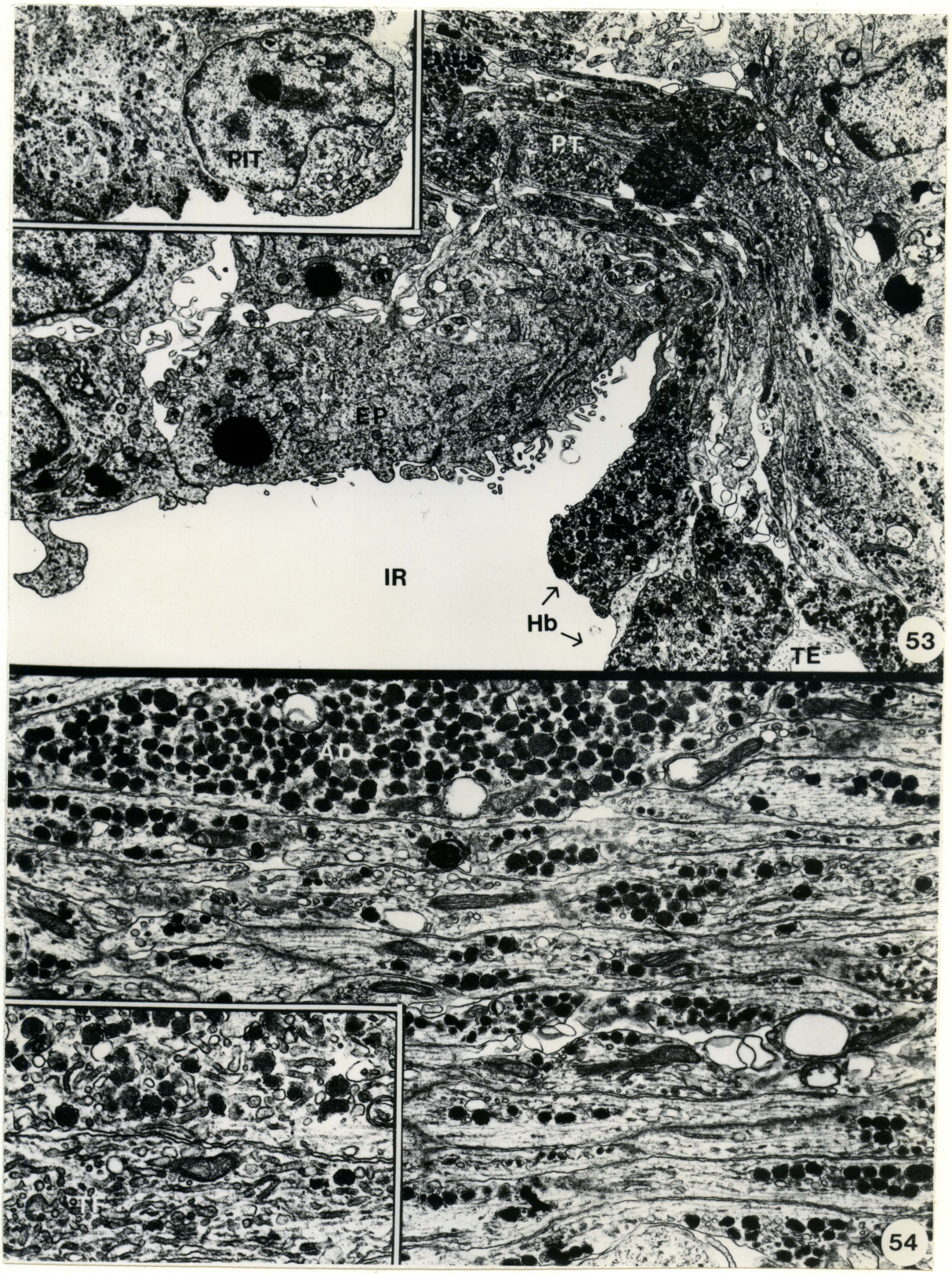

RIT
PT
EP
IR
Hb
TE
53
AD
54

Plate 16

Fig. 55. Preoptic neurons of an intact frog, arrows indicate HRP reaction products.
a. In a multivesicular body (arrow) and in dense bodies (arrow heads), 4 h. after the injection X 21,000
b. Within dense bodies, 4 h. after the injection X 28,000
c. Twenty-four hours after the injection X 42,500
d, e. Four hours after the injection. Labeled vacuole (arrow head) X 60,000

