Ultrastructure of Neurons in the Arcuate Nucleus of the Rat [1]

GUILLERMO JAIM ETCHEVERRY [2] AND
AMANDA PELLEGRINO DE IRALDI
Instituto de Anatomía General y Embriología, Facultad de Medicina — Universidad de Buenos Aires, Buenos Aires — República Argentina

ABSTRACT In the arcuate nucleus of the normal rat there are two types of neurons. One of them is characterized by a dense cytoplasmic matrix, dilated endoplasmic reticulum cisternae and well developed Golgi complexes; the other cell type by a paler overall appearance. In both of them granulated vesicles of about 1000 Å in diameter with a core of variable density are present. The frequent association between these vesicles and the Golgi complex suggests that they are formed in relation to this organoid. The presence of lysosomes and multivesicular bodies and their close relationship with granulated vesicles is analyzed.

The neuropil of the nucleus is constituted by axons, dendrites and glial processes. The majority of the axon profiles and nerve endings contain a mixed population of clear and granulated vesicles, the latter having a diameter ranging between 600 to 1300 Å.

The finding of granulated vesicles in arcuate neurons is discussed in relation with the known monoamine content of them and the dopaminergic nature of the fibers that form the tuberoinfundibular tract. It is suggested that these granulated vesicles contain primary monoamines.

Carlsson, Falck and Hillarp ('62) using the histochemical fluorescence method for catecholamines described a group of small nerve cells having weak green fluorescence located in the lateral walls of the third ventricle above the median eminence. Histochemical and pharmacological criteria for the specificity of the fluorescence reaction led to interpret these neurons as containing mainly dopamine and probably noradrenaline (Fuxe, '63; Dahlström and Fuxe, '64). Anatomically such nerve cells belong to the arcuate nucleus and to the ventral part of the anterior periventricular nucleus, both forming group A 12 of Dahlström and Fuxe ('64). These neurons give rise to fluorescent nerve fibers that form a tract, probably identical to the tuberohypophyseal tract (Spatz, '51; Szentagothai et al., '62), ending in the external zone of the median eminence in relation with the primary capillary loops of the hypophyseal portal plexus. In amphibians it was recently demonstrated that some of these fibers give monoaminergic nerve supply to the intermediate hypophyseal lobe (Enemar and Falck, '65; Iturriza, '66; Enemar et al., '67). The histochemical characteristics of such nerve fibers and terminals devoid of neurosecretory material (aldehyde fuchsin negative) also suggest that they contain dopamine and probably noradrenaline (Fuxe, '63, '64, '65). The existence of such a dopaminergic tuberoinfundibular system has recently received support from experimental work in which results of pharmacological treatment and lesions of the median eminence were studied (Fuxe and Hökfelt, '66).

The ultrastructure of the distal part of this dopaminergic system has been extensively studied (see DISCUSSION), but its proximal part, i.e. the neurons of the arcuate and anterior periventricular nuclei has received less attention.

In this paper we will describe the ultrastructural characteristics of the arcuate nucleus of the normal rat particularly in relation to the neuronal perikarya. "Dark" and "pale" neurons both containing granulated vesicles will be described.

MATERIAL AND METHODS

Adult rats of the Wistar strain weighing 150–200 gm were killed by decapitation.

[1] This work has been supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas, República Argentina and United States Air Force (AF-AFOSR 963-66).

[2] Fellow from the Consejo Nacional de Investigaciones Científicas y Técnicas, República Argentina.

Frontal brain slices of about 1–2 mm containing the anterior part of the arcuate nucleus were cut and immersed in cold 3% glutaraldehyde (Sabatini et al., '63) in 0.1 M phosphate buffer pH 7.4. After varying periods of time, with the help of the stereotaxic atlas of König and Klippel ('63), the arcuate nucleus was identified in the slice and dissected under a binocular stereomicroscope at 20 ×. The slices were also examined under a light microscope and the zone under study more exactly confirmed (fig. 1). After being washed in 0.3 M sucrose in the same buffer, the blocks were "refixed" in 1.5% buffered osmium tetroxide, transferred to a 2% aqueous solution of uranyl acetate, dehydrated in ethanol and embedded in Epon 812 according to the procedure of Luft ('61). All the above mentioned procedures were carried out at 0–4°C. The sections were stained with lead citrate (Reynolds, '63) and examined in a Zeiss EM 9-A electron microscope.

OBSERVATIONS

At low magnification of the electron microscope the arcuate nucleus shows a variety of nerve cell perikarya, glial cells and a neuropile containing axons, dendrites, synaptic endings and glial processes (fig. 2). Two types of neurons may be distinguished on the basis of some morphological parameters. In one cell type there is a rather dense cytoplasmic matrix which confers to the perikaryon an overall "dark" appearance. The nucleus is rather irregular with a folded and often dilated nuclear envelope. There are numerous polyribosomes free in the matrix and some attached

