THE UNIVERSITY OF MANITOBA

THE POST-NATAL DEVELOPMENT OF THE OLFACTORY BULB OF THE RAT

A LIGHT AND ELECTRON MICROSCOPIC STUDY

by

DYAL NARAIN PERTAB SINGH

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Dedicated to my wife, son (Davindra) and parents.

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ABSTRACT

Olfactory bulbs of Sprague-Dawley albino rats were fixed in aldehydes by both vascular perfusion and immersion. Araldite was used as the embedding medium and sections were examined with light and electron microscopes. The development of blood vessels, neurons and glial cells was studied from birth to five weeks postnatal.

Light microscopic studies showed that blood vessel densities and width of the external plexiform layer attained their maximum growth at 21 days postnatal.

Differentiation of Blood Vessels:

Both primitive and mature looking blood vessels designated types I and II respectively, were encountered in all time periods studied. The type I possessed a slit like lumen and a thick endothelial cell wall surrounded by the watery cytoplasm of an astrocyte. The basal lamina was relatively thin. These were encountered predominantly during the first week. Further development resulted in the enlargment of the lumen and attenuation of the endothelial cell. These were ascribed as type II blood vessels. The basal lamina was well defined and wider than those in type I blood vessels. Astrocytic, neuronal and oligodendritic processes were related to these mature vessels. Type II vessels appeared in large numbers during the second and third week of development. Pericytes were encountered in both types of blood vessels.

Neuronal Differentiation.

Mitral and granule cells were studied as representative neuronal

cells. Mitral cells at birth showed a well defined perikaryon in which free ribosomes were the predominant cytoplasmic organelle. Short and thick dendritic processes extended into the adjacent neuropil. At the end of the first week, the cytoplasmic area had increased significantly and there was a considerable elaboration of rough endoplasmic reticulum and Golgi complexes. Occasional lysosomal bodies were observed. Concomitantly, the apical dendrite had also increased in length. Subsequent development revealed that the rough endoplasmic reticulum, Golgi complexes and dense lipofuscin granules continued to increase in numbers and complexity.

Immature granule cells have been observed in clusters with apposition of cytoplasmic membranes. The nucleus occupied the major portion of the perikaryon and was surrounded by a thin rim of cytoplasm. Free ribosomes were the major cytoplasmic organelle. Few synapses were observed on the soma. The differentiating granule cell showed a nucleus in which the chromatin tended to aggregate at its periphery. There was little change in its cytoplasm.

Differentiation of Astrocytes and Oligodendrocytes:

Astrocytes:

Astrocytes were observed as satellite cells to nerve fibers, blood vessels and neurons. The nucleus has an irregular contour and uniform distribution of chromatin. Astrocytic processes surrounding Type I or immature blood vessels had a clear and watery cytoplasm and few cytoplasmic organelles. Astrocytic processes in relation to type II vessels appeared less watery. Few astrocytic processes

displayed filaments in 21 day and older animals.

Oligodendrocytes:

In one day old rat, oligodendrocytes are typified by a dense large nucleus, a thin rim of cytoplasm and few processes. Differentiating cells displayed a cytoplasm in which the organelles increased in numbers and complexity. Clumping of chromatin occurred at the nuclear membrane. By 21 days postnatal oligodendrocytes have reached maturity retaining a dense nucleus and an equally dense cytoplasm showing numerous ribosomes and well organized granular reticulum.

INTRODUCTION

The olfactory system of man and primates is relatively small in comparison to lower forms of animals. Vertebrates, from acordata, Amphioxus sp., to mammalia, display a wide morphological variety of olfactory systems each particularly adapted to suit the functional need of the animal. Documentation and description of vertebrate olfactory systems are fully treated by Wiedersheim and Parker, (1907); Matthes, (1934); Allison, (1953b).

The morphology and cytoarchitecture of the olfactory bulb were first studied by Golgi, (1875) using his newly developed bichromate-silver impregnation technique. Later Cajal, (1890); van Gehuchten and Martin, (1891), and several other investigators described the cytology of the olfactory bulb. Since the work of Cajal, (1911) little has been added until Allison and Warwick, (1949) and Allison, (1953a, b) made some quantitative observations on the olfactory system of the rabbit. The problem of olfaction is still to be elucidated at the anatomical, physiological and biochemical level so much so that reports on the olfactory system contained many suppositions and few factual statements.

The primary olfactory structures are found in the rhinencephalon and consist of the olfactory nerves, bulbs, tracts, subcallosal gyrus, anterior perforated substance and uncus. Fiber connections between the primary centres and the secondary olfactory centres in the central nervous system are numerous, complex and extensive. The anatomy of the secondary olfactory centres is described (Curtis et al., 1972:

p. 429-436) as the emotional, vital, visceral brain and limbic system. These structures are physiologically related to vital senses that contribute to the survival and food habits of the animal in its surrounding.

In primitive animals, smell is employed as the initial sensory mechanism to trigger other senses such as gustatory, vision and auditory. However, in highly developed animals, birds and man, perception supercedes the sense of smell, and in others, such as the rat, a combination of smell and perception is used. Early postnatal rats employ almost exclusively the sense of smell to carry out their feeding habits. As development progresses a combination of smell and perception is involved.

As the olfactory bulb is an integral part of the central nervous system, its development consisting of differentiation, migration and maturation of cellular components may be expected to follow a pattern as depicted in other areas of the central nervous system. Bearing this in mind, it is the purpose of this investigation to use the olfactory bulbs to study histogenesis and/or neuronogenesis in rats, from birth to five weeks, at the light and electron microscopic level.

STATEMENT OF THE PROBLEM

The morphology of vertebrate olfactory systems has been described by several investigators (Wiedersheim and Parker, 1907; Matthes, 1934; Allison, 1953a, b).

Light microscopic studies on the olfactory bulb of various animals, including the mouse, rat and rabbit, have been carried out by Cajal (1890, 1909); Hortega (1920); Altman (1969); Hinds (1968, 1972). Similarly, the fine structure has been described by Andres (1965, 1970); De Lorenzo (1970); Hinds (1970, 1972).

In spite of several electron microscopic studies on the development of elements in the central nervous system, the early development of neurons relating to their differentiation from proliferating cells, their migration to definitive positions and subsequent development of characteristics, axons and dendrites, is poorly understood in most regions of the vertebrate nervous system (Hinds 1972).

In the present study, investigations were carried at the light and electron microscopic level to determine regional and cellular changes including neuronal, neuroglial and vascular development in the olfactory bulb of rats from birth tofive weeks. Ultrastructure studies undoubtedly will provide the ultimate identification of morphological features. However, light microscopy gives the most reasonable and practical means of determining the overall development in the nervous system (Allerand, 1971).

The goal of this study is to establish the pattern of neuronal, glial and vascular development of the olfactory bulb in the rat. This

information may then serve as a basis with which morphological changes observed in experimental situations could be compared.

Literature Review

Morphology:

The olfactory system is made up of the olfactory epithelium, olfactory bulb and associated centres in the brain (Figure 1).

The olfactory epithelium is located on the superior concha and adjacent part of the nasal septum. Sensory, sustentacular cells and Bowman's glands make up its histology. Extending from the sensory cell is a short dendrite which gives rise to several villi. At the opposite pole of the sensory cell projects an axon which pierces the cribiform plate and enters the olfactory bulb (Figure 2). These axons represent the first cranial nerve or olfactory nerve.

Olfactory bulbs are paired structures located anterior to the cerebral hemispheres in the rat and ventral in human. The shape and size of the bulb are directly related to the reliance of the animal on the sense of smell. The olfactory bulb is connected with the olfactory centers of the brain by means of the olfactory tract. Most, if not all, of the axonal fibers constituting the olfactory tract originate from the mitral and probably tufted cells.

The centres of olfaction in the brain have not been fully elucidated. However, it is generally accepted that fibers from the medial portion of the tract make synaptic connections in the septal region and/or continue into the anterior commissure. Fibers from the intermediate component of the tract synapse in the anterior perforating substance while those of the lateral portion of the olfactory tract make synaptic contacts in the uncus and cortico-amydaloid nucleus (Curtis *et al.*, 1972),

Figure 1. Diagram to show the morphology of the olfactory system in mammals and the fiber connections between the olfactory bulb and centres in the cerebral cortex (Modified after Noback, 1967).

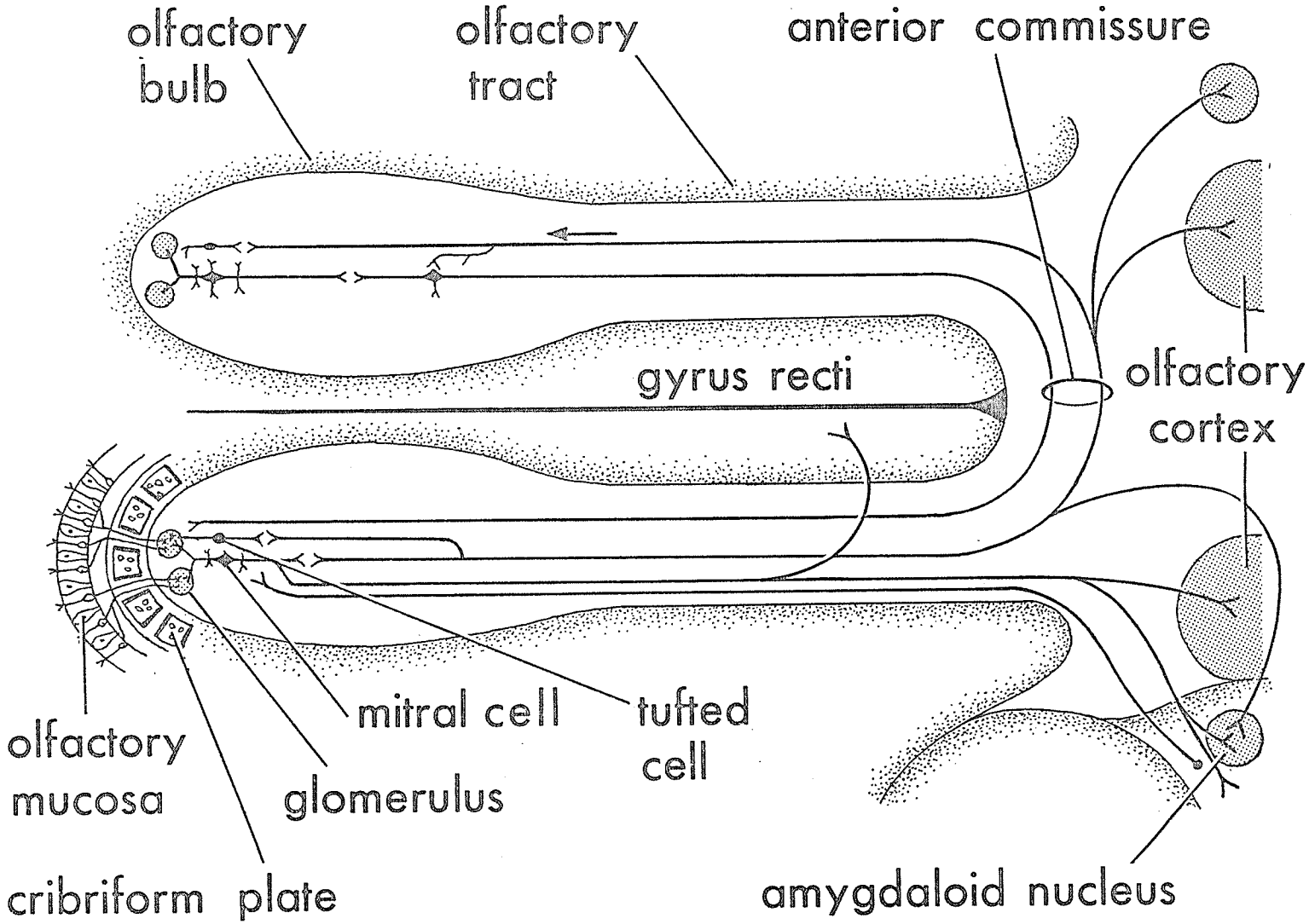

olfactory bulb
olfactory tract
anterior commissure
gyrus recti
olfactory cortex
olfactory mucosa
mitral cell
tufted cell
glomerulus
cribriform plate
amygdaloid nucleus

Figure 2. This diagram depicts the relationship between the olfactory epithelium, receptor axons and olfactory bulb (Modified after Price and Powell, 1970a).

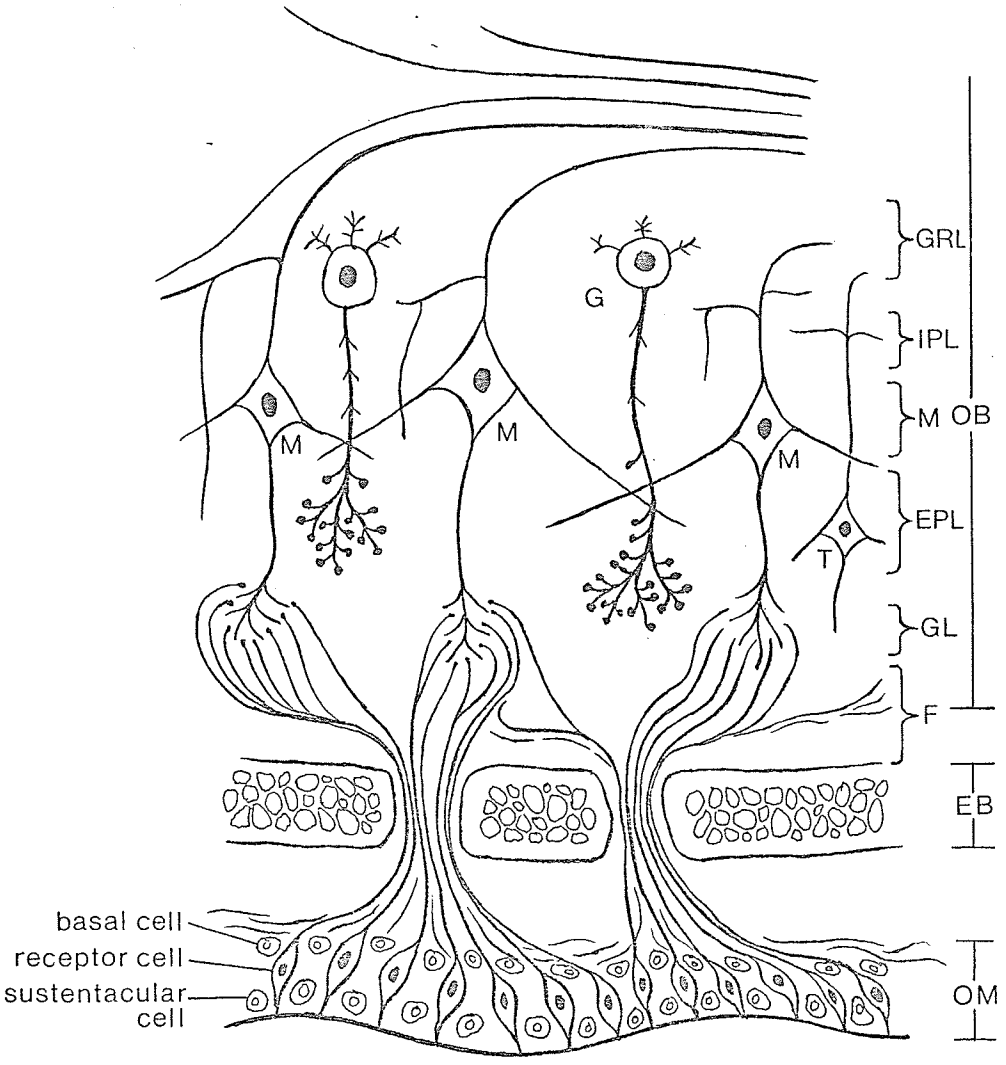


Fig. 2 – Drawing showing the olfactory mucosa and its relationship to the olfactory bulb.

OM = olfactory mucosa
EB = ethmoid bone
OB = olfactory bulb
T = tufted cell
G = granule cell
M = mitral cell
F = fiber layer
GL = glomerular layer
EPL = external plexiform layer
M = mitral layer
IPL = inner plexiform layer
GRL = granule layer

Modified after Price and Powell (1970a)

(Figure 1).

Microscopic Anatomy:

The histology of the olfactory system has been described in several texts (Bloom and Fawcett, 1968; Copenhaver et al., 1971) and in review articles (Allison, 1953a,b).

Olfactory Epithelium:

The olfactory epithelium lines the superior conchae and part of the superior portion of the nasal septum in humans and mammals. Although it appears, in light microscopy, as being of the pseudo-stratified epithelium, several authors have described it as being a modified epithelium. Three cell types are identified in the olfactory epithelium and include sustentacular or supporting cell sensory or receptor cell and basal cell (Figure 2).

Supporting cells are more numerous and superfically located. These cells contain pigment and granules arranged in longitudinal rows. A centrally located nucleus is present in the cytoplasm and micro-villi are observed beneath the luminal surface. Close association between receptor cells and supporting cells results in the formation of tight junctions (desmosomes).

Sensory or receptor cells are located below the supporting cells and are classified as being bipolar with a single dendrite and axon. The dendrites are short and extend between the supporting cells to the luminal surface where they expand to form bulb-like structures which contain basal bodies. Cilia extend from the basal bodies and become modified to form villi.

These structures are believed to be the receptive site for odour producing molecules. Stimulation produced by chemicals is transduced into electrical impulses which travel along the receptor cell axon to the glomerular layer of the olfactory bulb (Figure 2).

A third cell type has also been described. This cell type occupies the basal part of the epithelium and is believed to be capable of undergoing differentiation to form both the receptor and supporting cell. Graziadei (1973), using a combination of ultrastructural and autoradiographic studies, has shown that basal cells are capable of replacing degenerated sensory and supporting cells in the olfactory mucosa of the frog, Rana pipiens and Rana catesbeiana.

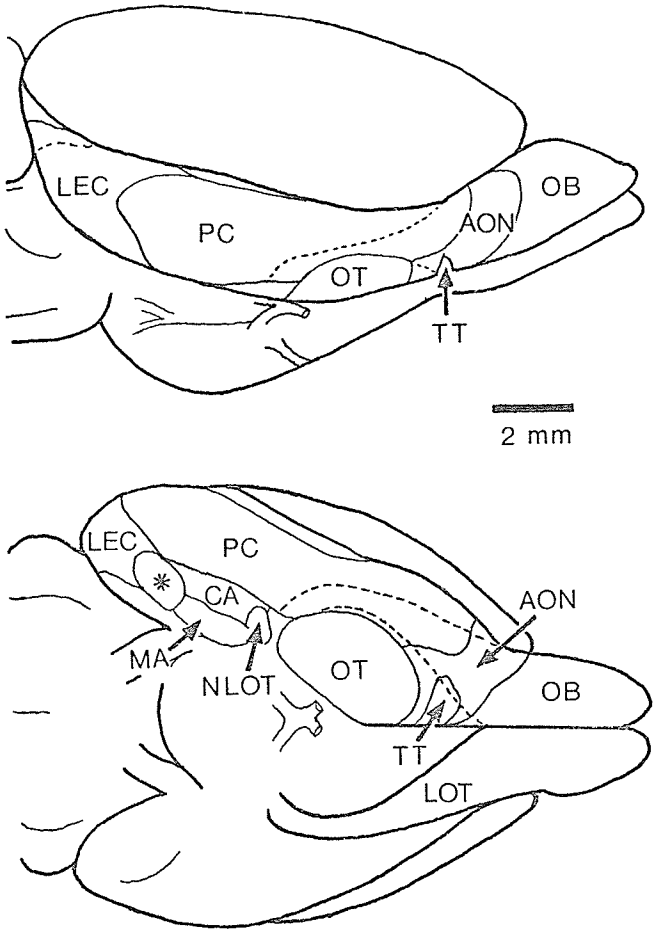
Glandular structures have been observed to be present below the olfactory epithelium. These glands, Bowman's, have been described as containing a body, fundus and duct. Secretions from Bowman's glands are discharged at the luminal surface and give the mucosa its yellowish colour.

Olfactory Bulb:

The olfactory bulb is spherical in shape and by means of the olfactory tract attaches to the cerebral hemispheres. Six cellular layers have been described in the cortex of the olfactory bulb. These are, from pial surface to olfactory ventricle, the fiber, glomerular, outer plexiform, mitral, inner plexiform and granular layer.

Axons of sensory cells make up the fiber layer. These axons synapse at the glomerular layer with the dendrites of mitral and tufted cells. Subsequently, the axons of mitral and tufted cells leave

Figure 3. Diagram of the olfactory bulb and related surface connections of the basal forebrain of the rat (Modified after Price, 1973).



AON = ANTERIOR OLFACTORY NUCLEUS
CA = CORTICAL NUCLEUS OF THE AMYGDALA
LEC = LATERAL ENTORHINAL CORTEX
LOT = LATERAL OLFACTORY TRACT
MA = MEDIAL NUCLEUS OF THE AMYGDALA
NLOT = NUCLEUS OF THE LATERAL OLFACTORY TRACT
OB = OLFACTORY BULB
OT = OLFACTORY TUBERCLE
PC = PREPIRIFORM CORTEX
TT = TENIA TECTA OR ANTERIOR RUDIMENT OF THE HIPPOCAMPUS

MODIFIED AFTER PRICE (1972)

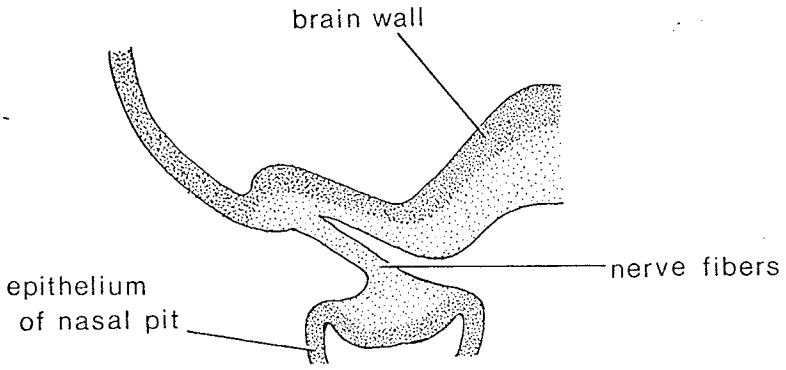
the olfactory bulb and form the olfactory tract to relay impulses to the relevant centres in the cerebral hemispheres (Figure 3). The olfactory cortex consists of those areas in the basal forebrain which receives fibres directly from the olfactory bulb. These areas are several and include, the anterior olfactory nucleus, the olfactory tubercle, the prepiriform cortex, the nucleus of the lateral olfactory tract, the cortical nucleus of the amygdala, and the lateral entorhinal cortex (Lohman, 1963; White, 1965; Scalia, 1966; Price, 1973). Studies involving Golgi impregnation techniques have demonstrated that fibers from the olfactory bulb terminate in the plexiform layer of the anterior olfactory nucleus and in the prepiriform cortex.

Embryology of the Olfactory Bulb and Central Nervous System.

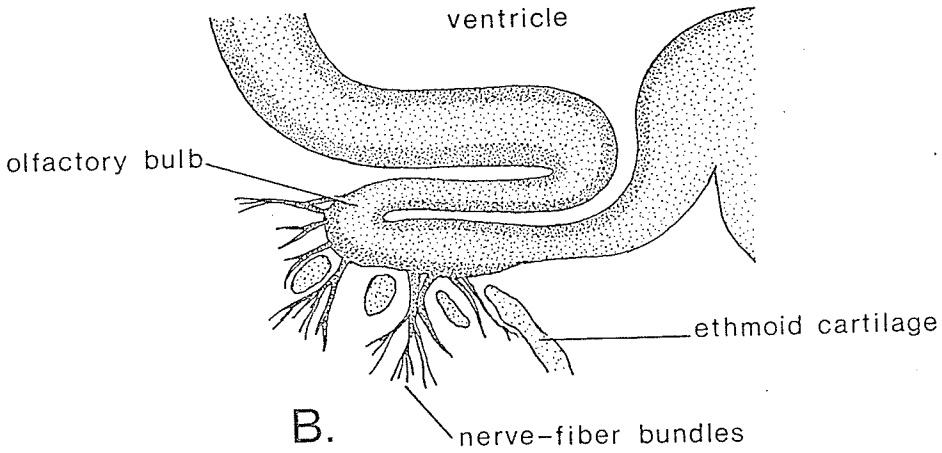
In humans, the primordium of the olfactory lobes appears during the sixth week of gestation (Hamilton et al., 1959; Moore, 1973). At first, these appear as swellings on the ventral surface of the cerebral hemispheres. Later, these swellings enlarge into spherical shaped olfactory bulbs. Subsequently, as development proceeds, the bulbs become separated from the cerebral hemispheres by means of the olfactory tract (Arey, 1966), (Figure 4).

Development of the central nervous system takes origin from a thickened mass of cells, the neural plate, located along the mid-dorsal line of the embryo. The primordium of the nervous system appears during the third week of development in man and is made up of immature and undifferentiated cells. Subsequent development produces two cell types, neuroblast and spongioblast. Neuroblasts and spongioblasts have been described to differentiate into nerve cells and neuroglial cells respectively (His, 1889).

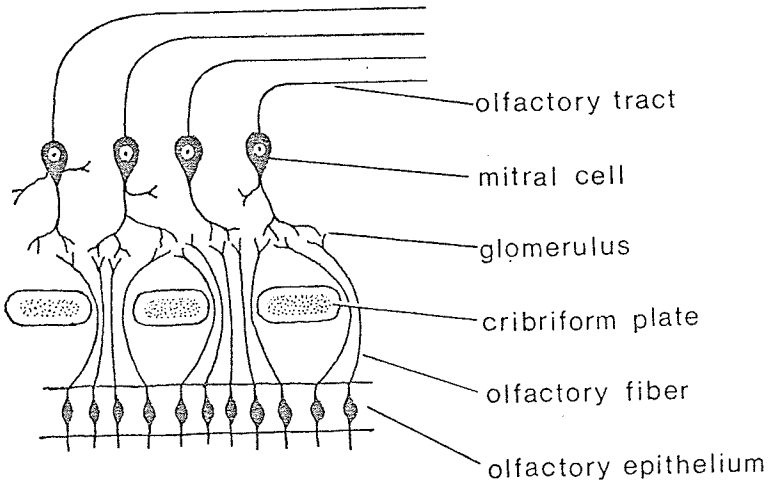
Figure 4. Schematic diagrams, A, B, C to illustrate the growth of the olfactory bulb and subsequent connections with olfactory epithelium in man (Modified after Arey, 1966).



A.



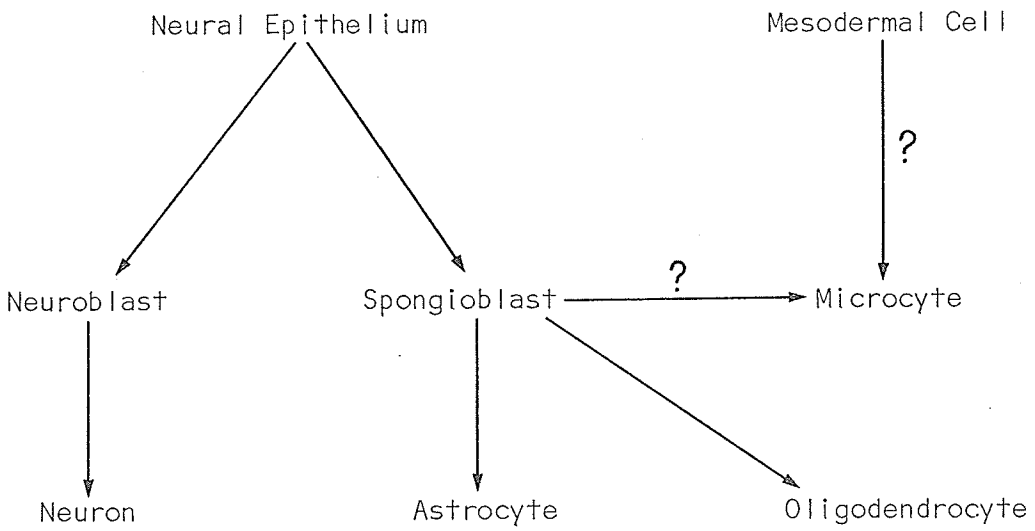
B.



C.

Modified after Arey (1966)

Schematic of Origin and Differentiation of Cells in the Nervous System



The primitive neural epithelium has been described as made up of two cell types, neuroblast and spongioglast (His, 1889). However, Schaper (1897) observed that His's neuroblast and spongioblast were differentiated cells of a common cell type. Subsequently, several investigators using different areas in the central nervous system, Sauer (1935) in the neural tube; Kershman, (1938) in medulloblastoma; confirmed Schaper's findings on cellular differentiation. Further studies incorporating the use of autoradiography in mouse embryos (Sidman et al., 1959) and in chick embryos (Fujita, 1962) have shown that the primitive neural tube is made up of a homogeneous cell population. Caley and Maxwell (1968) with the use of the electron microscope have also shown that at birth the cerebral cortex, in rat, consisted of undifferentiated cells.

Cellular Components of the Olfactory Bulb:

The cellular components of the olfactory bulb consist of morpho-

logically distinct neurons, neuroglial cells and their processes.

Three distinct cell types make up the neuronal population. These are the large cells of the mitral cell layer, the tufted cells of the external plexiform and lower glomerular layer, and the cells of the granular layer. Except for few minor differences, the periglomeruler cell of the glomerular layer is morphologically similar to the granule cell.

Astrocyte, oligodendrocyte, microcyte and Schwann cells make up the neuroglial cell types. Not unlike other areas in the nervous system, neuroglial cells are distributed in a random fashion.

Neuron in General:

The morphology and cyto-architecture of neurons in general appear to be more complex than any other basic cell type in the body. However, in spite of this diversity, all neurons have similar physiological properties, namely: specialized ability to respond, assimilate, modify and transmit the information to other neurons, muscle cells and glandular cells. In conformity to this comprehensive idea, the structure of neurons will be reviewed on a general basis.

Hyden (1960) described the neuron as a large cell made up of a cell body from which extend several dendritic processes and a single axon. On the basis of dendritic spread, and arborization, neurons have been classified by light microscopists into various types.

A large portion of the cytoplasm of the nerve cell body is occupied by the nucleus. Most nuclei are round and centrally located in the perikaryon. The nucleus is limited by a membrane which has

been reported to contain numerous pores which facilitate the passage of materials. Electron microscopic studies have shown that the nucleoplasm is made up of chromatin granules and a dense spherical body, the nucleolus. Chromatin granules have been observed to aggregate at the inner surface of the nuclear membrane.

The cytoplasm of nerve cell body has been observed to contain organelles which include rough-endoplasmic reticulum, smooth endoplasmic reticulum, free ribosomes, Golgi complex, lysosomes, mitochondria, lipofuscin granules, cilia and centrioles, neurofilaments and microtubules (Lentz, 1971).

Endoplasmic Reticulum

Endoplasmic reticulum in nerve cells has been described by several investigators (Key and Retzius, 1876; Fleming, 1882; Nissl, 1892; 1894a, b, c; 1896) and more recently by Deitch and Murray (1956); Deitch and Moses, (1957); Peters et al., (1970).

Light microscopic studies involving the use of several dyes, including Toluidine blue, Creysl violet, have demonstrated that the cytoplasm and dendrites of nerve cells contains fine granular material which has been described as the Nissl substance (Nissl, 1892). These studies have also reported that the axon-hillock and axon are devoid of the Nissl substance. The physiological role of Nissl substance has been postulated by several investigators (Held, 1895; Cajal, 1904; Heidenhain, 1911). These studies have indicated that synthesis of materials is associated with the Nissl substance.

Electron microscopic studies have shown that the major portion of

the Nissl substance is composed of rough endoplasmic reticulum. Palay and Palade (1955) provided the first description of the rough endoplasmic reticulum. Several reports have shown that rough endoplasmic reticulum is in continuity with smooth endoplasmic reticulum. It is generally accepted that materials synthesized by the rough endoplasmic reticulum flow into the smooth endoplasmic reticulum and are subsequently secreted through the Golgi apparatus.

Smooth endoplasmic reticulum has been reported to be present in all cells including those of cortical neurons (Rosenbluth, 1962b), neurons of the posterior horn of the spinal cord (Nathaniel and Nathaniel, 1966), and in spinal ganglion cells (Nathaniel and Nathaniel 1973). Free ribosomes and mitochondria have been observed to be associated with smooth endoplasmic reticulum.

Mitochondria:

Michaelis (1900) using Janus Green B as a supra-vital stain identified structures similar to that of mitochondria. Champy (1912), Lewis and Lewis (1914; 1915), using tissue culture techniques, confirmed the identity of mitochondria and dispelled the belief that mitochondria were fixation artifacts.

The ultrastructure of mitochondria was described by Palade, (1952; 1953), Sjostrand, (1953), Sjostrand and Rhodin, (1953). The fine structure of mitochondria is similar in all vertebrate cells (Palade, 1953; Fawcett, 1966a). Neuronal mitochondrial configuration resembles mitochondria in other tissues and consists of an outer smooth membrane in an inner folded membrane. The mitochondria have

been described as small, round granules or slender rodlets varying in diameter from 0.1 micron to 20 microns. Mitochondria have been observed as being plastic and polymorphic. Karlsson (1966b) observed that in the lateral geniculate nucleus of the cat mitochondria were of the branching type. Tissue culture studies have shown that mitochondria are capable of motion, alteration of shape, size and location within the same cell (Pomerat et al., 1967).

Biochemical studies have demonstrated that mitochondria are the organelles exclusively involved in the process of oxidative phosphorylation. Using differential centrifugation and absorbancy measurements, it has been shown that the carriersa and a_3 of the cytochrome system, and if not all of the enzymes in the citric acid cycles, are located on the inner membrane of the mitochondria. Other biochemical processes including the oxidation of fatty acids, amino acids and choline have also been shown to be carried out by mitochondria.

Although the metabolic role of mitochondria has been demonstrated to be the same for all cells, neuronal mitochondria have been observed to show certain intrinsic metabolic phenomena. Fractions of brain mitochondria when deprived of ADP fail to swell. Peachey (1964) observed that neuronal mitochondrial inner matrix contains a few dense granules which are believed to be hydroxyapatite sequestered in the presence of Calcium (Ca++) ions metabolism (Lehninger, 1967). The paucity of these granules in brain mitochondria indicates the absence of stored energy in the brain and its greater dependence on the

immediate availability of glucose and oxygen.

Golgi Apparatus:

After a long history of controversy that the Golgi apparatus is a cytoplasmic organelle, Baker (1963) concluded from his studies of nerve cell that the Golgi apparatus does exist and is not an artifact.

The Golgi apparatus has been described as being pleomorphic and ill-defined. Light microscopic studies have shown that its general pattern is variable and is related to the metabolic activity of the cell. Structurally, it has been described as being filamentous, plate-like, or made up of a network of tubules arranged in a stack-like manner. Even in cells of a local population, the Golgi apparatus can be of different shape (Bourne, 1955).

The ultrastructure of the Golgi complex has been described by several investigators (Sjostrand, 1953; Beams and Tahmisian, 1953; Dalton and Felix, 1954). It is made up of a complex of broad flattened cisternae, with a surrounding population of vesicles. In most instances cisternae are piled one atop another. Anastomosis between adjacent cisternae occurs. The absence of ribosomes, free or attached, is a peculiar observation in the area occupied by the Golgi apparatus. At various points pores or fenestrae are present. At these locations vesicles have been observed to form. These vesicles are also believed to differentiate at a later time and give rise to several types of organelles.

The origin and formation of the Golgi apparatus is believed to be by three possible methods. These are de novo (von Bergen, 1904),

from pre-existing Golgi cisternae (Maruyama, 1965), and from other membranous structures (Hall and Wirkus, 1964) of the cell.

Further investigations, have associated the Golgi apparatus in a variety of complex biochemical reactions. Early light microscopic studies have shown that the apparatus is involved in elaboration and polymerization of secretory products. Addition of sugar moiety to protein molecules destined to be excreted is believed to take place at the Golgi apparatus level (Mandel and Ellison, 1963). Reports from several laboratories have shown that the Golgi apparatus is associated with additional metabolic processes including secretion of mucopolysaccharide (Spiro, 1963), excretion of synthesized protein (Sjostrand, 1962), formation of the acrosome (Clermont and Leblond, 1955), lysosome (Van Lancker, 1964), phospholipid (Cohn and Benson, 1965a, b), neurosecretions (de Robertis, 1962), and pigment formation (Dalton, 1959).

Experimental studies have shown that the Golgi apparatus is capable of undergoing plasticity. Cells that are grown in a rich metabolic environment have been observed to have a large Golgi apparatus (Falk, 1962; Kephart et al., 1966). Conversely, cells grown in poor metabolic medium undergo fragmentation and dissolution of Golgi apparatus (Becker et al., 1961; Becker, 1962). Hypertrophy of the Golgi complexes has been reported in neurons following injury to its neurites (Nathaniel and Nathaniel, 1973).

Lysosomes:

During his attempt to isolate mitochondria, de Duve (1955), found a class of particles, in his fractions, with different enzymatic properties. These enzymes were later isolated and classified as hydrolases. A few years earlier, some investigators were associating the activities of acid phosphatase and uricase with mitochondrial fractions (Schein et al., 1951; Palade, 1951). However, additional studies have shown that these enzymes belong to the microsome fraction and not to the mitochondrial fraction. The isolation of lysosomes is dependant on the centrifugation techniques (Tsuboi, 1952; Novikoff et al., 1953).

Lysosomes are present in all neurons. Under the light microscope, they have been confused with secretory granules, mitochondria, and Golgi apparatus (Palay, 1960b). Electron microscopic studies have depicted lysosomes to be electron dense bodies which are readily distinguishable from other cytoplasmic organells. Particles identified as lysosomes are usually spherical and membrane-bound. These particles, measuring about 0.25 to 0.5 μ in diameter, are filled with fine dense granular material. Large lysosomes 1 to 2 μ in diameter have been reported from cell types other than neurons. Variation in shape and size of lysosomes have also been observed.