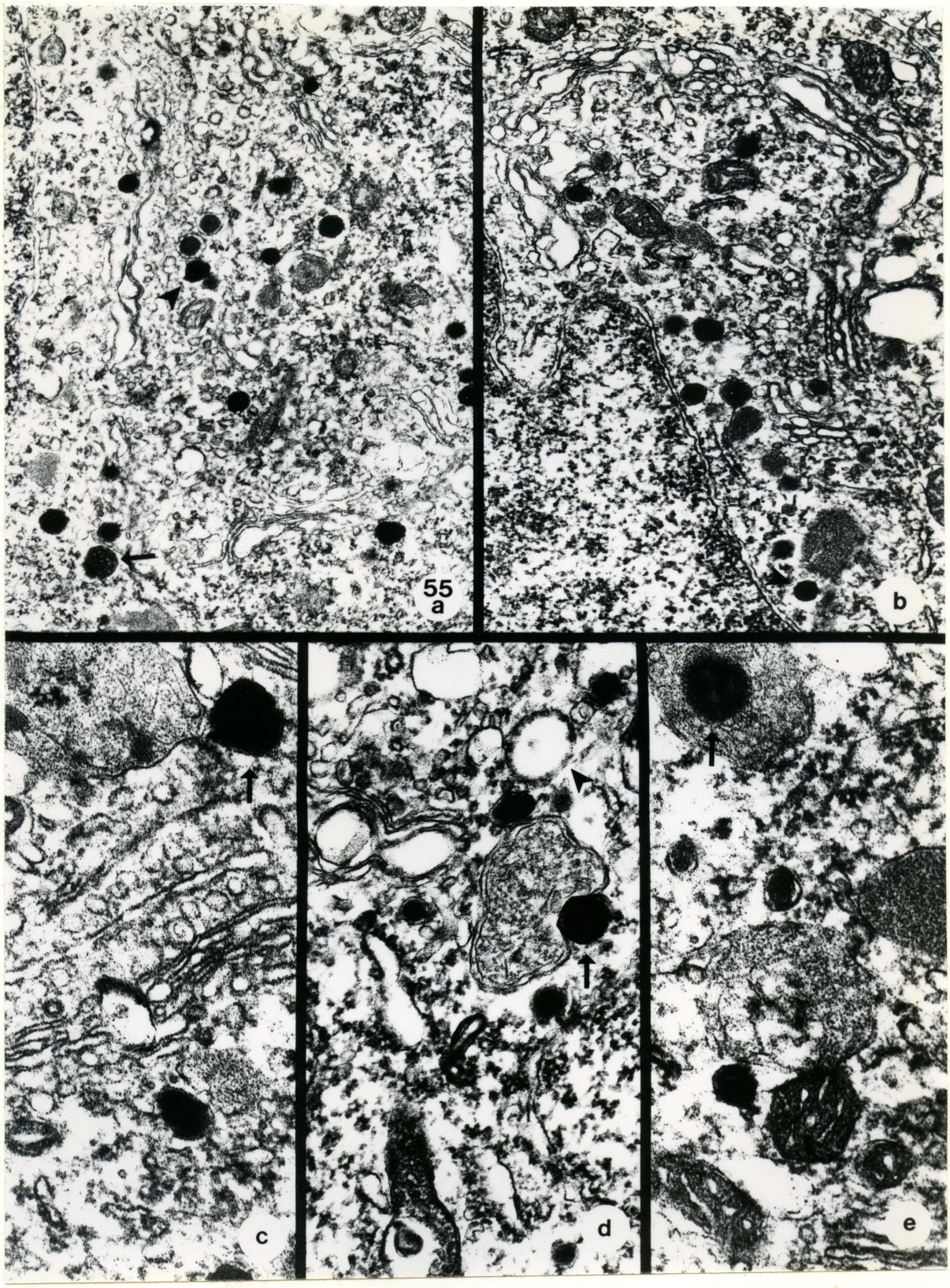

55
a
b
c
d
e

Plate 17

Fig. 56. Preoptic neurons of transected frogs.

a. Sixteen hours after the transection, HRP is localized in smooth cisterns (arrow). Note labeled pinocytotic vesicles (arrow head) X 20,400

b, c. HRP is localized in varicosed (b) and smooth (c) cisterns, 16 h. after the transection. HRP labeled dense bodies (arrow heads) X 21,000

d. Coated vacuole (arrow), 36 h. after the transection X 36,000

e. HRP is localized in a short smooth cistern (arrow) and in pinocytotic vesicles (arrow head), 16 h. after the transection X 27,000

