THE UNIVERSITY OF MANITOBA

THE POST-NATAL DEVELOPMENT OF THE POSTERIOR HORN OF THE RAT SPINAL CORD - AN ULTRASTRUCTURAL STUDY.

by

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Dedicated to my wife, sons and parents without whose encouragement this work would not have been possible.

"Of the cell, the wondrous seed
Becoming plant and animal and mind
Unerringly forever after its kind,
In its omnipotence, in flower and weed
And beast and bird and fish, and many a breed
Of man and woman, from all years behind
Building its future."

William Ellery Leonard

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Abstract

Dorsal segments of spinal cord from the cervical enlargement of Holtzman albino rats, were fixed by both perfusion and immersion in aldehydes in phosphate or cacodylate buffer and post-fixed in osmium tetroxide. Tissues were embedded in Araldite and were examined with light and electron microscopes. The differentiation and maturation of neurons, blood vessels and glial cells in the substantia gelatinosa was studied from birth to six weeks post-natal.

Differentiation of Neurons: Neuroblasts at birth were small with a large nucleus and scanty cytoplasm. The nucleus was generally oval without any irregularities in its contour. The cytoplasm revealed the presence of free ribosomes and mitochondria. Granular endoplasmic reticulum and Golgi complexes were absent or rudimentary. Electron dense, lysosomal-like bodies were seldom observed. By the end of the first week, evidences of differentiation and maturation of neuroblasts began to appear. The nuclei of several cells demonstrated early nuclear invaginations; cytoplasmic processes, some with growth cones and a well developed granular endoplasmic reticulum. At several points the channels of endoplasmic reticulum became continuous with the perinuclear space. In addition, a well developed Golgi complex was observed. By the end of the second week, differentiation of the neuroblasts was more advanced. More nuclei showed invagination of its contour. The cytoplasm revealed well developed granular endoplasmic reticulum and multiple Golgi complexes. Numerous vesicles and dense bodies were found adjacent to the Golgi. By the third week, features of neuronal differentiation, such as nuclear invaginations, granular endoplasmic reticulum, multiple Golgi

and dense bodies in the cytoplasm, became well established. Arrays of agranular endoplasmic reticulum also appeared during the third week.

Differentiation of Blood Vessels: The extracellular space which was quite conspicuous at birth was greatly reduced by the third week. Light microscopy revealed an increase in blood vessel profiles between one and three weeks post-natal. Ultrastructurally, at birth, there were few patent vessels, but endothelial cells enclosing a slit-like lumen were numerous. The endothelial cells contained a high concentration of free ribosomes and pinocytotic vesicles. Pseudopod projections of the endothelial cells into the vascular lumen were often present. The nuclei were very irregular and dense with clumped chromatin. The basal lamina varied in thickness. During the period between one and three weeks the nuclei of endothelial cells became less dense; the lumen became patent and the endothelial wall progressively thinner. The periphery of the vessel became completely surrounded by glial end feet and the basal lamina was regular and prominent. By three weeks, all blood vessels appeared to be mature. Pericytes were present at all stages of development.

Differentiation of Glial Cells: Glioblasts, which were represented the precursors of the oliogodendrocyte and astrocyte cell lines, were present during the first post-natal week. An observed decline in the numbers of glioblasts during the first week coincided with an increase in identifiable macroglia. Young astrocytes, which were present in the neonate animal, were characterized by relatively low nuclear and cytoplasmic densities and the numerous cytoplasmic processes forming perivascular end feet around blood vessels. The cytoplasm contained short, wide cisternae of granular endoplasmic reticulum, well developed Golgi,

numerous electron dense bodies, and a few scattered filaments. As development proceeded there was a decrease in cytoplasmic density and an increase in the number of filaments present. Young oligodendrocytes were first identifiable at three days post-natal. The young oligodendrocyte was characterized by a dispersed nuclear chromatin pattern and a large amount of organelle rich cytoplasm forming both broad and slender processes. As the oligodendrocytes approached maturity there was a subsequent loss of many of the cytoplasmic processes and nuclear chromatin became progressively more clumped in appearance. By six weeks post-natal both astrocytes and oligodendrocytes appeared mature.