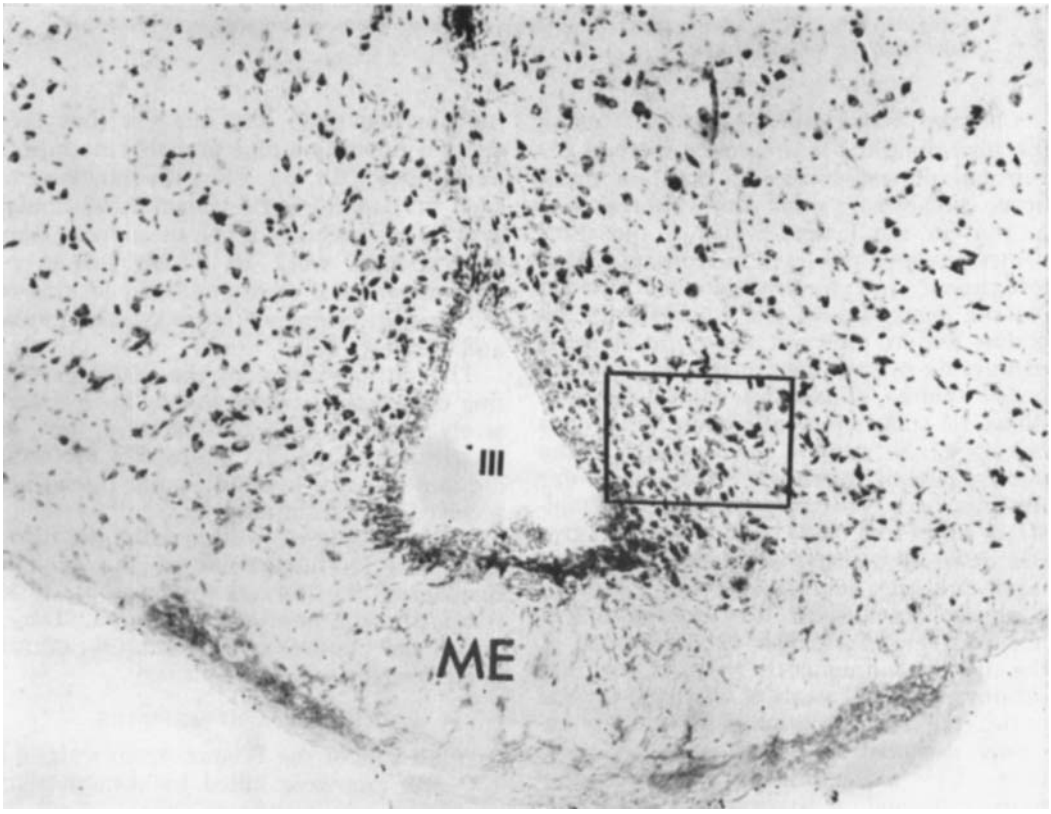


Fig. 1 Photomicrograph of a frontal section through the anterior hypothalamus of the rat involving the third ventricle (III) and the median eminence (ME). The area outlined in the square, corresponding to the arcuate nucleus was excised and studied under the electron microscope. Nissl stain. × 100.

to the cisternae of the endoplasmic reticulum. Another contrasting feature with the "pale" cell type is given by the dilatation of the cisternae of the vacuolar system. "Dark" neurons have large Golgi complexes located in the perinuclear zone and consisting of curved and dilated cisternae with a concentric disposition (figs. 3, 5). In close relationship with the Golgi complex there are dense cored vesicles of about 1000 Å in diameter having a clear rim around the granule. Few of such vesicles are observed in other areas of the cytoplasm. The density of the core varies considerably, especially in those vesicles more in proximity to the Golgi complex (fig. 5). Ocasionally an amorphous material is found within the Golgi cisternae. Such observation suggest a process of formation and maturation of the granulated vesicles related to the Golgi complex. Also in this neighborhood there are large dense bodies of 3–4000 Å having morphological characteristics of lysosomes. Some of these are uniformly dense but others are rather polymorphic and contain concentric membranes and even some granulated vesicles. Some lysosomes having clear vacuoles inside (fig. 6) may correspond to the vacuolated bodies or vacuolated dense bodies described in other cell types (Miller and Palade, '64; Holt and Hicks, '61). Also in the proximity of the Golgi complex some multivesicular bodies may be observed (figs. 5, 6).

The "pale" neuronal type has a more spherical nucleus and a less dense cytoplasmic matrix with fewer ribosomes (fig. 2). There are also fewer cisternae in the endoplasmic reticulum and these are rather flattened. In the perinuclear zone there are cisternae and vesicles of the Golgi complex but this is altogether less developed than in the "dark" type of neuron (fig. 4). Also here a few granulated vesicles and a variety of intermediate forms with the Golgi type of vesicle may be recognized. In this "pale" type of cell mitochondria appear to have a denser matrix and well defined crests, there are few typical lysosomes and less polymorphic ones.

The axons observed in the neuropil are predominantly unmyelinated with a diameter ranging from 0.2 μ to 2.2 μ. Some of these axons make synaptic contact with dendrites and perikarya of both nerve cell types and are characterized by a mixed population of vesicles: clear vesicles of about 400–500 Å in diameter and granulated vesicles with diameters ranging from 600 to 1400 Å with a mean of 1100 Å (fig. 6). These vesicles contain a dense core and a clear halo interposed between the dense core and the surrounding membrane. At higher magnification this membrane reveals a trilayered structure. The proportion of the two types of vesicles varies in the different endings but nerve terminals containing exclusively clear vesicles are ocasionally observed.

DISCUSSION

The present paper demonstrates the existence of "dark" and "pale" neurons in the arcuate nucleus of the rat. Both types contain granulated vesicles, while in the neuropil there are unmyelinated axons and endings with a mixed population of clear and granulated vesicles.

The existence of "dark" and "pale" neurons in the central nervous system has been a controversial matter since the light microscope demonstrated the presence of cells having a much more abundant ergastoplasm than others and such a discussion has also brought to the ultrastructural level (see Gonatas, '66). Recently "dark" and "pale" types of neurons were described in the ganglion cell layer of the retina (Pellegrino de Iraldi and Jaim Etcheverry, '67a). Since on purely morphological grounds it is difficult to interpret the significance of the two cell types of the arcuate nucleus, histochemical and experimental evidence is needed.

The close association of the Golgi complex, lysosomes and secretory vesicles is a problem of broad interest (see Novikoff et al., '64). A relationship between Golgi complex and the formation of catechol-containing droplets was postulated by De Robertis and Sabatini ('60) in the adrenomedullary cells of the hamster. The coexistence of Golgi complex elements, lysosomes and granulated vesicles in close vicinity was observed in the monoaminergic neurons of substantia nigra (Bak, '67) and of the retina (Pellegrino de Iraldi and Jaim Etcheverry, '67a). In arcuate

neurons these organelles may play some important role in the processes of synthesis and metabolism of the material contained in the granulated vesicles. The presence of such vesicles in close relation with lysosomes and multivesicular bodies in these neurons suggest the existence of a mechanism of intercellular disposal of secretory products in a way similar to that recently postulated by Smith and Farquhar ('66) in adenohypophyseal cells.