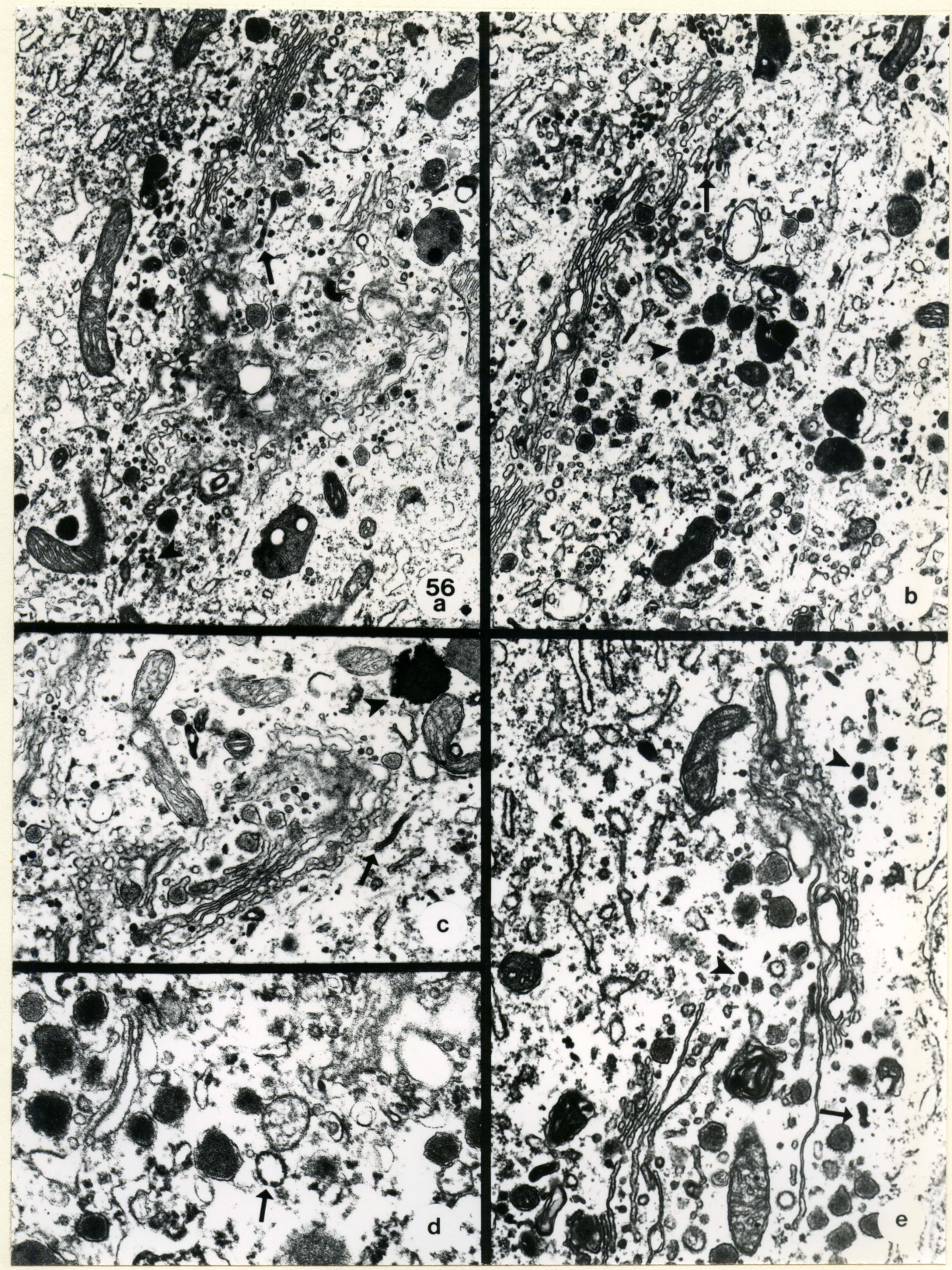

56
a
b
c
d
e

Plate 18

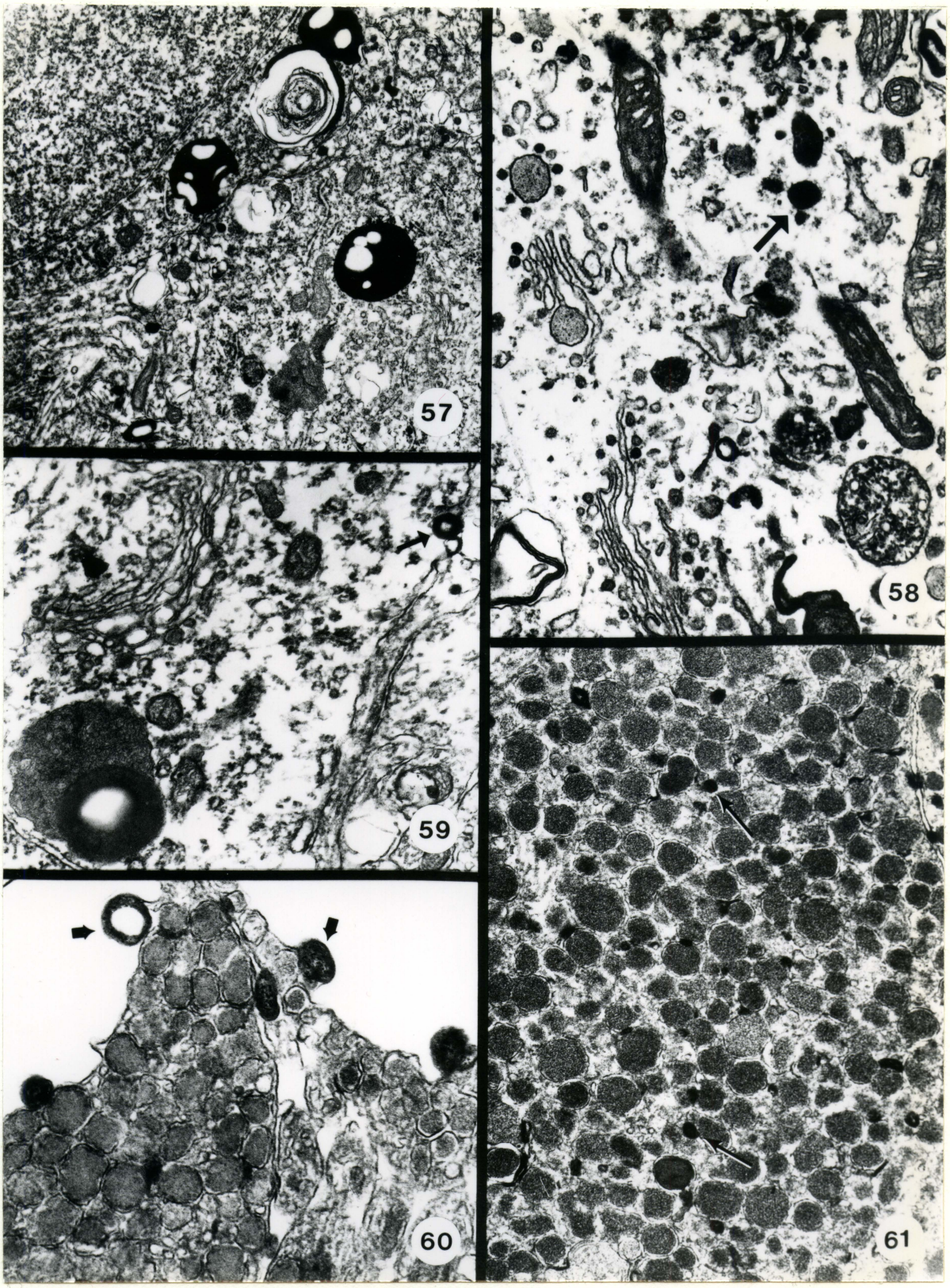
Fig. 57. A preoptic neuron containing labeled multivesicular bodies, 5 days after the transection X 18,000

Fig. 58. A preoptic neuron showing an HRP labeled dense body (arrow), 16 h. after the transection X 28,000