Lysosomes are involved in the lysis of cells and in sequestration of essential nutrients during starvation. The presence of strong hydrolytic enzymes in lysosomes enables this organelle to carry out the process of autophagy, whereby excess metabolic substances are

digested and harmful or poisonous ones removed. Lysosomal content has been reported to be increased in chromatolytic neurons (Nathaniel and Nathaniel, 1973).

The activities of lysosomes are dependent on a variety of drugs and chemical substances. Cortisone has been shown to decrease the metabolic activities of lysosome whereas Vitamin A increases these activities.

Multivesicular Bodies:

The presence of multivesicular bodies in neurons has been reported (Palay and Palade, 1955). Multivesicular bodies have been described as being spherical and membrane-bound within which are smaller membrane-bound alveolate vesicles. In addition, the presence of filaments, granules, irregular dense masses and membranes have also been observed within the matrix. Generally, multivesicular bodies have a translucent matrix, but transitional forms have been observed. Due to their close proximity to the Golgi apparatus, the origin and function of multivesicular bodies are believed to be related to those of the Golgi apparatus.

The uptake of substances by multivesicular bodies has been studied by Rosenbluth and Wissig (1964), using ferritin and Friend and Farquahar (1967), using horseradish peroxidase. In the initial stages, these substances are taken up by single membrane-bound alveolate vesicles.

Lipofuscin Granules:

Light microscopic studies have shown lipofuscin granules as brown in colour in unstained preparations and as dark bodies when stained with fat-soluble dyes. On exposure to ultraviolet light these granules are capable of exhibiting flourescence.

Several reports have suggested that lipofuscin granules might originate from the Golgi apparatus (Gatenby and Moussa, 1951), or might represent the final end product of the lysosomal digestive system (Essner and Novikoff, 1960; Samorajski et al., 1964).

Lipofuscin granules have been observed to increase with age (Bourne, 1957; Wilcox, 1959; de Robertis et al., 1960). This accumulation of lipofuscin is reported from studies of several organs.

Microtubules and Neurofilaments:

Microtubules and microfilaments have been described in several types of cells. The microfilaments have been observed in melanoma tumor cells which have been treated with mitotic inhibitors like colchicine (Loader and Nathaniel, 1972; 1973).

Electron microscopic studies have shown that microtubules are 200-600A° in diameter and have a dense outer wall, 60A° thick, which surrounds a translucent core with a centrally placed dot. Due to the high resolution of the electron micrographs, only small pieces of microtubules are observed. However, at the light microscopic level and with the use of silver impregnation techniques, they are observed to be very prominent in dendrites and axons.

Microtubules are believed to take part in the maintainence of cell shape and the transport of materials from the soma to processes and nerve endings.

Neurofilaments, described as microfilaments in non-neuronal cells, have a diameter of about 50A°. In cross section, neurofilaments have been observed to be bordered by a wall 30A° thick within which a light core exists. The distribution of neurofilaments parallel that of microtubules. Gray and Guillery (1966) observed that neurofibrils of light microscopy represent clumped microtubules and neurofilaments.

As in the case of microtubules, neurofilaments are believed to be associated with themaintainence of cell shape and the flow of materials from the soma to the outlying parts of the cell viz. the processes.

Methods and Materials

This study used postnatal albino rats, Sprague-Dawley, one day, one, two, three and five week old. A total of 40 rats was used in this study and was obtained from the animal laboratory of the Department of Dentistry, Faculty of Medicine, University of Manitoba.

The rats were anaesthetized by intraperitoneal administration of Sodium Pentobarbital (Nembutal) 35 mg/kg weight of rat. All animals were perfused with Karnovsky's (1965) fixative through the left ventricle using a 23 gl, Sterile Disposable Yale needle and a 25 cc syringe. Drainage was accomplished by incision of the right atrium. A total volume of 25 cc of perfusion fluid was used for 1 day old animals and between 75 to 100 cc for older animals. Perfusion time lasted between 5 to 10 minutes.

Following perfusion, craniectomy was performed by surgical method previously described by Singh (1971). A sagittal mid-line incision was made on the scalp and the integument and underlying muscles removed. A bone rongeur was used to expose the brain and olfactory bulbs in two weeks and older animals. In one day and one week old animals a pair of scissors was used to expose the brain and olfactory bulbs. The area was kept moist by the fixative fluid. A No. 11 scalpel blade fitted on a No. 3 blade handle was used to separate the olfactory bulbs from the cerebral hemispheres. Olfactory tissues were sectioned into small pieces (1 mm^2) and placed in Karnovsky's (1965) fixative for 4 hours in the refrigerator.

Subsequently, the tissues were washed several times in Millonig's

Phosphate buffer and immersed in 1% osmium tetroxide for post-fixation. Following osmication, tissues were washed several times in Millonig's Phosphate buffer and dehydrated in graded series of alcohol. Tissues were cleared in propylene oxide and embedded in araldite (see procedure below).

Procedure:

1. Karnovsky fixative for 4 hours in refrigerator.
2. Wash in buffer several times.
3. Place in2% osmium tetroxide (aqueous) and 0.2M cacodylate in 0.4M sucrose, equal parts, for 2 hours.
4. Wash in buffer several times.
5. 50% alcohol 10 minutes. 1 change
6. 70% alcohol 10 minutes. 1 change
7. 95% alcohol 20 minutes. 2 changes
8. 2% uranyl acetate in absolute alcohol 30 minutes. 3 changes
9. Propylene oxide 30 minutes. 3 changes
10. Equal parts of propylene oxide and araldite. Leave overnight on a stirrer.
11. 75% araldite and 25% propylene oxide mixture on the stirrer for 2 hours.
12. Pure araldite mixture 1 hour
13. Embed in pure araldite placed in plastic capsules and leave in incubator 40-60°C for 3 days.

Preparation of Solutions:

1. Aldehyde fixative, modified after Karnovsky, (1965) was used as the perfusion fluid and prepared by the following method. Eight grams of paraformaldehyde were added to 100 ml of distilled water and the mixture brought to 65°C temperature. 1 N sodium hydroxide (NaOH) was added drop by drop until a solution free from precipitate was obtained. To this solution 40 cc of a 25% glutaraldehyde solution were added. Subsequently, the volume was made up to 200 cc with phosphate buffer and the pH adjusted to 7.2..

2. Millonig's phsophate buffer (500 cc).:

Solution A: 2.25% $NaH_2 PO_4 H_2O$

(10.1 grams in 450 cc H_2O)

Solution B: 2.52% NaOH

(2.52 grams in 100 cc H_2O)

Solution C: 5.40% glucose

(2.70 grams in 50 cc H_2O)

Solution D: 415 cc of solution A and 85 cc of solution B.

Final Mixture: 450 cc of solution D.

50 cc of solution C.

500 cc of phosphate buffer.

3. Araldite mixture: (50 ml batch)

araldite 29.4 grams

D.D.S.A. 23.0 grams

D.M.P. 1.0 ml.

LIGHT MICROSCOPY:

Araldite blocks were removed from their plastic capsules and trimmed with single edge blades (Tek). Thick sections, 0.5 microns, were cut with glass-knives on a Reichert Nr 318 423/E ultramicrotome and mounted on frosted precleaned glass slides. After drying on a hot plate, the slides were stained for 3 minutes with Toluidine blue. The sections were studied with the aid of an optical microscope with a two-fold objective. The first was to establish correct orientation of the tissue and the second to evaluate the vascular and neuronal development at the light microscope level. Photomicrographs demonstrating these findings were taken at representative time sequence and at appropriate magnification.

ELECTRON MICROSCOPY:

Thin sections, approximately 700A° in thickness, were also cut from representative blocks on the Reichert Nr. 218 423/E ultramicrotome and mounted on copper grids. These grids were stained with a saturated solution of aqueous uranyl acetate for 30 minutes followed by lead citrate for 3 minutes. Electron micrographs were taken on a Philips 300 electron microscope.

LIGHT MICROSCOPY OF OLFACTORY BULB

-AN OVERALL VIEW

Studies on the development of the olfactory cortex havebeen carried out using 0.5 μ thick araldite sections stained with Toluidine blue for light microscopy and gold and silver coloured sections, 60 mμ, for electron microscopy. The paradigm used to present the findings is divided into four sections. Firstly, regional and cellular morphology of the olfactory bulb will be presented at the light microscopic level. Secondly, the development of the vasculature in the olfactory bulb will be dealt with at the light and electron microscope levels and its implications discussed. The third portion of the dissertation will describe the ultrastructural patterns of neuronogenesis followed by a discussion of its significance. Finally the differentiation and maturation of neuroglial cells will be presented and discussed.

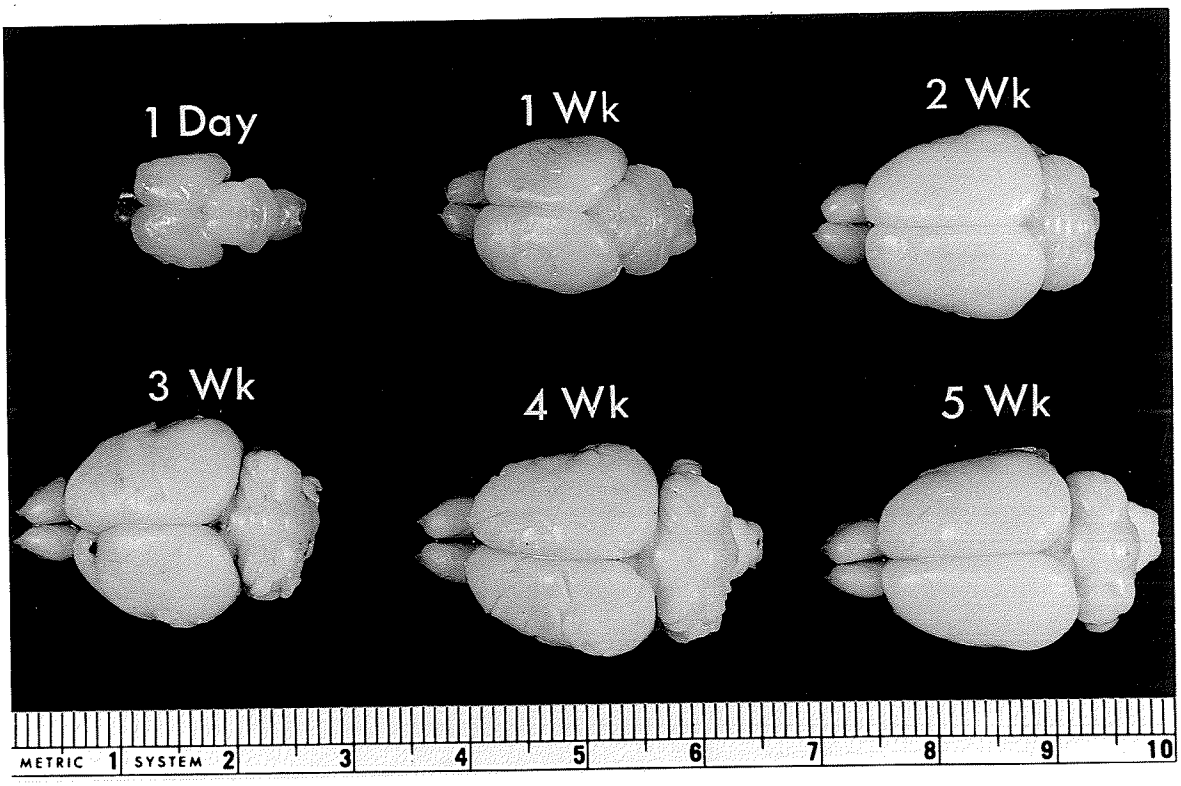
Gross Observation

Perfused brains and including the olfactory bulb, from postnatal rats ranging in age from one day to five weeks have been observed to reach their adult size at three weeks of age (Figure 5). In all age groups, the olfactory bulb is oval in shape and is located at the rostral end of the cerebral cortex.

Light Microscopic observations on the olfactory bulb:

The six layers of the olfactory bulb are easily recognized in adult rats, and are termed fiber layer, granular layer, external plexiform layer, mitral cell layer, inner plexiform layer. These layers are also distinguishable in neonatal and one day old animals.

Figure 5. This photograph illustrates the brains and associated olfactory bulbs from postnatal rats ranging in age from one day to five weeks. The olfactory bulb is rostral to the brain and oval in shape. The olfactory tracts are hidden by the cerebral cortex. Cardiac perfusion.


1 Day
1 Wk
2 Wk
3 Wk
4 Wk
5 Wk
METRIC 1 SYSTEM 2 3 4 5 6 7 8 9 10

However, these layers do not show clear cut demarcation and adjacent layers do show certain degree of overlap. Of the six layers, the mitral cell layer and the inner plexiform layer are least defined. The inner plexiform layer manifests its identity more obviously in one week old animals (Figure 7).

Fiber Layer:

This layer is made up of axons from receptor cells, the primary olfactory nerves (PON). The width of the fiber layer is variable, being wider at the anterior than in the posterior part of the olfactory bulb. Darkly stained nuclei of Schwann cells are observed and are related to the unmyelinated axons (Figure 8). At birth the full number of nerve fibres are present. Hinds (1972) reported that in the mouse olfactory nerve, axons make connections with the olfactory primordium at day 12 of gestation.

Glomerular Layer:

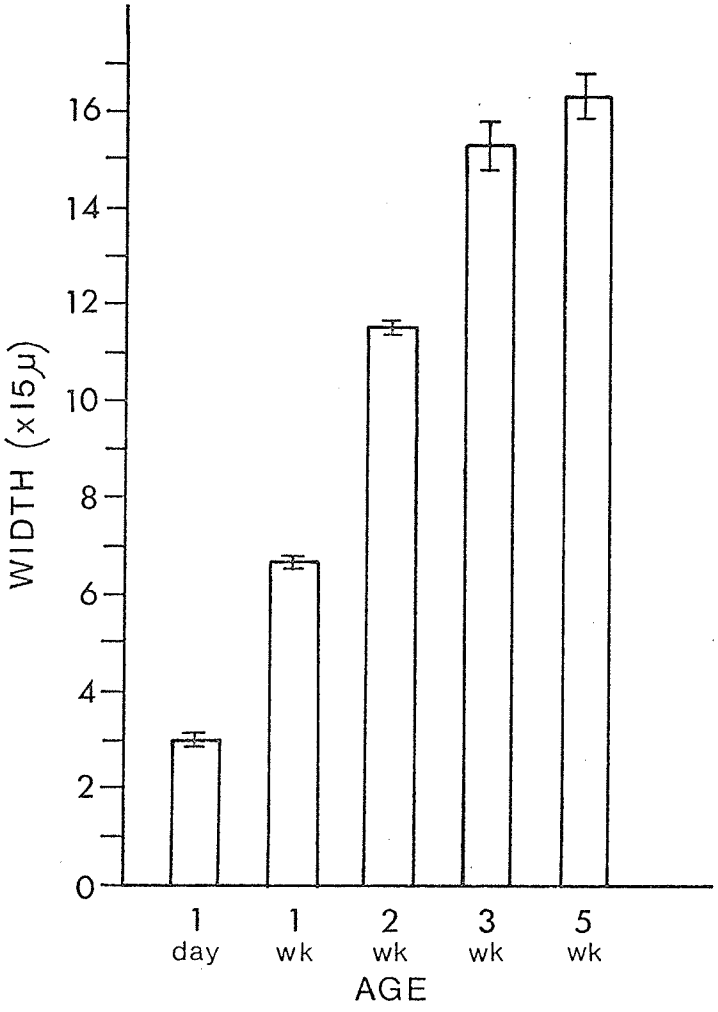
This is a heterogeneous layer of nerve fibers, neurons, nerve endings and neuroglia. This layer is observed to be made up of islets of nerves surrounded by periglomerular neurons and neuroglia (Figure 7).

External Plexiform Layer (EPL):

In this study, the maximum increase in the width of the olfactory bulb has been observed in the first three weeks of postnatal development (Figure 7). Studies to ascertain factors contributing to the increase in the size of olfactory bulb revealed that this was principally due to growth in width of the external plexiform layer as the animal

Figure 6. Histogram to illustrate the width of the external plexiform layer in one day, 1, 2, 3, 5, weeks old rats olfactory bulb. Observations from this study showed that the external plexiform layer increased in width up to 3 weeks of age. No significant increase has been found in animals beyond 3 weeks of age.

EXTERNAL PLEXIFORM LAYER GROWTH



AGE	WIDTH OF EXTERNAL PLEXIFORM LAYER (X15μ)
1 day	3.055± 0.154
1 week	6.700± 0.420
2 weeks	11.50 ± 0.273
3 weeks	15.30± 0.366
5 weeks	16.33± 0.527

became older. The external plexiform layer was 45 microns in thickness at birth and 325 microns in three week old animals (Figure 6). Concomitant with development of the external plexiform layer was the appearance of dendrites and axons. One day old animals showed a layer that was diffuse and uniformly hyperchromatic (Figure 7). Subsequent age groups studied showed that the occurrence of translucent areas was more frequent and was made up of dendrites of several cell types including mitral, tufted and granule cells. In two and three week olfactory cortex, dendrites became more extensive and have been traced to enter the glomerular layer. Several reports have shown that these dendrites make synaptic connections with the primary olfactory nerves (Andres, 1965; Reese and Brightman, 1970; Pinching and Powell, 1971a, b, c; White, 1972). Similar observations have been made in the adult cat's olfactory bulb (Willey, 1973). Concomitant with the increase in width of the external plexiform layer there was also a significant increase in blood vessels (Singh and Nathaniel, 1973), (Figure 7). This observation is described more fully in the vascular section of this thesis.

Mitral Cell Layer:

This layer was easily recognized because of the presence of large mitral cells (Figure 7 & 8). At birth, mitral cells were observed to be round or oval in shape. These cells have a relatively large nucleus and a small amount of cytoplasm. Chromatin content of the nucleus was uniformly distributed with occasional aggregation. In a few cells, cellular processes have been identified and they

projected towards the glomerular area.

One week and older animals showed mitral cells with processes polarized towards the external plexiform and glomerular layer. The cytoplasm was more extensive when compared to younger animals. The nucleus was centrally located and showed clumping of chromatin.

Granular Layer:

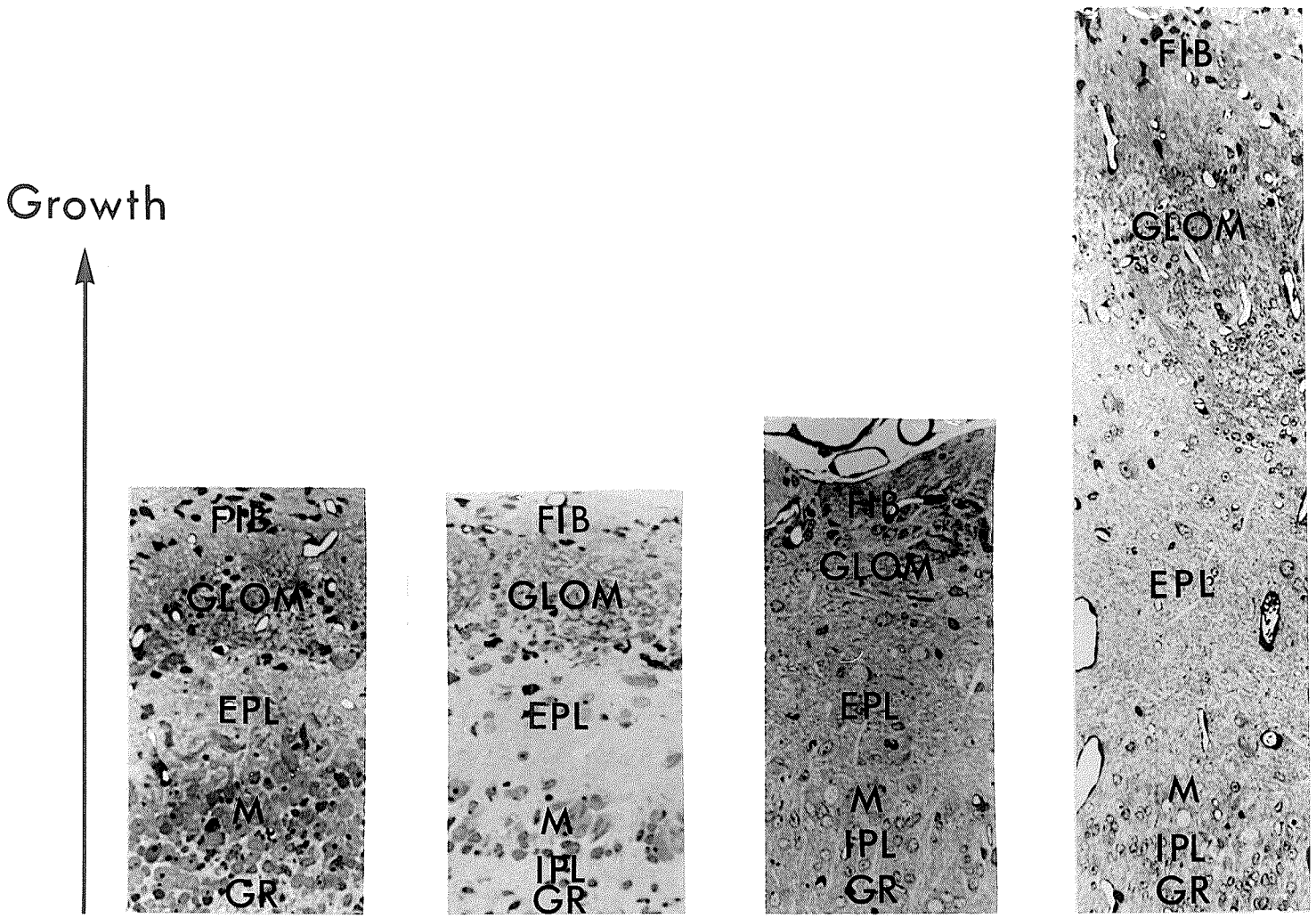
Nuclei of other cells have been observed to lie peripheral to the mitral cells (Figure 8). In one day animals, these nuclei have a homogeneous distribution of chromatin granules. Older animals, including one week to five weeks, showed nuclei with peripheral aggregation of chromatin. Reese and Brightman (1970) have described these cells as granule or short-axon neurons and is consistent with the findings of Willey (1973).

SUMMARY

1. Regional and cellular morphology of the olfactory bulb, using 0.5 μ araldite sections, was studied at the light microscopic level.
2. The six layers of the olfactory bulb were distinguishable in neonatal and one day old animals.
3. The olfactory bulb reached its adult or mature size at 21 day postnatal. This enlargement of the olfactory bulb diameter was directly related to the increase in width of the external plexiform layer.
4. Concurrently, a significant increase in blood vessel density occurred during the second and third week of postnatal development. Subsequently, no further increase in blood vessel density appeared during the fourth and fifth week.
5. At the end of the first week of postnatal development, the mitral layer was well delineated and the mitral cells showed definite polarity towards the glomerular layer. At this stage mitral cells were characterized by a large nucleus and copious cytoplasmic area. In addition, other neuronal cells are observed to reach their definitive position.

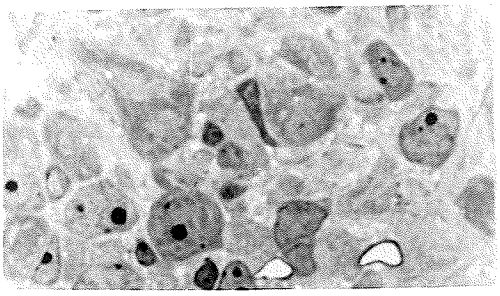
Figure 7. Photomicrographs of olfactory bulb of rats aged one day, 1, 2 and 3 weeks old. Each of the micrograph extends from the fiber layer (pial) to the granular layer. Comparative studies were carried out on the thickness of the olfactory bulb, cell and blood vessel densities and on the maturation of the layers in the bulb. Numerical studies in the present investigation have shown that in the 21 day old rat the width of the olfactory bulb and the blood vessel density have reached their adult stage.

X 400

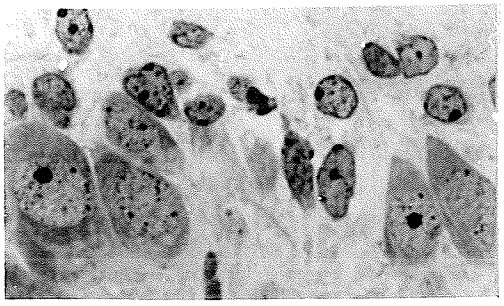


Photomicrographs of Growth in Rat Olfactory Bulb

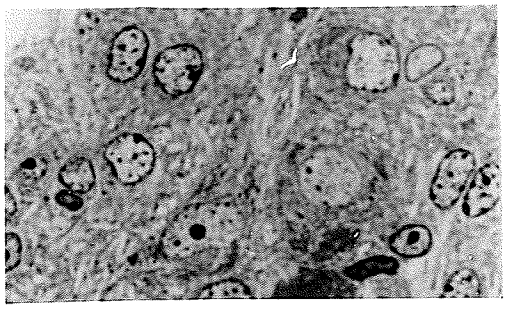
Figure 8. Photomicrographs of the mitral cell layer in one day, 1, 2 and 3 weeks old rats illustrating the differentiation of the mitral cell. In one day old rats mitral cells are round to oval in shape with few cells having observable processes. Subsequent differentiation in older groups showed a definite polarity of the mitral cells towards the glomerular layer.
X 1600



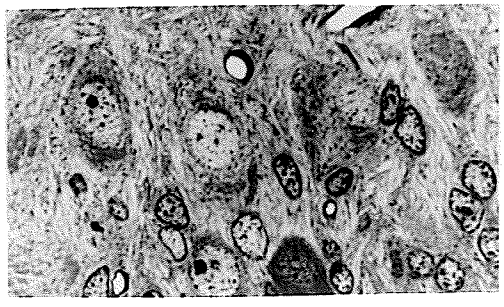
1day



1wk



2wk



3wk

Photomicrographs of Mitral Cell Layer

SECTION ON BLOOD VESSELS

1. Review of Literature
2. Observations:
   a. Light microscopic studies.
   b. Electron microscopic observations
3. Discussion
4. Summary

REVIEW OF LITERATURE

The vascular components of the central nervous system occupy a unique structure-function relationship and are very different from those in organs having a connective tissue framework. This significance was appreciated as early as 1851 and led several investigators to study the structure of the capillaries of the nervous system. Virchow (1851) demonstrated the presence of a perivascular space. Subsequently, researchers added more information, and the space became known by several names viz. Virchow space, Virchow-Robin space, Space of His, and Space of Key and Retius. Early studies using silver staining and light microscopy failed to produce conclusive and true morphological data relating to the perivascular spaces due to poor fixation.

With the introduction of the electron microscope and better fixation and embedding methods, morphologists were able to study, with much more reliability and success, the fine structure of the capillaries of the nervous system.

Capillaries of the central nervous system are lined by a continuous layer of endothelial cells, surrounded completely by a basal lamina which divides to incorporate the adjacent pericytic cell, when present. Electron microscopic studies also showed that in the adult nervous system, astrocytic processes surround entirely the basement membrane and pericytic cell.

Structurally, endothelial cells of the capillaries in the central nervous system show features that resemble those present in other

tissues with slight variations. The endothelial cells of the central nervous system do not show as many pinocytic vesicles, although they may be of the same shape and size and have been observed to open into both the luminal and abluminal surfaces. In addition, adjacent endothelial surfaces form tight junctions of the zonulae occludentes type. Adherence between these apposing membranes can be found at several places or radially along the entire length of the adjoining membranes.

Ever since Ehrlich (1885) and Goldmann (1913) showed that certain dyes injected into the systemic circulation penetrate the tissues and cells of most organs but not the brain, investigators began to search for the mechanism governing this phenomenon later to be known as the blood brain barrier (BBB). Tschirgi (1950) showed that dyes, excluded from the brain tissue, were bound to plasma-proteins and formed dye-protein complexes. Horseradish peroxidase, isolated by Straus (1958), was used repeatedly by several investigators to study the blood-brain barrier mechanism (Karnovsky, 1965b, 1967; Reese and Karnovsky, 1967; Bodenheimer and Brightman, 1968).

The relationship between capillaries, perivascular spaces and neuroglia cells has been described (Bairati, 1958), which is related to the morphological problem of the blood-brain barrier mechanism. This part of the present study is devoted to the ultrastructure of developing capillaries in the olfactory bulb of post-natal rats.

Differentiation and maturation of capillaries in the central nervous system have been reported by several laboratories (Stern and

Peyrot, 1927; Behnsen, 1927; Bakay, 1956; Donahue and Pappas, 1961; Caley and Maxwell, 1970; Hannah and Nathaniel, 1972, 1974).

Development of capillaries has occupied the attention of several investigators mainly because of its relation to the blood brain barrier system (BBB). Grazer and Clemente (1957) have reported that trypan blue enters the nervous system of embryos more readily than it does in adult animals. Subsequently, several chemical dyes (Bodenheimer and Brightman, 1968), radioactive phosphate and proteinaceous materials (Klatzo et al., 1962) have been used to study the permeability of endothelial cell wall. These studies confirmed the findings of Grazer and Clemente (1957).

The ultrastructure of developing capillaries has been studied by Donahue and Pappas (1961), Caley and Maxwell (1970), and Bar and Wolff (1972) in the cerebral cortex of rat; by Phelps (1972), Hannah and Nathaniel (1972, 1974) in the rat spinal cord and by Weschler (1965) and Delorme et al., (1968) in the avain brain.

The above studies in general, have indicated that the endothelial cells at birth are relatively thick with abundant cytoplasm which contain smooth endoplasmic reticulum and are composed of dilated canaliculi and vesicles. Cytoplasm is also rich in free ribosomes distributed singly or in rosettes. Apposing endothelial cell membranes form tight junctions of the zonula occludens type. On the other hand, adult animals have shown attenuated endothelial cells with fewer vesicles and ribosomes which are free as well as attached to membranes.

The significance of the basal lamina, located between the endothelial cell and the glial cell processes, as a morphologic

component of the blood brain barrier and its probable functional role in relation to the diffusion of metabolites is well recognized. The ultrastructural studies referred to in the preceeding paragraphs have revealed that the thickness of the basement membrane increases with the postnatal age of the animal (Donahue and Pappas, 1961; Caley and Maxwell, 1970; Bar and Wolff, 1972; and Hannah and Nathaniel, 1972, 1974) and is dependent on the astrocytic investment of the vessel (Caley and Maxwell, 1970; Hannah and Nathaniel, 1972, 1974).

OBSERVATIONS

Light Microscopic Studies:

One micron (1 μ) thick araldite sections stained with Toluidine Blue were used to study the population density and related distribution of capillaries in the olfactory bulb of the rat.

At birth, few blood capillaries were present in the olfactory cortex. These capillaries were round, oval, and irregular, depending on the shape of the lumen. Distribution of capillaries was random and they have been observed to occupy spaces adjacent to cell soma and processes. Comprehensive studies at the light microscopic level, to identify unopened capillaries or site of future capillaries, were not successful.

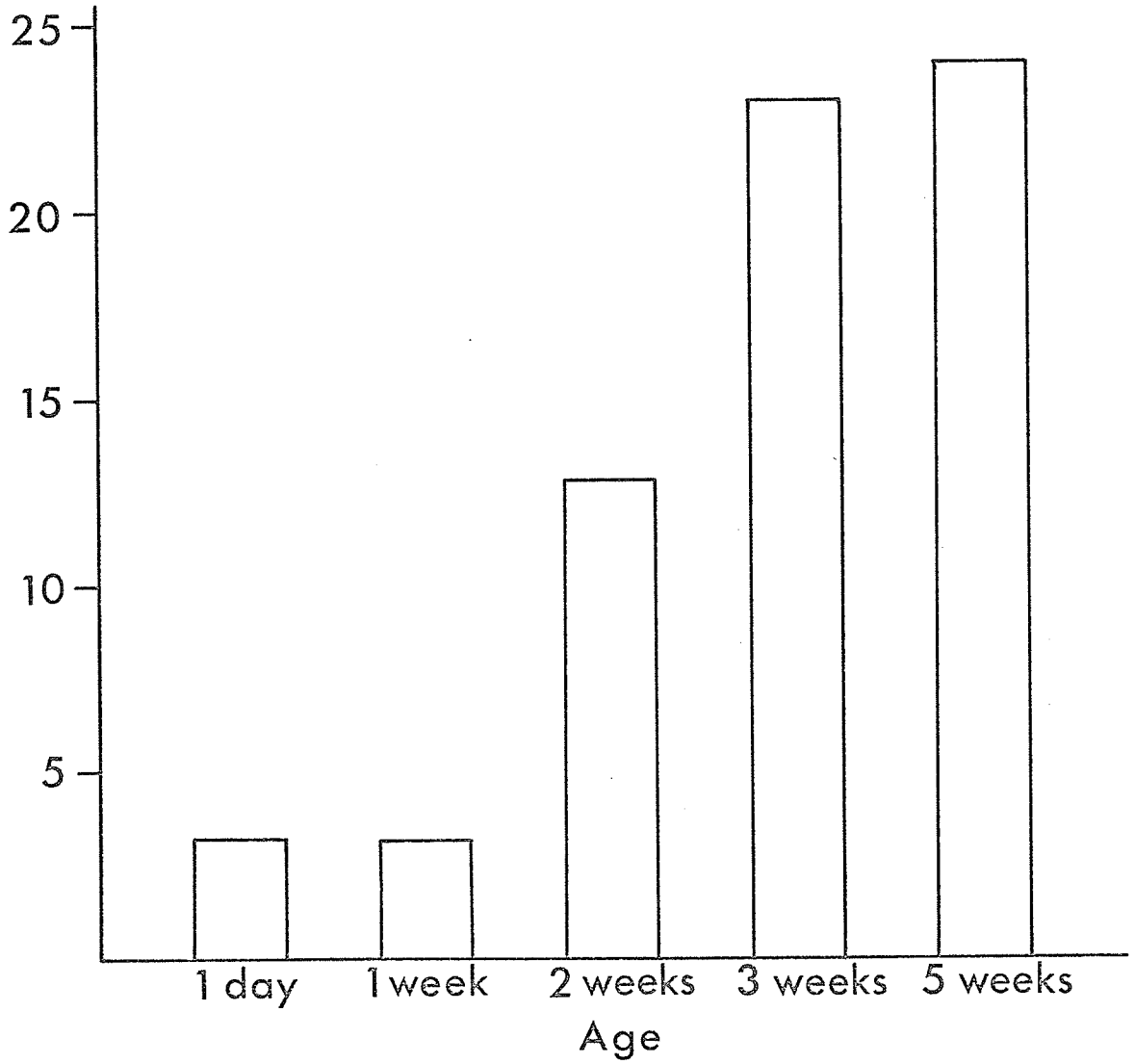
One week old olfactory cortices did not show any significant increase in capillary densities when compared to one day old animals. Development and differentiation appear to be one of degree. Three and five week old animals exhibited a significant increase in capillary density when compared to two week and younger animals (Figure 6). No significant differences have been observed between three and five week old animals (Figure 9).

No attempt will be made to describe structural differences of the endothelial cell at the optical level.

Electron Microscopic Observations:

Blood vessels observed in neonates, and in animals ranging from one to five weeks postnatal period, possessed morphological features which ranged from a primitive pattern to an adult pattern. Rather

Figure 9. Histogram to show the relative blood vessel densities in the olfactory bulb in different age groups. The relative calculated densities and standard deviations are inserted inthe lower section of this figure. Maximum blood vessel density has been reached at 3 weeks postnatal.



Age	Relative Blood Vessel Densities per mm^2
1 Day	34.2 ± 1.7
1 Week	32.9 ± 00.00
2 Weeks	129 ± 16.6
3 Weeks	230 ± 4.4
5 Weeks	245 ± 8.9

than describe the two extreme ranges of vascular patterns exhibited at each time sequence, it is felt a more meaningful picture may emerge from this study if one were to deal with the development and maturation of blood vessels as a whole, irrespective of the postnatal period of the animal from which the tissue was obtained. Following this presentation a brief reference will be made to blood vessels of each time sequence to emphasize special features, if present.

The blood vessels present in newborn and one to five week old postnatal rats may be broadly categorized into two categories designated as Type I and Type II.

Fine Structure of Type I blood vessel:

Basically, the vessels belonging to this category possess a primitive appearance. They were small in size and composed of one to three endothelial cells limiting a lumen which varied from a mere slit (Figures 10, 11) to one of a distinct but small lumen (Figures 12, 13, 14). Surrounding these primitive vessels was the large, clear cytoplasm of astrocytes containing organelles such as mitochondria, small amounts of endoplasmic reticulum and particulate material which is considered to be glycogen (Figures 10-14).

Endothelial cell walls of this type of vessel were thick, and demonstrated a variety of cytoplasmic organelles. Most of the ribonucleic particles occurred as free ribosomes (Figures 10, 11, 12). However, a few ribosomes were associated with membranous structures giving rise to granular reticulum (Figures 13, 14). The major portion of the endoplasmic membrane was of the agranular variety. Mitochondrial

vesicles, lysosomes, Golgi complexes were also observed within the endothelial cell cytoplasm. Considerable numbers of pinocytotic vesicles (Figures 10, 11) and cytoplasmic projections (Figures 11-14) into the vascular lumen were observed in these immature blood channels. Centrioles were also observed within the endothelial cells (Figure 10). Well defined junctional complexes were present between adjacent endothelial cells (Figure 10). The nucleus was prominent and occupied a large portion of the endothelial cell cytoplasm. There was a considerable margination of chromatin (Figures 11, 12, 13 & 15). A nucleolus was often present.

These capillaries were surrounded by an ill-defined basement membrane which had an amorphous appearance. Several of the primitive blood vessels were invested by pericytes (Figures 10-13). A well defined basement lamina separated the pericyte from the endothelial cell on the inner aspect and from the neurophil on the external aspect.