The granulated vesicles have been identified as the site of storage of norepinephrine in various territories. Different authors demonstrated that catecholamines are stored in electron-dense vesicles or droplets of large size (900–2500 Å) in the adrenomedullary cells of different species (Hillarp et al., '53; Lever, '55; Sjostrand and Wetzstein, '56; De Robertis and Vaz Ferreira, '57). Much smaller granulated vesicles (400–600 Å) were described and interpreted as the site of storage of norepinephrine by De Robertis and Pellegrino de Iraldi ('61a, b) in sympathetic nerves. That these vesicles also found in other autonomic territories (Lever and Esterhuizen, '61; Richardson, '62, '64) contain norepinephrine was demonstrated in a series of investigations from this laboratory (Pellegrino de Iraldi and De Robertis, '61, '63; see Pellegrino de Iraldi et al., '64; Pellegrino de Iraldi and Zieher, '66) which received supporting evidence from other experimental approaches (Wolfe et al., '62; Van Orden et al., '66). Granulated vesicles of the intermediate type (800–1700 Å) were first described in axons and nerve endings of the anterior hypothalmus of the rat by Pellegrino de Iraldi, Farini Duggan and De Robertis ('63) and interpreted as the possible site of storage of norepinephrine. Cell fractionation studies of the hypothalmus (De Robertis et al., '65) as well as pharmacological treatments (Matzuoka et al., '65; Ishii et al., '65; Hashimoto et al., '65), autoradiographic studies on the incorporation of labeled amines (Aghajanian and Bloom, '66) and histochemical studies (Wood, '66) gave further experimental evidence to that hypothesis.

Using fluoresence microscopy Fuxe ('65) described noradrenergic fibers in the arcuate nucleus which is also in line with the presence of granulated vesicles in axons and nerve endings.

The localization of monoamines in perikarya of the central nervous system is still a matter of controversy. In various nuclei several authors (Fuxe et al., '65; Lenn, '65; Fuxe et al., '66) have failed to observe a correlation between granulated vesicles and monoamine content. In perikarya of dopaminergic neurons of substantia nigra (Bak, '67) and retina (Pellegrino de Iraldi and Jaim Etcheverry, '67) granulated vesicles were observed. In these cases as well as in the presently described neurons of the arcuate nucleus they corresponded to the intermediate type of Pellegrino de Iraldi and De Robertis ('64).

Since it is well demonstrated (Spatz, '51; Szentagothai et al., '62; Fuxe and Hökfelt, '66) that numerous axons from the arcuate nucleus end in the external or palisade zone of the median eminence, it is appropiate to mention that various investigators have found in it unmyelinated axons with clear and granulated vesicles, these ranging in size between 800–1300 Å and which can be distinguished from the larger neurosecretary granules of the hypothalmus-neurohypophyseal tract (Barry and Cotte, '61; Dufy and Menefee, '65; Mazzuca, '65; Röhlich et al., '65; Seitz, '65; see Kobayashi, '64; Kobayashi et al., '66; Rinne, '66; Monroe, '67; see Streefkerk, '67). This finding together with the high concentration of monoamines in this region (Carlsson et al., '62; Fuxe, '64) has been interpreted as evidence supporting the monoaminergic nature of these granulated vesicles (see Kobayashi, '64; Röhlich et al., '65; Kobayashi et al., '66; Rinne, '66; see Streefkerk, '67). Pharmacological experiments also tend to support this view (Rinne and Arstila, '66; Streefkerk, '67; Matsui, '67; Pellegrino de Iraldi and Jaim Etcheverry, '67b) although some doubts have been introduced by the results of Mazzuca ('65) and Monroe ('67). The latter concludes that there is not conclusive evidence in the correlation between the granulated vesicles and monoamines, stating that "the possibility that the dense cored vesicles contain specific releasing factors is still quite real." If this were the case the granulated vesicles of the arcuate

neurons would represent the secretion of such polypeptides.

Although it seems early to establish a definite association between the granulated vesicles of arcuate neurons and the high monoamine content revealed by fluorescence microscopy (Dahlström and Fuxe, '64), all the evidences in favor of the monoaminergic nature of these vesicles are highly suggestive.

This study is intended to serve as a basis for future work on the morphological aspect of these neurons in different physiological and experimental conditions.

ACKNOWLEDGMENTS

We are deeply indebted to Professor Eduardo De Robertis for his valuable advice during the preparation of the manuscript, and to Miss Haydeé Agoff, Miss Nequer Vegliante and Mr. Basilio Rodríguez for their skillful technical assistance.

We also want to express our gratitude to Carl Zeiss, Argentina, for having provided the Zeiss EM 9-A electron microscope used throughout this investigation.

LITERATURE CITED

Aghajanian, G. K., and F. E. Bloom 1966 Electron-microscopic autoradiography of rat hypothalmus after intraventricular H^3-norepinephrine. Science, *153:* 308–310.

Bak, I. J. 1967 The ultrastructure of the substantia nigra and caudate nucleus of the mouse and the cellular localization of catecholamines. Exp. Brain Res., *3:* 40–57.

Barry, J., and G. Cotté 1961 Etude preliminaire, au microscope electronique, de l'éminence médiane du cobaye. Z. Zellforsch., *57:* 714–724.

Carlsson, A. B., B. Falck and N. -Å. Hillarp 1962 Cellular localization of brain monoamines. Acta Physiol. Scand., *36:* Suppl. 196.

Dahlström, A., and K. Fuxe 1964 Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand., *62:* Suppl. 232.

De Robertis, E., and A. Pellegrino de Iraldi 1961a A plurivesicular component in adrenergic nerves. Anat. Rec., *139:* 299.

——— 1961b Plurivesicular secretory processes and nerve endings in the pineal gland. J. Biophys. Biochem. Cytol., *10:* 361–372.