Fig. 59. A preoptic neuron showing an HRP labeled dense body, and a ring-shaped HRP reaction product (arrow), 4 h. after the transection X 27,500

Fig. 60. Neural lobe, 5 days after the transection, with HRP reaction products in the axoplasm (arrows) X 42,500

Fig. 61. The neural lobe of the intact frog, 1 h. after HRP injection, showing HRP reaction products associated within NGVs (arrows) X 30,000


57
58
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61

Plate 19

Fig. 62. A pituicyte of the intact frog, 24 h. after the injection of HRP, showing an HRP labeled vacuole (arrow) and a multivesicular body (arrow head) X 14,000

Fig. 63. A dense complex of the HRP reaction product is seen in the capillary endothelium of the intact neural lobe (arrow), 1 h. after the injection. Blood capillary (Cap) X 51,000

Fig. 64. An HRP-labeled multivescular body (arrow head) in the pituicyte of an intact frog, 24 h. after the injection of HRP. Note the labeled vesicle found in the axon near the pituicyte (arrow) X 16,800

Fig. 65. A pituicyte, 36 h. after the transection, showing an HRP labeled multivesicular body and a coated vacuole (arrow head) X 23,800

Fig. 66. a. Neural lobe, 24 h. after the transection, showing HRP labeling the electron-lucent tails of the NGVs (arrows) X 90,000
b. One hour after the transection. HRP reaction product in the extracellular space (arrows) X 47,500

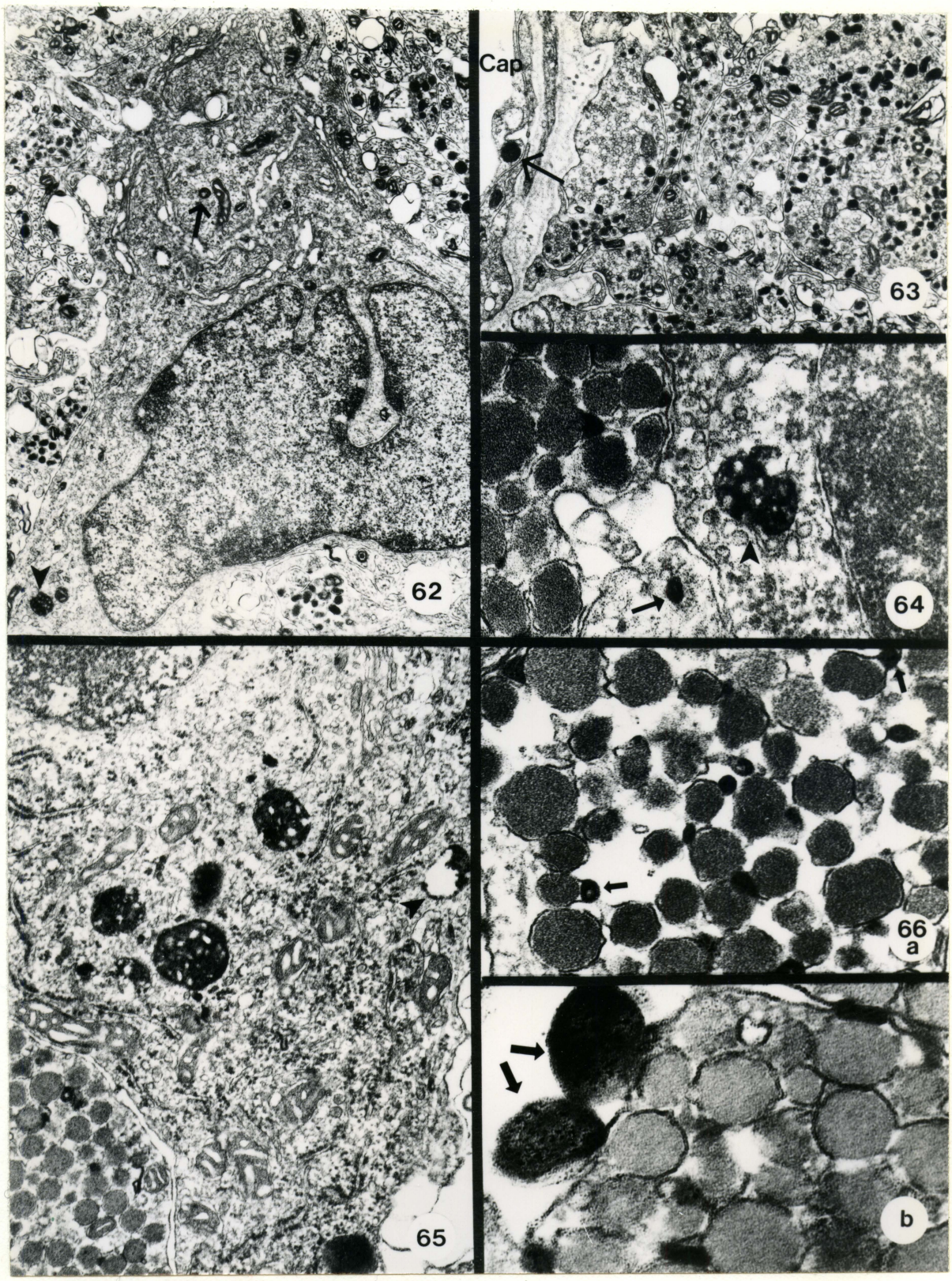

Cap
62
63
64
65
66
a
b

Plate 20

Fig. 67. The infundibulum of the intact frog, 1 h. after the HRP injection
a. HRP reaction product in ring-shaped forms (arrow) and in dense complex (arrow heads) X 19,500
b. A ring-shaped form of HRP reaction product (arrow). See dense molecules associated with NGVs (arrow heads) X 54,000