Fine Structure of Type II Vessels:

This group of blood vessels appeared to have reached a later stage of development when compared to Type I. Two distinct features appear to characterize these vessels: one is the presence of well defined lumen limited by attenuated endothelial cells, and the second being the absence of large watery cytoplasm of the astrocyte surrounding the entire blood vessel. In Type II blood vessels the processes of other cellular elements of the nervous system, such as oligodendrocytes and neurons, intermingled with those of the astrocyte to form an investment for these channels (Figures 15, 16, 17, 18, 19, 20).

The vascular lumen was distinctly patent and was limited by a variable number of endothelial cells (Figures 15-20). The configuration of the lumen is generally oval (Figures 15, 16, 18-20), and only occasionally irregular (Figure 17). The endothelial cells forming the vessel wall has a smooth contour with only an occasional cytoplasmic process, or fold, projecting into the lumen (Figure 17). The nuclei of these cells is flattened and takes the general form of the vasculature. Marginal accumulation of chromatin is distinct in some endothelial cell nuclei (Figures 17, 19). Rough endoplasmic reticulum was identified much more readily in the endothelial cells of these vessels compared to Type I vessels (Figures 15-20). Large numbers of free ribosomes were present as well. Other organelles such as mitochondria, Golgi complexes, vesicles and electron dense bodies, presumably lysosomes, were observed within the endothelial cells. Junctional complexes were observed between.

The basement lamina in Type II blood vessels was more defined than in Type I vessels. Well developed pericytes (Figure 19) were separated from the endothelial cells on the inner aspect, and the surrounding neuropil on the external aspect by well defined basement laminae. Sections passing through the nuclei of the pericytes showed elongated nuclei whose long axis was generally parallel to that of the vascular channel. These nuclei also showed a conspicuous accumulation of chromatin along its margin (Figure 19). The cytoplasm of the pericytes possessed mitochondria, Golgi complexes, free ribosomes and granular endoplasmic reticulum. In a few pericytes were found earlier

electron-dense particles, measuring about 200-300 A° in diameter, which were considered to be glycogen (Figure 15). The number of pericytes in relation to blood vessels was variable.

Blood vessels of neonatal and one day old animals have been observed to be ensheathed by cellular processes which appeared to be clear and watery, with few organelles. Electron dense particles presumed to be glycogen were found in these cellular processes, which were considered to belong to the astrocytes. These features were observed in relation to both Type I and Type II blood vessels in tissues fixed by vascular perfusion and immersion fixation. The latter technique is considered to eliminate or decrease the shrinkage of nervous tissue. The small amount of perivascular space observed in one day old animals suggest earlier maturation and differentiation of the vasculature in the olfactory bulb compared to that reported in other areas, such as the cerebral cortex and spinal cord of the rat. The earlier maturation observed in the olfactory bulb may be related to the fact that the modality of olfaction is essential to the feeding habits of animals, especially rodents, and is called upon to function early in life.

Blood vessels in one week old animals did not show significant structural differences from those of one day old animals. Both type I and type II vessels were observed. However it appeared that type I vessels were more numerous.

Vascular channels observed in two and three week animals also belonged to both type I and type II categories. However, by the

third week the majority of the blood vessels were of the type II variety. These vessels possessed a large lumen limited by attenuated endothelial cells. The basement membrane was well defined and invested by glial and neuronal processes.

In five week old animals, almost all vessels were of the mature type II pattern.

The preceeding description of blood vessels in one day, and one to five week old animals incorporates a general morphological differentiation and maturation pattern. However, it should be envisaged that within each of the survival periods studied there is an overlapping of morphogenesis. Several blood vessels studied present morphological characteristics that are present in consecutive survival periods. This would suggest that development and maturation of vasculature is not exclusively related to postnatal age of the animal, but is a continuous process. In general, primitive vessels made up the greater portion of vessels seen in one week animals, while in three to five week animals the majority of vessels belonged to the mature variety.

DISCUSSION

The development of capillaries in the olfactory bulb was studied in postnatal rats from day one to five weeks. Differentiation and maturation of capillaries in the central nervous system are the morphological problems associated with the blood brain barrier system whereby many dyes and drugs fail to leave the capillaries in the central nervous system.

At the light microscopic level, relative capillary densities were calculated in the olfactory bulb. The present study shows that there is a significant increase in the number of capillaries in two week and older animals when compared to younger animals. However, 21 days and older animals, the capillary densities appear to reach their maximum value.

Blood capillary densities in the cerebral cortex of rats have been reported by Sugita (1917; 1918), Craigie (1925), Caley and Maxwell (1970; 1971). These studies have shown that the major portions of capillaries develop over the second 10 day period of postnatal development. The present study shows that, in the olfactory bulb, the major portion of capillaries develop during the second and third week of maturation. This would indicate that the development of capillaries in the olfactory bulb parallels the development in the cerebral cortex.

The sudden increase in densities of capillaries in two week and three week old animals can only be explained at the electron microscopic level. The same material used at the light microscopic

level was also used at the electron microscopic level of investigation.

In one day and one week old animals electron microscopic studies show the presence of both primitive and well developed capillaries. Primitive blood vessels in both one day and one week old animals have an ill-defined basement membrane, a thick endothelial cell wall and slit-like lumen. The cytoplasm of the endothelial cell appears immature and possesses numerous free ribonucleic particles. The identification of capillaries with slit-like lumens is not possible with light microscopy and would be missed in any numerical estimates of capillary density. However, in two week and older animals these vessels would possess a patent lumen and would become observable. This might explain the sudden increase of blood vessels during the second ten day period of development.

Strong (1961) observed that blood vessels in the rabbit spinal cord have non-patent lumina at first, but over a short period of time these vessels became patent. Investigations of Craigie (1925), Klosovski (1963), have indicated that in the immature cortex, blood vessels develop as solid cords of cells possessing a primitive non-patent lumen. As the cortex increases in size and dimension, these vessels become filled with blood and assume a patent lumen. These changes occur over a short period of time, probably hours (Caley and Maxwell, 1971).

The type II group of blood vessels, observed in one day and one week old olfactory bulb have a well defined basement membrane, a large and patent lumen and an attenuated cell wall. These vessels

are structurally similar to those reported for two week and older animals. Donahue and Pappas (1961) and Caley and Maxwell (1970; 1971), in their studies on the development of capillaries in the rat cerebral cortex, did not describe any mature blood vessels during this period. However, observations made on the photomicrographs in the paper by Caley and Maxwell (1971) showed that there were some vessels with well defined patent lumina which could be compared to type II vessels of the present study. It is conceivable that the approach Caley and Maxwell (1971) took in treating vascular development was distinct from the present study. These authors described the pattern of vascular development in a sequential manner commencing with the most primitive looking blood vessel to a well differentiated mature vascular channel. In the present study, the pattern of vascular differentiation is treated in an alternate manner where the two distinct vascular patterns present in any stage of development are discussed simultaneously.

Several reports have indicated that the permeability in the immature nervous tissue is greater than in mature cortex. The alterations in the permeability of the blood-brain barrier in developing neonatal brain has been investigated by several investigators utilizing a range of substances: glutamic acid (Himwich and Himwich, 1955; cholesterol (Dobbing and Sands, 1963); chloride and insulin (Vernadakis and Woodbury, 1965) and sodium (Luciano, 1968). In early postnatal animals the majority of blood vessels are of the non-patent or primitive type with only few mature blood vessels. These immature

blood vessels have larger population of pseudopodial projections and a thin basement membrane. The presence of larger amounts of cytoplasmic projections in immature blood vessels bear some relationship to its higher degree of permeability. As development continues, the endothelial cell becomes attenuated, pseudopodia fewer, the lumen enlarges and the nucleus is extended to accomodate to the shape of the capillary wall. These structural changes are probably associated with the decrease in permeability of substances in mature blood vessels.

The perivascular spaces in the olfactory bulb, in postnatal animals, are completely occupied by cellular processes and in some cases by perikarya of glial and neuronal origin. This finding is in agreement with observations made by Phelps (1972) in the ventral horn of the rat cervical spinal cord and in the rat cerebral cortex by Donahue and Pappas (1961); Tennyson and Pappas (1962); Voeller. Pappas and Purpura (1963); Pappas and Purpura (1964). However, reports from several laboratories indicate the presence of perivascular spaces in the immature cortex of the rat (Del Cerro and Snider, 1967; Pysh, 1967; Caley and Maxwell 1968a, b; Hannah and Nathaniel, 1974). This disagreement in the electron microscopic literature as to the presence of extracellular spaces in immature nervous tissue would need further investigation. However, the possibility exists that there are areas in the central nervous system where development and maturation is more advanced than in other areas. This is certainly applicable to the development of the olfactory bulb where the sense of smell is essential for initial feeding habits of animals. This is especially true in the case of rodents,such as rats and mice.

SUMMARY

Electron microscopic studies have revealed that two types of blood vessels, categorized Type I and Type II, are present in newborn and one to five week old postnatal rats.

Basically, Type I blood vessels possess a primitive appearance and show the following features:

1. Small size, with a lumen which varies from a mere slit to one of a distinct but small lumen.
2. They are surrounded by the watery astrocytic cytoplasm.
3. Endothelial cell walls are thick, and contain a variety of cytoplasmic organelles mostly free ribosomes.
4. Pinocytotic vesicles and cytoplasmic projections are present in considerable numbers.
5. An ill-defined basement membrane which had an amorphous appearance surrounds these capillaries.

Type II Blood Vessels:

This group of blood vessels appear to have reached a later stage of development when compared to Type I. Type II blood vessels are characterized by:

1. A distinctly patent vascular lumen, generally oval in shape.
2. The vessels are ensheathed by astrocytic as well as neuronal and oligodendrocytic processes.
3. The attenuated endothelial cells contain few pinocytotic vesicles and cytoplasmic projections.

4. Rough endoplasmic reticulum is seen much more readily in these endothelial cells than in Type I vessel.
5. A well defined basement membrane is related to the endothelial cells and pericytes.
6. Type II blood vessels are found predominantly in two weeks and older rats.

Figure 10. Electron micrograph of an extremely primitive type I vascular channel from olfactory bulb of 7 day old animal. Observe a very narrow slit-like channel (arrows). The wall of the vessel is made up of two endothelial cells as indicated by the presence of two junctional complexes (JC). One of these endothelial cells displays a large, irregular nucleus with considerable accumulation of chromatin along its margin. Free ribosomes, segments of smooth endoplasmic reticulum and vesicles may be observed within the endothelial cell ctyoplasm. A tangentially sectioned centriole (C) is located adjacent to the U shaped nucleus. The cytoplasm of a pericyte (P) may be observed partly enclosing the lower half of the vessel. An ill-defined basement lamina (bl) is seen enclosing the vessel. Surrounding the primitive blood vessel is the watery cytoplasm of an astrocyte (As) with few particulate material considered to be glycogen. X 34,400

Figure 11. Electron micrograph of a primitive Type I capillary in the olfactory bulb of 7 day animal. Observe the irregular slit-like lumen enclosed by two endothelial cells. One of these cells displays a large irregular nucleus with peripheral aggregation of chromatin, a cytoplasm containing free ribosomes, granular endoplasmic reticulum and vesicles. Two junctional specializations (JC) may be observed. A pericyte (P) with very dense cytoplasm may be seen in relation to a part of the blood vessel. A basal lamina (bl) can be seen external to the pericyte and the endothelial cell. Astrocytic process (As) encircles a large portion of the vessel.

X 29,400

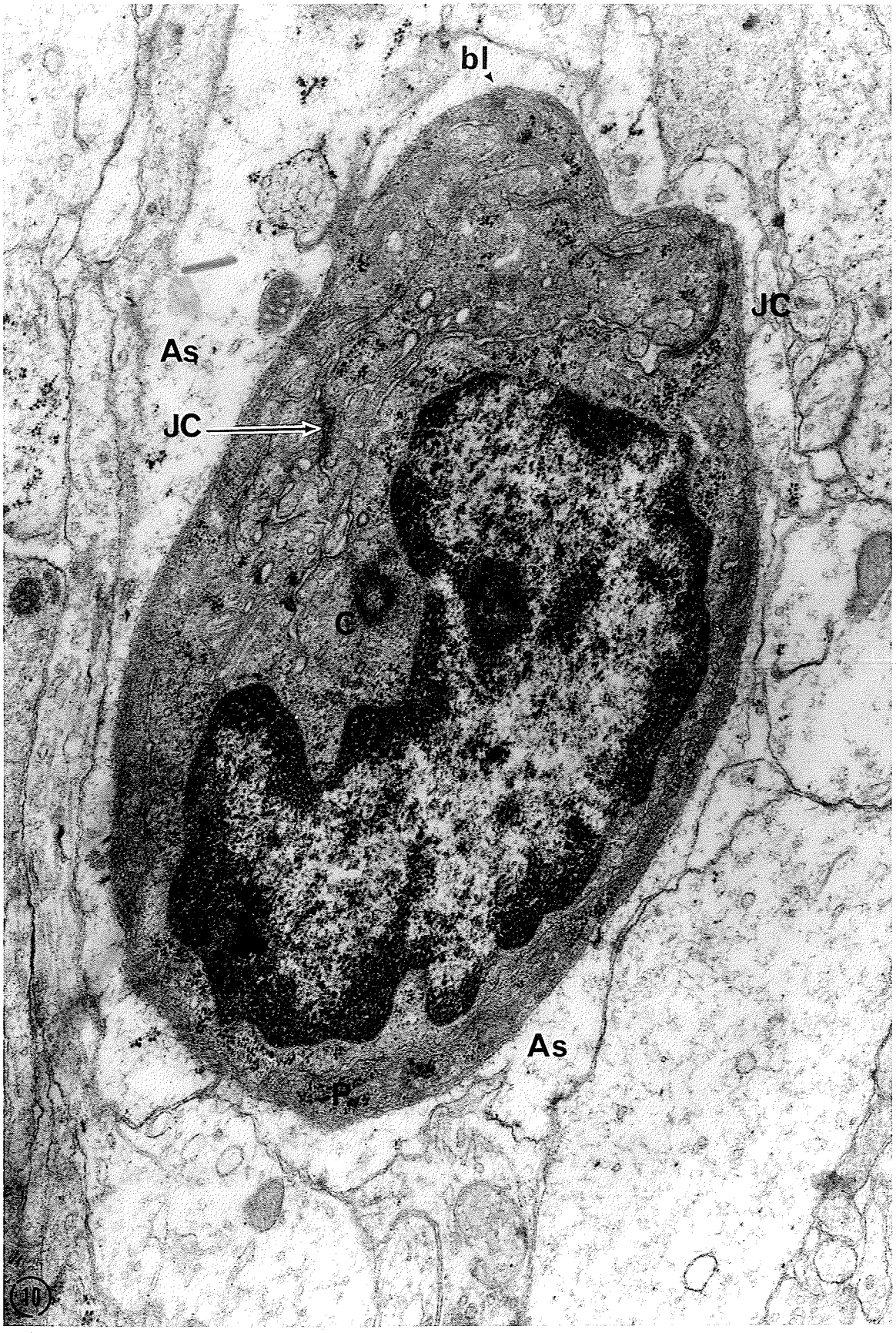
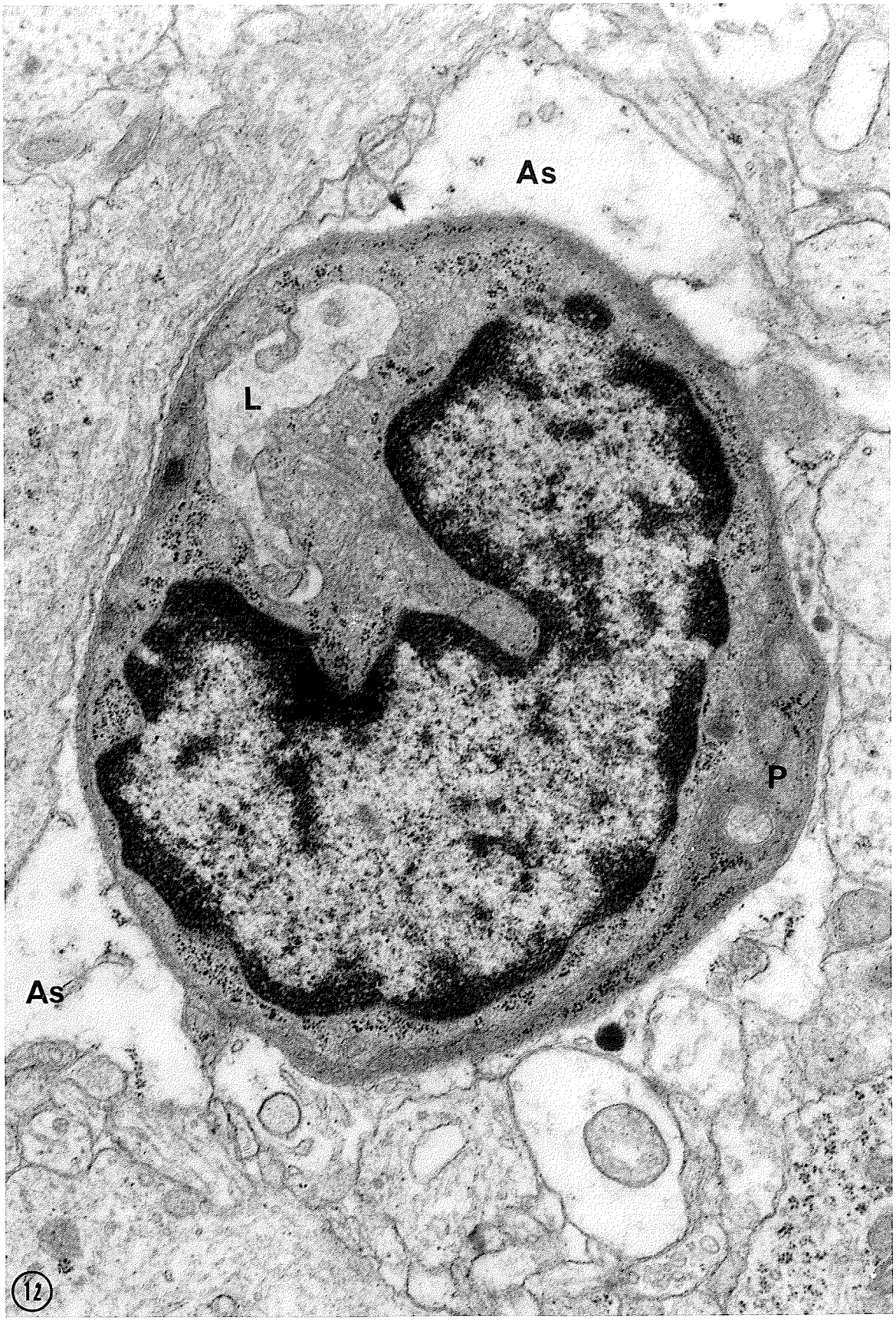

bl
JC
As
JC
G
As
P
10

Figure 13. Low power electron micrograph of the external plexiform layer showing a primitive blood vessel from a seven day old olfactory bulb. Endothelial cells surround a small lumen (L) containing a granular material which may represent coagulated blood plasma. Several vesicles, free ribosomes, very little rough endoplasmic reticulum and mitochondria are identified within the endothelial cells. The basal lamina surrounding the endothelial cell is poorly defined. Attenuated pericyte (P) cytoplasm encircles the vessel. A basement lamina separates the pericyte from the surrounding glial and neural elements. Astrocytic (As) process encircles a considerable portion of the primitive blood vessel. The right half of the primitive blood vessel is related to a cytoplasmic process which contains fine filamentous material interspersed with ribosomal clusters. This process is considered to belong to an astrocyte (As).
X 20,000

Figure 12. Cross section of a primitive blood vessel from the olfactory bulb of one week old animal. Note the thick endothelial cell possessing a highly irregular nucleus with a marked aggregation of chromatin along its margin. The endothelial cytoplasm is dense and has numerous vesicles (V) and short segments of agranular reticulum. This vessel is presumed to be formed by a single endothelial cell enclosing a small but distinct lumen (L) into which project pseudopodial projections of the cytoplasm. An ill-defined basement lamina surrounds the pericyte (P) which encloses this primitive vascular channel. The watery cytoplasm of an astrocyte (As) encloses the blood vessel.
X 30,000


As
L
P
As
12

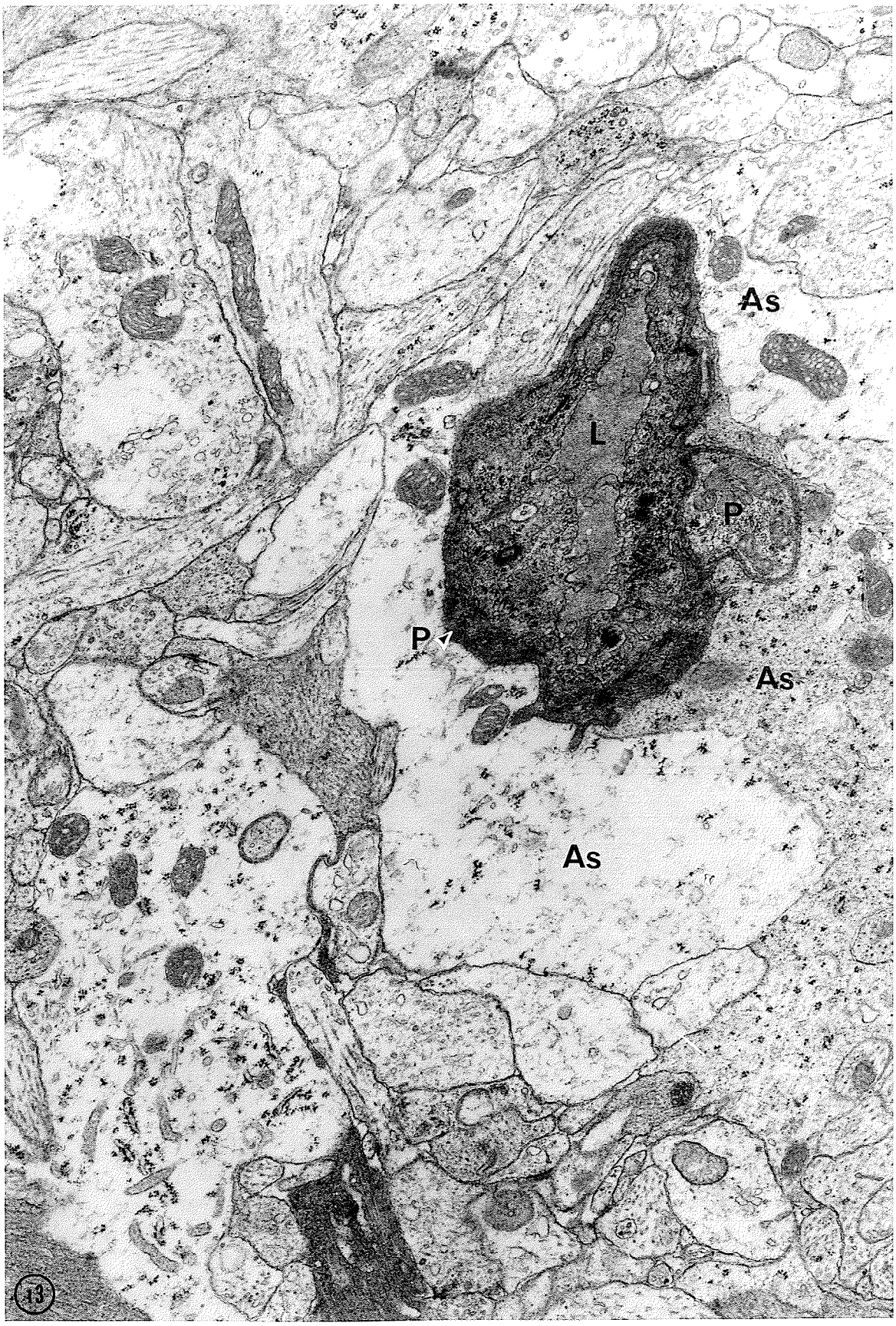

As
L
P
P
As
As
13

Figure 14. A type I primitive blood vessel seen in the External Plexiform Layer of the olfactory bulb of a seven day animal. Observe the vessel to be completely enveloped by astrocytic (As) process containing mitochondria and smooth reticulum. The vessel is enclosed by two endothelial cells as indicated by two junctional specializations (arrows). The nucleus is irregular with marked peripheral localization of chromatin. The cytoplasm is dense with many free ribosomes. Numerous profiles of cytoplasmic projections may be seen within the lumen. A portion of a pericytic (P) cytoplasm is in relation to the upper aspect of the vessel wall. A periglomerular cell (Pg) is found in the lower portion of the figure.
X 22,800

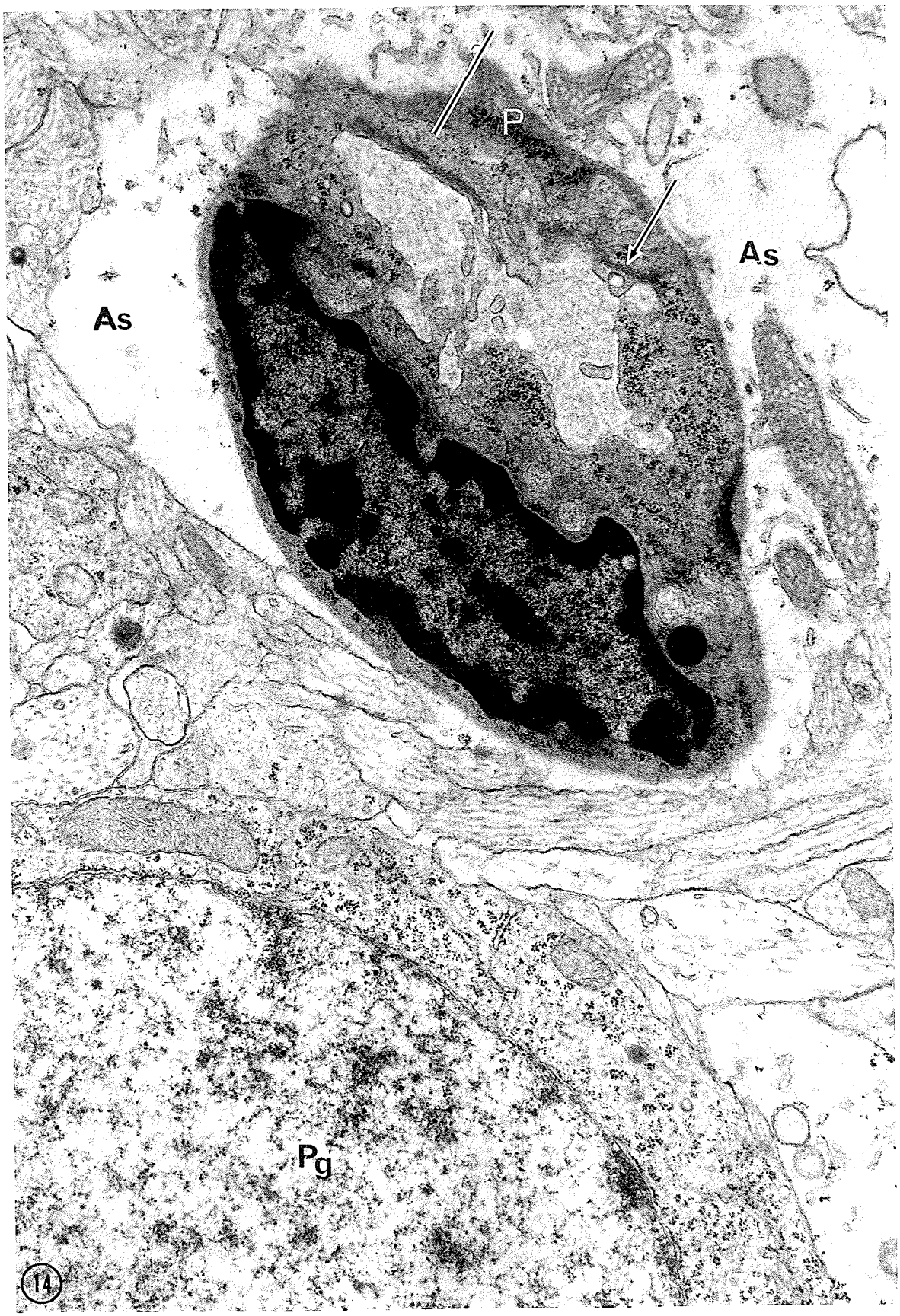
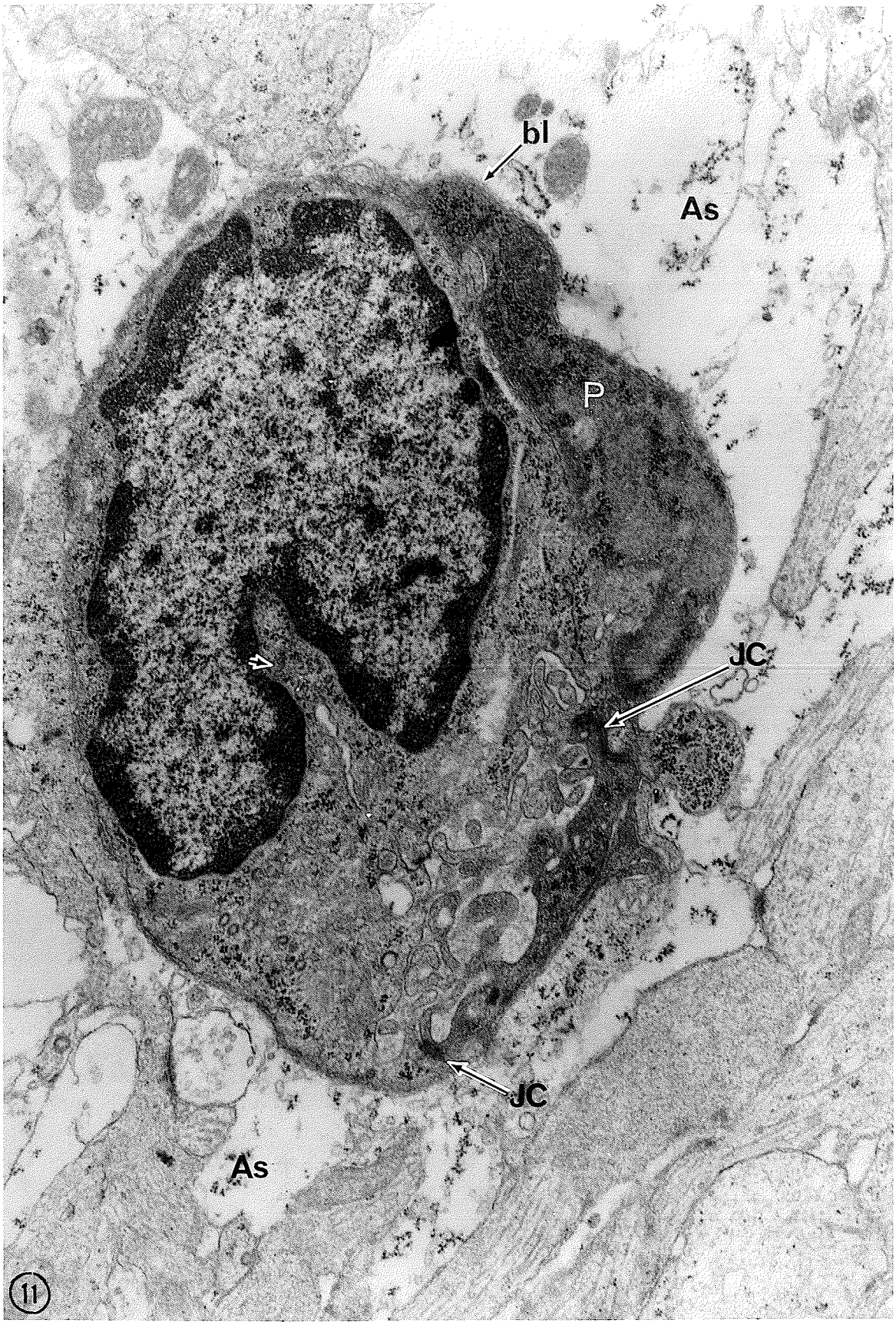

P
As
As
Pg
14

Figure 15. Electron micrograph of a mature blood vessel from a 1 day olfactory bulb. Observe well defined basement lamina (bl) separating the endothelial cells from the overlying pericytic (P) processes. The endothelial cell cytoplasm possess numerous free ribosomes, granular endoplasmic reticulum and mitochondria. Large electron dense granules considered to be glycogen (gly) can be seen in the pericyte. Segments of cytoplasmic processes of astrocyte (As) are also visualized. The cytoplasm of the cell, located in the lower portion of the micrograph, contains well developed Golgi complex, segments of rough endoplasmic reticulum and free ribosomes and is considered to belong to a glial cell, possibly an astrocyte (As).

X 29,400


bl
As
P
JC
JC
As
11

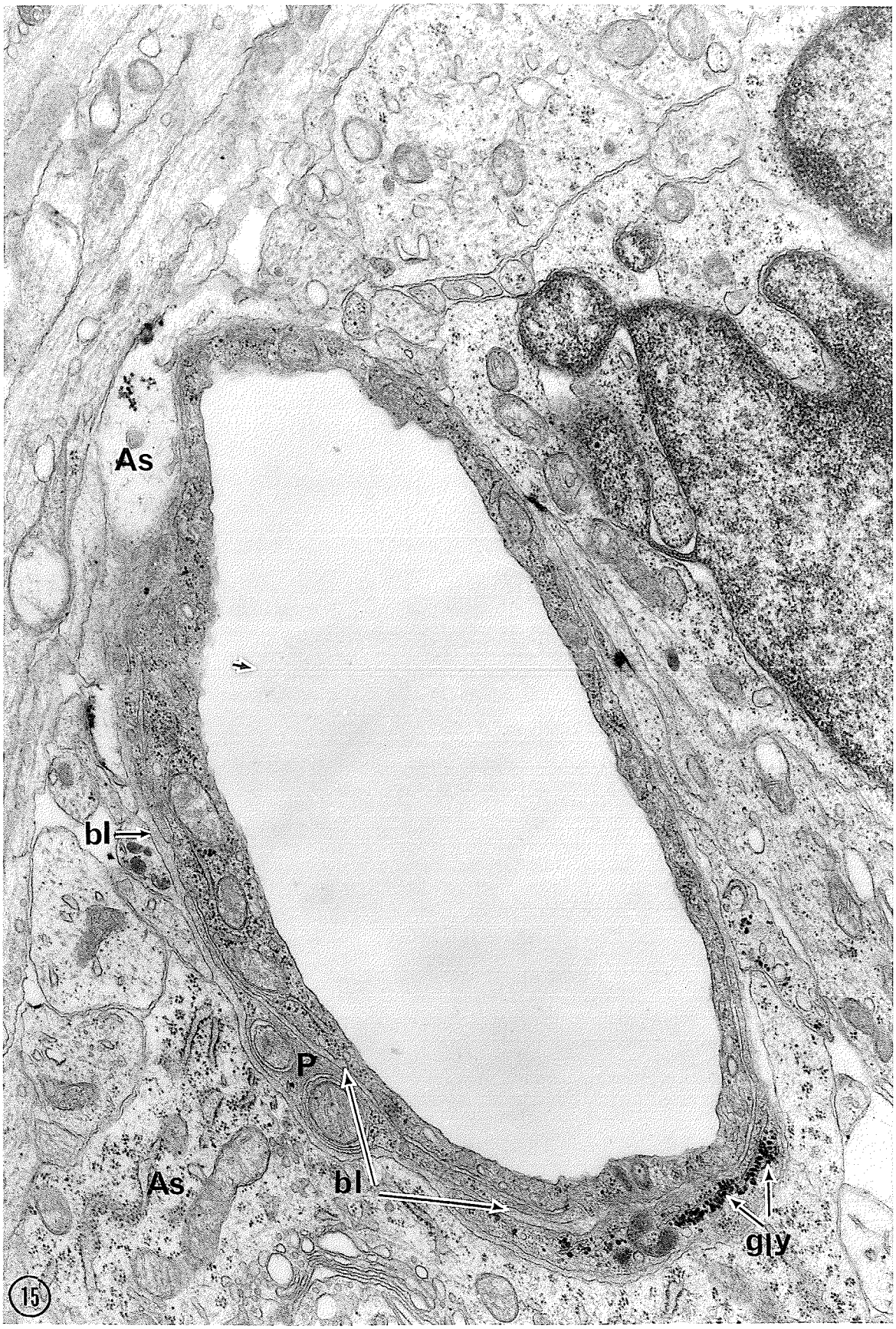

As
bl
P
As
bl
gly
15

Figure 16. Illustrates a large type II vessel with higyly attenuated endothelial cells limiting the lumen. Portions of pericyte (P) may be observed as well. Distinct basement lamina (bl) can also be seen. Note that processes of neurites, neurons (Ne), and glial cells are aligned around the vascular channel. Specimen from one day old olfactory bulb.