Materials and Methods

All of the animals used in this study were Holtzman albino rats. Groups of at least three animals were killed at each of the following times: Birth, 2,3,6,9,14,21,28,36 and 42 days post-natal.

Perfusion Technique

Animals one week post-natal and older were anaesthetized with Nembutal. The thoracic cavity was opened to expose the heart. A hypodermic needle (18 gauge) was inserted through the left ventricle into the aorta and secured. The perfusate was administered with a hypodermic syringe utilizing minimal pressure. Perfusion was maintained until the liver showed signs of clearing. Most animals under one week of age were cooled to approximately 4^{o} C, utilizing an ice pack. The spinal cord was exposed while immersed in fixative. At least one animal at each time period during the first post-natal week was perfused utilizing the technique performed on the older animals.

Removal of Tissue Sample

The dorsal surface of the cervical spinal cord was exposed and the region of the cervical enlargement was removed in one piece and immediately immersed in fixative. Utilizing a razor blade, transverse slices of approximately one mm. thickness, were removed from the central portion of the cervical enlargement. The slices were then divided into dorsal and ventral halves and the dorsal halves were again divided in the midline.

Method of Fixation

The fixative found to give the most satisfactory results consisted of 3% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2. The pieces of dorsal spinal cord remained immersed in fixative for one hour. The tissues were then rinsed in 0.1 M cacodylate buffer. The samples were then post-fixed in cacodylate buffered 1% osmium tetroxide for 45 minutes.

Method of Embedding

After fixation the samples were rinsed in cacodylate buffer and dehydrated in a graded ethanol series to 100%. The tissue samples were then transferred through a graded series of propylene oxide and alcohol to 100% propylene oxide. A series of mixtures of propylene oxide and Araldite were utilized to bring the tissue to 100% Araldite, at which time the tissue was embedded in Beem capsules.

Light Microscopy

The Araldite embedded material was sectioned at 0.5u on a Reichert Om U2 ultra microtome utilizing glass knives. Sections were mounted on glass slides and routinely stained with toluidine blue. The substantia gelatinosa Rolandi was studied in 0.5u sections, utilizing an optical microscope. Light micrographs were taken with a Zeiss photomicroscope on Kodak Panatomic-X film.

Electron Microscopy

The blocks were further trimmed leaving only the area at the tip of the posterior horn, thereby including the area of the substantia gelatinosa.

Thin sections were cut on a Reichert Om U2 ultramicrotome utilizing glass knives. They were placed on uncoated copper grids and stained with uranyl acetate and lead citrate. Thin sections were visualized with a Phillips 300 electron microscope operated at 60 Kv. All electron micrographs were recorded on Dupont Ortho S Litho sheet film and developed in Kodak D-19.

Introduction - General

With this study, the author has attempted to establish a base line for the "normal" development of the posterior horn of the rat spinal cord and more specifically the substantia gelatinosa Rolandi. A broad understanding of the developmental processes and interaction between the various structures present, would allow for the subsequent testing of how the system would react to various insults, such as metabolite depletion, hormonal variations or mechanical injury.

Very little research has been reported at the ultrastructural level in the field of neuroembryology. There are, however, many reports dealing with the clinical problems of the central nervous system (C.N.S). It was the feeling of the author that a better understanding of the developmental process might produce insight into the etiology of many neurological disorders.

The developing posterior horn encompassed many interactions between the different cellular elements present. The study of teratology has demonstrated the very delicate balance maintained between intrinsic and extrinsic elements in all developing systems. However, the author decided for the purpose of clarity, to divide the description of the developing substantia gelatinosa into three major sections: neurons, blood vessels and neuroglia. Each section included the appropriate literature review, observations, discussion and micrographs.