De Robertis, E., A. Pellegrino de Iraldi, G. Rodríguez de Lores Arnaiz and L. M. Zieher 1965 Synaptic vesicles from the rat hypothalamus. Isolation and norepinephrine content. Life Sci., *4:* 193–201.

De Robertis, E., and D. D. Sabatini 1960 Submicroscopic analysis of the secretory process in the adrenal medulla. Fed. Proc., *19:* 70–78.

De Robertis, E., and A. Vaz Ferreira 1957 Electron microscope study of the excretion of catechol-containing droplets in the adrenal medulla. Exptl. Cell Res., *12:* 568–574.

Dufy, P. E., and M. Menefee 1965 Electron microscopic observations of neurosecretory granules, nerve and glia fibers, and blood vessels in the median eminence of the rabbit. Am. J. Anat., *117:* 251–286.

Enemar, A., and B. Falck 1965 On the presence of adrenergic nerves in the pars intermedia of the frog, Rana temporaria. Gen. Comp. Endocr., *5:* 577–583.

Enemar, A., B. Falck and F. C. Iturriza 1967 Adrenergic nerves in the pars intermedia of the pituitary in the toad, Bufo arenarum. Z. Zellforsch., *77:* 325–330.

Fuxe, K. 1963 Cellular localization of monoamines in the median eminence and in the infundibular stem of some mammals. Acta Physiol. Scand., *58:* 383–384.

——— 1964 Cellular localization of monoamines in the median eminence and infundibular stem of some mammals. Z. Zellforsch., *61:* 710–724.

——— 1965 Evidence for the existence of monoamine neurons in the central nervous system. II. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand., *64:* Suppl. 247.

Fuxe, K., and T. Hökfelt 1966 Further evidence for the existence of tubero-infundibular dopamine neurons. Acta Physiol. Scand., *66:* 245–246.

Fuxe, K., T. Hökfelt and O. Nilsson 1965 A fluorescence and electronmicroscopic study on certain brain regions rich in monoamine terminals. Am. J. Anat., *117:* 33–45.

Fuxe, K., T. Hökfelt, O. Nilsson and S. Reinius 1966 A fluorescence and electronmicroscopic study on central monoamine nerve cells. Anat. Rec., *155:* 33–40.

Gonatas, N. K. 1966 The significance of "pale" neurons in human cortical biopsies. An electron microscopic study. J. Neuropath. Exp. Neurol., *25:* 637–645.

Hashimoto, Y., S. Ishii, Y. Ohi, N. Shimizu and R. Imaizumi 1965 Effect of DOPA on the norepinephrine and dopamine contents and on the granulated vesicles of the hypothalmus of reserpinized rabbits. Jap. J. Pharmacol., *15:* 395–400.

Hillarp, N. -Å., S. Lagerstedt and B. Nilsson 1953 The isolation of a granular fraction from the suprarrenal medulla containing sympathomimetic amines. Acta Physiol. Scand., *29:* 251–263.

Holt, S. J., and R. M. Hicks 1961 The localization of acid phosphatase in rat liver cells as revealed by combined cytochemical staining and electron microscopy. J. Biophys. Biochem. Cytol., *11:* 47–66.

Ishii, S. N., M. Shimizu, M. Matzuoka and R. Imaizumi 1965 Correlation between catecholamine content and number of granulated vesicles in the rabbit hypothalmus. Biochem. Pharmacol., *14:* 183–184.

Iturriza, F. 1966 Monoamines and control of the pars intermedia of the toad pituitary. Gen. Comp. Endocr., *6:* 19–26.

Kobayashi, H. 1964 Histochemical, electron microscopic and pharmacologic studies on the median eminence. In: Proc. 2nd. Internat. Congr. Endocrinology, London. Excerpta Medica International Congress Series N° 83, p. 570–576.

Kobayashi, H., Y. Oota, H. Uemura and T. Hirano 1966 Electron microscopic and pharmacological studies on the rat median eminence. Z. Zellforsch., *71:* 387–404.

König, J. F. R., and R. A. Klippel 1963 The rat brain. A stereotaxic atlas of the forebrain and lower part of the brain stem. The Williams and Wilkins Co., Baltimore.

Lenn, N. J. 1965 Electron microscopic observations on monoamine-containing brain stem neurons in normal and drug-treated rats. Anat. Rec., *153:* 399–406.

Lever, J. D. 1955 Electron microscopic observations on the normal and denervated adrenal medulla of the rat. Endocrinology, *57:* 621–635.

Lever, J. D., and A. C. Esterhuizen 1961 Fine structure of the arteriolar nerves in the guinea pig pancreas. Nature, *192:* 566–567.

Luft, J. H. 1961 Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol., *9:* 409–414.

Matsui, T. 1967 Effect of reserpine on the distribution of granulated vesicles in the mouse median eminence. Neuroendocrinol., *2:* 99–106.

Matzuoka, M., S. Ishii, N. Shimizu and R. Imaizumi 1965 Effect of Win 18501–2 on the content of catecholamines and the number of catecholamine containing granules in the rabbit hypothalamus. Experientia, *31:* 121–123.

Mazzuca, M. 1965 Structure fine de l'éminence médiane du cobaye. J. Microscopie, *4:* 225–238.

Miller, F., and G. Palade 1964 Lytic activities in renal protein absorption droplets. An electron microscopical cytochemical study. J. Cell. Biol., *23:* 519–532.

Monroe, B. G. 1967 A comparative study of the ultrastructure of the median eminence, infundibular stem and neural lobe of the hypophysis of the rat. Z. Zellforsch., *76:* 405–432.

Novikoff, A. B., E. Essner and N. Quintana 1964 Golgi apparatus and lysosomes. Fed. Proc., *23:* 1010–1022.

Pellegrino de Iraldi, A., and E. De Robertis 1961 Action of reserpine on the submicroscopic morphology of the pineal gland. Experientia, *17:* 122–123.

——— 1963 Action of reserpine, iproniazid and pyrogallol on nerve endings of the pineal gland. Int. J. Neuropharmacol., *2:* 231–239.