X 21,800

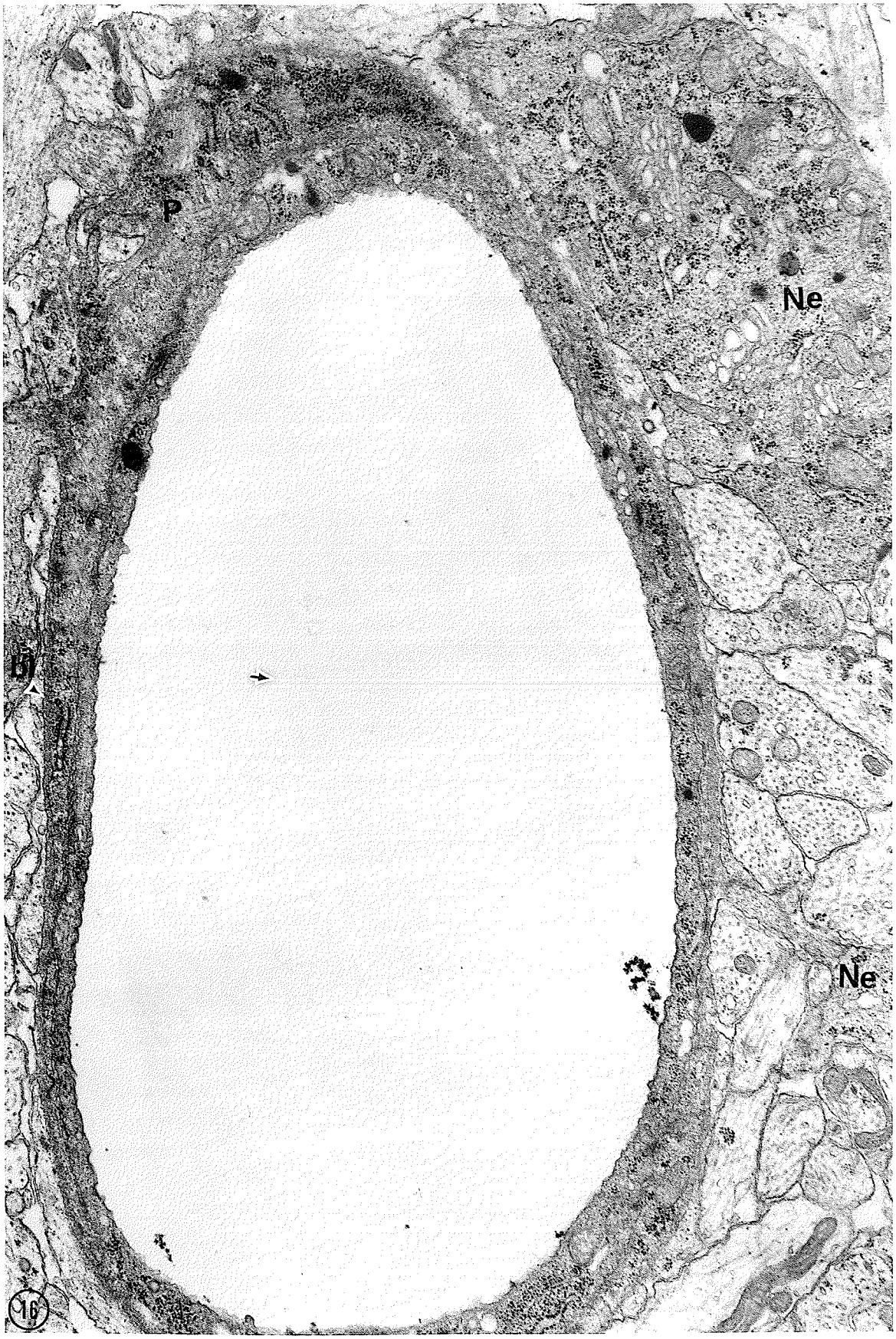
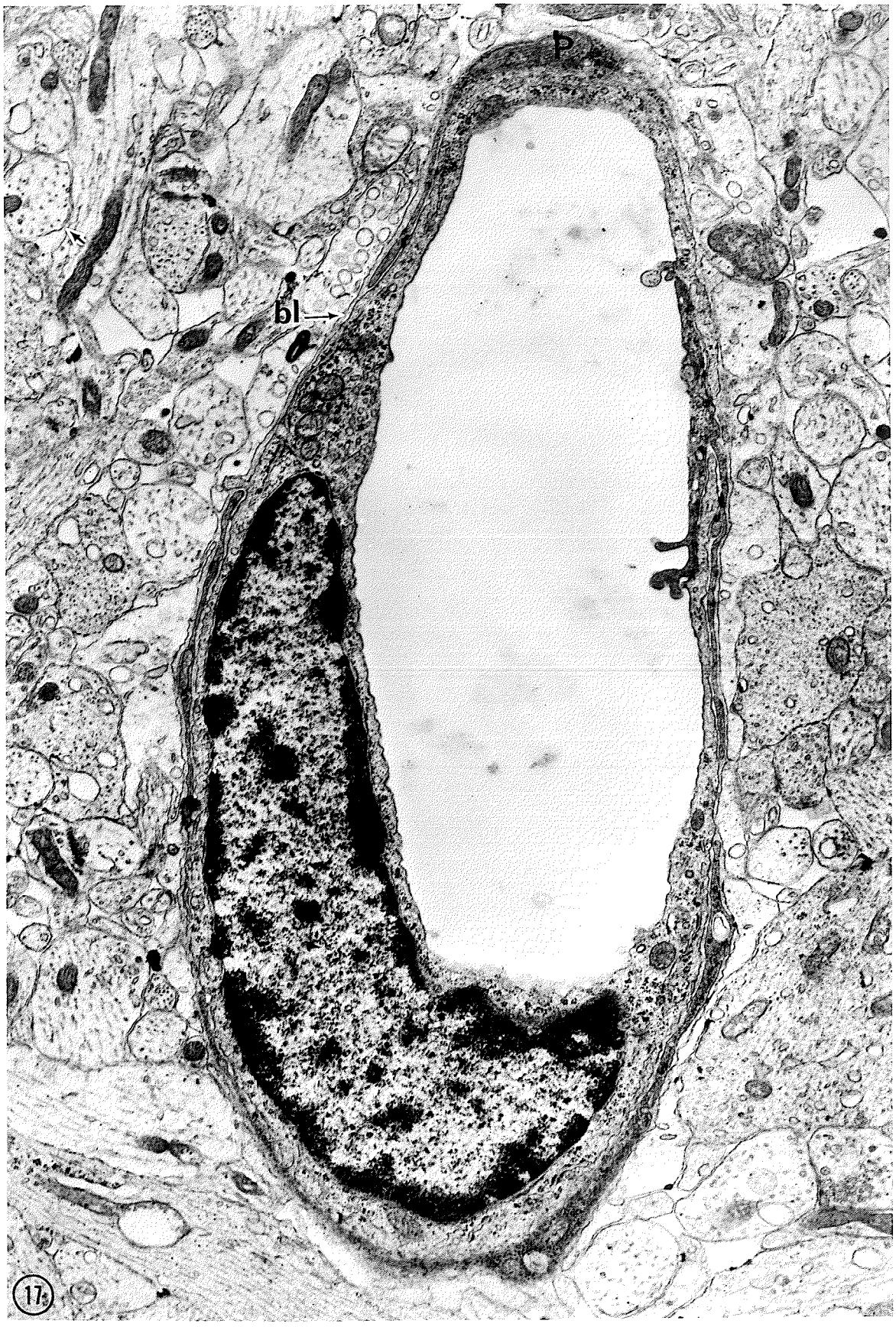

P
Ne
Ne
BM
16

Figure 17. This electron micrograph illustrates a well developed or type II blood vessel found in both neonatal and one week old olfactory bulb. The nucleus is elongated and the cytoplasm attenuated. Short projections extend into the vascular lumen→ Numerous ribosomes, mitochondria and vesicles are present in the endothelial cell. A distinct basal lamina (bl) surrounds the vessel. Portions of pericytic cytoplasm (P) can be observed surrounding the endothelial cells.

X 18,500



17

Figure 18. Electron micrograph of a well developed or Type II blood vessel from a 35 day old olfactory bulb. A patent lumen containing two red blood cells and attenuated endothelial cells make up its morphology. The basal lamina (bl) is thick and well defined. The endothelial cytoplasm contains several segments of rough endoplasmic reticulum, free ribosomes, mitochondria and few vesicles. A tight junction (arrow) is also present. Note that the astrocytic processes (As) do not envelope the vessel to the extend as seen in type I vessel. Large numbers of profiles of neuronal and neural processes lie adjacent to the basement lamina.
X 24,200

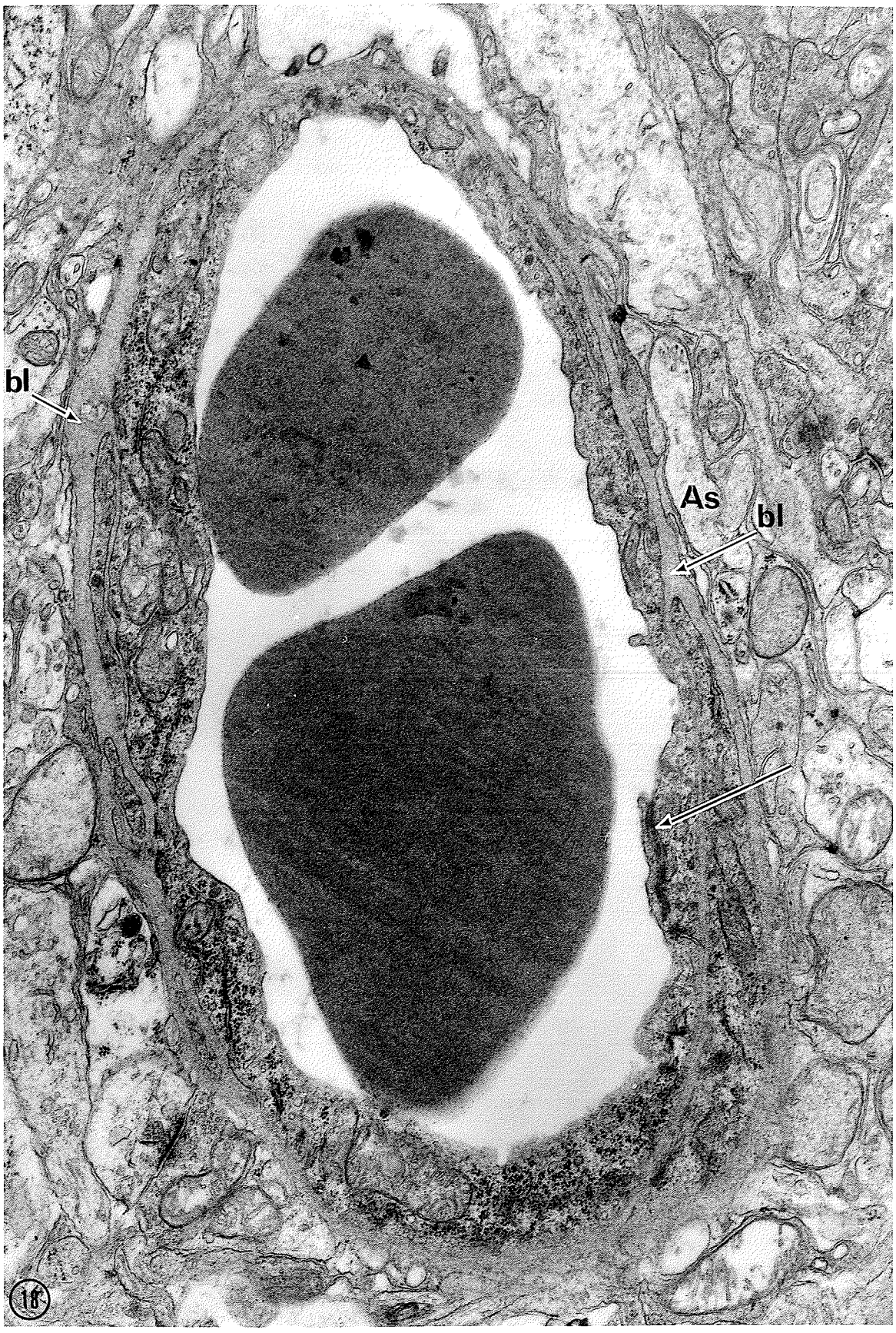

bl
As
bl
1f

Figure 19. This figure shows a mature blood vessel and an associated pericytic cell. The endothelial cell is attenuated and a basal lamina is present. The presence of a single junctional complex indicates that the channel is formed by a single endothelial cell at this point. The pericyte is closely associated with the vessel and is related, on both the external and internal aspects, to a basement lamina (bl). The cytoplasm of the pericyte contains several cytoplasmic organells viz, Golgi complexes, free ribosomes, mitochondria, dense bodies and a small amount of rough endoplasmic reticulum. Blood vessel from 35 day old olfactory bulb. X 23,300

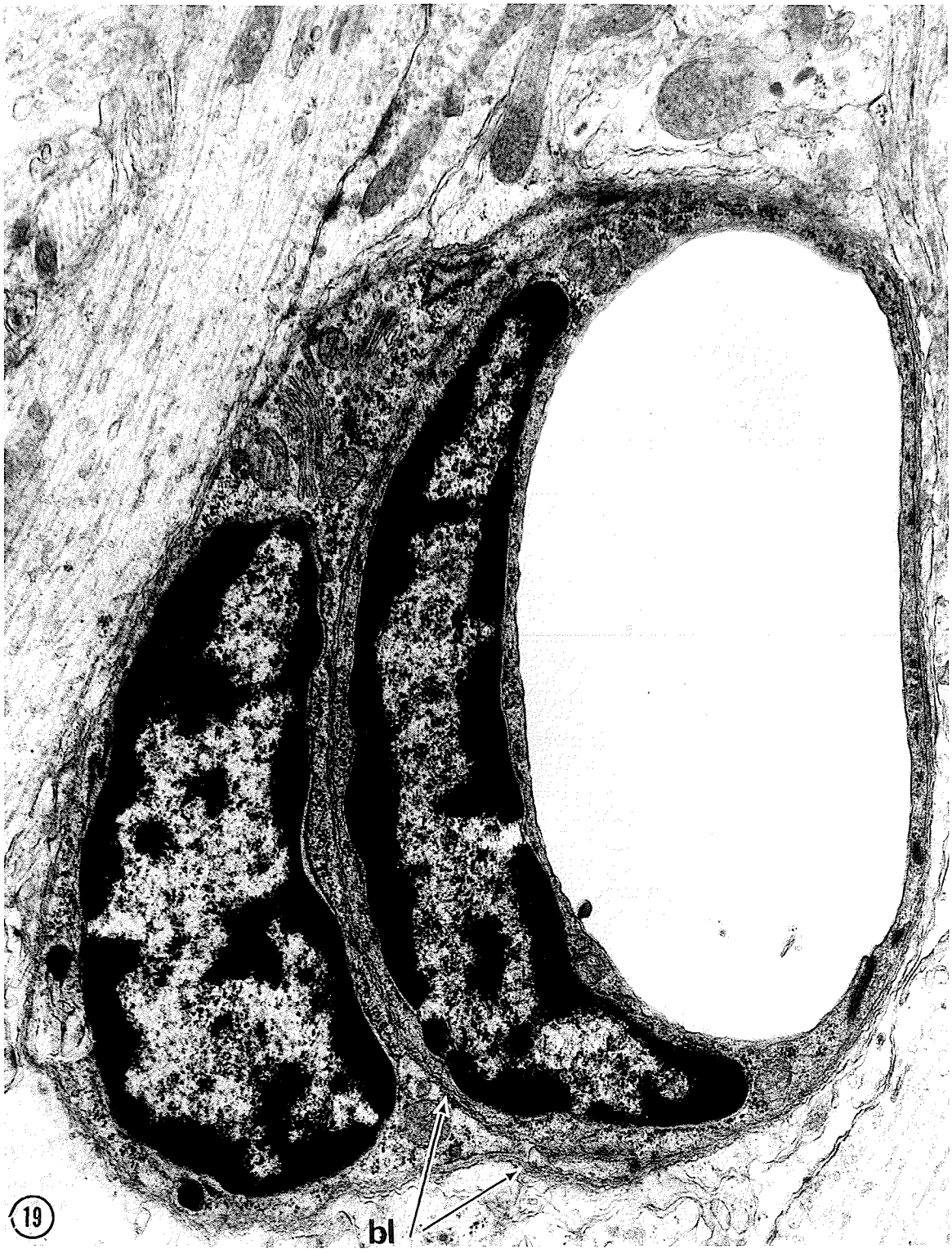
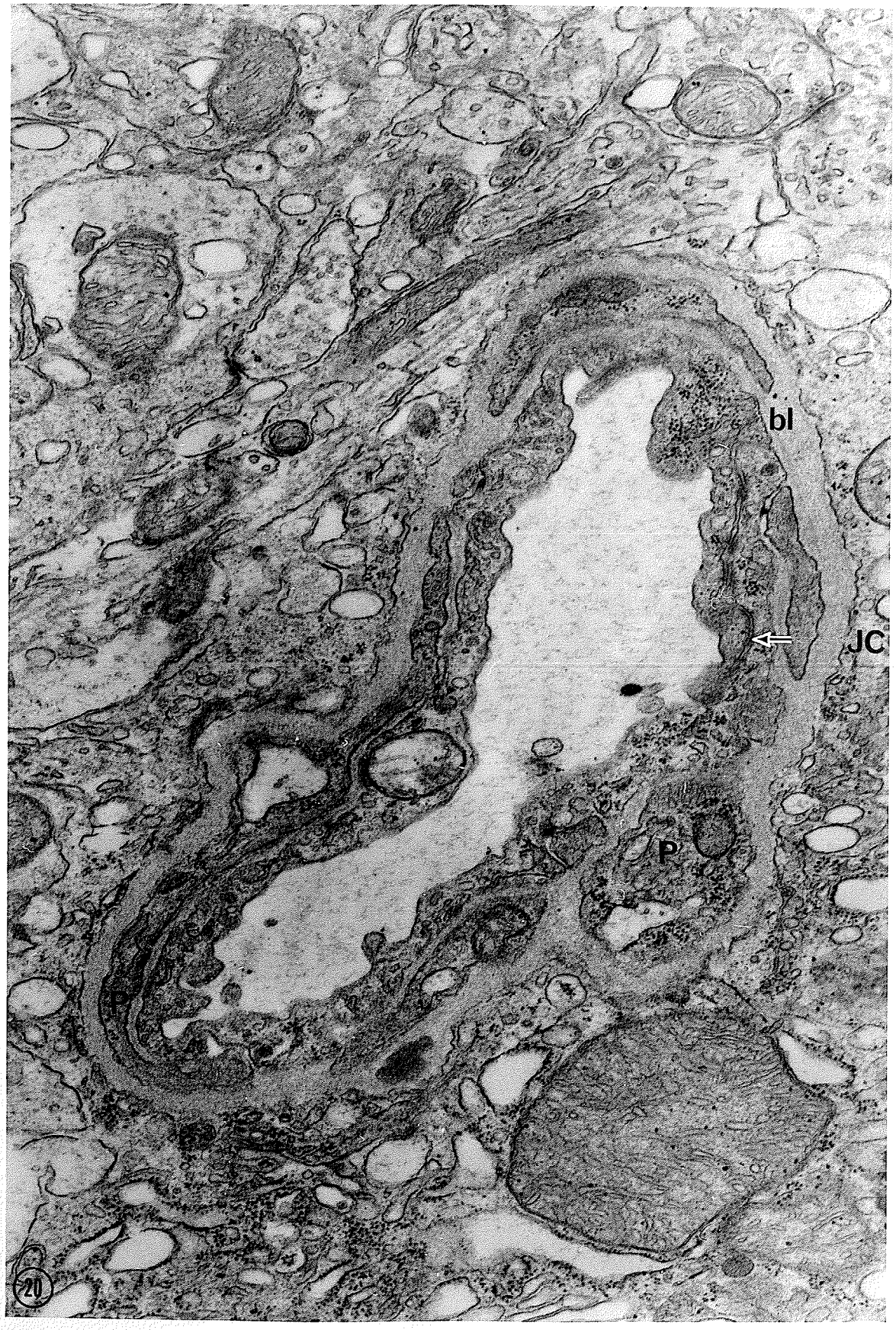

19
bl

Figure 20. Mature, Type II, blood vessel from olfactory bulb of a 35 day old animal. Note the markedly thickened basement lamina (bl) enclosing the endothelial cell as well as the pericyte (P). Junctional complexes (JC) between adjacent endothelial cells may be seen.
X 34,400


bl
JC
P
P
20

SECTION ON NEURONS

1. Review of Literature.
2. Electron microscopic observations:
   a. Fine structure of the layers of olfactory bulb
   b. Differentiation and maturation of neurons of olfactory bulb
3. Discussion.
4. Summary.

REVIEW OF LITERATURE

In the early period of comparative neurology (1890-1920) it was generally believed that the answer to the evolution of the forebrain was associated with the olfactory system. The intrinsic structure, relating to the arrangement of the neurons in the olfactory bulb, has been described (Calleja, 1893; Kolliker, 1896; Blanes Viale, 1897)). Vertebrate olfactory systems have a constant morphology. For this reason, any description of a specific species of animal could be related to any other vertebrate olfactory system. A narrative on its morphology, histology and embryology has already been briefly accounted for in the general review part of this thesis.

The olfactory system of different animals has been used to study several aspects of nervous system elements and their development.

Light microscopic studies have been used in quantitative estimations of olfactory bulb elements in Norway rats (Holt, 1917); in wild Norway and albino rats (Smith, 1928) and in human (Smith, 1940). Norway rats were found to have 75,000 mitral cells in each olfactory bulb, wild Norway rats have 5,600,000 granule cells and 37,000 mitral cells and albino rats 3,200,000 internal granule cells and 55,000 mitral cells. Similar numerical studies were carried out on the rabbit olfactory system by Allison and Warwick, (1949). These investigators found that there were, on the average, 50,000,000 receptor cells in each nasal cavity, 1,900 glomeruli, 45,000 mitral cells and 1,300,000 tufted cells in each olfactory bulb, and 60,000 fibres in each olfactory tract.

Using semithin sections and light microscopy, Andres (1970) described the configuration of the olfactory bulb in lamprey elasmobranchs and teleost fishes. From these studies it became apparent that, except for the presence of the external plexiform layer in land-living vertebrates, there is a constant cytoarchitecture in the olfactory bulb of all vertebrates.

The fine structure of the cellular entities of the olfactory system has been studied with the electron microscope. However, most of the studies have been concerned with the sensory input into the cell, with emphasis on the differences in synaptic arrangement (Price and Powell, 1970 a, b, c, d; Pinching and Powell, 1971, a, b, c; White, 1972; Willey, 1973).

The fine structure of the olfactory epithelium in young male squirrel monkey is described in detail by De Lorenzo (1970). Similar studies have been carried out on the frog (Bloom, 1954), mouse (Frisch, 1964), rabbit (De Lorenzo, 1957) and in primates and man (De Lorenzo, 1968). These studies have shown that there is a degree of uniformity in the morphology of the olfactory epithelium in vertebrates.

Based on earlier electron microscopic findings of the olfactory bulb in different mammals, Andres (1970) concluded that the basic structure of the olfactory bulb or system remains relatively constant from cyclostones to primates. The cell types and their processes of the olfactory bulb, at the light microscopic level, have been described in an earlier section of this thesis. These elements have been investigated under the electron microscope in various mammals (Rall

et al., 1966; Price, 1968). The most recent study by Willey (1973), in the adult cat olfactory bulb, has shown that the olfactory bulb contains six layers, namely: peripheral olfactory nerve layer, glomerular layer, external plexiform layer, mitral layer, internal plexiform layer and granular layer. The cellular components are neurons and glial cells. Four types of neurons were described and included the periglomerular neuron in the glomerular layer, the tufted neuron in the external plexiform layer, the mitral cell in the mitral cell layer and the granule cell in the granular layer. Willey (1973) observed from cytological and structural criteria, that both the tufted and mitral cells were similar except for size and location and the periglomerular and internal granule cells resembled each other.

A fifth neuronal cell type, the stellate, was occasionally present in glomerular, external plexiform and granular cell layer (Willey 1973). In contrast to tufted and mitral cells, the stellate neurons possessed very irregular nuclei and were free of somatic reciprocal synapses.

In recent years a combination of autoradiography, Golgi impregnation and electron microscopic techniques have been employed to elucidate certain phenomena of neuronal development. Altman (1969), using autoradiographic studies in rats, ranging in age from newborn to adult, has postulated that cells produced by mitotic divisions in the sub-ependymal layer of the lateral ventricle were destined to reach the granular layer olfactory bulb. Eventualy, these cells were believed to differentiate and replace degenerated cells in the olfactory bulb.

Similar techniques using tritiated thymidine-H^3 were employed by Hinds (1968) to study the time of origin of neurons and neuroglial cells in the mouse olfactory bulb, of ages ranging from gestation period to adult animals. In a subsequent investigation, using the Golgi impregnation technique and electron microscopy, Hinds (1972 I, II) elucidated the differentiation of neurons in the mouse fetuses.

However, there have been no systematic studies on the development and maturation of the olfactory bulb in postnatal rat.

ELECTRON MICROSCOPIC OBSERVATIONS

This section will initially describe the ultrastructural features of the six layers encountered in the olfactory bulb, and subsequently proceed to the consideration of the neuronal development.

Fine Structure of the layers of the olfactory bulb:

It was seen in the earlier section of the thesis, designated 'light microscopy of olfactory bulb', that the olfactory bulb cortex shows distinct stratification or layers which become well defined by fiveweeks postnatal. These layers, commencing from the pial or surface layer and proceeding into the interior, consist of the following: the fiber layer, the glomerular layer, the outer plexiform layer, the mitral cell layer, the inner plexiform layer and finally the granule cell layer.

Fiber Layer:

This layer is made up of axons of olfactory receptors and their satellite cells (Figures 21-23). These axons, composed of a large number of unmyelinated fibers, are grouped into bundles or fasciculi related to satellite cells. The satellite cell, associated with axons located in the outermost region of the olfactory bulb, exhibited the features of a Schwann cell by having a basement lamina and collagen fibers in the extracellular space. However, as the primary olfactory nerves entered deeper into the olfactory bulb the satellite cells did not resemble the conventional Schwann cells, being devoid of basement lamina. In addition, the extracellular space and the collagen

were no longer present. These satellite cells are considered by some as astrocytes (Andres, 1970) and by others as Schwann cells (Willey, 1973).

The satellite cells have elongated nuclei and generally possesses considerable accumulation of chromatin, especially localized along the nuclear margin (Figure 21). Few nuclei have less chromatin. The cytoplasm of these cells possesses distinct amount of free and membrane attached ribosomes, and mitochondria. The cytoplasmic processes of these cells are long and attenuated extending in various directions and separating groups of axons (Figure 23).

The axons are of the unmyelinated variety and contains microtubules and mitochondria (Figures 21-23). Some of the larger caliber axons possesses lamellated figures as well as intact mitochondria (Figure 21). Longitudinally oriented axons, as well as cytoplasm of satellite cells, separates the clusters of cross sectional profiles of unmyelinated nerve fibers (Figure 22). No synaptic complexes are observed in this layer (Figures 22 & 23).

Glomerular Layer:

This area has been investigated repeatedly in the rat and mouse to study its architecture and synaptology (Hirata, 1964; Rall et al., 1966; Price, 1968; Hinds, 1970; Pinching, 1970; White, 1972 and Willey, 1973).

When viewed with the electron microscope, each glomerulus was found to be composed of clusters of dendrites and axons (Figure 24). The criteria used to differentiate dendrites and axons are those

described by Peters et al., (1970). Dendrites and axons were found to intermingle freely, producing at the same time a vast number of synapses.

Synapses were similar to those described in other areas of the vertebrate nervous system. Three criteria were used to study and identify the morphology of the synapses viz: (1) Presynaptic terminal and membrane, (2) Postsynaptic terminal and membrane, (3) synaptic cleft. The presynaptic terminals were found to be composed mainly of axonal endings, containing synaptic vesicles and mitochondria. The dendrites of these axo-dendritic synapses contained numerous tubules. The membrane specialization at the synapse was most marked on the post-synaptic membrane. The post synaptic terminal contained large numbers of microtubules. However, occasional dendro-dendritic and somato-dendritic contacts were observed.

Studies on synapses in the glomerular layer, at subsequent periods, did not reveal any further changes, indicating that the axons of first order primary neurons establish extensive contacts at birth and are probably functional. Any intrinsic changes might be subtle and would require various forms of morphometric and stereology studies (see Bodian, 1966; Vrensen and DeGroot, 1973; Vaughn and Grieshaber, 1972). Studies of Wannamaker et al., (1973) related to the development and maturation of synapses have shown that the ultrastructure of synaptic complexes isolated from different age groups in man, 2 to 78 years, were similar; indicating that development and maturation of synapses is essentially completed as early as 2 years of life in the human.

At the periphery of each glomerulus are periglomerular neurons (Figure 25) and glial cells. Processes from these cell types have been identified in the glomerulus. Periglomerular neurons outnumbered glial cells. Differentiation and maturation of these cell types will be described in a later section of this thesis.

External Plexiform Layer:

Dendrites from tufted, mitral and internal granule cells constituted the major part of the external plexiform layer (Figure 26). Intermingled within this maze of cytoplasmic projections were axons of tufted, periglomerular and stellate cells. Tufted neurons were the main cell type of this layer and associated with these neurons were neuroglial cells and their processes.

Similar to other areas in the central nervous system, dendrites were larger and more empty looking than axons. Most of the large dendrites are considered to belong to tufted and mitral cells. Smaller diameter dendrites are presumably from internal granule cells. The structure of these dendrites except for differences in size, was similar and included intracellular organelles such as microtubules, mitochondria and smooth reticulum. Synaptic contacts of the types axo-dendritic, dendro-dendritic and reciprocal are ubiquitous in the external plexiform layer.

In the newborn rat, dendritic processes from mitral and tufted cells have been observed to project for short distances into the external plexiform layer. These dendritic tips or buds were conical in shape and contained the usual intracellular organelles. The width

of the external plexiform in neonatal and one day old animals has been calculated to be 45 microns and in one week old animals 100 microns. Consonant with the increase in the thickness of this layer was the tremendous growth of apical dendrites of mitral cells. The dendrites transversed the entire width of the external plexiform layer and entered the glomerular layer (Figure 24) where synaptic contacts were established. Along the length of the dendrites, small spinal projections and synapses were present. Ensheathment by glial and other cell type processes have been observed.

In two weeks and older animals extensive display of dendritic contours was observed. They were tightly packed and the external plexiform layer acquired a mature disposition when compared to younger animals. In summary, the increase in width of the external plexiform layer with age was related to the growth and increase in length of the dendrites of mitral and tufted cells.

No myelinated fibres were found in one day and one week old olfactory bulb. However, in two weeks and older animals, small caliber myelinated axons were observed.

Mitral Cell Layer:

This layer was easily identified because of the conspicuously large soma of mitral cells (Figure 8). In the olfactory bulb the mitral cells are the largest neurons. These neurons occupied a well delineated layer.

Arranged in small clusters around the mitral cellsoma were small neurons that resembled, at the ultrastructure level, the granule cells.

Normally these cells are found in the granular layer. Cellular processes form granule cells projected between mitral cells to reach the external plexiform layer.

Internal Plexiform Layer (IPL):

At birth it was very difficult to identify this layer. However, older animals showed an area identifiable as the inner plexiform layer (Figure 7 & 8). This layer was devoid of mitral cell perikarya. Internal granule cells and their processes make up this layer.

Granule Cell Layer:

Granule cells and their processes constituted the major components of the granule cell layer. Oligodendrocytes with dense chromatin and cytoplasm were observed between clusters of granule cells. Cellular processes of other neurons and glial cells were present between the soma of cell types found in this area.

Synapses on the soma and processes of granule cells were observed in all age groups. However, synaptic contacts were not as numerous and as extensive when compared to the glomerular and external plexiform layer.

Few myelinated fibres, of small diameter, were observed in two weeks and older animals.

Differentiation and Maturation of Neurons of the olfactory bulb.

Differentiation of neuronal cells in the olfactory bulb have reached a well developed stage when compared to neuronal differentiation in the cerebral cortex. Apparently, in the olfactory bulb the neuroblasts differentiate at a precocious pace to give the rat a functional capacity at birth. Hinds and Hinds (1972) have reported that in the mouse olfactory bulb mitral neuroblast is formed between day 11 and day 13 of gestation period. Of the smaller neurons, the tufted cell differentiated between day 13 and 18, while the granule cell differentiation extended well into the postnatal period.

In the present investigation four basic types of neurons were encountered. These consisted of mitral cells, tufted cells, periglomerular and granule cells. The criteria for initially identifying these neurons is based on the previous light microscopic studies of Allison (1953a, b) Ramon Y Cajal (1955), Freeman (1972a) and others. As indicated earlier, the purpose of this study was to describe the neuronal development and maturation of mitral cell in particular, with reference to other neurons in less detail. This account will therefore describe initially the developmental features of mitral cell followed by an account of tufted, periglomerular and granule cells. It may be stated that the descriptive pattern will follow a temporal sequence, starting with an account of the primitive neuron in one day postnatal animal and culminating in a description of the adult neuron seen three weeks after birth. However, it should also be realized that at any time period studied, neurons of one population exhibiting different

stages of development will be encountered.

MITRAL CELLS:

These cells are the largest neurons of the olfactory bulb and the axons of these cells constitute the olfactory tract.

At birth, mitral cells have a well defined perikaryon in which a large nucleus is observed. Chromatin distribution was variable with an occasional clumping below the inner nuclear membrane. One and/or two reticulated nucleoli were present within the nucleoplasm. The cytoplasm contained abundant amounts of free ribosomes. Short segments of rough endoplasmic reticulum were found as well. Smooth endoplasmic reticulum was sparse. Well defined Golgi complexes composed of stacks of membranes and associated vesicles were observed within these cells (Figure 27). In several instances the Golgi complexes have been identified at the apical end of the mitral cell, and extended into the large apical dendrite. Dendrites are identified on the premise that they contained ribosomes and/or rough endoplasmic reticulum. Cytoplasmic organelles of these varietes are absent in axons.

Mitral cells in one week old olfactory bulb showed features which suggested further development (Figures 28-32). These were most striking in the cytoplasm. The nucleus showed no significant changes when compared to one day old animals. The chromatin was evenly distributed. The nuclear configuration was slightly undulated and continuity between the perinuclear space and granular endoplasmic reticulum was observed in several mitral cells (Figure 29). Nuclear pores were also seen in some mitral cells.

The cytoplasm of the mitral cells revealed features indicating progressive neuronal development. These changes were not only evident in the perikaryon of the cell (Figures 28, 29, 30) but also in their neuritic processes; namely the axons (Figure 30) and dendrites (Figures 31 & 32), especially the latter. The rough endoplasmic reticulum consisted of parallel, organized segments of ribosome studded tubules which showed branching (Figures 28 & 31). Rosettes of ribosomes were scattered throughout the cytoplasm. Multiple Golgi complexes and electron dense lysosomes were found in the perikaryon close to the nucleus (Figure 28).

Mitral cells in one week old olfactory bulb showed a remarkable increase in the growth of the apical dendrite (Figures 30-32). Dendritic processes have been traced for considerable length in the external plexiform layer. Throughout their length, these dendrites were ensheathed by other dendrites from internal granular cells, axonal processes, and glial processes. Dendro-dendritic, axo-dendritic and reciprocal synapses have been observed on the surface of mitral cell dendrites. Organelles including free ribosomes, mitochondria, vesicles, microtubules and neurofilaments were present. Except for a short portion at the base of the dendrite, rough endoplasmic reticulum was absent.

Elaboration of the Golgi complexes cisternae at the base of the apical dendrite was extensive (Figures 30 & 31). Intermingled between the Golgi cisternae were free ribosomes, portions of rough endoplasmic reticulum, mitochondria, membrane bound vesicles and few dense lipofuscin granules.

The cytoplasm of mitral cell in two week animal contained well organized granular endoplasmic reticulum and well developed Golgi complex and lysosomal bodies (Figure 33).

By the third week of postnatal development, the elaboration of rough endoplasmic reticulum has reached a peak (Figure 34) and a conspicuous increase in electron dense bodies. While the small, amorphous dense granules resembling lysosomes were found in immediate relation to Golgi complexes, large dense bodies, several of which possessed clear vacuolated areas within, were located further away and were comparable to the lipofuscin granules. Willey (1973) has shown that these granules are typical features in the adult cat mitral cells. In the present study numerous mitral cells in five week old olfactory bulb (Figure 35) possessed lipofuscin granules.

Tufted cell:

This cell type is the main cellular component of the external plexiform layer. Tufted cells were also observed in the deeper aspects of the glomerular layer.

At birth, tufted cells are not as differentiated as mitral cells. However, they have a large nucleus and a nucleolus. The cytoplasm is well defined and contains the regular organelles. There are equal portions of rough endoplasmic reticulum and free ribosomes. Mitochondria, lysosomes, microtubules, neurofilaments and Golgi complexes are distributed in the cytoplasm.

In one week and older animals, the rough endoplasmic reticulum became more elaborate and extensive (Figure 36). Golgi complexes

were distributed extensively within the cytoplasm. Cisternae were also found to extend into the apical dendrite of the cells. There was also an increment in the number of lysosomes and lipofuscin granules.

Periglomerular and Granule Cells:

Except for some minor differences, periglomerular and granule cells have similar ultrastructural morphology and will be discussed together. These cell types were found in the glomerular, mitral, internal plexiform and granule cell layers.

In one day old olfactory bulb, granule cells were observed to occur in clusters of 3 or 4 cells, with closely apposed cell membranes (Figure 37). Granule cells have a large nucleus and a thin rim of cytoplasm. Chromatin was dense and evenly distributed and a nucleolus was often present.

Free ribosomes occupied a large section of the cytoplasm and no rough endoplasmic reticulum was observed (Figures 38 & 39). There was a paucity of mitochondria and other cytoplasmic organelles.

After one week of development, granule cells showed no significant morphological changes except for the thinning out of the chromatin material.

Two week old granule cells have a relatively unchanged nucleus. However, the chromatin was less dense than in earlier cells and short segments of rough endoplasmic reticulum were observed in the cytoplasm.

Changes in 3 week and older granule cells include: the nucleoplasm was paler than in younger cells; there was an aggregation of chromatin

material below the inner nuclear membrane and a pale cytoplasm (Figure 39).

Granule cells have not been confused with oligodendrocytes which have a darker nucleus and denser cytoplasm.

DISCUSSION

Neuronal Differentiation:

Differentiation and development of neurons in postnatal olfactory bulb of the rat have been studied in age groups extending from one day to five weeks.