The posterior spinal grey matter was studied in the cat by Rexed (1964) using cytoarchitectonic methods and was found to consist of four discrete areas or laminae. Functionally, laminae I to IV were described as the primary sensory area of the spinal cord, collecting exteroceptive sensory information from the trunk and extremities. Rexed (1964),

identified lamina II as the substantia gelatinosa Rolandi composed of small, tightly packed cells and crossed by many large nerve fibers from the dorsal fasciculus. In a light and electron microscope study of the substantia gelatinosa involving dorsal root section, Ralston (1965) concurred with Rexed (1964) that the substantia gelatinosa was confined to lamina II in the cat. The substantia gelatinosa cell was described as small, spindle shaped and lacking an organized Nissl substance. The neuropil contained many small non-myelinated axons and astrocytes were the predominant glial element.

Szentagothai (1964), by morphological comparison and Wall (1965) by electrophysiological techniques, grouped laminae II and III into the area defined as the substantia gelatinosa.

Scheibel and Scheibel (1968) in cat spinal cord observed a structural difference between laminae II and III but considered the area as one functional unit.

To the best of the author's knowledge there have been no comprehensive studies on the lamination of the posterior grey horn of the rat spinal cord.

The only ultrastructural study on the neurons located adjacent to the entrance of the posterior root fibers in the dorsal grey column of the rat was by Nathaniel and Nathaniel (1966a). These authors showed that sensory neurons possessed pale, highly irregular nuclei. The cytoplasm was dense with elaborate smooth surfaced membrane systems. The granular endoplasmic reticulum was relatively small in amount and did not possess an orderly arrangement. In the absence of studies on lamination of rat posterior grey column and on the basis of the description of the location of the sensory neurons described by Nathaniel and Nathaniel (1966a) it appeared that they were describing neurons in

the substantia gelatinosa of the rat spinal cord.

The substantia gelatinosa and its sensory mechanisms remained a controversial issue and for the purpose of this thesis, only the more recent papers were discussed.

A controversy existed in the literature as to whether dorsal afferent fibers entered the substantia gelatinosa. Szentagothai (1964), Schiebel and Schiebel (1966, 1968) and Heimer and Wall (1968) have produced strong evidence that dorsal root fibers terminated on cells of the substantia gelatinosa. In contrast, Ralston (1965) and Sterling and Kypers (1967) reported that no significant number of the dorsal root afferents terminated in lamina II.

Szentagothai (1964) described the substantia gelatinosa of the cat as a closed neuron system in which all its axons turned back to terminate within the system. Both large and small calibered afferent fibers fed into the substantia gelatinosa as did dendrites from the large neurons of lamina IV. Szentagothai suggested that the functional significance of such a system was a modulatory effect on impulses going to the neurons of lamina IV.

Scheibel and Scheibel (1968) described the gelatinosal complex as composed of neuropil sheets, formed by coarse cutaneous afferents. Each neuropil sheet represented a linear area on the body surface. Gelatinosal cells of laminae II and III were immersed in the neuropil sheets and exerted presynaptic control of the incoming cutaneous fibers.

Melzak and Wall (1965) implicated the substantia gelatinosa in a "gate control" system for perception of pain. The authors proposed that the substantia gelatinosa was involved in a feed back system that modulated impulses proceeding from dorsal root fibers to the larger neurons

in the dorsal horn. The afferent impulses were carried in both small and large calibered fibers. The small fibers produced an inhibitory effect on substantia gelatinosa cells while the large fibers produced exitation. Such dual input allowed the substantia gelatinosa to control the amount of stimulation received by cells higher in the pathway, thus determining the excitatory effect upon them.

Ralston (1965) agreed with other authors that the substantia gelatinosa maintained a modulatory effect. However, it was suggested that most dorsal root fibers entered lamina III and impulses reached the substantia gelatinosa polysynaptically.