——— 1964 Ultrastructure and function of catecholamine containing systems. In: Proc. 2nd. Internat. Congr. Endocrinology, London. Excerpta Medica International Congress Series N° 83, p. 355–383.

Pellegrino de Iraldi, A., H. Farini Duggan and E. De Robertis 1963 Adrenergic synaptic vesicles in the anterior hypothalamus of the rat. Anat. Rec., *145:* 521–531.

Pellegrino de Iraldi, A., and G. Jaim Etcheverry 1967a Granulated vesicles in retinal synapses and neurons. Z. Zellforsch., *81:* 283–296.

——— 1967b Ultrastructural changes in the nerve endings of the median eminence after Nialamide-DOPA administration. Brain Res., *6:* 614–618.

Pellegrino de Iraldi, A., and L. M. Zieher 1966 Noradrenaline and dopamine content of normal, decentralized and denervated pineal gland of the rat. Life Sci., *5:* 149–154.

Pellegrino de Iraldi, A., L. M. Zieher and E. De Robertis 1964 Ultrastructure and pharmacological studies of nerve endings in the pineal organ. In: J. Ariëns Kappers and J. P. Schadé, editors: Structure and Function of the Epiphysis cerebri. Progress in Brain Research, *10:* 389–422. Elsevier Publishing Co., Amsterdam.

Reynolds, E. S. 1963 The use of lead citrate as an electron dense stain in electron microscopy. J. Cell Biol., *17:* 208–212.

Richardson, K. C. 1962 The fine structure of autonomic nerve endings in smooth muscle of the rat vas deferens. J. Anat. (Lond.), *96:* 427–442.

——— 1964 The fine structure of the albino rabbit iris with special reference to the identification of the adrenergic and cholinergic nerves and nerve endings in its intrinsic muscles. Am. J. Anat., *114:* 173–205.

Rinne, U. K. 1966 Ultrastructure of the median eminence of the rat. Z. Zellforsch., *74:* 98–122.

Rinne, U. K., and A. U. Arstila 1966 Electron microscopic evidence on the significance of the granular and vesicular inclusions of the neurosecretory nerve endings in the median eminence of the rat. I. Ultrastructural alterations after reserpine injection. Med. Pharmacol. exp., *15:* 357–369.

Röhlich, P., B. Vigh, I. Teichmann and B. Aros 1965 Electron microscopy of the median eminence of the rat. Acta Biol. Acad. Sci. hung., *15:* 431–457.

Sabatini, D. D., K. G. Bensch and R. J. Barrnett 1963 Cytochemistry and the electron microscope. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol., *17:* 19–58.

Seitz, H. M. 1965 Zur Elektronenmikroskopische Morphologie des Neurosekrets im Hypophysenstiel des Schweins. Z. Zellforsch., *67:* 351–366.

Sjöstrand, F. S., and R. Wetzstein 1956 Elektronenmikroskopische Untersuchung der phäochromen (chromaffinen) Granula in den Markzellen der Nebenniere. Experientia, *12:* 196–199.

Smith, R. E., and M. G. Farquhar 1966 Lysosome function in the regulation of the secretory process in cells of the anterior pituitary gland. J. Cell Biol., *31:* 319–347.

Spatz, H. 1951 Neues über die Verknüpfung von Hypophyse und Hypothalamus. Acta neuroveget., *3:* 1–49.

Streefkerk, J. G. 1967 Functional changes in the morphological appearance of the hypothalamo-hypophyseal neurosecretory and catecholaminergic neural system and in the adenohypo-

physis of the rat. A light, fluorescence and electron microscopic study. Drukkerij Wed. G. van Soest N. V., Amsterdam.

Szentagothai, J., B. Flerko, B. Mess and B. Halasz 1962 Hypothalamic control of the anterior pituitary. Académiai Kiadó, Budapest.

Van Orden, L. S., F. E. Bloom, R. J. Barrnett and N. J. Giarman 1966 Histochemical and functional relationships of catecholamines in adrenergic nerve endings. I. Participation of granular vesicles. J. Pharmacol. Exp. Ther., *154:* 185–199.

Wolfe, D. E., L. T. Potter, K. C. Richardson and J. Axelrod 1962 Localizing tritiated norepinephrine in sympathetic axons by electron microscopic autoradiography. Science, *138:* 440–442.

Wood, J. G. 1966 Electron microscopic localization of amines in central nervous tissue. Nature, *209:* 1131–1133.

PLATE 1

EXPLANATION OF FIGURE

2 Low power electron micrograph of the arcuate nucleus. The neuron in the left of the field corresponds to the "pale" type of our description. The rounded nucleus (N) and the clear cytoplasm are characteristic of this cell. The one in the lower right corner corresponds to the "dark" type of neuron. It is characterized by an irregular nucleus (N) and a darker cytoplasmic matrix. In the Golgi zone (G) of this cell granulated vesicles are observed (arrows). In the upper right corner, a portion of a "dark neuron" is seen. Between the cells unmyelinated axons (A) having a mixed population of clear and granulated vesicles are present. × 11,850.