Development of mitral cells showed similarities to neurons that have been described in other areas of the nervous system: Bellaires (1959) in the chick embryo; Tennyson (1965), in the dorsal root ganglion of the rabbit embryo; Mugnaini and Forstroneu (1967), in chick embryo; Caley and Maxwell (1968), in the cerebral cortex of post-natal rats. This finding is not a surprising one since all neurons, irrespective of their location and shape, are destined to perform a common function of transmission of nerve impulses. However, each population of a common neuronal type demonstrates individuality singly or as a group. In addition, even in a homogeneous population, all cells do not develop at the same rate. These diversities are demonstrated by electrophysiological studies whereby nerve impulses from neurons of the same type are of different strength and duration. Due to these factors, studies involving the differentiation and development of nerve and neuroglial cells present an enormous amount of information in a realm of uncertainties.

Hinds and Ruffett (1973) using the Golgi impregnation techniques revealed that mitral cells make their appearance in the mouse olfactory bulb at day 14 of gestation. Subsequently, the mitral cells undergo a spatial reorientation from a tangential position to one of radial

orientation. This change in orientation would ensure that the apical dendrites of the growing cells would eventually make synaptic contacts with the receptor cell axons in the glomerular layer. Coupled with this manoever, was the appearance of Golgi complexes, rough endoplasmic reticulum, mitochondria, and numerous free ribosomes in the cytoplasm.

Such precocious development during gestation period ensures that the largest and most influential cell in the olfactory bulb would be in a functional capacity at birth, a factor that the young would need for survival. Similar investigation on monkey fetus spinal cord (Bodian, 1966) has revealed that particular reflex activities influence an early development of synaptic junctions in the neuropil.

In the present study, mitral cells revealed a fairly well differentiated perikaryon at birth. Rough endoplasmic reticulum, mitochondria, Golgi complexes and numerous ribosomes were found in large amounts in the cytoplasm. Primordium of cellular processes were observed to project into the neuropil tissue. Synaptic junctions were already well established on the perikaryon. Theoretically, the presence of synaptic contacts signify the establishment of reflex activity, an entity which enhances growth and differentiation (Bodian, 1966; 1970).

Subsequent differentiation and cytological reorganization showed that several cytoplasmic organelles increased in size and were more elaborate. In seven day old rats, a remarkable increase in size of the apical dendrite had taken place. Rough endoplasmic reticulum was widely distributed in the cytoplasm and continuity between

neighbouring cisternae of the same type and those of Golgi complexes, and perinuclear space were frequent. In addition, there was a significant increase in the number of mitochondria. During this reorganization, free ribosomes, the predominant and most numerous organells in young neurons, decreased.

This elaboration of cytoplasmic organelles proceeded to about 21 day postnatal. About this time, there was an observable increase in the numbers of lysosomes and lipofuscin granules which were frequently associated with Golgi complex cisternae. This finding is in agreement with those of Peters et al., (1970) who reported that as the animal aged there was an increase in the number of lysosomes and lipofuscin granules.

The change in configuration and elaboration of the cytoplasmic organelles in the mitral cell suggests that synthesis of materials necessary to produce growth has been embarked upon. This growth in the initial stage seemingly, is occupied with the production of macromolecules which are destined to be used as cytological structures within the same cell (Billings, 1972). Biochemical studies have shown that synthesis of protein takes place on the ribonucleic particles of the cytoplasm. The presence of a large number of free ribosomes, in the one day old rat, suggests that materials synthesized on polyribosomes would eventually be utilized for growth of cell.

On the other hand, the association of ribosomes to membranous channels would indicate that the protein synthesis in this instance would be related to specific functions of the neuron. It is conceivable that the transmitter substances synthesized by the neurons could be

conveyed along the channels of endoplasmic reticulum to the nerve terminals.

Segregation of ribosomes by membranous organelles has spurred biochemists to believe that each compartment is assigned the task of producing a specific protein. Later, as the membranous organelles increased, the compartments become more numerous suggesting that there was an increase in the numbers of different compounds produced.

The cytoplasm of young nerve cells or neuroblast has been reported to be filled with a wash of free ribosomes (Tennyson, 1965; Pannesse, 1968; Caley and Maxwell, 1968) and a few strands of endoplasmic reticulum. Materials synthesized by these ribonucleic particles, the Nissl substance of the light microscopist (Nissl, 1889, 1894), are those concerned in the increase in size of the perikaryon, processes, and internal structures. As maturity is reached the membranous organelles become more numerous and several compartments are created. Concomitantly, different compounds are synthesized to influence the physiology of the cell.

Morphological differentiation and maturation is correlated with functional maturation of nervous tissue. The electroencephalogram in the first seven days of postnatal rat cerebral cortex is irregular, low voltage and intermittent (Crain, 1952). During the second week rhythmicity is established and silent periods are seldom encountered. Similar findings have been reported in the immature cortex of the rat (Tilney, 1933; Flexner, 1955) and cat (Purpura et al., 1965).

To the author's knowledge, there are no electrophysiological studies

of this nature that were attempted on neonatal and one day old postnatal olfactory bulb of the rat or, for that matter, any other species. However, it should be noted that the cytoarchitecture of the olfactory bulb in one day old rat is at an advanced stage of development when compared to the cerebral cortex in the same animal as reported by Caley and Maxwell (1968; 1971). However, similar cytological features would exist in the olfactory bulb during the fetal period and presumably would produce electrical recording observed in one day old cerebral cortex.

Granule Cell:

Differentiation and development of granule cell occur well into the postnatal period. This cell type shows cytological features unlike those of classical neurons such as the mitral and tufted cell. Autoradiographic studies (Hinds 1968) on the mouse olfactory bulb showed that the granule cell is the last of the neurons to go into maturation and differention. The zone occupied by the granule cells corresponds to the subventricular or ventricular area as stated by the Boulder Committee (1970). The subventricular or ventricular zone replaces His's (1889) germinal or ependymal layer of the developing nervous system. Eventually, cells from this layer differentiate to produce neuroblast and spongioblast, the primordia of neurons and macroglia respectively. Since this is the most primitive of all layers in the central nervous system the observation that granule cell is at an early stage of development compliments previous findings (Hinds, 1968).

At birth, granule cells in the present study had a large nucleus which occupied most of the perikaryon. Chromatin material is evenly distributed except for some clumping below the inner nuclear membrane. A nucleolar area is sometimes present. The cytoplasm is scanty and the major portion of this is at the poles where cellular processes originate. Elsewhere, a thin rim of cytoplasm with few organelles is present. At the end of the first week of development lysosomes, mitochondria and microtubules increase in numbers. The cytoplasm is still a thin rim but appears to widen somewhat. Ribosomes, present at birth, remained isolated and membrane free. Rough endoplasmic reticulum seldom made its appearance. No future changes seem to occur. However in 35 day bulb multivesicular bodies were not only present but appeared to be in the process of formation. In the nucleus, chromatin aggregates below the nuclear membrane leaving a relatively translucent karyoplasm.

Changes in the cytology of the granule cell differ from those of the mitral cell or tufted cell in that there is an absence or paucity of membranous organelles, chiefly rough endoplasmic reticulum and Golgi complexes. In other neuronal cell types, these organelles are responsible principally for the entire biochemistry and metabolic processes as mentioned earlier. Although free ribosomes exist at all ages their numbers are inadequate to account for any major anabolic or synthetic activities. The increase in numbers of lysosomes, mitochondria and multivesicular bodies are synonymous with the increase in age (Peters et al., 1970; Del Cerro and Snider, 1972).

Germinal cells of the external granular layer of the cerebellum bear some resemblance to cytology and general morphology of granule cells in the olfactory bulb. Del Cerro and Snider (1972) have described the germinal cells as having a large nucleus which occupied most of the space in the perikaryon. At all ages, there is a thin rim of cytoplasm in which are distributed few mitochondria, lysosomes and ribosomes. Cisternae of rough and smooth endoplasmic reticulum are scarce. Also these cells are found in groups and clusters, and apposition between cytoplasmic membranes is a common feature.

Unlike the germinal cells, no membrane specialization, desmosomes and fascia occludentes, were located on the granule cell cytoplasmic membranes.

Autoradiographic studies (Altman, 1969) revealed that cells from the ventricular or ependymal and granule layer of the olfactory bulb were capable of replacing degenerated cells. Such a function would account for the persistence of granule cell in a semi-undifferentiated state. This availability of a special cell type which can differentiate later to produce other neuronal cells is especially required in the olfactory bulb which is easily accessible to infection and toxic substances present in the air. Olfactory axons degeneration and associated destruction of olfactory neuron perikarya have been observed to be a common phenomenon in garfish, Lepisosteus sp, (Easton, 1971). The presence of toxic substances in the environment has been suggested to be responsible for this degeneration.

SUMMARY

1. The superficial portion of the olfactory bulb or fiber layer was made up of unmyelinated axons which were grouped into fasciculi by satellite cells and their processes.

2. Each glomerulus was composed of clusters of dendrites and axons and a large population of synaptic junctions.

3. The increase in width of the olfactory bulb, observed during the first three weeks of postnatal development, was principally related to the growth of the external plexiform layer composed of the apical dendrites of mitral and tufted cells. These neuronal cell types have been observed to be similar in their cytoarchitecture except for their size and location.

4. At birth, mitral cells have a well defined perikaryon with a centrally placed nucleus and short dendritic processes. Cytoplasmic organelles consisted of a wash of free ribosomes, Golgi complexes, sparsely distributed mitochondria and rough endoplasmic reticulum. At the end of the first week of development, mitral cells showed a remarkable increase in size of the perikaryon and apical dendrite. Concomitantly, rough endoplasmic reticulum, Golgi complexes were increased in numbers and complexity. As development proceeded, lysosomes and lipofuscin granules began to appear.

5. Development of the granule cell was slower than that of the mitral and tufted cell. At birth, these cells showed a large nucleus and a thin rim of cytoplasm in which free ribosomes outnumbered other organelles. By the second week, the chromatin became less dense and short segments of rough endoplasmic reticulum were observed. Granule cells observed in three and five week old olfactory bulbs did not exhibit further significant changes.

Figure 21. Electron micrograph of olfactory bulb fiber layer in a one day old rat. Traversely (AX) and longitudinally ($A X_1$) cut unmyelinated axons and two satellite cells considered to be astrocytes (As) are identified. Microtubules, mitochondria and a translucent axoplasm give the axons their cytology. Note the absence of synaptic junctions.

Astrocytes, identified as such because of the absence of a basal lamina and collagen, send processes between the axons. Their perikarya are located between axonal fasiculi. Free ribosomes, rough endoplasmic reticulum, mitochondria and vacuoles are observed in the cytoplasm. Chromatin is fairly evenly distributed except for a slight aggregation at the nuclear membrane.

X 21,800

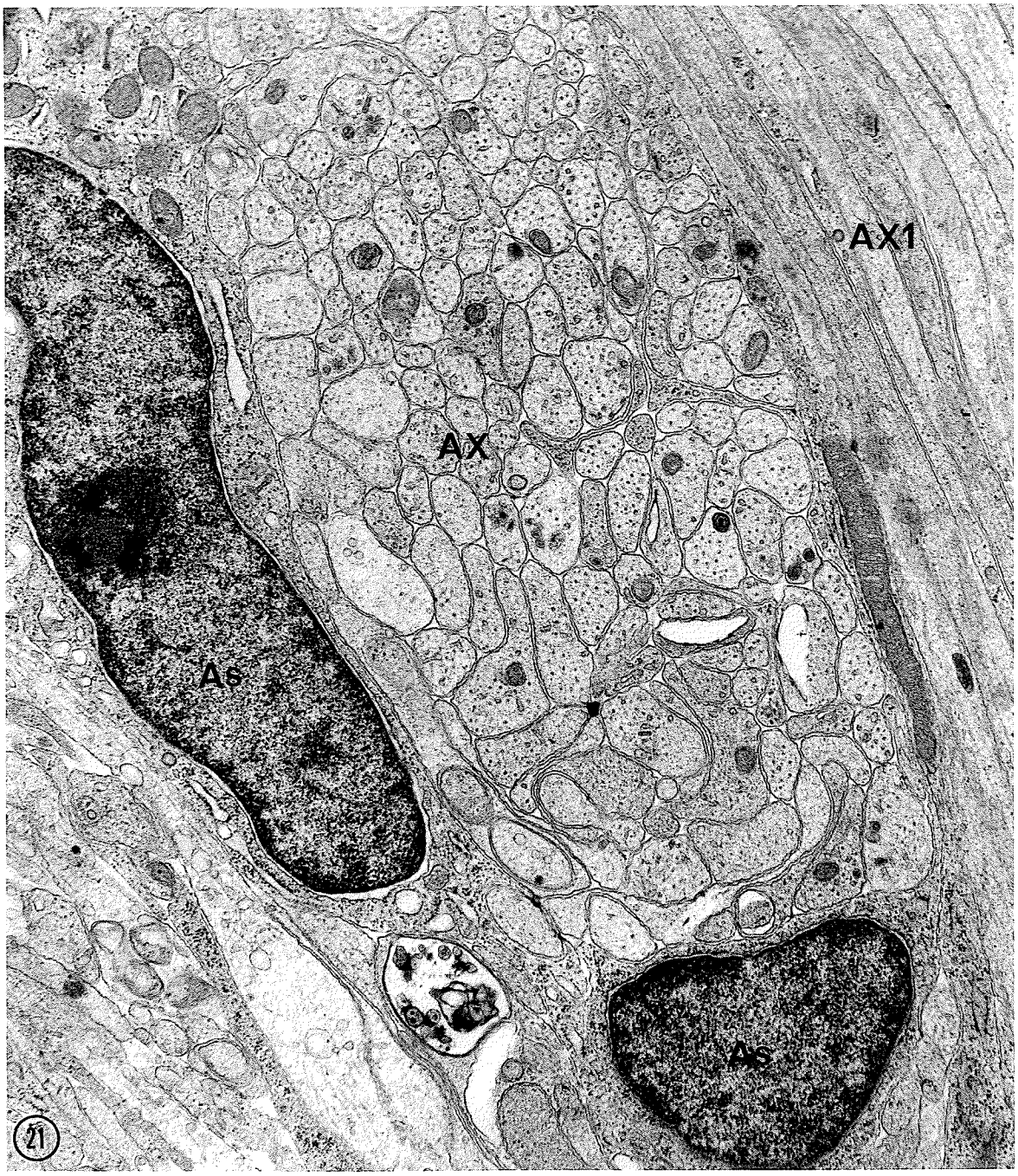

AX1
AX
AS
AS
21

Figure 22. Representative electron micrograph of fiber and glomerular layer in seven day old rat. Fasiculi of unmyelinated axons are located in the upper left hand portion of micrograph constituting the fiber layer. Glial processes surround each fasiculus and also insinuate into the centre. The glomerular layer is identified in the right half of the micrograph. It contains axo-dendritic synapses (arrows). X 18,400

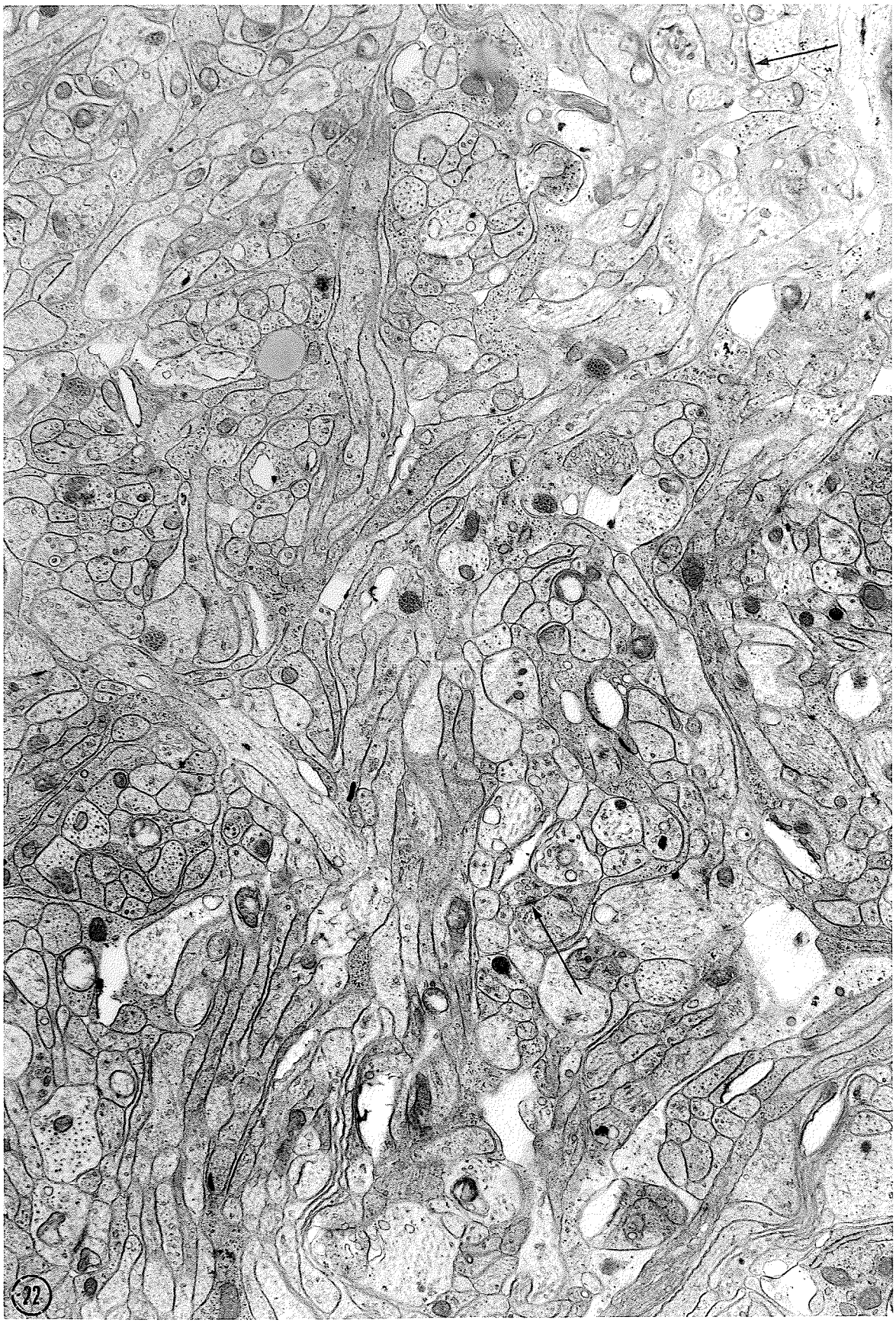

22

Figure 23. Higher magnification of unmyelinated axons and glial processes in the fiber layer of a seven day old olfactory bulb. Three fasciculi (F_1, F_2) are bordered by glial cell processes which contain free ribosomes, mitochondria, ribosomal and glycogen-like particles

X 35,900

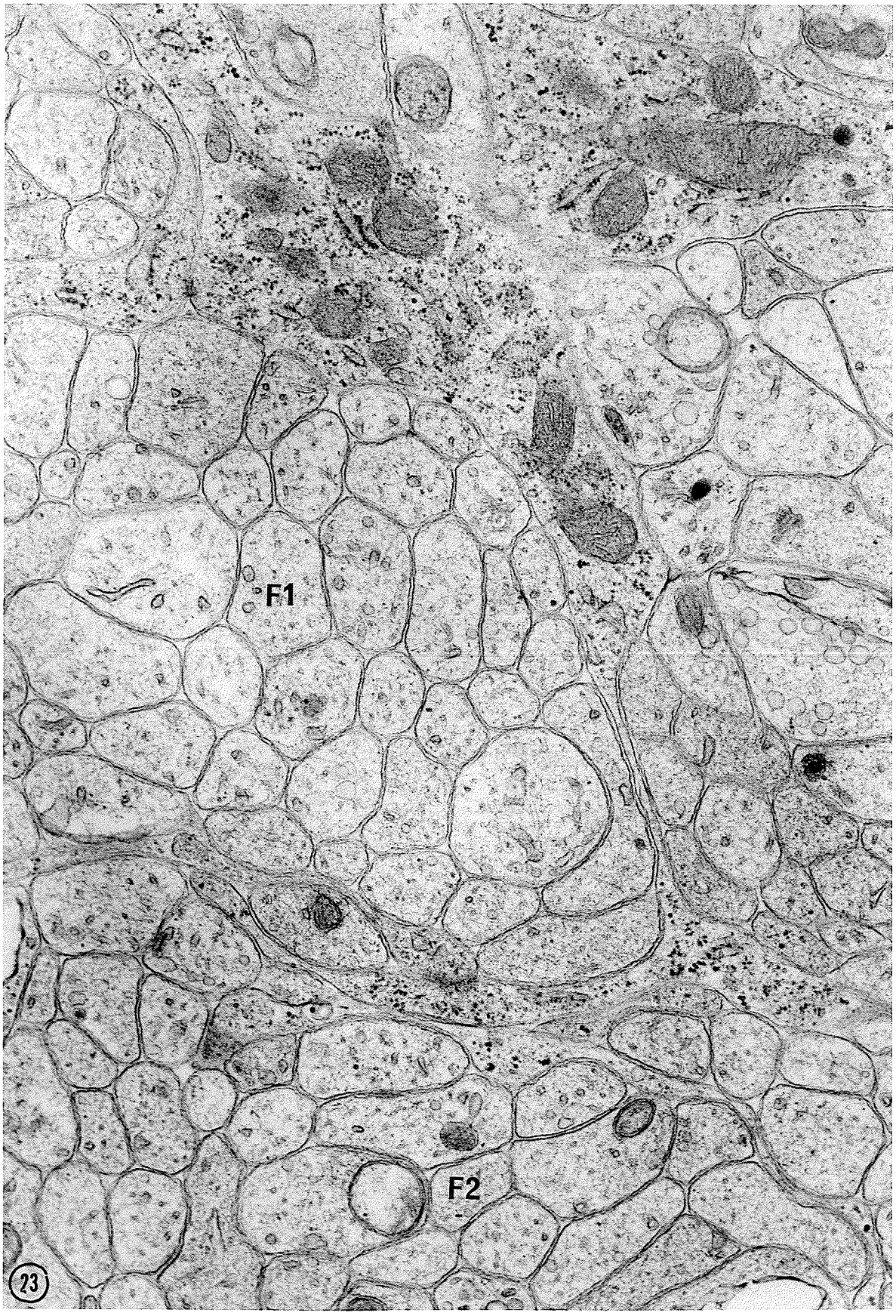

F1
F2
23

Figure 24. Illustrates glomerular area with several synapses (arrows) of axo-dendritic variety in 14 day olfactory bulb. A type II or mature capillary with two perivascular astrocytes (A_S) is identified. Portion of a periglomerular neuron (Pg) is seen to the left.

X 10,400

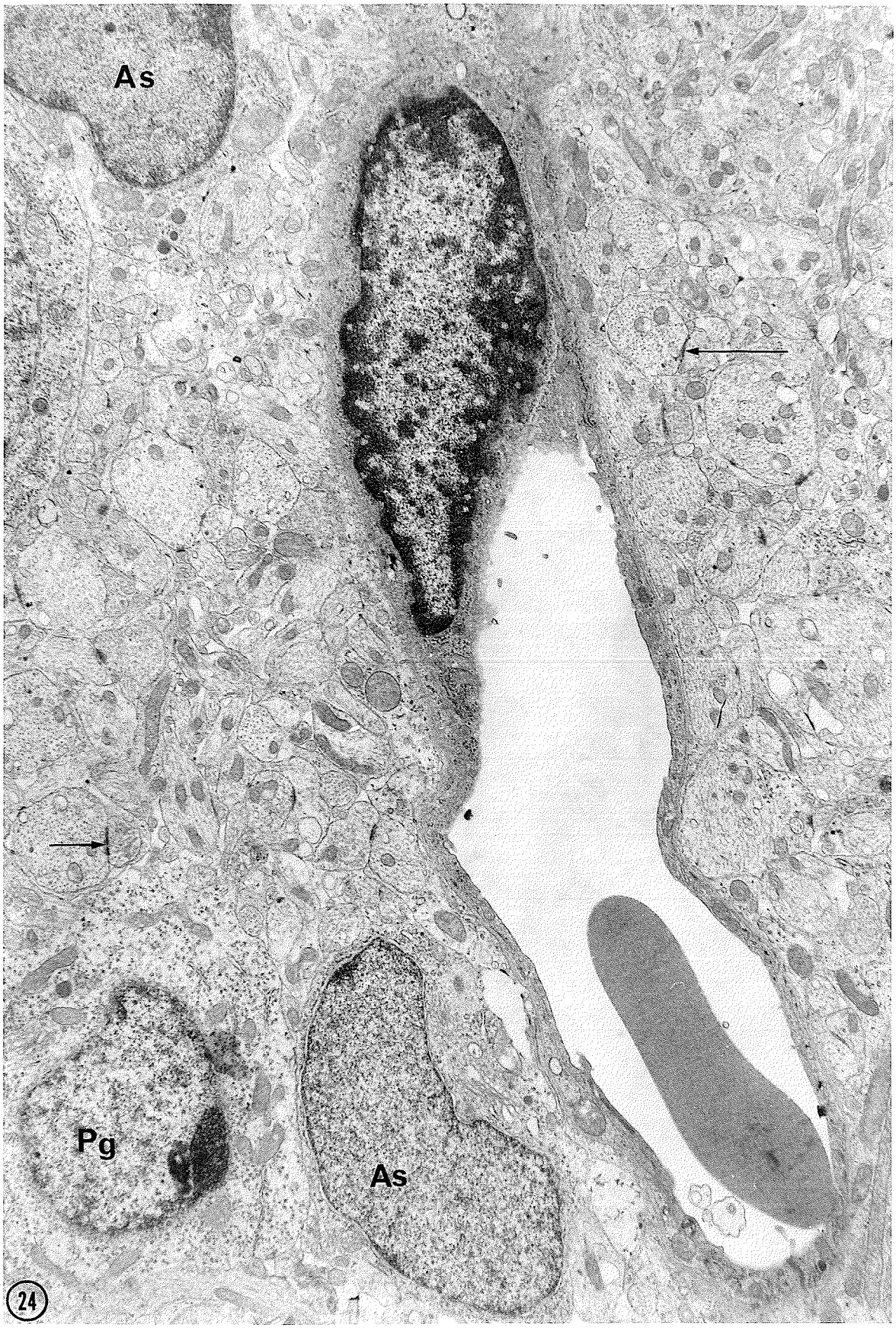

As
Pg
As
24

Figure 25. Electron micrograph of a periglomerular neuron in seven day old rat. A large nucleus (N) with uniformly distributed chromatin occupies the major portion of the perikaryon. The cytoplasm contains clusters of ribonucleic particles, few segments of rough endoplasmic reticulum (er) mitochondria and a lone lysosomal-like-body (L), Note the continuity between the perinuclear space and rough endoplasmic reticulum (arrow)

X 14,700

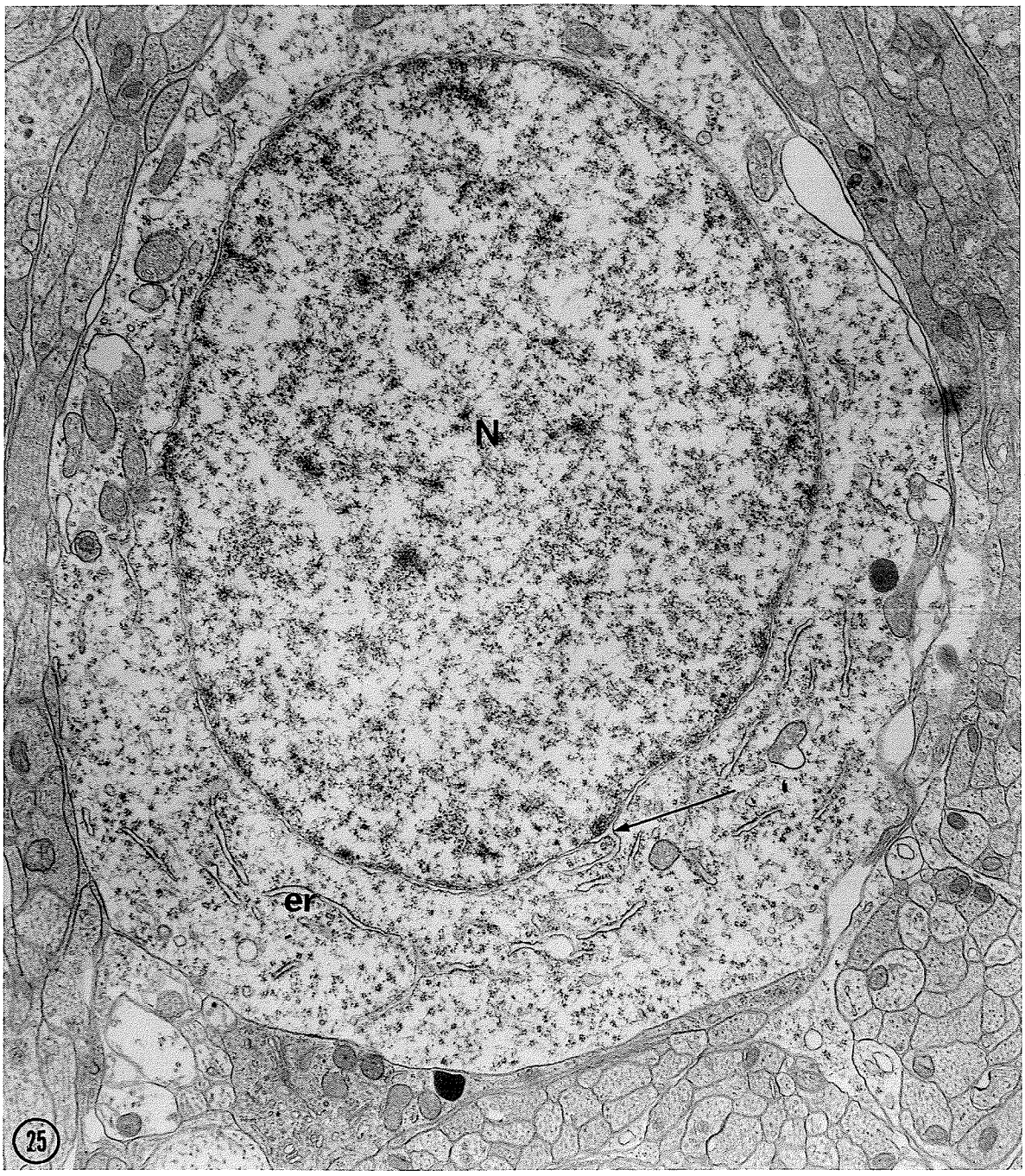

N
er
25

Figure 26. Illustrates external plexiform layer in seven day old rat. Neuronal and glial cell processes intermingle with each other.

Axons (AX), relatively, are dense than dendrites (D) and contain mitrotubules. Dendrites are large in size with a translucent matrix in which are observed ribosomes, mitochondria and few microtubules. Note the presence of synaptic junctions (arrow).

X 18,600

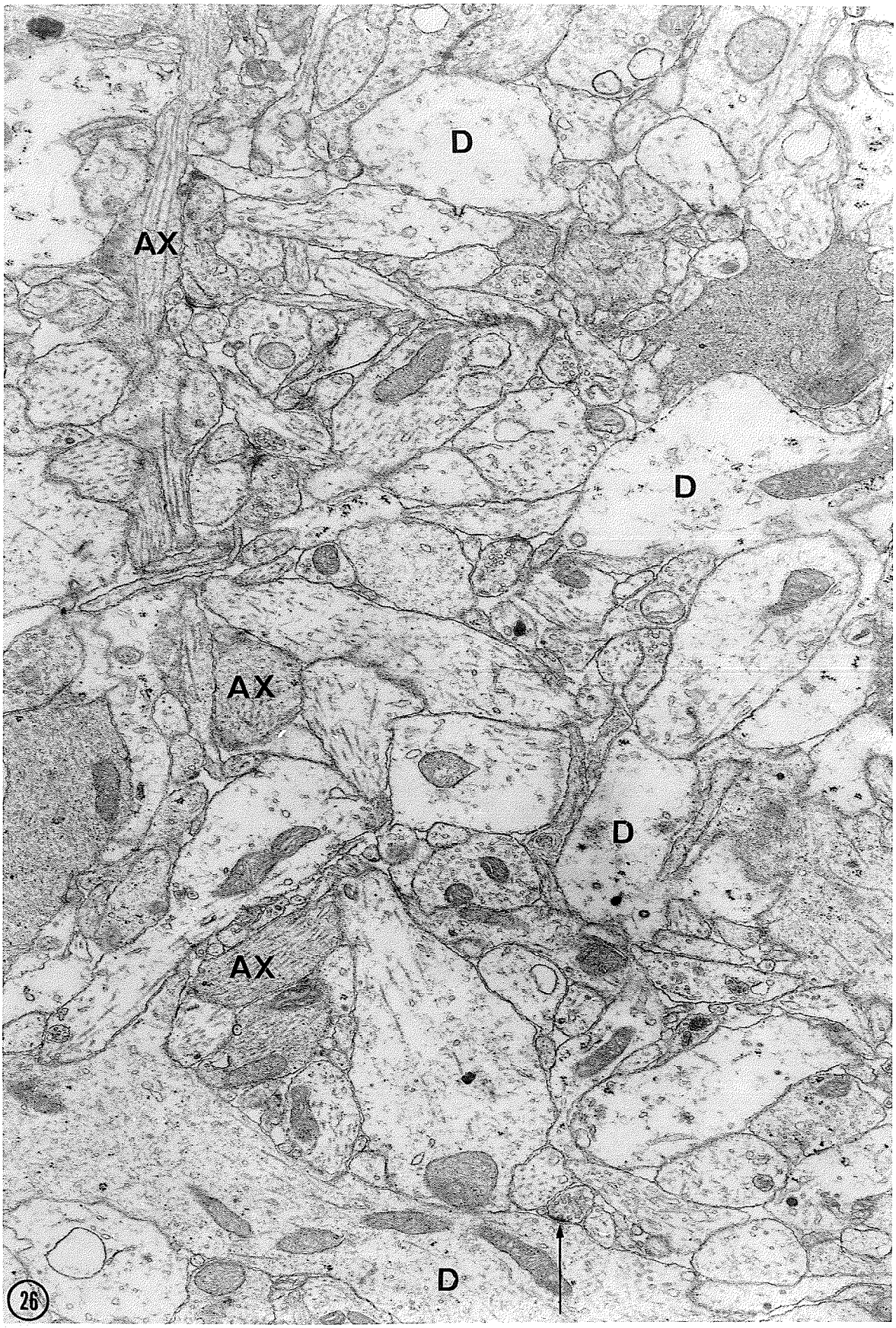
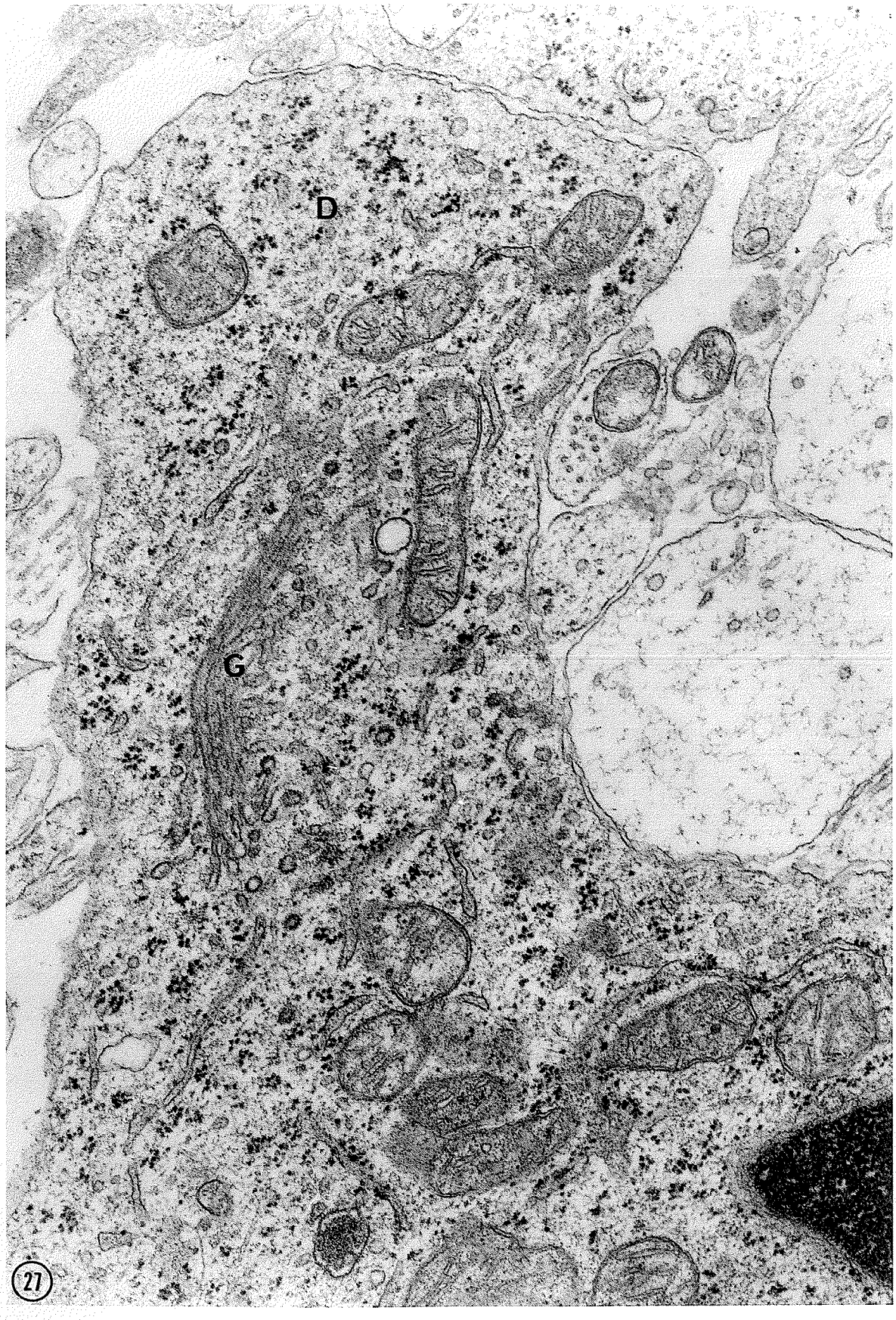

D
AX
D
AX
D
AX
D
26

Figure 27. Micrograph of a young mitral cell in one day old olfactory bulb showing a short dendrite (D) and portion of the cell cytoplasm and nucleus. Abundant amounts of free ribosomes are randomly distributed in the cytoplasm. Short segments of cisternae partly studded with ribosomes are also present. The Golgi complex (G) is well defined with associated vesicles. Several mitochondria are seen randomly distributed, X 21,900



27

Figure 28. Illustrates part of a mitral (M) and granule (Gr) cell in 7 day old rat. The nucleus of the mitral cell has a scanty distribution of chromatin granules. The organelles in the cytoplasm are relatively well differentiated. Although rosettes of free ribosomes are widely distributed, rough endoplasmic reticulum (er) is very prominent. Several Golgi complexes (G) associated with vesicles and dense lysosomal-like bodies are frequently encountered. The granule cell has a large nucleus and a thin rim of cytoplasm.