Review of the Literature-Neuron

After a brief description of basic neuronal development, the review of the literature was presented in terms of individual cellular components rather than the neuron as a whole.

Neuronal Differentiation - General

Cajal (1929) in a classical interpretation of the histogenesis of the central nervous system, described three types of cells; the germinal cells of His lying under the internal limiting membrane, and being the stem cell for the neural tube; spongioblasts, constituting the "primitive ependymal layer", and precursors of the neuroglia; and neuroblasts scattered between the germinal cells and spongioblasts.

Cajal's interpretation has been questioned by many authors. Fujita (1962b), proposed the name of "matrix cell" for a group of cells, to include both "spongioblasts" and "germinal cells", and the term "matrix layer" as an alternative for the "primitive ependymal layer". Utilizing autoradiography Fujita (1963) stated that matrix cells are a homogenous cell population composing the entirety of the matrix layer and different cell types reported by earlier authors was one cell in different stages of the cell cycle. The matrix cell first produced neuroblasts which migrated into the mantle layer. Matrix cells then ceased to produce neuroblasts, lost their mitotic ability and differentiated into both neuroglia and ependymal cells.

The general description of neuronal differentiation is agreed upon by most investigators, however, the terminology used to describe the different stages of development varied with the author. Wecheler (1966), Meller et al (1966) and Pannese (1968) in the chicken, Tennyson (1965)

in the rabbit, Karlsson (1967), Caley and Maxwell (1968a) and Hannah (1972) in the rat, and Kornguth et al (1967) in the monkey, described a primitive cell with an ovoid nucleus and scanty cytoplasm containing free ribosomes. The earliest identifiable neuroblast contained small segments of endoplasmic reticulum and a primitive Golgi complex. As development proceeded there was a concommitent increase in the amount of rough endoplasmic reticulum and Golgi complex cisternae and a decrease in the number of free ribosomes. Secondary structures, such as lysosomes and multivesicular bodies appeared later in the developmental process.

Nuclear Differentiation

Radouco-Thomas et al (1971) published a comprehensive survey of nuclear development in neurons, utilizing the Purkinje cell of the rat as a model. The nucleus of the undifferentiated cell displayed condensed chromatin, which progressively dispersed during the early neuroblast stage to attain a homogeneous appearance, with chromocenters and chromonemata in older neuroblasts representing a gradual change from an active to inactive state. Caley and Maxwell (1968a) also noted this dramatic change in nuclear chromatin, and suggested that the change may represent a loss of mitotic potential or change in RNA synthesis. The author utilized this change as a primary indication for discriminating between the neuroblast and other cells in the developing CNS.

In the undifferentiated cell, the nucleolus was composed of a compact mass of fibrillar elements surrounding the presumed nucleolar organizer, with the granular element sometimes present at the periphery. A "segregation-like" phenomena, possibly a transitory state with the appearance of "nucleolar microbodies" was encountered. The nucleolus appeared mature very early in development, (Radouco-Thomas et al 1971).

In an earlier investigation, Hay (1968), working with frogs observed that the fibrillar component of the developing nucleolus was initially more obvious than the granular component. Radouco-Thomas et al (1971), described a transient "coarse chromatin-like entity" they designated "granular mass" in the nucleus at the beginning of differentiation. They also described the presence of a "spotted body", which was present throughout development, being fibrillar in nature and resembling the pars fibrosa of the nucleolus. It was suggested that the "body" originated from the nucleolus and was probably RNP in nature. This same structure has been observed in mature cells by Monneron and Bernhard (1969) in pancreatic, hepatic and HeLa cells and Hardin et al (1969) in rat trigeminal neurons.

Radouco-Thomas et al (1971) also described a nucleolar organizer comprised of light fibrillar areas, which were present at all stages of neuronal differentiation, and encircled by the dense fibrillar part of the nucleolus.