Fig. 68. The infundibulum of the transected frog, showing an axon diffusely filled with HRP reaction product
a. One hour after the injection X 42,000
b. Four hours after the injection X 26,000

Fig. 69. Hilar region of the transected frog neural lobe, 4 h. after the injection, showing axons diffusely filled with HRP X 45,500

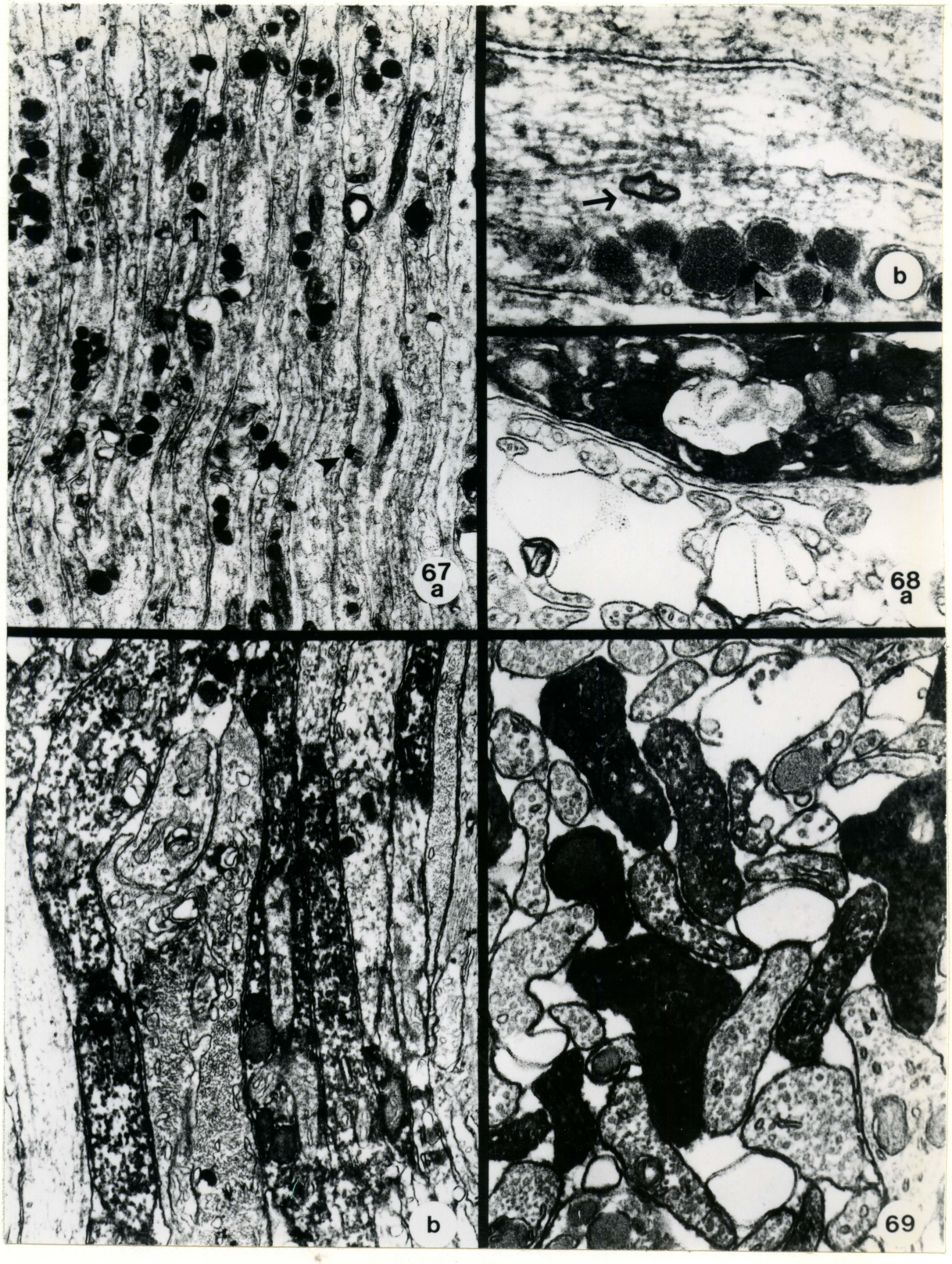
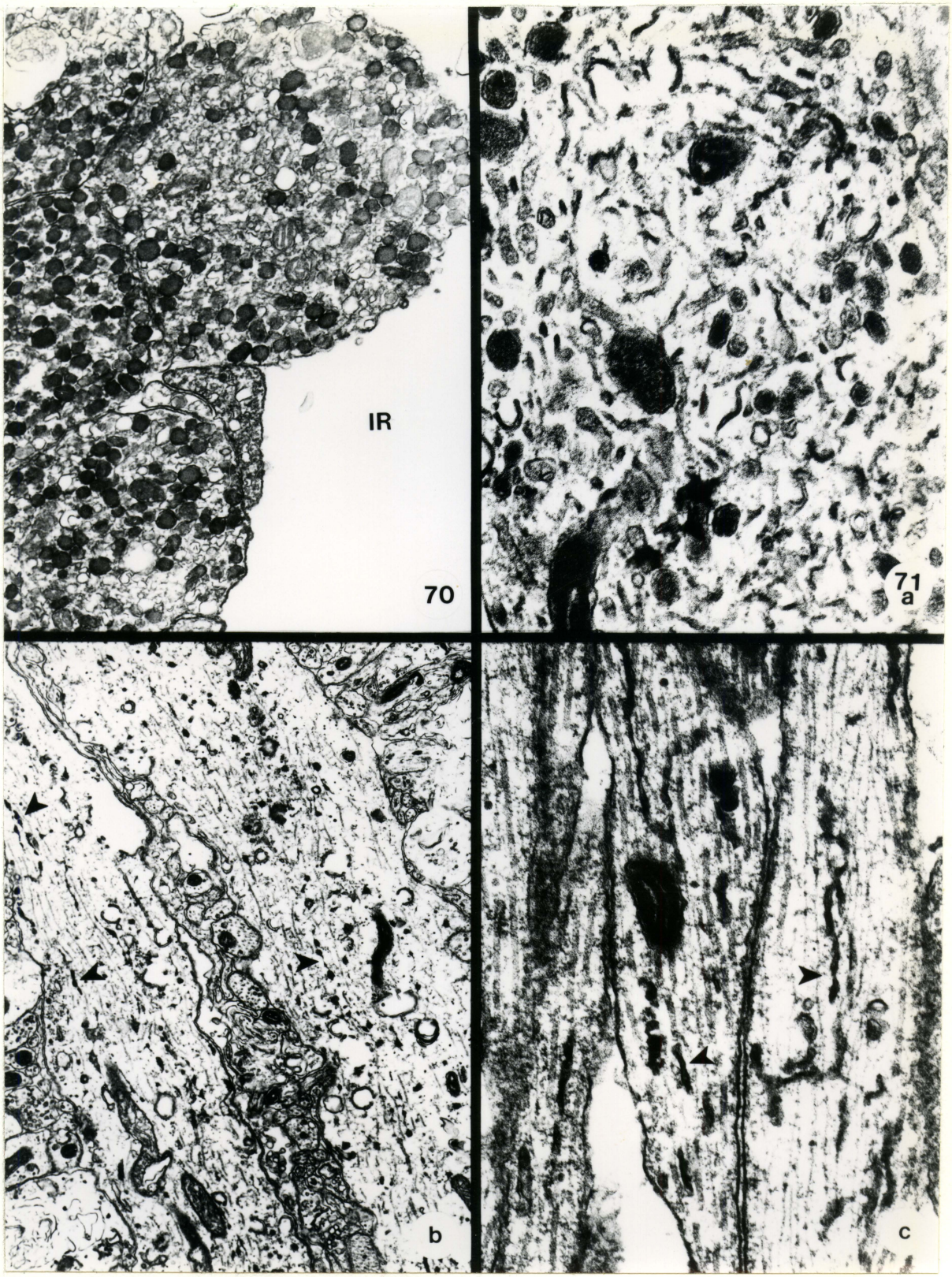