X 21,900

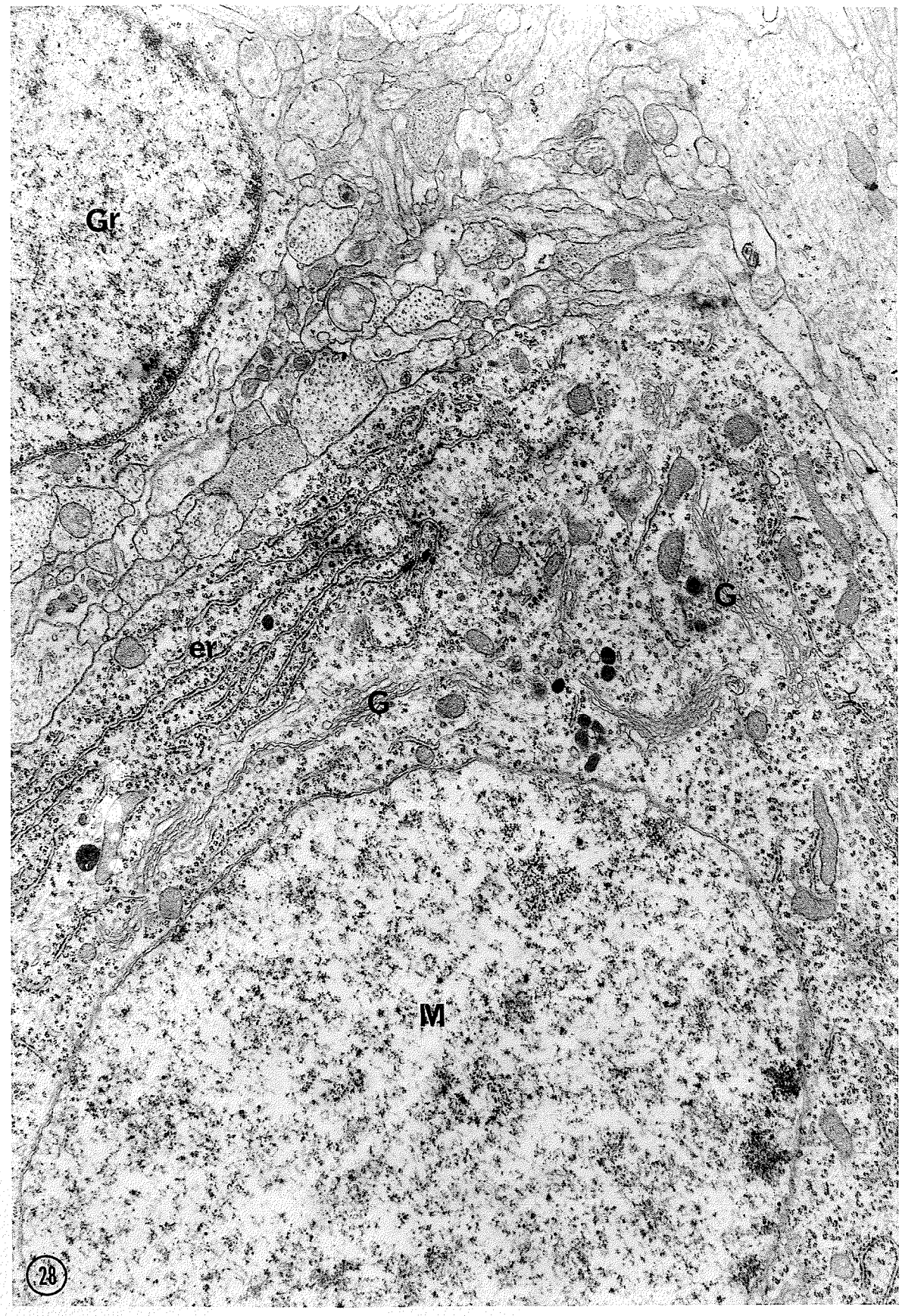

Gr
G
er
G
M
28

Figure 29. Electron micrograph of two mitral cells (M) and a granule cell (Gr) in seven day old olfactory bulb. Mitral cell cytoplasm has an abundance of free ribosomes and several strands of rough endoplasmic reticulum. Several mitochondria (M) are observed. Note that a single process is making synaptic contact (arrows) with both mitral cells. The axon (Ax) leaving a mitral cell may be seen in the lower part of the figure. The granule cell (Gr) has a large nucleus and a thin rim of cytoplasm which contains an abundance of free ribosomes.

X 14,000

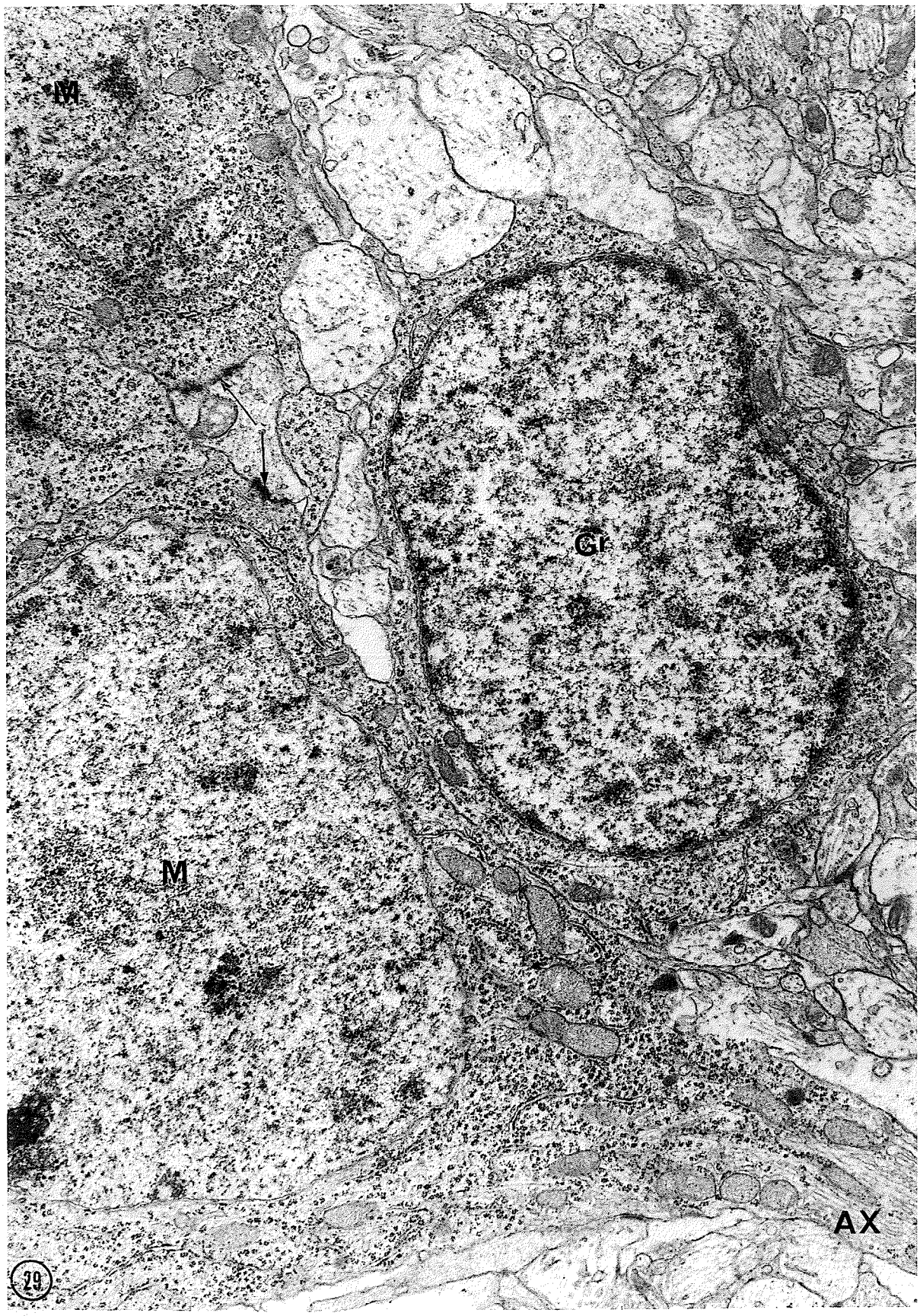

M
Gr
M
AX
29

Figure 30. Montage of a mitral cell (M) perikaryon and its apical dendrite (D) in a 7 day old olfactory bulb. In the perikaryon, rough endoplasmic reitculum (er) is well represented in the cytoplasm but sparse in the dendritic process. Rosettes of ribonucleic particles are distributed throughout the cytoplasm of the cell. Golgi complexes (G) are numerous and are associated with vesicles and a few lysosomes (L). A dendritic spine (DS) may be observed. Note the presence of a primitive or Type I blood vessel (V) surrounded by the watery cytoplasm of an astrocyte (As). Portions of astrocytic (As) nuclei showing uniform distribution of chromatin may be seen on either side of the apical dendrite. The perikaryon of these cells shows the usual organelles.

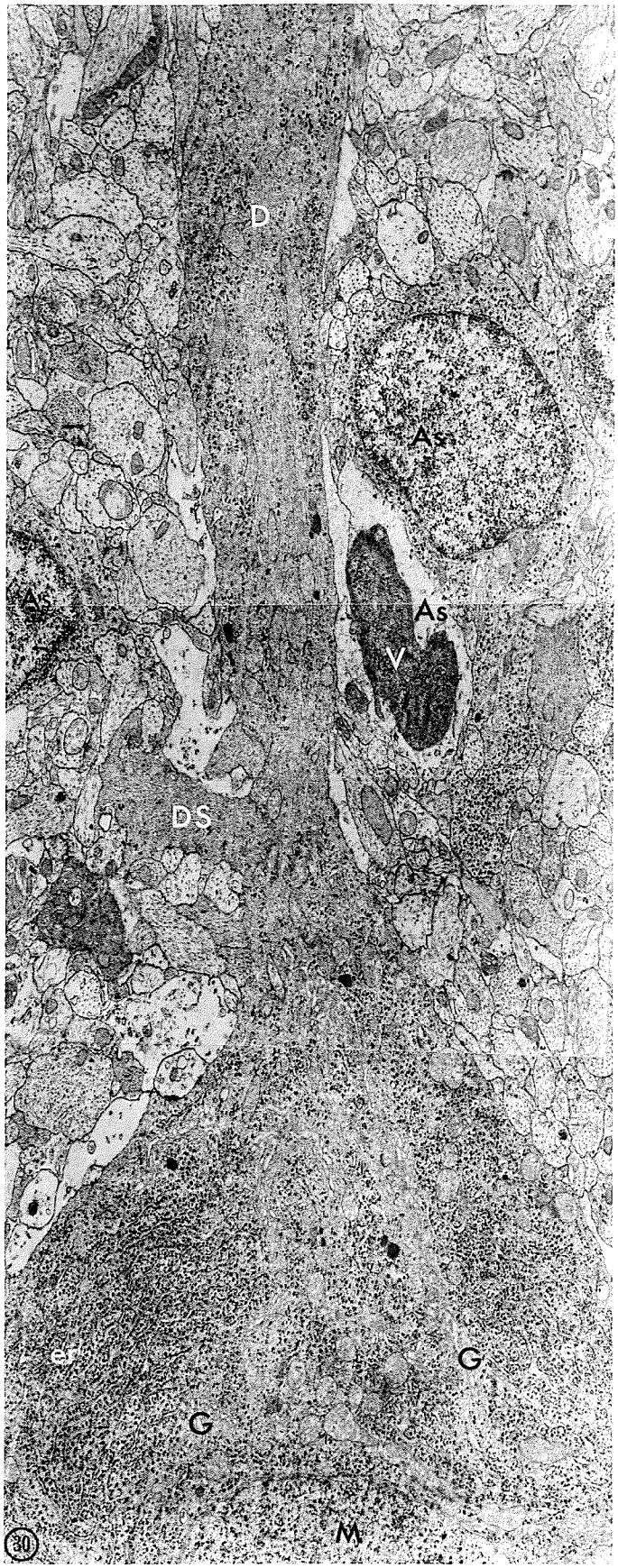

D
As
As
As
V
DS
er
G
G
M
30

Figure 31. Electron micrograph of mitral cell (M) and its apical dendrite (D) used to construct the composite of Firuge 30. Free ribosomes and rough endoplasmic reticulum are equally and well represented. Golgi complexes (G) are widely distributed in the cytoplasm and at base of the apical dendrite. Free ribosomes, microtubules and mitochondria are present in the dendritic matrix (D).

X 13,700

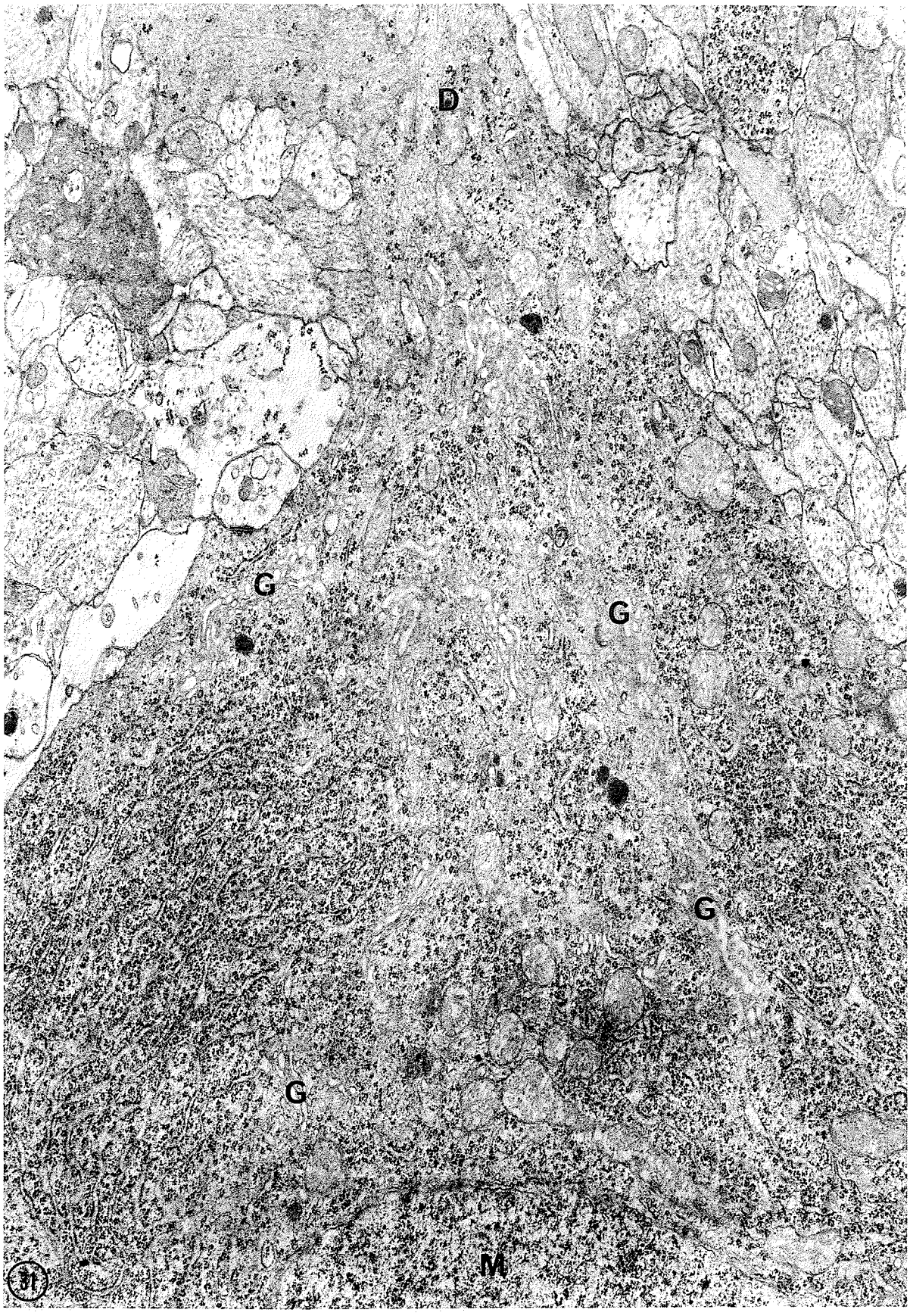
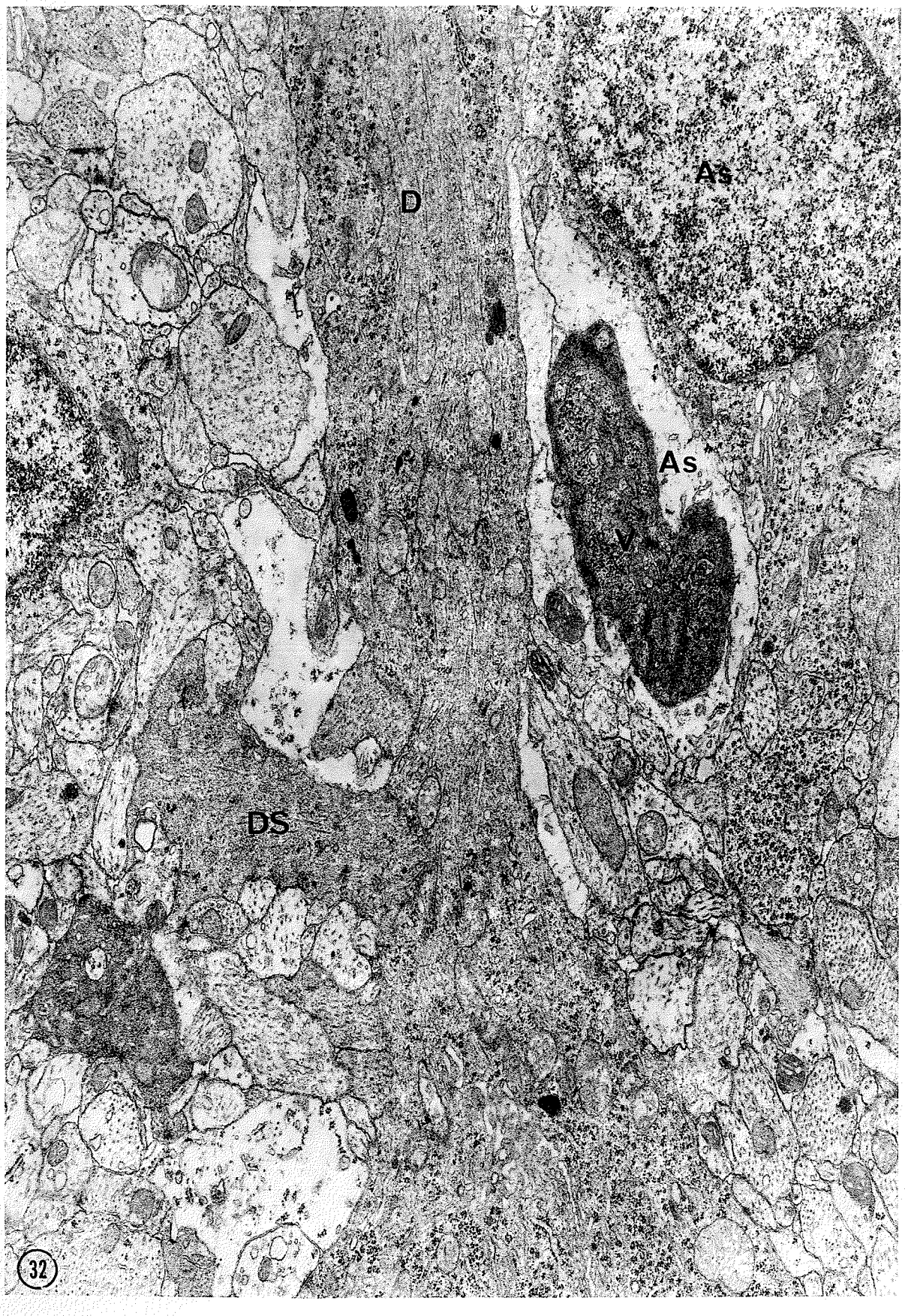

D
G
G
G
G
M
31

Figure 32. Illustrates apical dendrite (D) of mitral cell shown in Figures 30 and 31. Microtubules, free ribosomes, mitochondria and dense lysosomal bodies are observed in the matrix of the dendrite. At the base of the dendrite is what may be a dendritic spine (DS) which shows identical cytology to the main dendritic shaft.
Note the presence of a type I or immature blood vessel (V) surrounded by the watery cytoplasm of an astrocyte (As). Portions of two cells which may be astrocytes (As) are also observed in the figure.
X 15,400



32

Figure 33. Micrograph representing the cytoplasm and nucleus (N) of a mitral cell in 14 day old olfactory bulb. Chromatin granules are evenly distributed in the nucleus. In this cell the Golgi complexes (G) are well developed and consist of stacks of smooth cisternae with associated vesicles and dense lysosomes (L). Rough endoplasmic reticulum is widely distributed in the cytoplasm and continuity between cisternae exist. Free ribosomes are distributed among the rough endoplasmic reticulum.
X 30,800

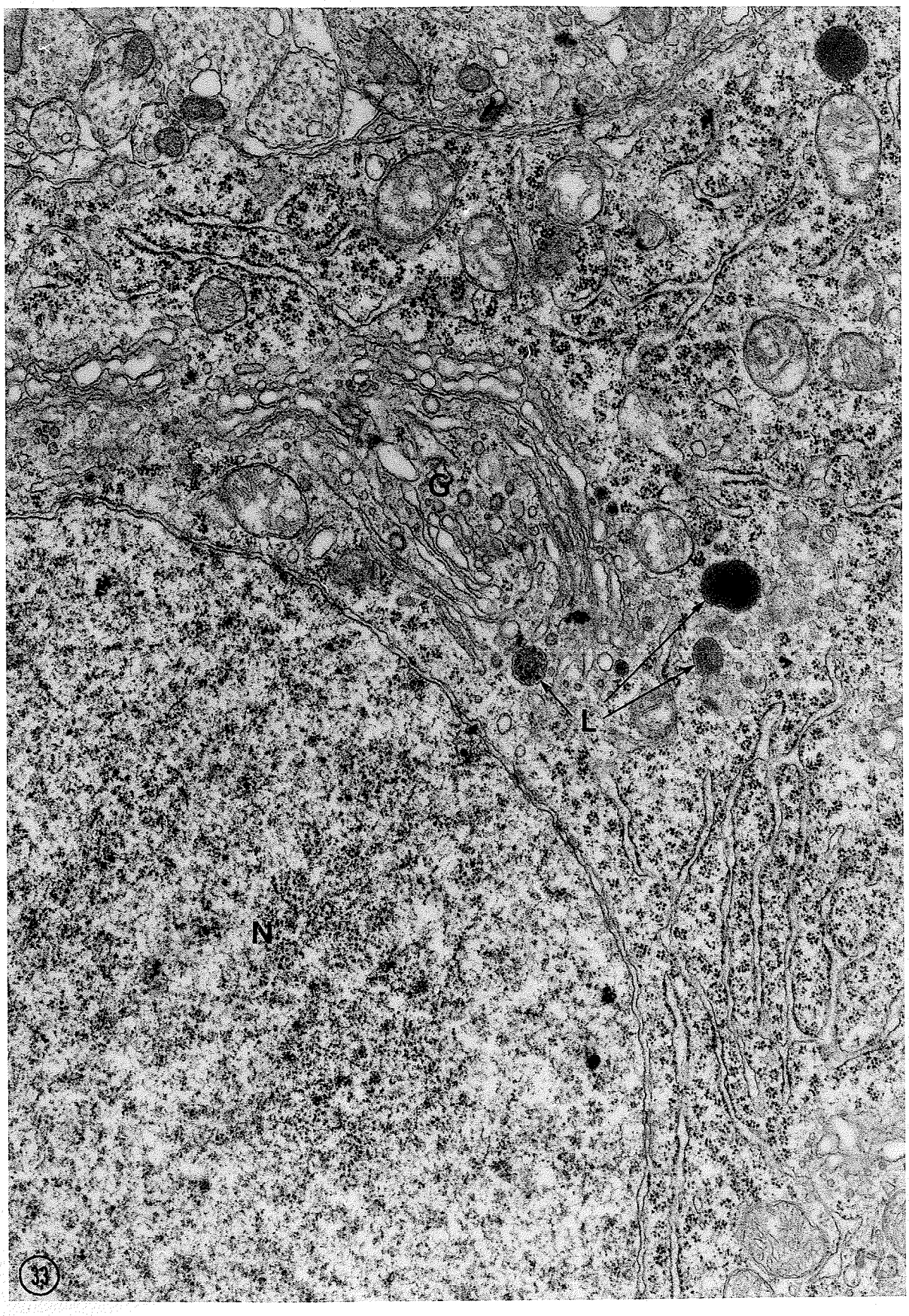
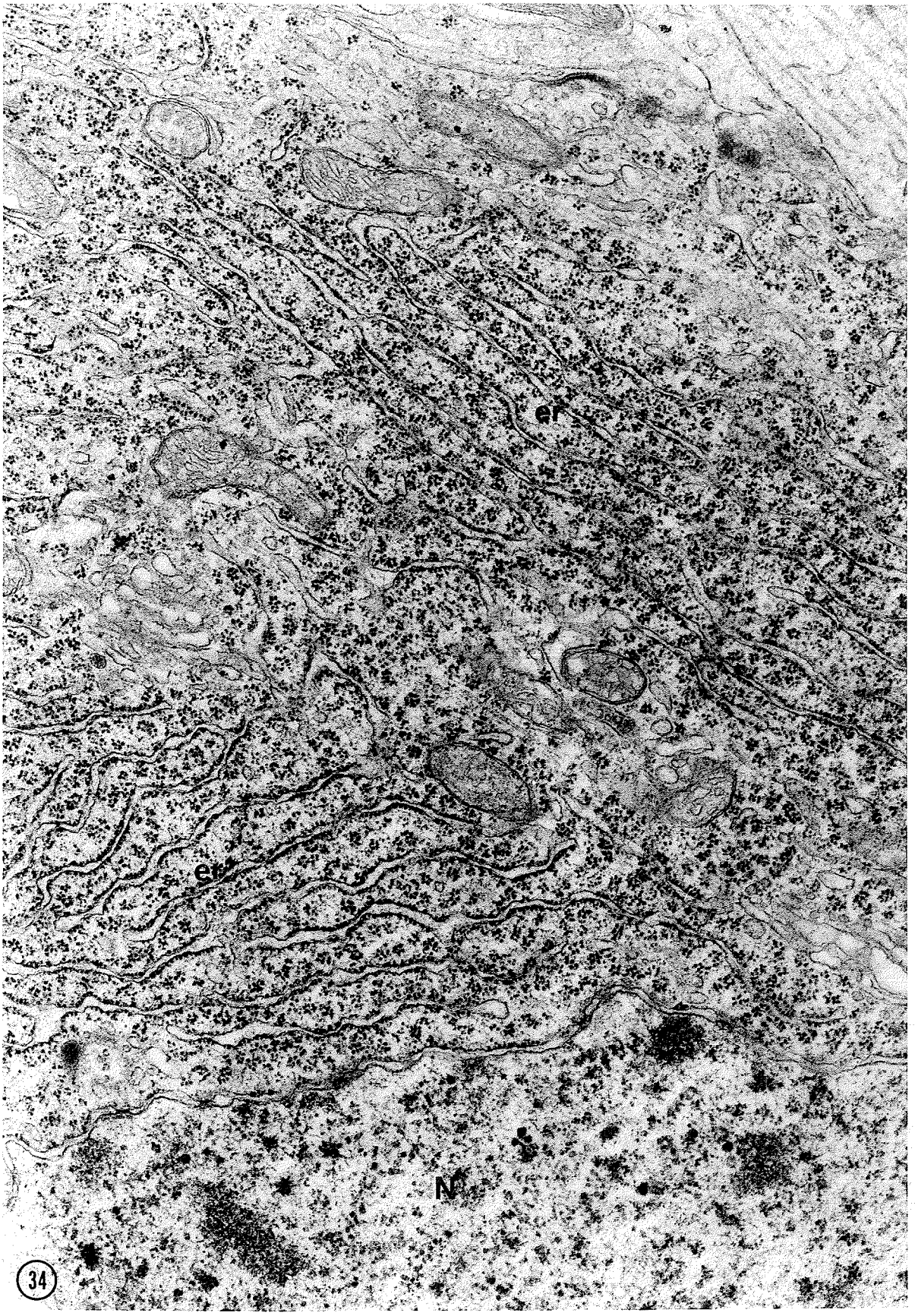

G
L
N
13

Figure 34. Electron micrograph of cytoplasm and nucleus (N) of a mitral cell in 21 day old rat. Rough endoplasmic reticulum (er) has increased in numbers and distribution. Free ribosomes in the form of rosettes are distributed between the rough cisternae. Mitochondria, and Golgi complexes are also present in the cytoplasm.
X 33,400



34

Figure 35. Illustrates a 35 day old mitral cell. In addition to the increase in rough endoplasmic reticulum (er), mitochondria and Golgi complexes (G), there is a significant increase in the numbers of lysosomes (L) and lipofuscin (LF).
X 17,300

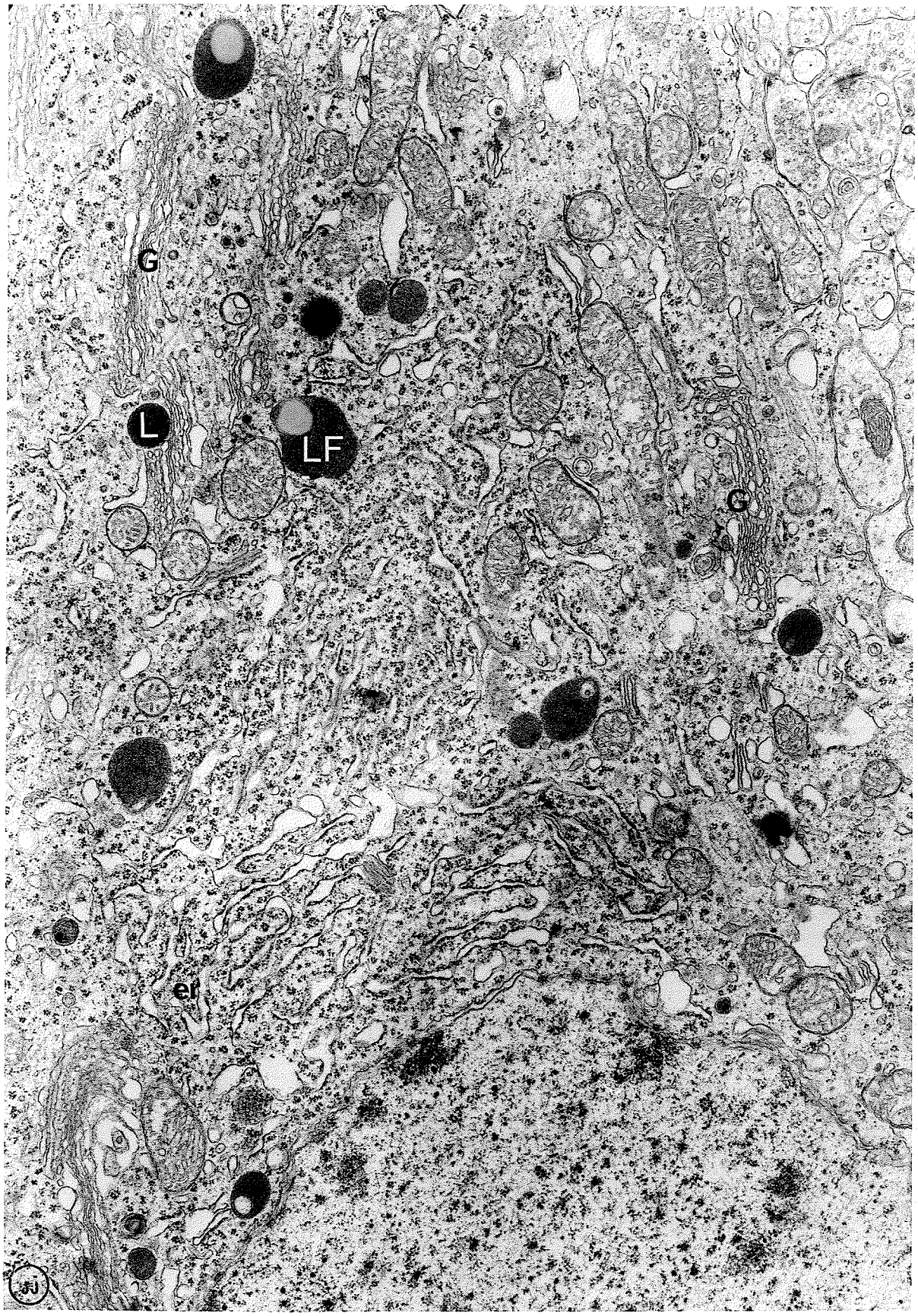

G
L
LF
G
er

Figure 36. Electron micrograph to illustrate part of a tufted neuron (T) and an adjacent dendrite (D) of a mitral cell in a 7 day old olfactory bulb. In the tufted cell rough endoplasmic reticulum (er) is well represented. Also observed are free ribosomes, mitochondria and Golgi complexes (G).

In the dendritic process, rough endoplasmic reticulum, free ribosomes, microtubules (MT) and mitochondria are observed.