Biochemical analysis of the nucleolar organizer by such investigators as Birnstiel (1970) and Miller and Beatty (1969), has shown that it is composed of DNP and acts as a template for ribosomal RNA.

The nuclear membrane undergoes several alterations during development. In the "early" neuroblast, Radouco-Thomas et al (1971) reported "blebs" or expansions of the external nuclear membrane which became detached and released into the cytoplasm as inclusions or vesicles, which they suggested could be a mode of protein transfer.

Numerous authors have reported prominent connections between the nuclear membrane and granular endoplasmic reticulum for a limited time interval during differentiation; Meller (1966), Kornguth et al (1967), Caley and Maxwell (1968a), Pannese (1968), Nosal and Radouco-Thomas (1971)

Hannah (1972).

Endoplasmic Reticulum

Several hypotheses have been elucidated as to the biogenesis of the endoplasmic reticulum. Fawcett (1955) and Whaley et al (1964) in plant cells proposed a"de novo"synthesis; beginning with small smooth vesicles coalescing into cisternae of endoplasmic reticulum. Hay (1958), in cartilage, Wohlfahrt-Botterman and Moericke (1959) in salivary glands of invertebrates, Tennyson (1965) in neuroblasts of rabbit embryoes and Staubli et al (1966) in midgut epithelial cells of mosquitoes, stated that endoplasmic reticulum originated from the Golgi complex. Parks (1962) in parotid acinous cells, Behnke and Moe (1964) in paneth cells and Pannese (1968) in chickens, proposed that endoplasmic reticulum was derived from the nuclear membrane.

Wechsler (1965) in chick embryo neuroblasts proposed a dual scheme of endoplasmic reticulum formation, consisting of nuclear membrane expansion and"de novo"synthesis in the cytoplasm. A dual mechanism was also proposed by Meller et al (1966), implicating both the Golgi complex and the nuclear membrane in endoplasmic reticulum development.

The undifferentiated cell reported in the cerebellum by Nosal and Radouco-Thomas (1971), Pannese (1968) in chick spinal ganglia and Meller et al (1966) in primative neuroblasts of chick spinal cord, was characterized by the absence or paucity of the granular endoplasmic reticulum. This was also a characteristic of the primitive neural cells (indifferent cell) reported by Caley and Maxwell (1968a) in rat cerebral cortex, and Fujita and Fujita (1963) in "matrix cells" of the optic lobes of chicks. The first appearance of the endoplasmic reticulum allowed for the discrimination of "early" neuroblasts from

glioblasts along with other features with respect to nuclear texture and configuration, Caley and Maxwell (1968) and Pannese (1968). A direct connection existed between the developing endoplasmic reticulum and the nuclear membrane, in the "early" neuroblast.

In the "intermediate" neuroblast, most authors reported a substantial proliferation of endoplasmic reticulum cisternae. Caley and Maxwell (1968a), and Nosal and Radouco-Thomas (1971) reported a transient swelling of the endoplasmic reticulum at this stage. As neuronal development proceeded the endoplasmic reticulum assumed its adult configuration which varied with the cell type.

Smooth-surfaced endoplasmic reticulum appeared later than the rough endoplasmic reticulum and was often observed in continuity with it, Pannese (1968). Many authors reported the early emergence of subsurface cisterns connected to the endoplasmic reticulum, Radouco-Thomas et al (1970), Tennyson (1965), Pannese (1968), Caley and Maxwell (1968a).

Golgi Apparatus

Several hypothesis have been advanced as to the biogenesis of the Golgi complex. Ward and Ward (1968) in frog ova, suggested that the Golgi formed "de novo" from aggregates of fine filaments fusing into cisternae. The formation of Golgi from the endoplasmic reticulum was proposed by Novikoff (1962), Favard (1969), Claude (1970) in hepatic cells and Jamieson and Palade (1967) in the pancreas.