67
a
b
68
a
b
69

Plate 21

Fig. 70. The terminal end of the transected infundibulum, 97 h. after the transection, showing no uptake of HRP by the Herring bodies. Infundibular recess (IR) X 23,800

Fig. 71. The preterminal region of the transected frog, 16 h. after the injection
a. A Herring body, showing cisterns of smooth AER filled with HRP X 48,000
b. Varicosed labeled cisterns are seen in axons in the preoptic region (arrow heads) X 14,000
c. Neural lobe of the transected frog, showing labeled smooth cisterns with HRP reaction product X 45,000


IR
70
71
a
b
c

DISCUSSION

The uptake of exogenous protein in the amphibian hypothalamo-neurohypophysial system has been investigated by using enzyme cytochemical techniques for HRP. By labeling the axonal and perikaryal organelles with HRP, the mechanism by which information reaches the nerve cell body from transected axons can be studied. The HRP labeled organelles from the preoptic nucleus, infundibulum, and neural lobe will be discussed separately.

Preoptic nucleus

Our experiment showed that exogenous peroxidase was taken up by the intact and transected neurons of the frog and became localized in a variety of neuronal organelles. Similar studies to those we performed had shown that peroxidase was incorporated readily by the damaged neurons (Litchy, 1973; Kristensson and Olsson, 1974; DeVito et al., 1974; Adams and Warr, 1976). In both cases, the HRP reaction product was found in multivesicular bodies, small vacuoles, membrane-delimited cisterns, small dense bodies and pinocytotic vesicles which were scattered throughout the cytoplasm.

Pinocytotic vesicles were seen in control non-injected animals, but these did not contain peroxidase. Similar vesicles were described by Teichberg et al. (1975), and

Holtzman et al. (1977). They are considered passively endocytosed by the membrane retrieved from the cell surface for enzymatic degradation in the lysosomes, as these vesicles accumulate near the Golgi complexes. HRP-labeled dense bodies are considered secondary lysosomes already present in the cell body (Holtzman, 1966; Novikoff, 1967; Sellinger and Petiet, 1973).

Broadwell et al. (1980) found that HRP reaction product undergoing retrograde transport was predominantly localized in endocytotic structures such as vacuoles and cup-shaped organelles. In our experiments, stimulation of the frogs for release of hormones has not been performed, therefore, vacuoles of endocytotic origin were infrequently present in the perikarya.

HRP-labeled cisterns, either smooth or varicosed, were infrequently seen in our experiment. Labeled cisterns were also similar to the results reported by Broadwell and Brightman (1979), who found HRP was localized in membrane-delimited cisterns in the perikarya of SON of the rat. By virtue of their morphology and in agreement with Broadwell et al. (1980), those cisterns are believed to represent an elongated form of lysosomes rather than being elements of the agranular reticulum.

Exogenous peroxidase does not appear to stimulate the neurons to increase their concentration of lysosomes. This

result is compatible with that from studies on the uptake of exogenous peroxidase in the PNS, where it was found that the concentration of lysosomes remains the same whether or not the cells are injected with HRP (Rosenbluth and Wissig, 1964; LaVail and LaVail, 1974a, b; Broadwell and Brightman, 1979).