X 28,400

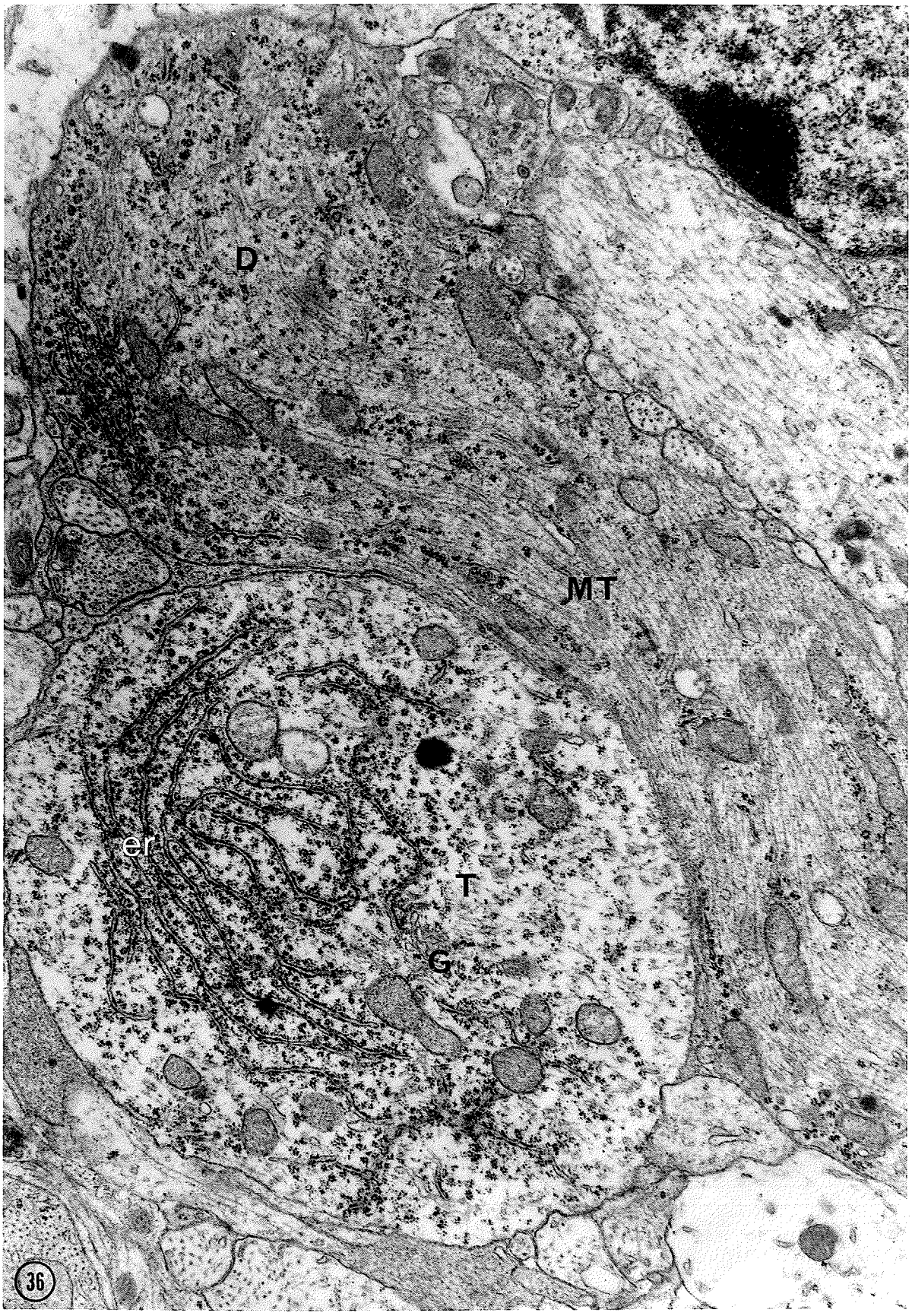

D
MT
er
T
G
36

Figure 37. Electron micrograph to illustrate a cluster of six early postnatal granule cells (Gr) in the granular layer of one day old olfactory bulb. These cells occur in groups and show apposition of cytoplasmic membrane. The nucleus is large and is surrounded by a thin rim of cytoplasm. Chromatin granules are evenly distributed with minimal aggregation under the nuclear membrane. Also represented in this micrograph are two immature oligodendrocytes (Ol) with a dense nucleus and cytoplasm. X 14,000

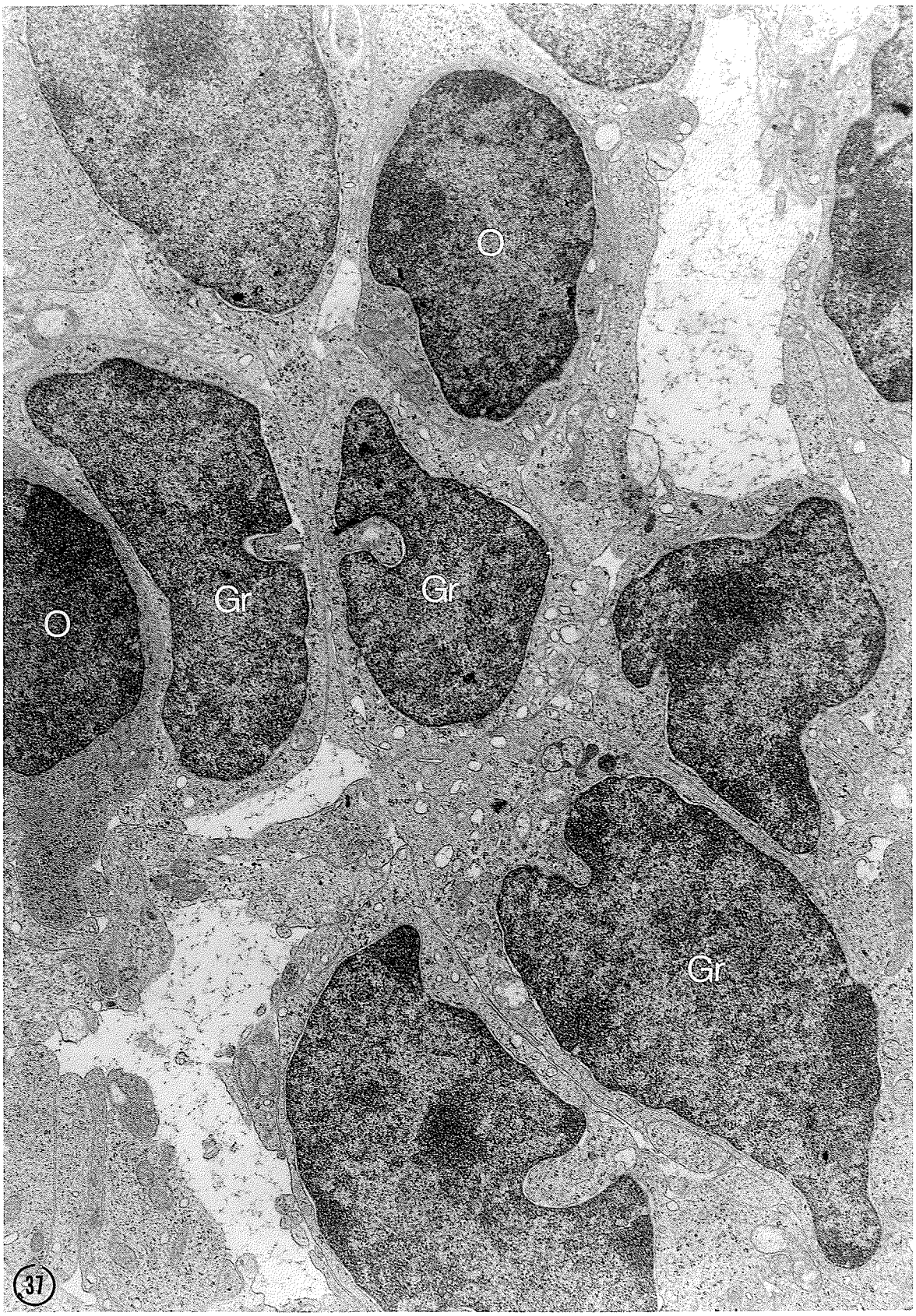

O
Gr
Gr
O
Gr
37

Figure 38. Electron micrograph of one day old olfactory bulb illustrating developing granule cells (Gr) similar to Figure 39. These cells occur in clusters and show apposing cytoplasmic membranes.

The nucleus occupies the major portion of the perikaryon. Cytoplasmic granules present are chiefly free ribosomes and few mitochondria.

X 19,400

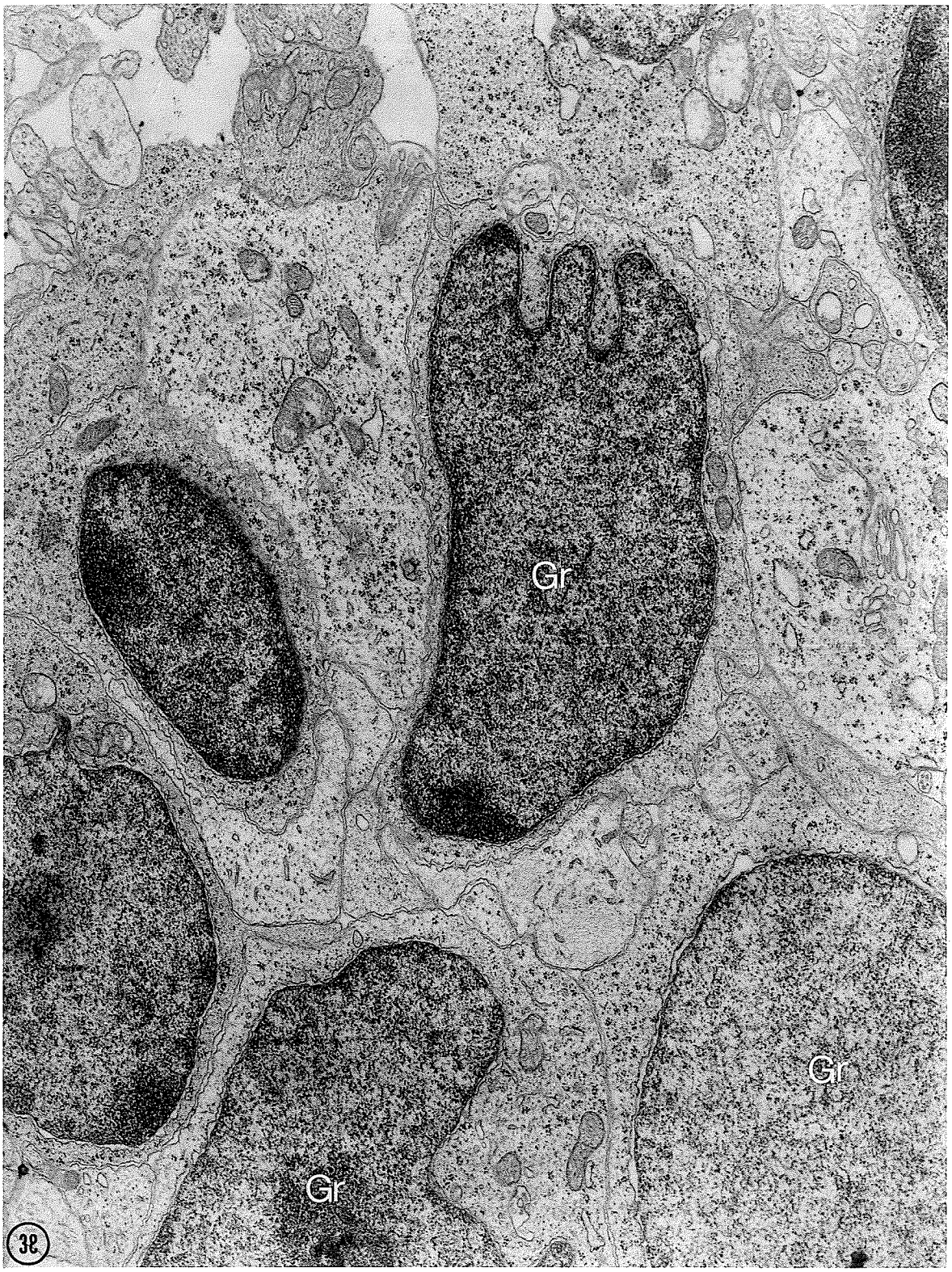
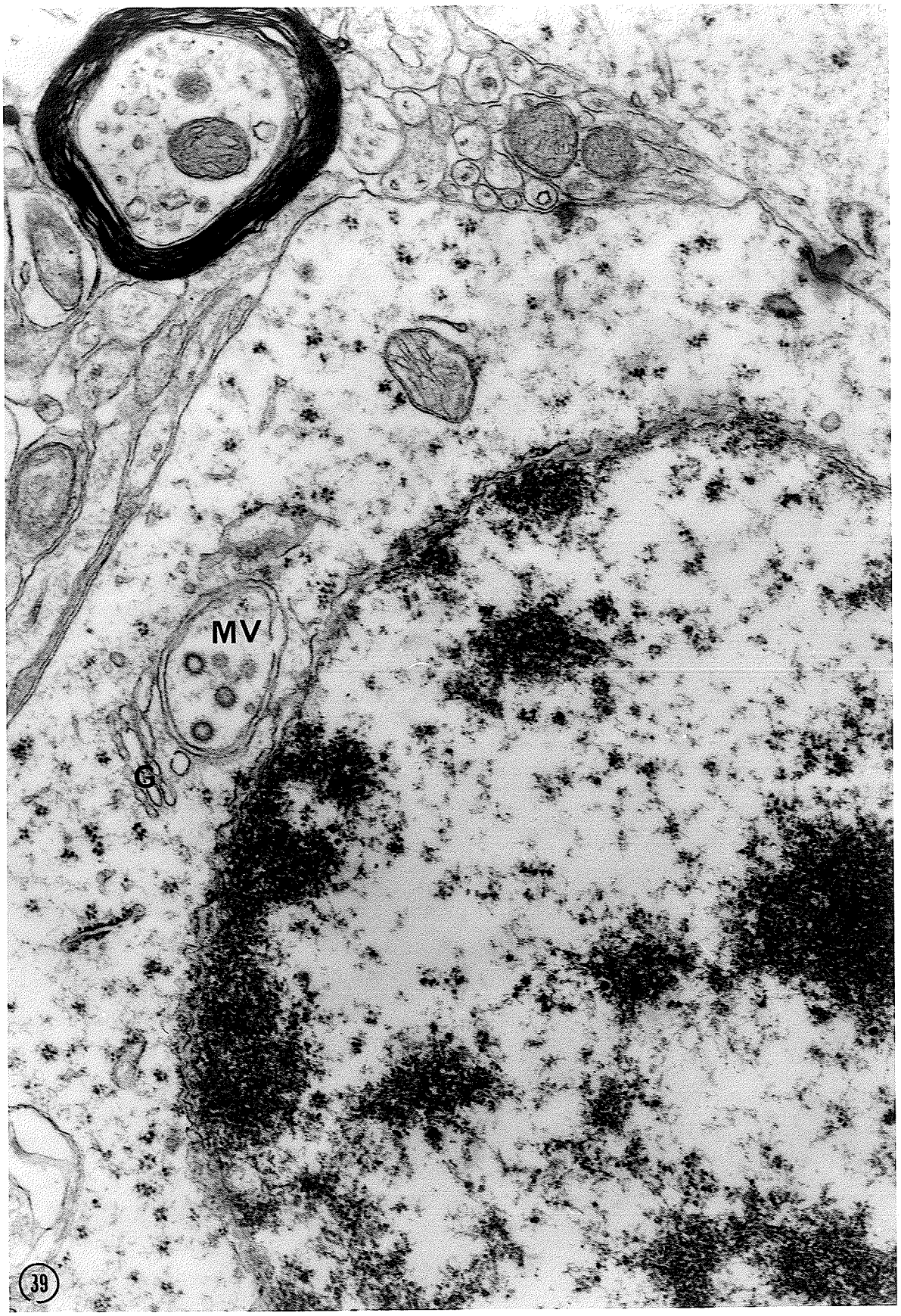

Gr
Gr
Gr
38

Figure 39. Illustrates a granule cell from a 35 day old olfactory bulb. The nucleus still occupies a major portion of the perikaryon.

The cytoplasm is clear and watery and contains sparsely distributed ribonucleic particles. A multivesicular body (MV), which appears to be in the process of formation, is associated with a few smooth cisternae which bear all resemblance to a Golgi complex (G).

X 42,600



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SECTION ON NEUROGLIA

1. Review of Literature
2. Electron microscopic observations
   a. Astrocytes
   b. Oligodendrocytes
3. Discussion
   a. Astrocytes
   b. Oligodendrocytes
4. Summary

REVIEW OF LITERATURE

The discovery of the neuroglial cells is ascribed to Virchow (1846). These cells were believed to form part of the interstitial space of the nervous system and to separate nervous tissue from blood vessels. With the advent of the electron microscope, neuroglial cells were observed to be the interstitial material of nervous tissue.

Using metallic impregnation techniques, Cajal, (1913; 1916) and del Rio Hortega, (1919; 1921) described the morphology of neuroglial cells. Further description at the light microscopic level was carried out by Penfield (1932); Glees (1955) and Windle (1958).

Neuroglial cells are classified as two types, macroglia and microglia. Astrocytes and oligodendrocytes are considered to be macroglia and microcytes the only microglial cell type. The neuroglial cells, at the electron microscopic level, can be delineated as astrocytes and oligodendrocytes based on the descriptive criteria set forth by Farquahar and Hartman (1957), Schultz, Maynard and Pease (1957) and De Robertis et al., (1954).

Astrocytes:

Astrocytes have been described at the light microscopic level as star-shaped cells (Cajal, 1913). Several reports have shown that the morphology of astrocytes, in the gray matter, is somewhat different from its counterpart in the white matter. These cells were designated protoplasmic atrocytes when located in the gray matter and fibrous astrocytes in the white matter. Weigert (1906) demonstrated that fibrous astrocytes are associated with a large population of fibrils, while the

protoplasmic astrocytes have few fibrils. In addition, fibrous astrocytes were observed to have long, slender processes, while protoplasmic astrocytes have short, thick processes. Processes of both cell types form physical contacts with blood vessels, forming the 'peri-vascular feet', and also contribute to the formation of the glia limiting membrane ("glia limitans").

Electron microscopic studies have shown that fibrous astrocytes are generally pale in appearance when compared to other cell types. The nuclei are oval to irregular in shape, with the nucler membranes thrown into folds (Farquhar and Hartman, 1957; Schultz, et al., 1957; and Peters et al., 1970). The nucleoplasm is homogeneous in appearance although clumping of nuclear chromatin has been observed beneath the nuclear membrane. The outer surface of the nuclear membrane has been observed to be studded with ribosomes.

The presence of numerous fibrils, 75A° in diameter, in the cytoplasm have been reported. These fibrils have been shown to contain alpha keratin (Bairati, 1958). Free ribosomes and short segments of granular endoplasmic reticulum are found in the astrocyte. The Golgi apparatus is made up of a few empty cisternae and is mainly confined to some cytoplasm.

Mictochondria have been observed in every part of the astrocytic cell and have been reported to exist in a variety of shapes. Mugnaini and Walberg (1964) have shown that mitochondria followed the same distribution as gliosomes of light microscopic reports.

Lysosomes, glycogen particles, centrioles, cilia and a small

population of microtubules have been reported to be present in the mature fibrous astrocytes. However, in the immature optic nerve, fibrous astrocytes have been found to contain numerous microtubules (Peters and Vaughn, 1967; Vaughn and Peters, 1967), which suggested that fibrils of mature astrocytes are derived from the walls of the microtubules.

Protoplasmic astrocytes have also been observed to contain fibrils in the cytoplasm. These fibrils are distributed in bundles and have been reported to be absent in a specific protoplasmic cell type of the cerebellum (Herndon, 1964; Palay, 1966).

The cytoplasm of the protoplasmic astrocytes contain organelles similar to those of fibrous astrocytes. Short cisternae, few free ribosomes, glycogen particles, mitochondria, microtubules, centrioles and cilia, have all been observed.

Weigert (1895) proposed the idea that astrocytes are involved in structural support in the central nervous system. Astrocytic processes were observed by Cajal (1913) to form physical connections between blood vessels and neurons. Ramon-Moliner (1957) made similar observations using a dichromate method. These contacts were interpreted as having formed a type of diffusion channel for the passage of materials from blood capillaries to neurons. Nakai (1963) and Polyak (1965) attributed the blood brain barrier mechanism to these physical contacts.

Experimental studies involving stab wounds (Schultz and Pease, 1959) and cortical isolation (Weisman and Singh, 1971) in the cerebral

cortex, have shown that astrocytes are capable of undergoing multiplication and are involved in the process of healing. Reactive astrocytes in the rat spinal cord have been reported to play an active role in the removal of degenerating myelin (Nathaniel and Nathaniel, 1973).

Oligodendrocytes:

Hortega (1921) with the use of metallic impregnation technique described oligodendrocytes as having few processes. These cells have a spherical or oval soma and their processes are more delicate than those of fibrous astrocytes. Light microscopic studies have shown that oligodendrocytes are located between myelinated fibers and as satellite cells to the perikaryon of nerve cells.

Electron microscopic studies have shown oligodendrocytes to be dark cells (Mugnaini and Walberg, 1964; Kruger and Maxwell, 1966; Wendel-Smith et al., 1966). The nuclear chromatin is frequently observed to clump adjacent to the nuclear membrane. In the rat cerebrum, Kruger and Maxwell (1966) observed that the lighter coloured Golgi apparatus and granular endoplasmic reticulum are easily discernible. The cisternae of these cytoplasmic organelles are irregular and several reports have indicated that they are capable of forming stacks. Multivesicular bodies and bodies similar in appearance to lysosomes and lipofuscin granules have also been described. Microtubules, 250A° in diameter, are very prominent in the cytoplasm and processes (Vaughn and Peters, 1967).

In the cerebral cortex of the rabbit, oligodendrocytes were

observed to be situated adjacent to neuronal perikarya and were termed perineuronal satellites (Polyak, 1965; Ham, 1969; King, 1968). This close relationship has led many investigators to postulate that there is a symbiotic mechanism between oligodendrocytes and neurons (Hyden, 1960).

In the central nervous system oligodendrocytes are believed to be responsible for the laying of myelin sheath. Several investigators (Maturana, 1960; Bunge, et al., 1962; Peters, 1964b) have reported that the disposition of oligodendrocytes and its proximity to developing myelin sheath, substantiate the observation of Hortega (1928) and Morrison (1931). However, there are no instances in the literature where a continuity between the myelin sheath and oligodendrocytes has been clearly demonstrated.

Microcytes:

Hortega (1919) by means of his silver carbonate technique, described the morphology of microcytes. These cells were observed to be smaller than astrocytes and oligodendrocytes and to have an elongated soma which stain dense with basic dyes. Microcytes have been described as present in white and gray matter and are observed to be perineuronal and occupy spaces adjacent to blood vessels.

Hortega (1919, 1932) from his light microscopic observations proposed that microcytes have a mesodermal origin and proliferate from adventitia and vascular elements. However, several reports ascribed to the belief that microcytes are transitional cells in the nervous system.

In the optic nerve of adult rats, Vaughn and Peters (1967) described a third neuroglial cell. This cell type has been observed to have morphological features intermediate to those of oligodendrocytes and astrocytes. Few microtubules are present in the cytoplasm and neurofilaments and glycogen particles are absent. Rough endoplasmic reticulum elaboration was sparse. Unlike astrocytes and oligodendrocytes, dense bodies are easily observed in the cytoplasm.

Several attempts to equate morphological findings of electron microscopy with those of light microscopy proved to be unsuccessful. Several explanations have been suggested to account for this difficulty. Because of the plasticity of microcytes and their ability to proliferate in response to injury (Cammermeyer 1965a, b; Konigsmark and Sidman, 1963; Blinzinger and Kreutzberg, 1968) electron microscopists find it difficult to define morphological entities that would satisfy the description of Hortega (1919, 1932) microcytes.

ELECTRON MICROSCOPIC OBSERVATIONS

Astrocytes:

Astrocytes with processes projecting in various directions from the perikaryon have been observed in all the layers of the olfactory bulb. These processes radiated between groups of axons, dendrites, cellular components and encircled vascular channels to a varying extent, forming astrocytic end-feet. Consequently, the course of these astrocytic processes was highly complicated.

The following description of astrocytic development and differentiation is based on observations made on one day, and one to five week old rats. The delineation of astrocytes into fibrillary and protoplasmic, while useful, is not absolute since the amount of fibrils within an astrocyte varies considerably, depending on the plane of section. It appears from this study that the astrocytes encountered in the olfactory bulb are most readily identified in two locations. The first being in relation to vascular channels forming perivascular feet. The second in the most superficial layer of the bulb where they are subpial in position and between the fascicles of unmyelinated axons.

Perivascular Astrocytes:

These astrocytes invest vascular channels and the extent of investment varies considerably. The astrocytes related to primitive, type I vascular channels possessed watery, clear cytoplasm containing few organelles, and enclosed the greater portion of the capillaries (Figures 10-14 - section on blood vessels). On the other hand the astrocytes related to blood vessels which are mature and belong to

type II variety, exhibited a distinctly different morphology. The cytoplasmic investment of the capillaries was considerably diminished and was intermingled with the processes of neurons, neurites and oligodendrocytes (Figures 15-19). The nucleus was slightly irregular with a uniformly distributed chromatin (Figure 40). The astroglial cytoplasm was less watery and contained more organelles. In passing it may be mentioned that filaments were seldom observed and consisted of fine wisps.

Subpial, interfascicular and perineuronal astrocytes:

These astrocytes have a similar morphology irrespective of their location. The subpial astrocyte was limited externally a well defined basal lamina which separated it from the extracellular collagen and pial cells (Figure 41). The nuclear contour was variable with uniform distribution of chromatin. A few nuclei showed slight marginal condensation which, however, was not dense enough to be mistaken for an oligodendrocyte. The perikaryon contained rosettes of ribosomes, Golgi complexes and mitochondria. The astrocytic processes, however, possessed less organelles.

The astrocytes observed in the first layer of the olfactory cortex, namely the fiber layer composed of clusters or fasciculi of unmyelinated axons, may be designated interfascicular astrocytes. These cells which separate groups of axons, have been considered by some as Schwann cells (Willey, 1973) and others as astrocytes (Andres, 1970) comparable to the Muller cells of the optic nerve (Lasansky, 1965). As indicated in the previous paragraph only an occasional astrocytic

process contained filaments (Figure 42).

In some situations, cells with nuclei showing uniform distribution of chromatin and no evidence of clumping were observed lying adjacent to neurons (Figures 43 & 44). These cells, on the basis of the nuclear morphology, are considered to be astrocytes and their cytoplasm contained rosettes of ribosomes, mitochondria and well developed Golgi complex (Figure 44).

Oligodendrocytes:

Oligodendrocytes in the rat olfactory bulb are characterized by a round dense nucleus, dense cytoplasm and equally dense processes. There is relatively little perinuclear cytoplasm. These prominent features permitted easy identification of the oligodendrocyte.

In one day old rat, photomicrographs showed young oligodendrocytes as dark nuclei which were satellite to lighter stained cells. Under the electron microscope these cells were observed to have a large dense nucleus surrounded by a thin rim of dense cytoplasm (Figures 45 & 46). Chromatin material, although being distributed fairly evenly though heavy, showed several aggregations not only in the core of the nucleoplasm but also at the nuclear membrane. The matrix of young oligodendrocyte cytoplasm was dense, a feature that would remain with the cell to maturity. Cytoplasmic organelles were predominatly free ribosomes and a few mitochondria. Portions of cisternae studded with a few ribosomes were also present.

As development proceeded, cellular processes were observed to radiate from the perikaryon and project into the neuropil (Figure 47).

Compared to a very young oligodendrocytic or oligodendroblast there was an increase in the numbers and distribution of cytoplasmic organelles. Free ribosomes, few mitochondria and short portions of rough endoplasmic reticulum were sparsely distributed in a dense cytoplasm. No Golgi complexes have been observed. Indented at several points along the cytoplasmic membrane are unmyelinated axons (Figure 41). However, no observations were made of an axon being completely surrounded by the cytoplasmic membrane of an oligodendrocyte.

By 14 days postnatal oligodendrocytes appeared to have reached maturity. They were large in size. Chromatin aggregation was very prominent along the inner nuclear membrane (Figure 48). The cytoplasmic area and the numbers of organelles were both increased. The cytoplasmic matrix was denser in some cells than in others. However, most of the oligodendrocytes observed in the present study were of the dark type described by Mori and Leblond (1970). Rough endoplasmic reticulum became much more numerous. In most instances the cisternae were completely studded with ribosomes (Figure 49). Free ribosomes, mitochondria and Golgi complexes were randomly distributed throughout the cytoplasm. Although Golgi complexes were observed at several locations they appeared to be centered at the bases of cytoplasmic processes. In the proximity of Golgi complexes were vesicles and a few dense lysosomal and lipofuscin granules.

Several cytoplasmic membrane adhesions were found. These adhesions were dense and were found between the oligodendrocyte cytoplasmic membrane and membranous processes of different cell types (Figure 49).

The association of myelinated fibers with mature oligodendrocytes has also been observed. Several of these myelinated fibers were enclosed partially by oligodendrocyte cytoplasmic membrane. This would support the generally accepted opinion that oligodendrocytes are responsible for the process of myelination in the central nervous system.

DISCUSSION

Astrocytes:

The development and differentiation of astrocytes have been studied in the optic nerve (Vaughn and Peters, 1967) and in the cerebral cortex (Caley and Maxwell, 1968) of the rat. Prior to these reports, only few scanty descriptions by Meller et al., (1966) on glioblast differentiation in the developing mouse cerebral cortex need mentioning. Recently, similar studies were carried out in this laboratory by Hannah, (1973) on the substantia gelatinosa of the rat spinal cord.

In the present study the differentiation of astrocytes and oligodendrocytes were perused in the olfactory bulb of postnatal rats.

Ensheathment of the blood vessels in the olfactory bulb, in the present study, was completed even in primitive blood vessels, there being little or no extracellular process in relation to the capillaries (Figures 10-14). It has been shown by Caley and Maxwell (1968) that considerable amounts of perivascular space are present in the cerebral cortex of one day old rat. Such variations can be related to, as stated earlier, the earlier development and differentiation of the olfactory bulb.

In the early stages of postnatal development in the rat optic nerve, astrocytes have well developed perikarya and their processes extended for considerable distances to enclose unmyelinated axons (Vaughn and Peters, 1967). This enclosure resulted in the formation of fascicles. Similar observations were made in the fiber layer of

the present study. Unlike the optic nerve immature astrocytes, the processes in the olfactory bulb are clear and watery. It is inconceivable to relate such differences in appearance to fixation simply on the criterion that the adjacent tissue are relatively healthy. However, at the outermost surface of the olfactory bulb cellular processes identified as astrocytes (Andres, 1970) and Schwann cells (Willey, 1973), (Figures 21-23) showed resemblance to the optic nerve immature astrocytes. We consider these cells to be astrocytes or modified astrocytes.

In the present study identification of microfilaments in the cytoplasm and processes was not made until 21 days postnatal, and was confined to small areas. Hannah (1973) in the substantia gelatinosa of the rat spinal cord showed filaments at 14 days postnatal. Vaughn and Peters (1967) reported that microfilaments make their presence in 21 day old rat optic nerve.

The appearance of microfilaments in astrocytic cells appeared to come after the maturation and differentiation of astrocytic cells have been completed. At this time the movement of materials within the cell becomes vital to its existence. Evidences suggesting that neurofilaments or microfilaments are associated with the transport of metabolites within the cell have been reported (Tilney and Gibbons, 1969; Pollard and Ito, 1970).

On the basis of morphological studies it is purely not feasible. to speculate on the function of microfilaments within mature astrocytes. However, other investigators have attributed functional roles to

microtubules and microfilament of various cell types. The appearance of microfilaments in the mature Mauthner cell in cyclostomes has prompted Billings (1972) to suggest that these structures have embarked on a role of selective movement of materials.

In early postnatal astrocytes the movement of ions and metabolites has been linked with microtubules since microfilaments are relatively absent. However, studies carried out on sperm tails (Pease, 1963) and on mitotic spindles (Barnicot, 1966) showed that microtubules are made up of at least 10 microfilaments. This would suggest that in reality microfilaments are the media by which cellular compounds are transported.

Oligodendrocytes:

Several reports have described oligodendrocytes as being smaller and denser than astrocytes (De Robertis and Gerchenfeld, 1961; Shultz, 1964; Stensaas and Stensaas, 1968). Similar observations were made in the present study.

On the basis of cytoplasmic and nuclear density Mori and Leblond (1970) have described three types of oligodendrocytes in the corpus callosum of young male rats. These are: (a) light oligodendrocytes with a pale nucleus and cytoplasm; (b) a medium-shade oligodendrocyte with moderately dense nucleus and cytoplasm and (c) a dark oligodendrocyte with a very dense nucleus and cytoplasm. In the olfactory bulb, oligodendrocytes were of the medium shade and very dense classes.

Differentiation and maturation of oligodendrocytes have been traced in the cerebral cortex of the rat (Caley and Maxwell, 1968b);

in the optic nerve of the rat (Vaughn, 1969); and in the kitten optic nerve (Blunt et al., 1972). These studies have shown that with the increase in age, oligodendrocytes became larger, there was also an increase in the numbers of cytoplasmic organelles and the perikaryon became spherical to oval. Concomitantly, there was an increase in denseness of the nucleus and clumping of chromatin granules.

In the present work, oligodendrocytes were observed to have large nuclei and a thin rim of cytoplasm in one day old rats. Few processes were observed. By seven days of development, the nucleus became denser, there was an increase in the cytoplasmic ratio and numbers of organelles. Processes were observed to radiate into the surrounding neuropil. This differentiation continued to about 21 days postnatal, following which no observable cytological changes were noted. These findings seemed to be in agreement with those of Caley and Maxwell (1968b); Vaughn (1969) and Blunt et al., (1972).

The changes in configuration of the nucleus and cytoplasmic contents in differentiating oligodendrocytes are indicative of the metabolic processes that are being employed. In conformity to the general task of development, immature cells, including oligodendrocytes, are primarily concerned with the synthesis of structural proteins which enhances the size of the cell and the numbers of cytoplasmic organelles. Initially, these proteinaceous compounds are synthesized on polyribosomes to which the mRNA strand is attached.

Changes in the rearrangement and distribution of chromatin from a diffuse state in the immature cell to a nucleus with several chromatin

aggregations have been interpreted as a slow down in the metabolism of the mature cell. It is highly speculative to suggest functional attributes purely on the basis of morphological observations. Reports from several sources have associated the formation of myelin sheaths with mature oligodendrocytes (Stensaas and Stensaas, 1968; Vaughn and Peters, 1971; Blunt et al., 1972). This observation would implicate that, in reality, the mature oligodendrocyte is an active cell which is concerned with the biochemistry of myelin formation, a process that draws information not only from the nucleus but also from the cytoplasm. However, Vaughn and Peters (1971) have described an "active" oligodendrocyte. In this cell type chromatin aggregations have been reported to be absent. In addition, Golgi complexes and microtubules become very apparent. From a theoretical point of view it may be possible that mature oligodendrocytes demonstrate plasticity by reverting to the "active" type. Also, Mori and Leblond (1970) have shown that tritiated thymidine, H^3, is incorporated in the nucleus indicating that the cell is capable of mitotic division, a process that requires some amount of energy.

In the present study, myelinated fibers have been observed to be few and randomly distributed in the olfactory bulb. Some of these fibers were observed to be adjacent to the cytoplasmic membrane of oligodendrocyte, indicating a functional relationship. However, the majority of oligodendrocytes were located as satellite cells to neurons, being more obvious in the granular layer. This finding suggests an additional function in maintaining the integrity of the neuron and which may be paramount to myelinogenesis, at least, in the olfactory bulb.

SUMMARY

Astrocytes:

These cells were observed in all the layers of the olfactory bulb and could be delineated into subpial, perivascular, interfasicular and perineuronal depending on their location.

Perivascular astrocytes which invested Type I and Type II blood vessels revealed different morphology. Those in relation to primitive Type I have clear water cytoplasm containing few organelles. In addition, these processes surrounded the greater portion of the vessel. Perivascular astrocytes which were in contact with mature Type II vessels possessed processes which were less watery and contained more organelles.

The subpial, interfascicular and perineuronal astrocytes displayed a similar morphology consisting of a nucleus with uniform distribution of chromatin and a perikaryon containing ribosomes, Golgi complexes and mitochondria. The morphology of these astrocytes did not appear to alter in a significant manner in different time periods studied. However, occasional astrocytes processes exhibited filaments.

Oligodendrocyte:

Immature oligodendrocytes were observed to have a large and dense nucleus and a thin rim of dense cytoplasm composed of free ribosomes. Few processes were observed to radiate from the cell soma.

Subsequent development revealed that cytoplasmic organelles increased in numbers and processes were observed to radiate from the

cell soma. By 14 to 21 days postnatal oligodendrocytes appeared to have reached maturity and displayed the usual organelles such as Golgi complexes, granular reticulum and free ribosomes. The nucleus and cytoplasm maintained their denseness. Most of the oligodendrocytes were located adjacent to neurons.