Evidence that the Golgi complex arose directly from the outer nuclear membrane was reported by Akerman (1962) in developing lymphocytic cells, Ziegel and Dalton (1962) in some protein secreting cells,

Fawcett and McNutt (1969) in heart muscle and Weston et al (1972) in numerous blood and tumour cells.

In the case of the nervous system, Caley and Maxwell (1968a) observed the Golgi complex in direct connection with the outer nuclear membrane of developing neurons of the rat cerebral cortex. Tennyson (1965) noted direct connection between the Golgi and the granular endoplasmic reticulum in rabbit spinal ganglion cells and implicated the Golgi in the possible source of granular endoplasmic reticulum.

Mitochondria

Fawcett (1966) stated that neuronal mitochondria were similar to mitochondria of other cells, in basic structure. Bunge et al (1965) described the mitochondria of fetal rat spinal cord as round and rod shaped and either bent, curved or branched. Karlsson (1966) observed that in the lateral geniculate nucleus of the rat 179 of 569 mitochondria were branched.

Mitochondria were abundant in neurons and were found mainly in the "roads" created by neurofilaments between the Nissl bodies in larger neurons (Bunge et al, 1967). Pomerat et al (1967), described the movement of mitochondria and other organelles and particles along the "roads" between discrete Nissl bodies. Barondes (1967) suggested that the neurofilaments and microtubules might be responsible for their propulsion.

Many authors, including Brightman and Palay (1963) and Bunge et al (1967), have reported the absence or near absence of the characteristic mitochondrial granules in neuronal mitochondria, which was perhaps a clue to their function. Lehninger (1967), identified these granules as

a calcium phosphate precipitate similar in nature to that of hydroxy-appatite or bone crystals.

Multivesicular Bodies

Multivesicular bodies were first described by Palay and Palade (1955). Palay (1963a), described these bodies in Pukinje cells as spherical (300-500mu in diameter), enclosing 1-12 small vesicles in a trilaminte wall. They occurred singly or in clusters in all parts of the cytoplasm. Frequently one side of the multivesciular body was flattened and a dense mass applied resembling a collapsed cisterna. This flattening was also reported by Pappas and Purpura (1961), who suggested that the thickening resembled those at the postsynaptic axodendritic sites. The small vesicles were described by Palay (1963b), as being 50-100mu in diameter with radially arranged striae extending from them, representing the walls of a honey-comb structure making up the limiting membrane of the vesicle. Palay (1960) described the vesicles found in secretory neurons of goldfish as two distinct types, each enclosed in a single membrane with a granular texture. Palay proposed that the small vesicles arose from the Golgi apparatus and the large vesicles from a gradual transformation of a multivesicular body. Herndon (1963), working with rat Purkinje cells, describing multivesicular bodies with varying amounts of dense granular material similar to the transitional forms described by Palay (1960). Barron et al (1967), described the presence of coated (alveolate) vesicles near the Golgi apparatus and multivesicular bodies with electron dense material partially occluding the vesicles suggesting a transitional form between multivesicular bodies and dense bodies in the lateral

geniculate nucleus of the cat.

Rosenbluth and Wissig (1964), injected ferritin into toads and observed the ferritin arriving at the neuronal surface. In the neuron it appeared in coated vesicles and then segregated into multivesicular bodies which suggested that the neuron acquired whole protein by pinocytosis. The authors suggested two possible explanations for the appearance of the tracer in the multivesicular bodies. Either the coated vesicles were multivesicular bodies in the process of being formed, with the limiting membrane of the multivesicular body formed by cell surface invagination or coated vesicles coalesed into an existing multivesicular body. Rosenbluth and Wissig (1964), also described two types of multivesicular bodies: dark with small vesicles in scant matrix of low density, and light with few vesicles in a large matrix of very low density.