Infundibulum

After transection of the hypothalamo-neurohypophysial tract, the accumulation of neurosecretory materials in the stalk and subsequent enlargement of the stump have been extensively described (Dellmann and Owsley, 1968; Dellmann and Rodriguez, 1969, 1971; Rodriguez and Dellmann, 1970; Daniel and Prichard, 1970; Raisman, 1973). There is a general assumption that the enlargement of the stump after regeneration explains the establishment of a new neural lobe-like structure. The question is when this new neural lobe has the first release of hormone. The use of HRP in our experiment possibly could clarify this problem. The involvement of axonal cisterns as a conductor for retrograde and anterograde transport of exogenous proteins has been described (LaVail and LaVail, 1974b; Sotelo and Riche, 1974; Nauta and Britz, 1974; Broadwell and Brightman, 1979; Broadwell et al., 1980; Tsukita and Ishikawa, 1980). It has been shown that HRP can be transported retrogradely in transected, crushed, or ligated nerves (Kristensson and Olsson, 1974, 1976; Frizell et al., 1976). Our results show that intact and

transected axons can take up HRP. In intact axons, HRP reaction product was present only in ring-shaped structures and in small dense complexes. This indicates that HRP was pinocytosed by axon terminals and is in agreement with Price and Fisher (1978). In transected axons, the HRP reaction product was found in labeled cisterns of smooth and varicosed forms 16 h. after the injection. In addition, HRP reaction product was found in dense complexes in axons of the preterminal region and in axons of the preoptic region. Similar cisterns have been reported in the perikarya of the rat SON by Broadwell et al. (1980), who believed they represent an elongated form of lysosome.

Diffusely filled axons were observed 1 to 4 h. after the injection in axons of the preterminal region. This result suggests that HRP is diffused through the cut end and transported retrogradely before the axons are healed (Kristensson and Olsson, 1976).

Neural Lobe

HRP reaction product was found in various organelles depending on the various experimental conditions. In the cut neural lobe, HRP reaction product has been demonstrated in pinocytotic vesicles by axon terminals (Price and Fisher, 1978), in vacuolar and C-shaped structures of a size comparable to or larger than NGVs (Castel and Hockman, 1976; Theodosis et al., 1976), and in axonal sER and multivesicular bodies (Tsukita and Ishikawa, 1980). Our results show that

HRP reaction product was not incorporated into vacuoles or lysosomes of the damaged axons. HRP reaction product was found either free as small dense molecules in the axoplasm, or labeling the electron-lucent tails of the NGVs. Labeled cisterns were also observed 16 h. after the injection, suggesting that it is part of perikaryal lysosome.

The release of neurosecretory materials is known to occur by exocytosis of the dense cores of membrane-bounded NGVs (Douglas, 1973; Dreifuss, 1975; Nordmann, 1976; Morris et al., 1978). During secretion of neural lobe hormones, the excess membrane is recaptured from the axon surface (Theodosis et al., 1976; Nordmann and Morris, 1976; Morris and Nordmann, 1978; Nordmann and Morris, 1980). Vacuoles are the major routes for membrane retrieval after hormonal release (Lescure and Nordmann, 1980). There was no incorporation of HRP in the axon terminals in the form of small molecules. This was presumably due to the fact that there was no release of the hormone from the damaged axons. HRP reaction product was likely to come through pinocytotic activity of axolemma. These results agree with Price and Fisher (1978), who described HRP pinocytosed by axon terminals.

The reason electron-lucent tails of the NGVs were labeled is not known. It has been hypothesized the pseudopodia represent bound calcium at the vesicle attachment sites (Boyne et al., 1974), or they may represent the active site on the

granular membrane (Castel, 1977). Axons of the hilar region diffusely filled with HRP were also observed 1 to 4 h. after the injection. This also suggests that HRP is diffused through the cut end before the axons are healed.

Pituicytes of the neural lobe take up HRP by endocytosis, and the reaction product was present mainly in lysosomal multivesicular bodies as early as 24 h. after the injection in intact animals. In the transected neural lobe, similar labeled multivesicular bodies were seen 36 h. after the injection. In both cases, endocytosis occurs normally in the pituicytes, which agrees with results obtained by Theodosis et al. (1976). They observed that the reaction product was sequestered in lysosomal multivesicular bodies and dense bodies, and they suggested that endocytosis normally occurs in pituicytes which appeared related to neurohypophysial secretion.

In conclusion, the use of HRP as an exogenous protein tracer in the hypothalamo-neurohypophysial system of the grass frog Rana pipiens, shows there is no uptake of HRP in the form of vacuoles, suggesting that there is no release of hormone from the transected end after regeneration during the survival period. Based on morphological evidence, labeled cisterns of smooth and varicosed form are presumed to be a form of lysosome. It is believed that the presence of these cisterns in the axons comes about through anterograde transport. Axons diffusely filled with HRP are observed as early as

1 to 4 h. after the injection. Throughout the experiment, HRP is found in a unique association with NGVs, although the reason for this is still unclear.

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SUMMARY AND CONCLUSIONS

The hypothalamo-neurohypophysial system of the grass frog Rana pipiens was described in normal, transected and after the injection of HRP.

The ultrastructural characteristics of neurons of the PON are similar to those described by Palay (1960) in the fish. The magnocellular neurons of the PON consist of large cells containing large spherical, eccentrically located nuclei that sometimes had a shallow indentation. Their cytoplasm could be divided into peripheral and central zones. In the peripheral zone, the rER occurred in dilated and undilated varieties. In the central zone, numerous spherical or elongated mitochondria, Golgi complexes, dense bodies, multivesicular bodies, autophagic vacuoles, and NGVs of variable sizes and electron density were observed.