Figure 40. Micrograph of a perivascular astrocyte (As) and a mature or type II blood vessel. The nucleus has a light appearance and chromatin granules are evenly distributed. The cytoplasm peripheral to the nucleus contains sparsely distributed free ribonucleic protein.
Note that there is no extra-cellular space between the astrocytic cytoplasmic membrane and basal lamina (bl).
Specimen from 7 day old olfactory bulb.
X 30,300

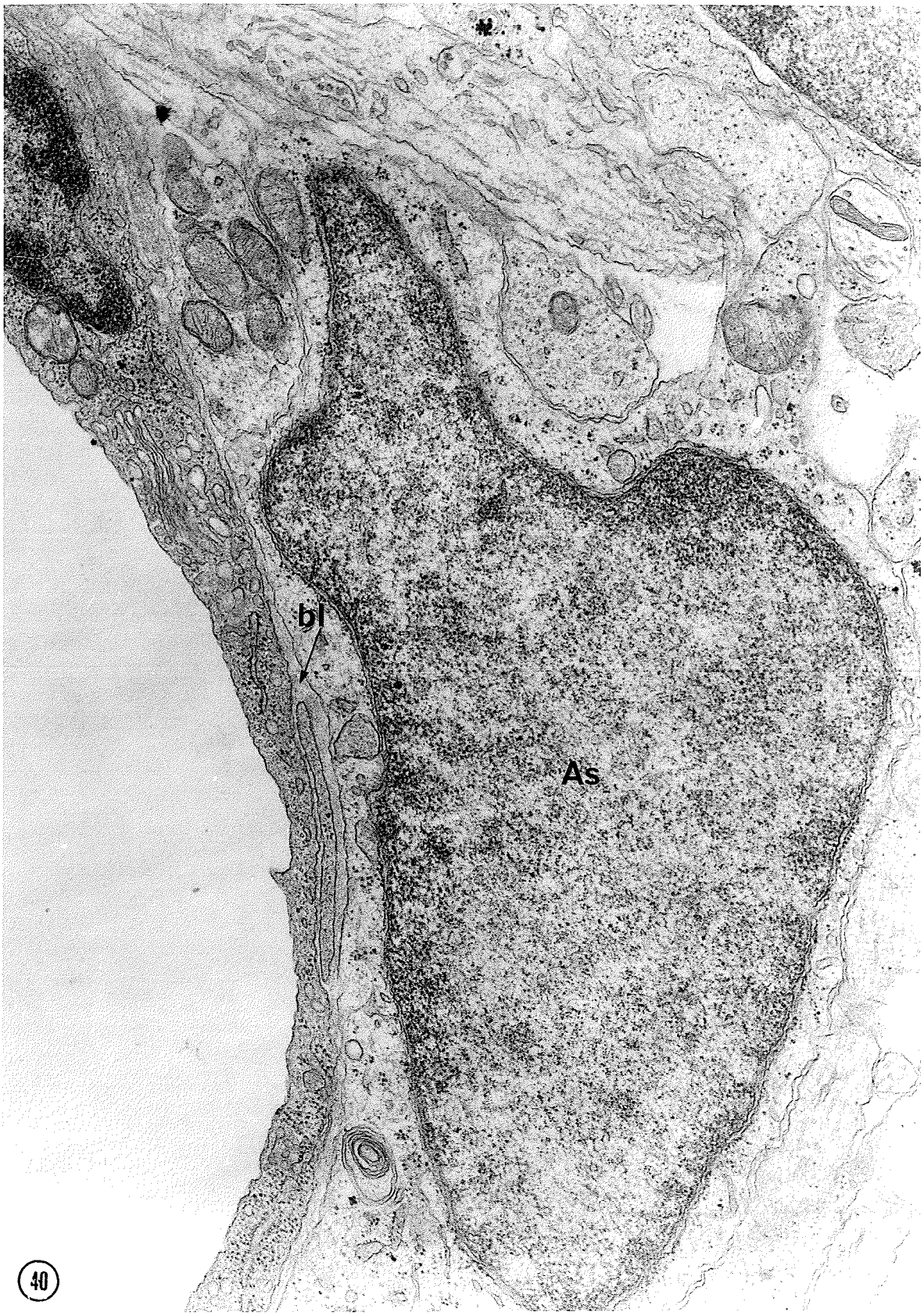
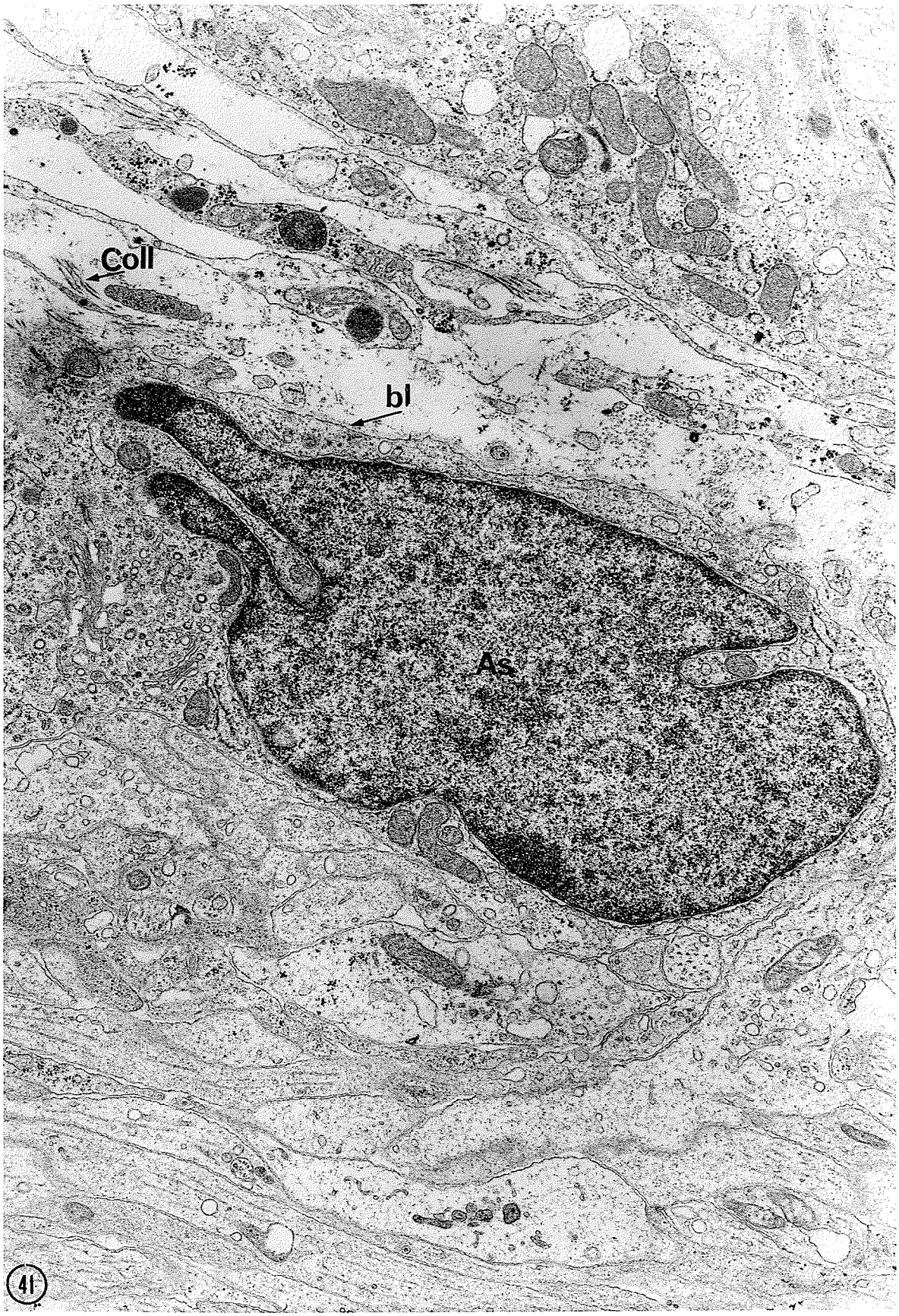

bl
As

Figure 41. Illustrates a subpial astrocyte (As) in one day old olfactory bulb. A basal lamina (bl) separates the astrocyte from the extra-cellular space collagen fibers (Coll). The nucleus is large and irregular. Chromatin granules are uniformly distributed, a criterion that has been used by several investigators to identify astrocytes. Free ribosomes, mitochondria and Golgi complexes are observed in the cytoplasm.

X 20,800



41

Figure 42. Electron micrograph of the superficial part of the fiber layer of olfactory bulb in a 35 day old rat. Observe a portion of a blood vessel (BV) and the pericyte (P) in the lower portion of the figure. Large cluster of unmyelinated axons containing microtubules and mitochondria occupy the upper half of the figure. Collagen (coll) may be observed in the extra cellular space between the pericyte and axons. Astrocytic processes, some having predominantly watery cytoplasm (As_1) and other fibrils (As_2), are also visualized. The presence of filaments have been interpreted as a sign of differentiation and maturation (Vaughn and Peters, 1967). However, in the olfactory bulb only wisps of these filaments have been observed in the astrocytic cytoplasm.

X 25,700

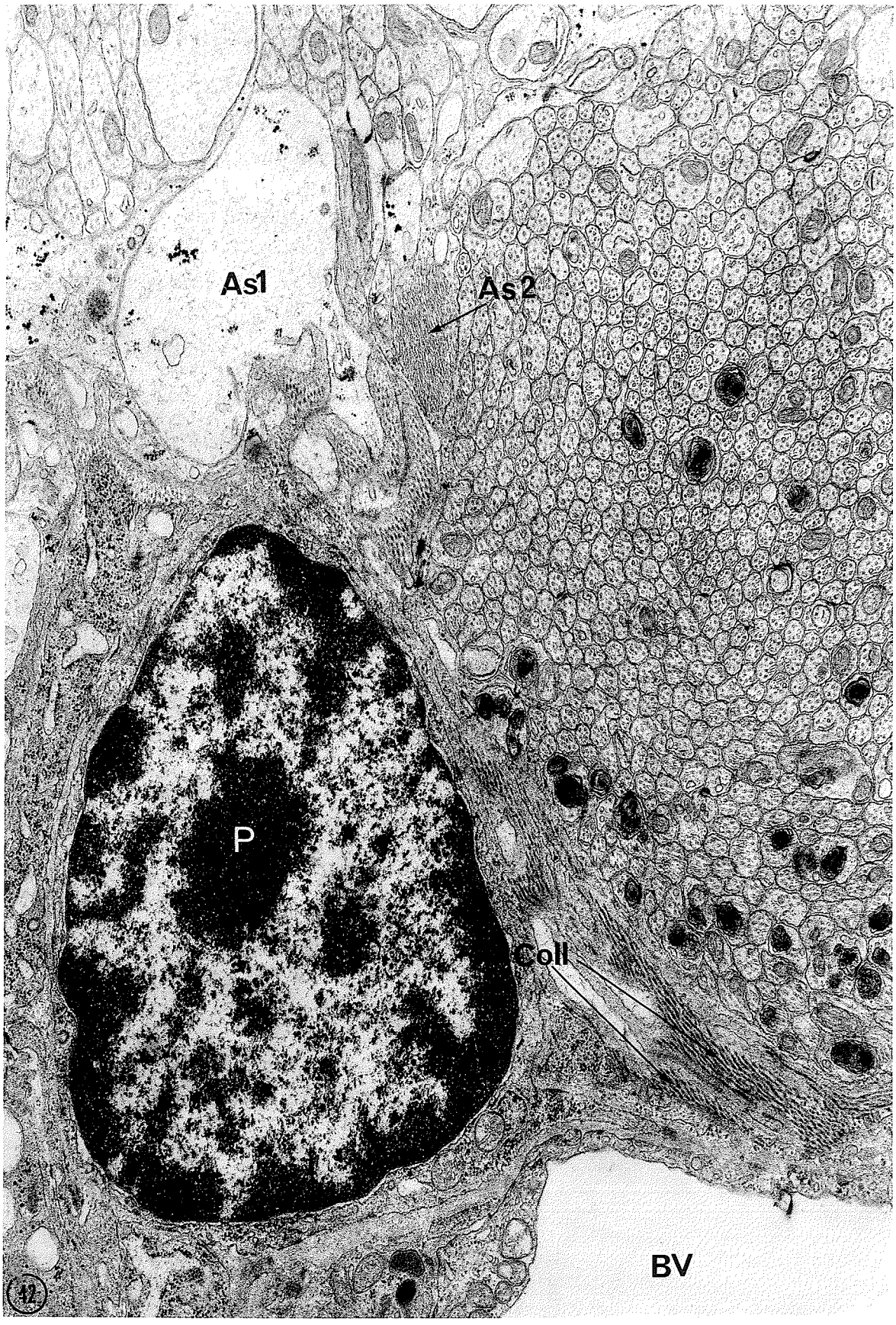
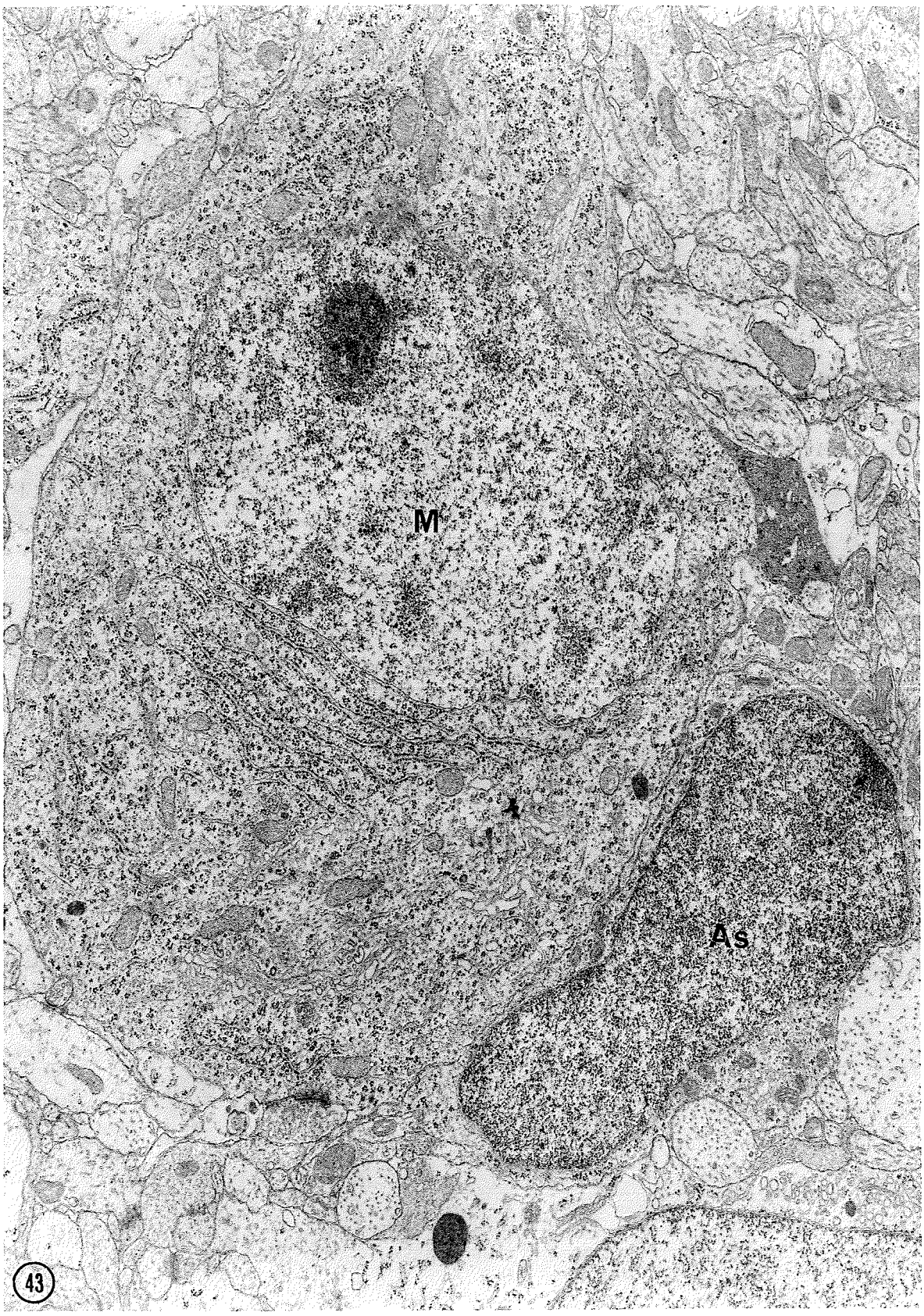

As1
As2
P
Coll
BV
12

Figure 43. Astrocyte (As) in a seven day old olfactory bulb situated adjacent to a mitral (M) cell. Similar to astrocytes found in other area of the olfactory bulb, there is a large nucleus with evenly distributed chromatin granules. The cytoplasm forms a thin rim around the nucleus and contains free ribosomes and a few mitochondria. Note that the mitral cell (M) is well developed at this stage and contains well developed endoplasmic reticulum, Golgi complexes and numerous mitochondria.
X 12,800



43

Figure 44. Electron micrograph of an astrocytic cell (As) which is satellite to a mitral cell (M) in a 7 day old olfactory bulb.

The nucleus of the astrocytic cell is relatively large, and on the basis of identification contains evenly distributed chromatin granules. Free ribosomes, a few mitochondria and a small Golgi complexes are observed in one portion of the cell. A small bundle of filaments (fil) may be observed adjacent to the astrocytic nucleus.

X 13,300

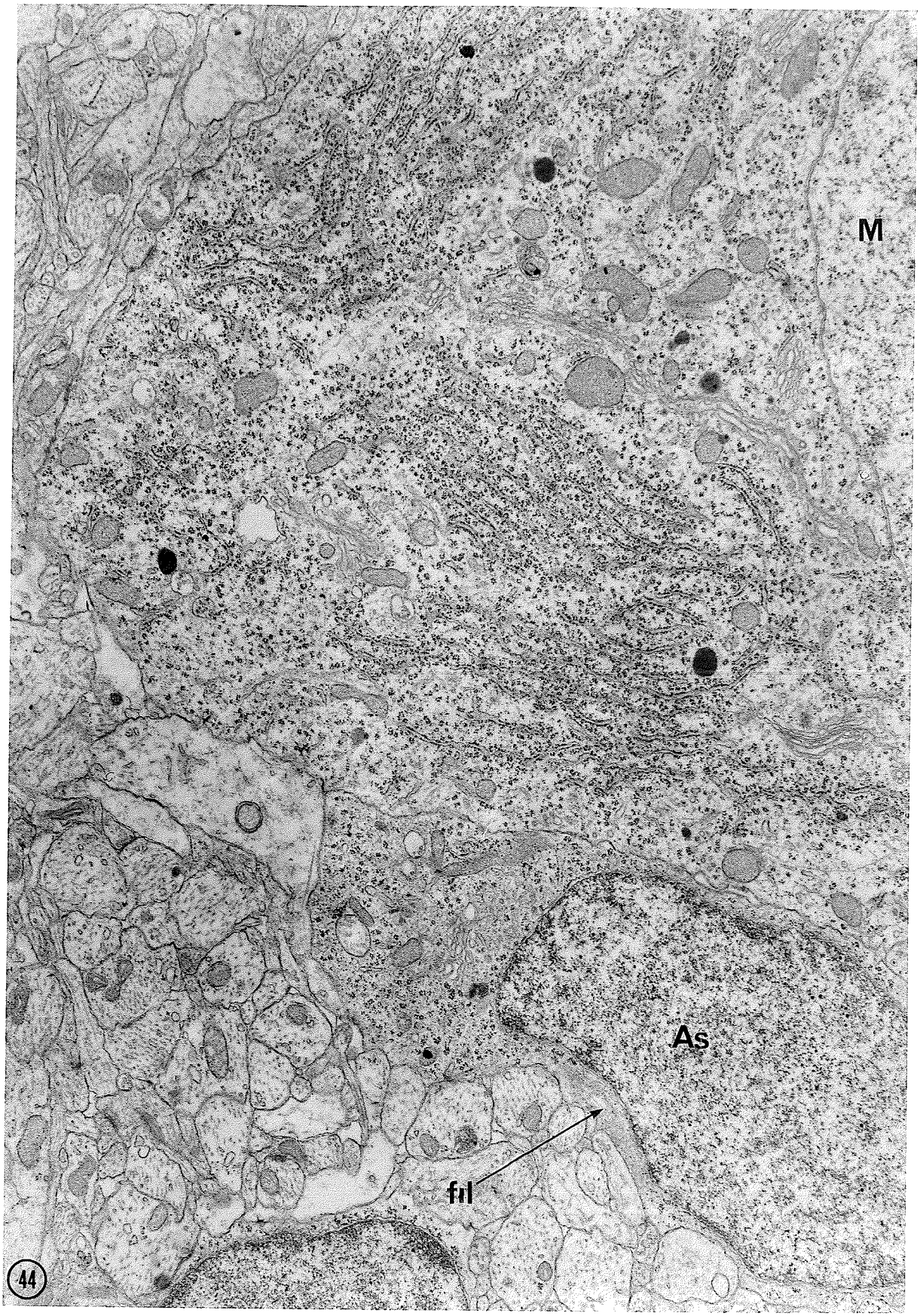

M
As
fil
44

Figure 45. Illustrates a pair of young oligodendrocytes (Ol) and granule cells (Gr) in a one day old olfactory bulb. The oligodendrocytes have large and dense nuclei. The cytoplasm of the oligodendrocytes is much more dense compared to that of the adjacent granule cell.
X 15,900

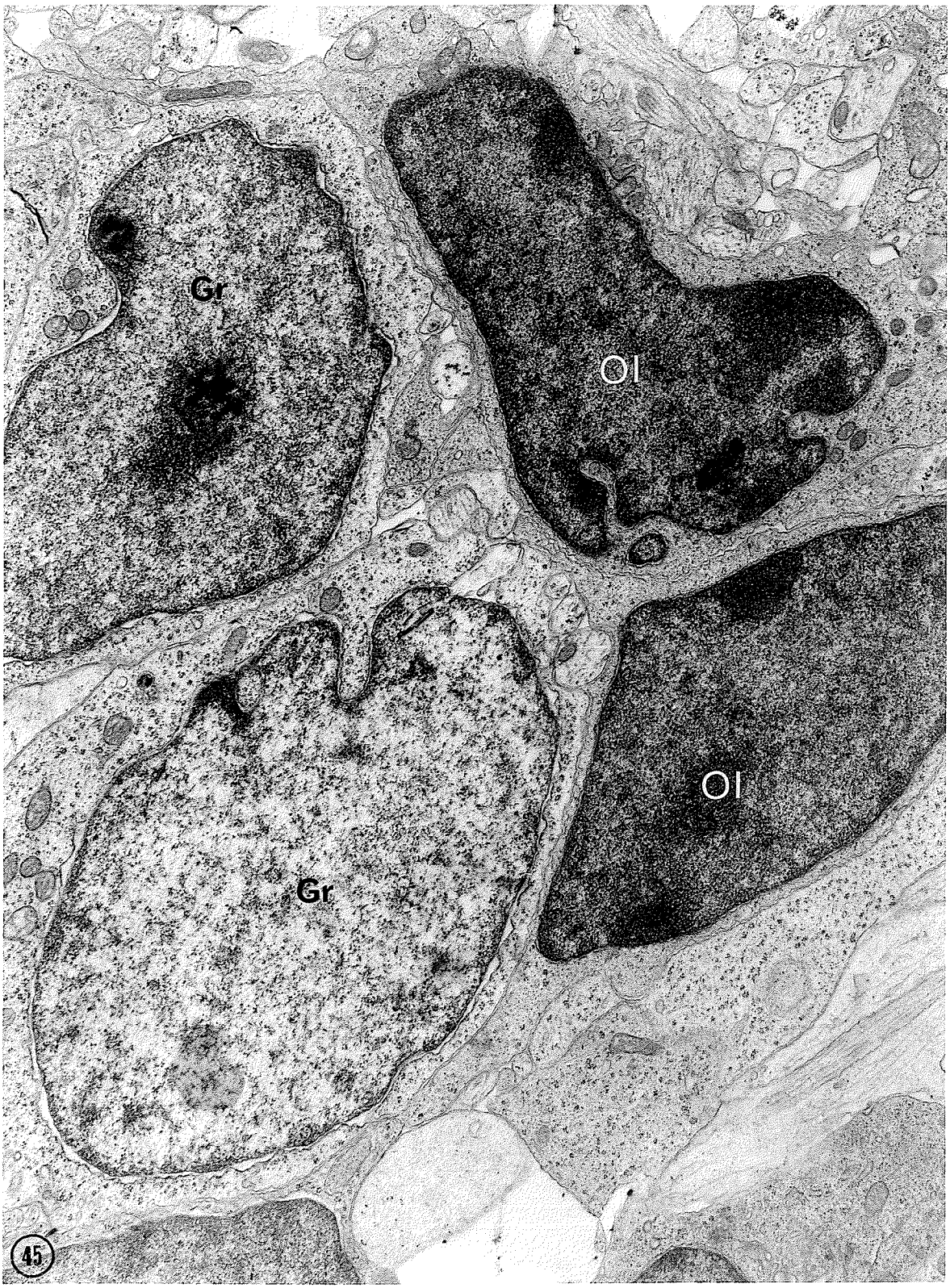

Gr
Ol
Ol
Gr
45

Figure 46. A young oligodendrocyte (OI), in a one day old rat, depicting a large nucleus and a thin rim of cytoplasm which is moderately dense. The cytoplasm contains free ribosomes and a few mitochondria. Note that the nucleus is moderately dark and may correspond to Mori and Leblond's (1970) type II oligodendrocyte. Portions of four granule cell (Gr) may be also seen in the micrograph. X 15,800

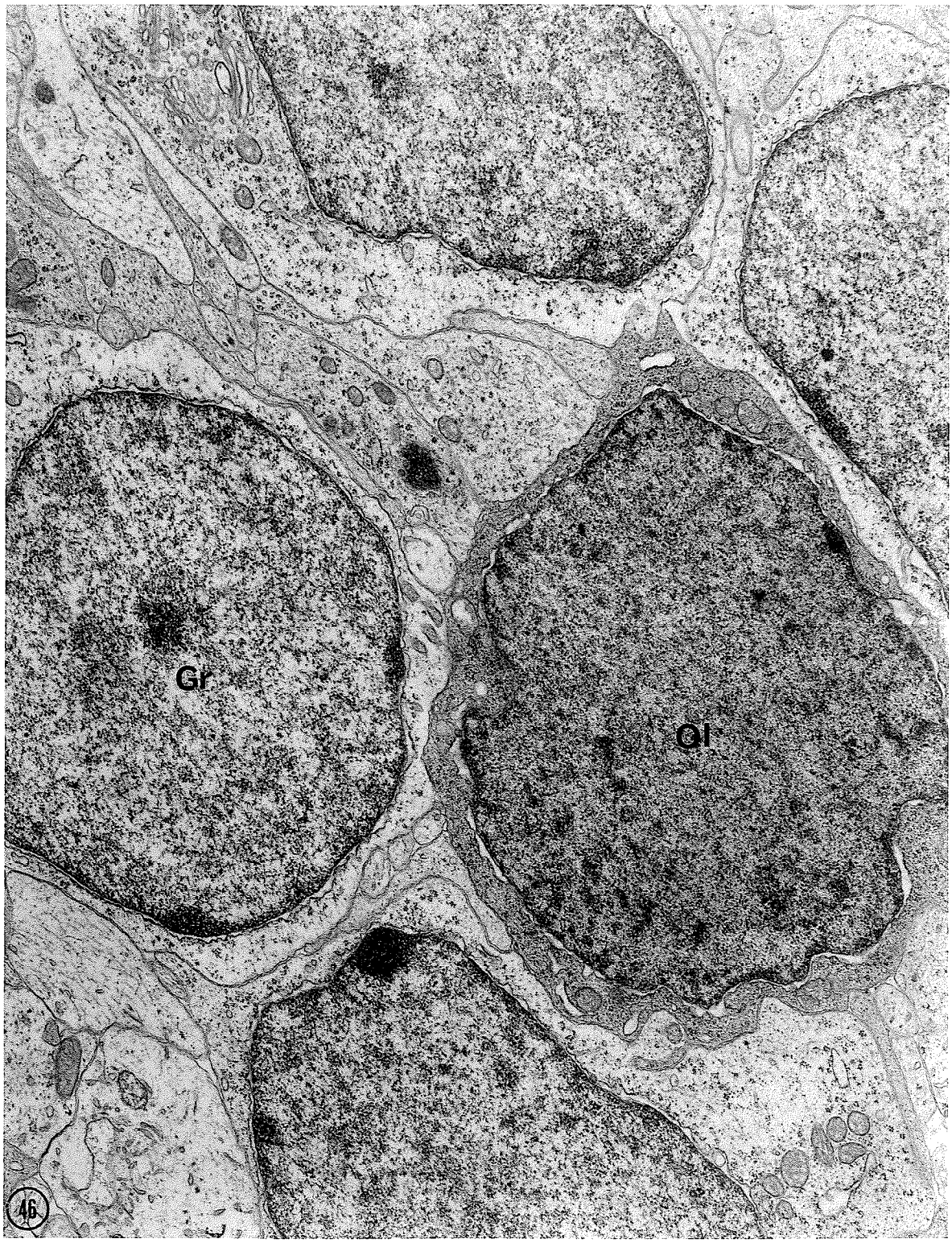

Gr
Gl
46

Figure 47. Seven day old olfactory bulb illustrating an oligodendrocyte (Ol) which is at a later stage of differentiation when compared to oligodendrocytes in Figure 45 and 46. The nucleus is large and dense and shows marked condensation of chromatin along the inner nuclear membrane. Present in the thin rim of cytoplasm are free ribosomes, mitochondria and lysosome. Note the cytoplasm of an adjacent neuron (Ne).

X 25,700

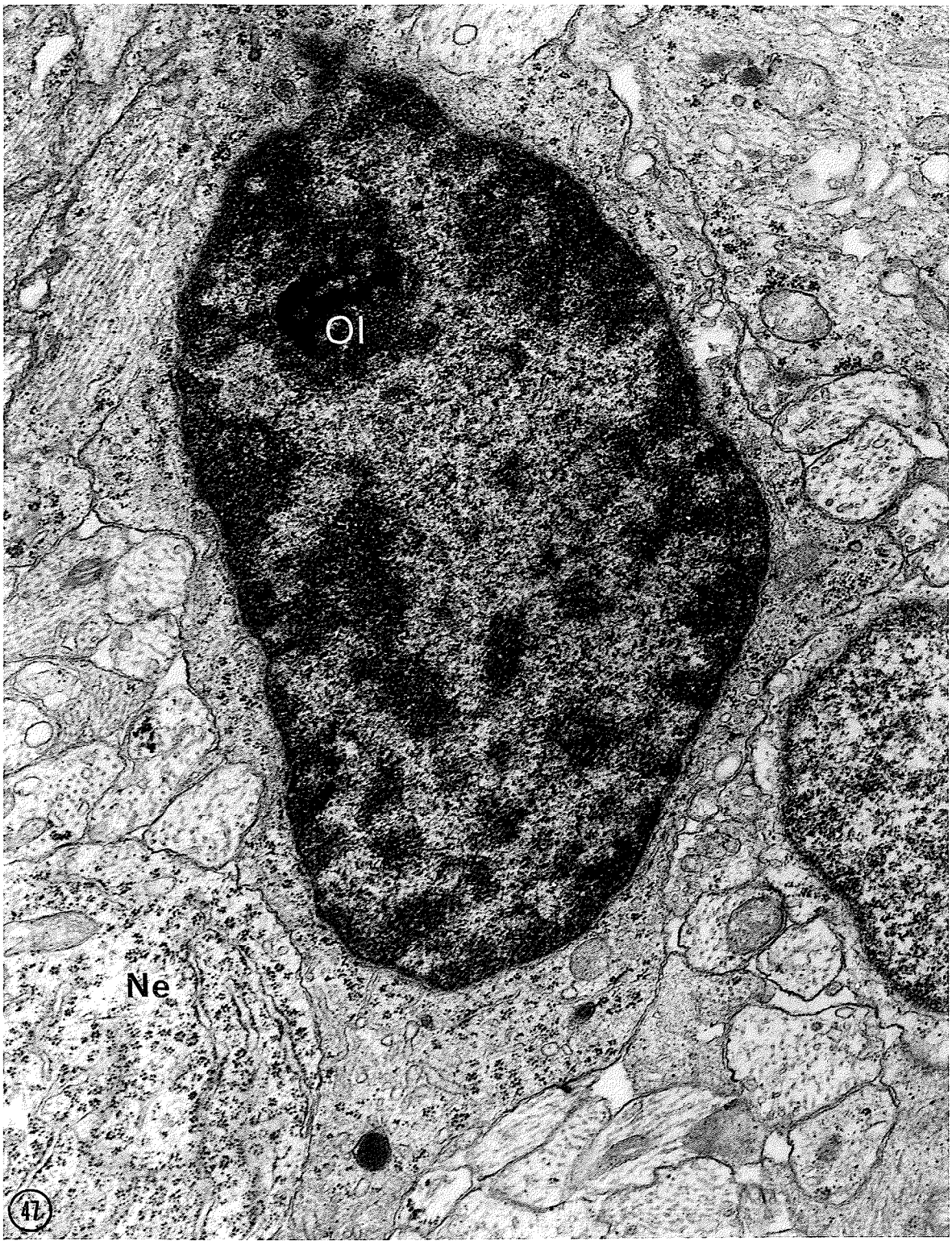
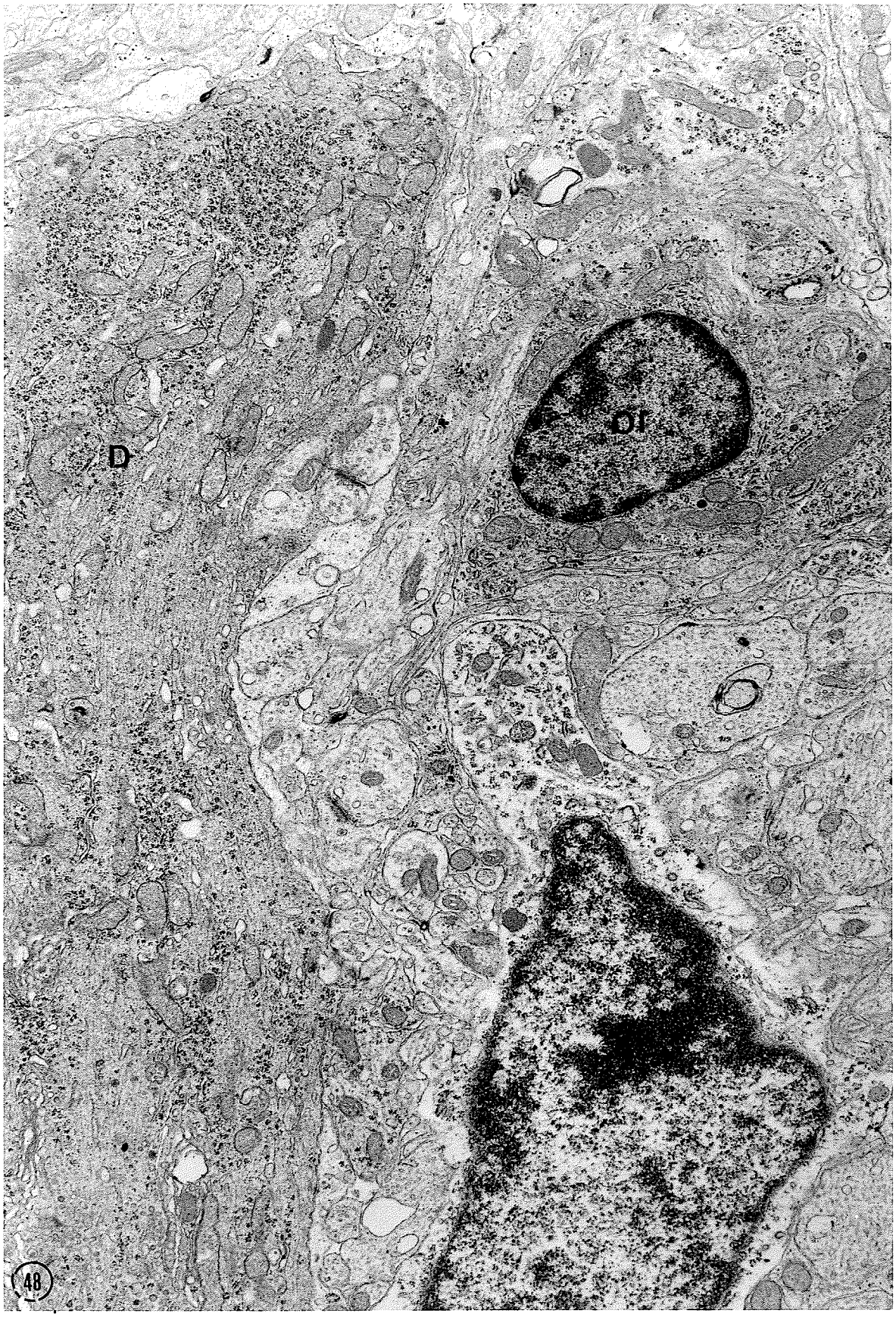

Ol
Ne
47

Figure 48. Electron micrograph of 21 day old rat illustrating an oligodendrocyte (OI). The nucleus shows a remarkable aggregation of chromatin granules below the nuclear membrane. The cytoplasm shows an increase in organelles, chiefly rough endoplasmic reticulum and mitochondria. The cytoplasmic matrix is dense. Note the dendritic process (D) which is presumed to originate from a mitral cell perikaryon.

X 15,100

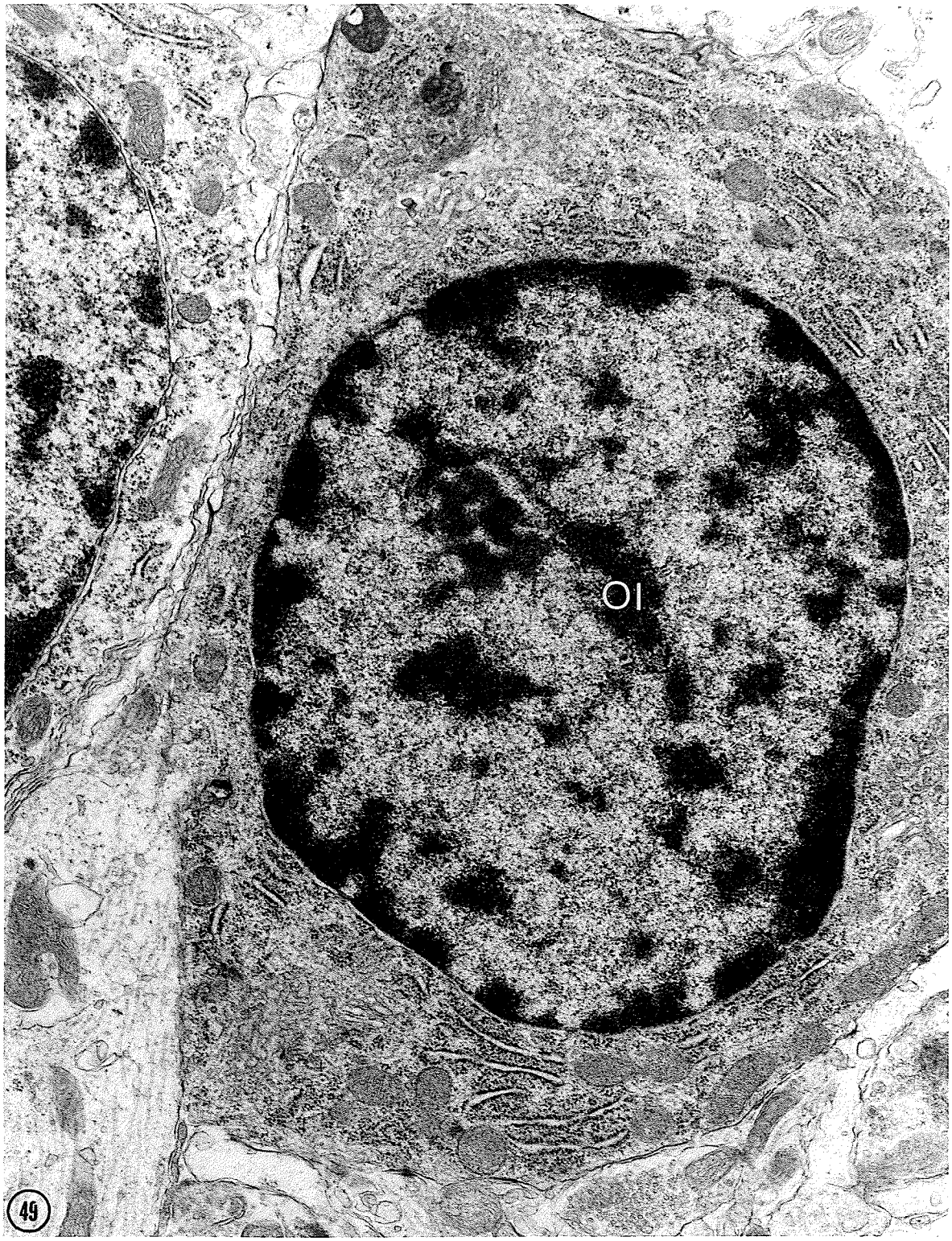


48

Figure 49. A mature oligodendrocyte (OI) in a 21 day old olfactory bulb. Both the nucleus and cytoplasm are intensely dense and correspond to the dark oligodendrocyte of Mori and Leblond (1970).

In this cell the nucleus is large with considerable aggregation of chromatin. The cytoplasm is dense and contains extensive amount of rough endoplasmic reticulum. Numerous mitochondria and Golgi complexes are also found in the cell.

X 23,800


OI
49

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