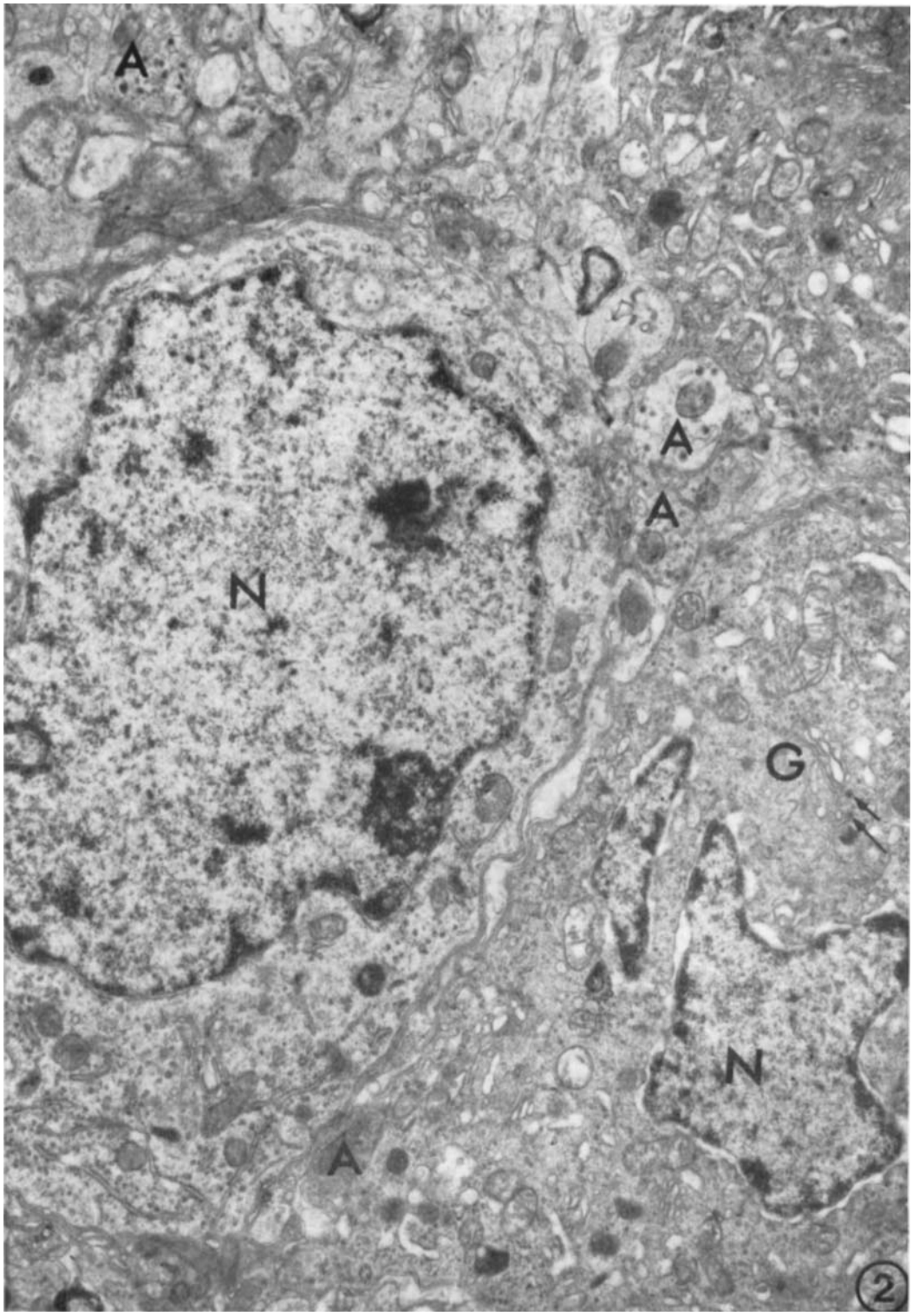

A
D
A
A
N
G
N
A
2

PLATE 2

EXPLANATION OF FIGURE

3 Arcuate nucleus nerve cell of the "dark" type. The irregular nucleus (N) and the dark appearance of its cytoplasm are prominent characteristics of this type of cell. The Golgi complex (G) is well developed and located in the perinuclear zone. Numerous polyribosomes and ribosomes attached to the dilated endoplasmic reticulum cisternae are seen. Granulated vesicles of varying densities (arrows) and with a diameter of about 1000 Å are present in the cytoplasmic matrix. Some are also associated with components of the Golgi complex. Mitochondria, lysosomes (Ly) and multivesicular bodies (Mv) are also seen. × 23,100.