Two periods were distinguished in the evolution of postoperative changes in the PON. Twenty-eight to 36 hours after the transection there was a remarkable increase in cell bodies of reactive secretory neurons. Their hypertrophied nuclei were deeply indented and contained two to five hypertrophied nucleoli. The Golgi complex was widely dispersed and hypertrophied. Large and small pleomorphic dense bodies scattered in the perinuclear region. The NGVs were aggregated mainly in the central zone facing the indented side of the nucleus.

Two to five days after the transection, an intermediate stage between reactive and recovered cells was also seen. These cells had widely dispersed fragmented forms of dilated cisternae of rER. The Golgi complex was hypertrophied, few NGVs and pleomorphic dense bodies and multivesicular bodies were observed. The reactive cells showed the same changes as in the previous period but well developed; there were massive accumulations of NGVs in the central zone. The nucleus was distorted and deeply indented. The rER consisted of either dilated short cisternae or long cisternae forming an incomplete zone close to the plasma membrane.

Two cells can be distinguished toward the end of second period, recovered and degenerated cells. The recovered cells were hypertrophied with eccentric nuclei. The nuclei were generally circular in profile but became flattened on the side facing the main mass of the cytoplasm. The Golgi complex appeared less active. The rER were organized into long non-dilated concentric cisternae.

The degenerated cells were shrunken, the nuclei were dense pyknotic and shrunken. The cytoplasm was packed with ribosomes and contained autophagic vacuoles and dense bodies. The rER consisted of dilated cisternae fused to form continuous channels that occupied most of the cytoplasm. Finally, it became difficult to identify the cellular organelles of the degenerated cells. The degenerated neurons also had NGVs

in their cytoplasm, suggesting that transection did not stop the synthesis of NGVs. The presence of dense bodies in reactive and recovered neurons is considered secondary lysosomes involved in intracytoplasmic resorption of cell constituents.

The uptake of exogenous protein in the hypothalamo-neurohypophysial system was also studied in the grass frog Rana pipiens under normal conditions and following transection of hypothalamo-neurohypophysial tract. HRP reaction product was taken up by the intact and transected neurons and became localized in multivesicular bodies, small vacuoles, membrane-delimited cisterns, small dense bodies as well as pinocytotic vesicles. The pinocytotic labeled vesicles were considered passively endocytosed by membrane retrieved from the cell surface. Similar vesicles were described by Teichberg et al. (1975) and Holtzman et al. (1977) which support our findings. Labeled dense bodies are considered secondary lysosomes already present in the cell body.

Retrogradely transported labeled vacuoles were infrequently present in the perikarya, due to the fact that stimulation of the frogs for hormone release has not been performed. HRP labeled cisterns, either smooth or varicosed, were considered an elongated form of perikaryal lysosome. The concentration of lysosomes remains the same whether or not the cells are injected with HRP. Therefore, HRP does not appear

to stimulate the neurons to increase their concentrations of lysosomes.

After transection of hypothalamo-neurohypophysial tract, there is no uptake of HRP in the form of vacuoles, suggesting that there is no release of hormone from the transected end after regeneration during the survival period. HRP reaction product was found in the form of dense molecules in intact and transected axons. Labeled cisterns of smooth and varicosed forms were observed 16 h. after the injection in axons of the preterminal region and in axons of the preoptic region. Diffusely filled axons were observed 1 to 4 h. after the injection in axons of the preterminal region. This result suggests that HRP is diffused through the cut end and transported retrogradely before the axons are healed.

In the neural lobe, HRP reaction product was not incorporated into vacuoles or lysosomes of the damaged axons. It was found either free as small dense molecules in the axoplasm or labeling the electron-lucent tails of NGVs. Labeled cisterns were also observed 16 h. after the injection, suggesting that it is part of the perikaryal lysosome. Diffusely filled axons were also observed 1 to 4 h. after the injection in axons of the hilar region. This also suggests that HRP is diffused through the cut end before the axons are healed. Pituicytes were taken up HRP by endocytosis, then the product was sequestered in lysosomal multivesicular bodies and dense

bodies which suggested that endocytosis normally occurs in pituicytes. Throughout the experiments, the presence of HRP in close association with NGVs remained enigmatic.

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ACKNOWLEDGEMENTS

I wish to express great appreciation to my previous advisor Prof. Dr. Horst-Dieter Dellmann, who, during my graduate career at Iowa State University, provided sound advice and counsel in coursework and research.

Deep appreciation is extended to my advisor Etsuro Uemura for his inspiration and guidance in the preparation of this thesis. His friendship and understanding throughout my master's program is most greatly appreciated.

I wish to thank the members of my graduate advisory committee, Dr. J. Carithers and Dr. J. Viles for their advice and constructive suggestions.

I am particularly indebted to Mrs. Susan Jennifer Woroch Stahl for her excellent preparation of thin sections and photographic assistance. Her sincere friendship cannot be forgotten and is greatly appreciated.

Gratitude is also expressed to the faculty and staff of the Department of Veterinary Anatomy for their assistance and suggestions.

Thanks are extended to fellow graduate students at Iowa State University who provided friendship, advice, and criticism which gave me education and understanding of life in general.

Special thanks are extended to Prof. N. G. Ghoshal, whom I associated closely with during my years of graduate work.

Special thanks are extended to Miss Susan Danks for typing this manuscript.

Thanks are extended to the Iraqi government for their financial support.

I wish to dedicate this to my mother Khadijah, for patience and encouragement. My wife Hoda has been involved lately in my many hardships. I look forward to being able to enjoy a more normal life with her and our future baby.