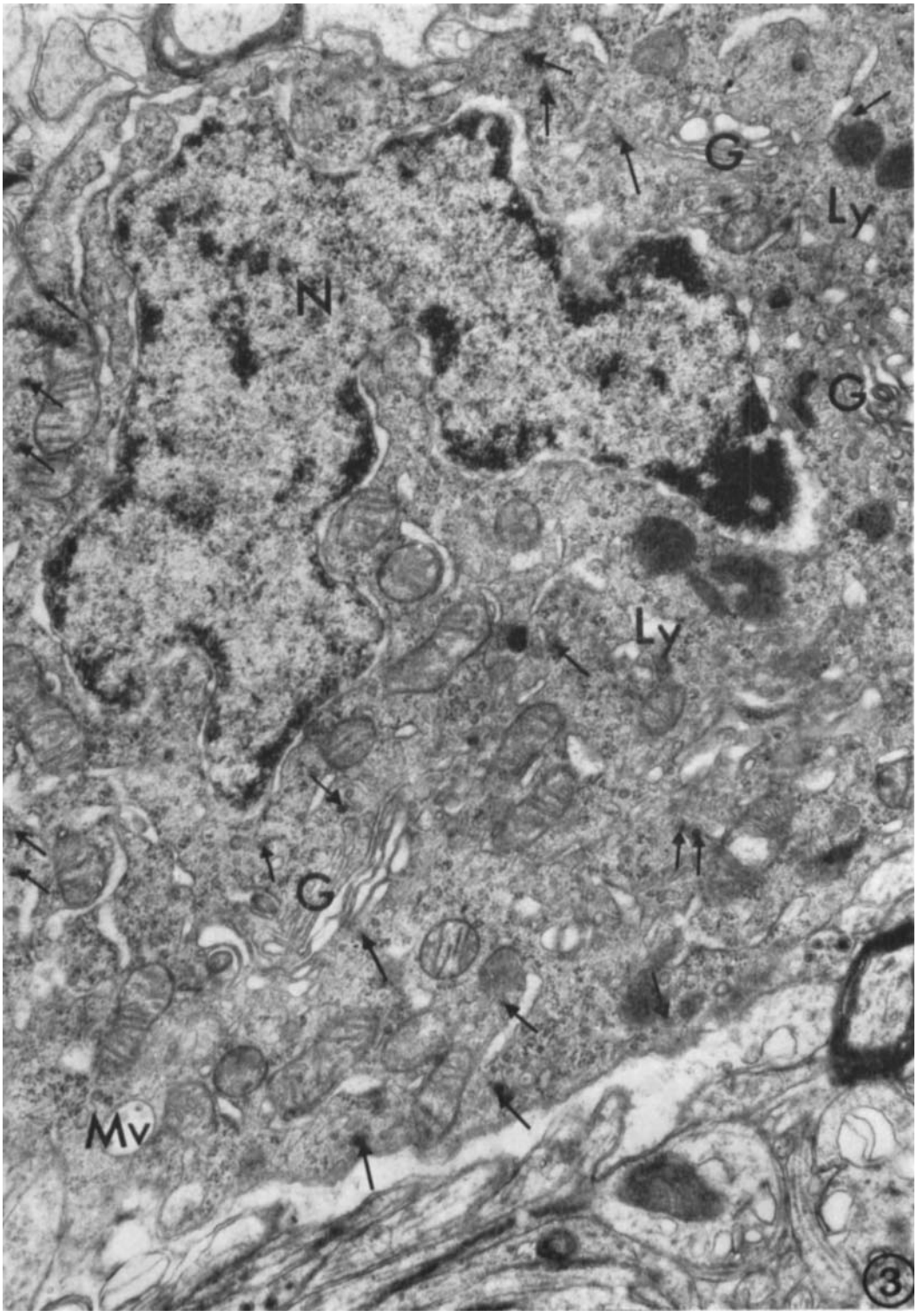

G
Ly
N
G
Ly
G
Mv
3

PLATE 3

EXPLANATION OF FIGURE

4 A "pale" neuron of the arcuate nucleus. The nucleus (N) exhibits a regular contour and the cytoplasmic matrix has an overall pale appearance. In the Golgi complex (G) located in the perinuclear zone granulated vesicles can be observed (arrows). Mitochondria, lysosomes (Ly) and multivesicular bodies (Mv) are also seen. A portion of the cytoplasm of a glial cell (GC) is observed in the left margin of the field. × 23,100.

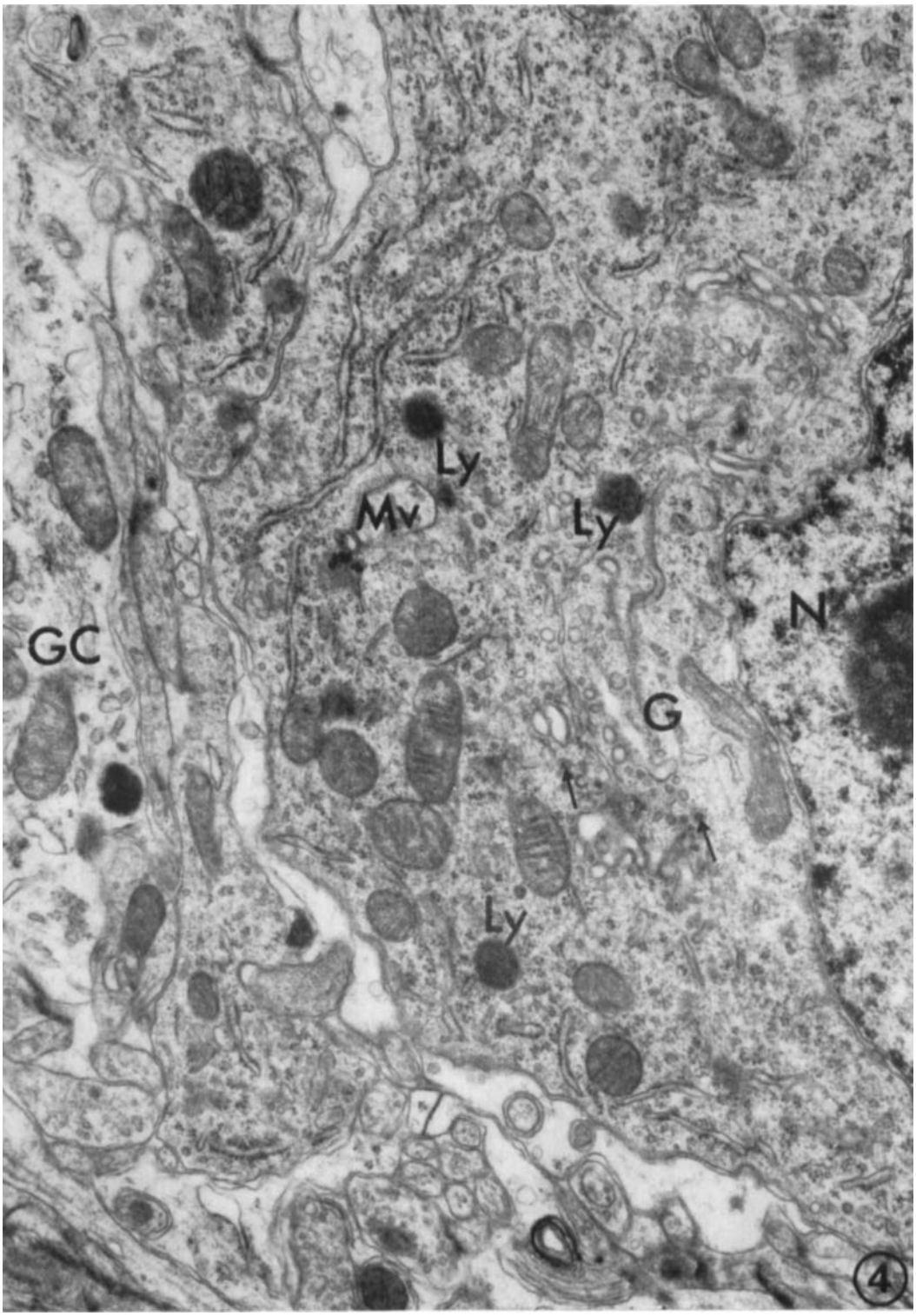
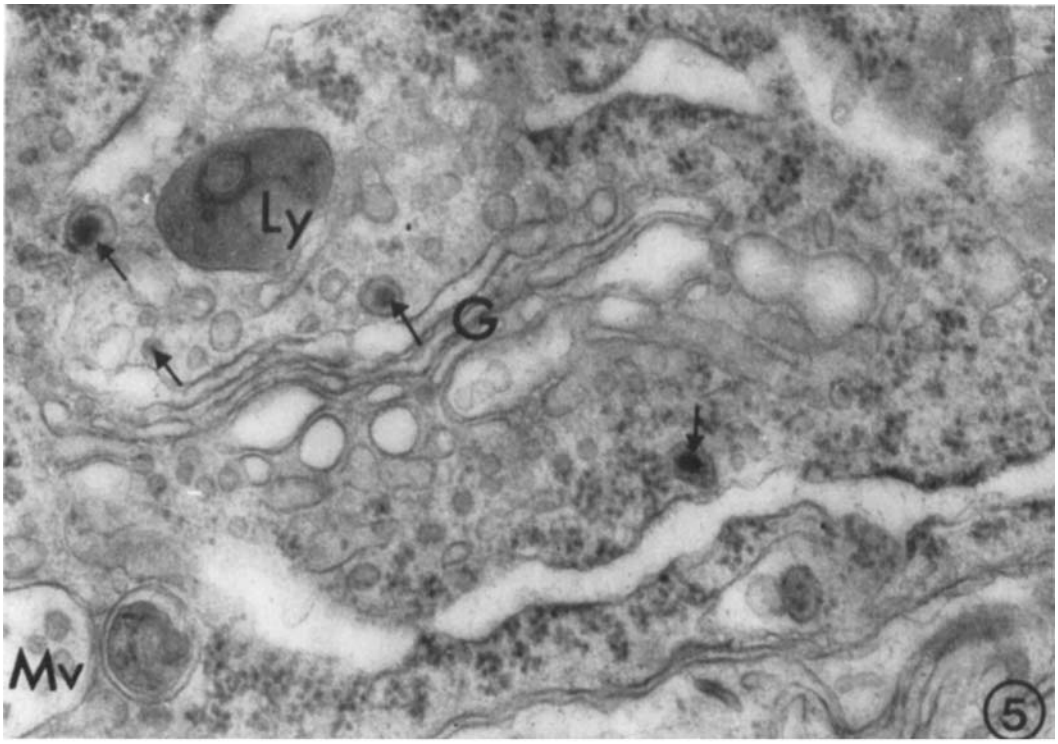

Ly
Mv
Ly
N
GC
G
Ly
4

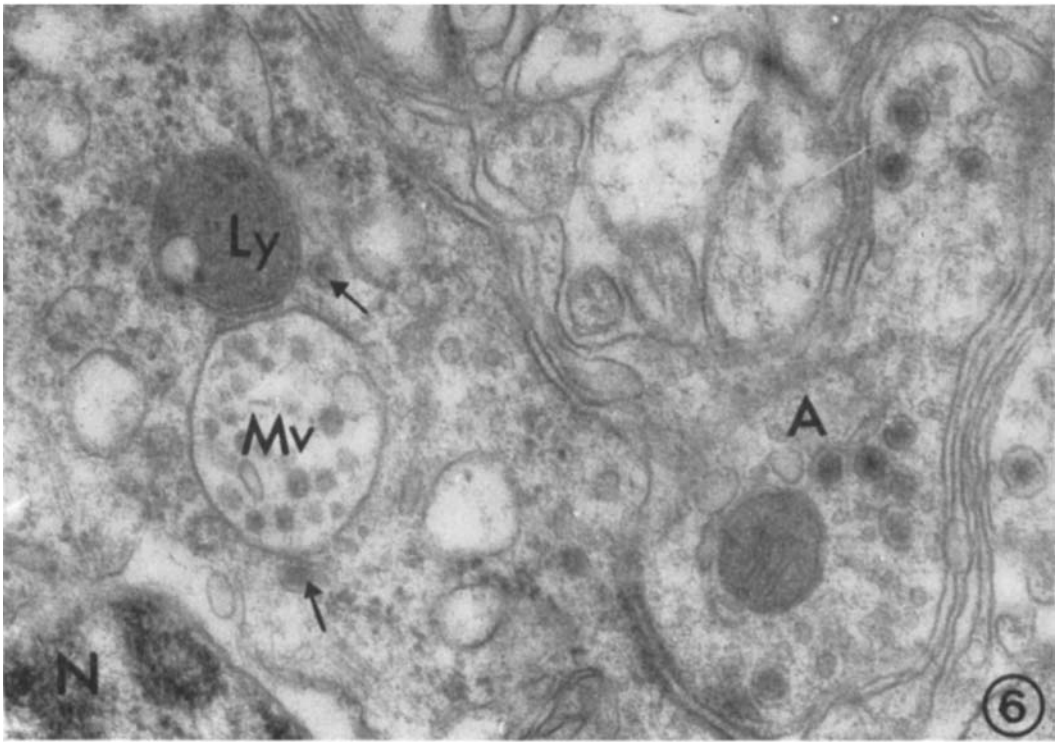
PLATE 4

EXPLANATION OF FIGURES

5 The Golgi zone of a "dark" type of neuron. Granulated vesicles (arrows) of varying size and density of the dense core appear in the vicinity of the Golgi complex (G). A polymorphous lysosome (Ly) and a multivesicular body (MV) are also present in this region. The cytoplasmic matrix of this cell is dense and the free polyribosomes and ribosomes attached to the endoplasmic reticulum cisternae are abundant. × 53,100.

6 A lysosome (Ly) and a multivesicular body (Mv) are observed in the cytoplasm of a "dark" cell type. The lysosome having an area of low density, corresponds to the so-called vacuolated dense bodies. The association of granulated vesicles (arrows) with lysosomes and multivesicular bodies is frequently seen in these neurons. On the right side of the figure an axon (A) containing granulated vesicles and making synaptic contact with the cell soma is observed. × 55,800.


Ly
G
Mv
5


Ly
Mv
A
N
6