Friend and Farquar (1967), studied peroxidase uptake in rat vas defferens and described two types of coated vesicles similar to those of Palay (1960). Large vesicles were found in the apical region of the cell and small vesicles in the Golgi region. The peroxidase was found only in the large vesicles and in multivesicular bodies. They proposed that the large coated vesicles transported absorbed protein to the lysosomes and the small coated vesicles from the Golgi complex served as primary lysosomes carrying enzymes to the multivesicular bodies. Holtzman et al (1967), confirmed that the small coated vesicles contain hydrolytic enzymes and emphasized the role of the Golgi associated agranular endoplasmic reticulum in the origin of lysosomes.

The lysosomal nature of the dense bodies has been well established; Novikoff (1960), Novikoff and Essner (1962), Novikoff, Essner and Quintana (1963, 1964b), Torack and Barrnett (1962), Osinchak (1964)

and Koenig et al (1964). In the previously mentioned papers, it was proposed that electron dense bodies arose from multivesicular bodies. However, Novikoff (1967) suggested another possibility; since he had observed multivesicular bodies in close proximity to smooth endoplasmic reticulum the enzymes might come directly from the endoplasmic reticulum bypassing the Golgi in some cases.

Agranular Endoplasmic Reticulum

Agranular endoplasmic reticulum may or may not have been a component of the laminated inclusions reported by authors in a variety of cells. Laminated inclusions have also been reported to exist in many different configurations.

Morales et al (1964) described a laminated body in the lateral geniculate nucleus of the cat. They observed alternating dense zones composed of tubules and lighter zones of a finely granular material. Along with the distinct absence of any ribsosomes each body enclosed some cytoplasm containing organelles. Similar inclusions have also been observed in the lateral geniculate nucleus by Peters and Palay (1966) and Barron et al (1967) and in the striate cortex by Kruger and Maxwell (1969).

In studies of the posterior horn neurons in the adult rat, Nathaniel and Nathaniel (1966a) observed that several smooth surfaced channels exhibited "lines" formed by the fusion of membranes limiting the channels resulting in elimination of the enclosed space. A similar arrangement in cortical neurons was observed by Rosebluth (1962) in the acoustic ganglion cells in the rat and Gulley and Wood (1971) in the medium sized neurons of the rat substantia nigra.

Similar configurations, but lacking "lines", were observed by

Adinolfi (1969) in the entopeduncular nucleus of the cat and by Anzil et al (1971) in rat striatal neurons.

Morales and Duncan (1966), described three types of laminated bodies in the cerebellum of the cat. Type 1 laminated inclusions in the stellate neurons were smaller, less complexly folded and fewer in number than those in the lateral geniculate nucleus, but were similar, with darker lines composed of tubules, (250Å) continuous with the endoplasmic reticulum. The second type were similar to those described by Nathaniel and Nathaniel (1966a) in posterior horn cells of rats. Two or more endoplasmic reticulum sacs in dense parallel apposition with dense material between them. No ribosomes were observed between the sacs but were occasionally numerous on the periphery. The sacs were continuous with both granular or agranular endoplasmic reticulum. Type 3 were described as knots of coiled tubules observed in Purkinje cells. This type was also observed by Sotelo and Palay (1968) in rat Purkinje cells.

Microtubules and Neurofilaments

Microtubules and neurofilaments were first described by Fernandez-Moran (1952, 1953).

Developmentally, it was observed by Bodian (1966) in monkeys and Peters and Vaughn (1967) in rats, that microtubules were present first and neurofilaments appeared secondarily. In rat optic nerves neurofilaments appeared as compact bundles, increased in number and became dispersed throughout the axoplasm (Peters and Vaughn, 1967).

The structure of neurofilaments was described by Palay (1964), Sanborn (1966) and Peters and Vaughn (1967). The most comprehensive assessment of neurofilament structure was proposed by Wuerker (1970).

Wuerker described neurofilaments as straight unbranched tubular structures of indefinite length, with a clear core and beaded wall. In cross section the wall was circular and formed by four globules and interconnected by 25Å thick crossbars. Fibrous sidearms radiated from the globules into the cytoplasm. The filaments were formed by stacking of these units. Glial filaments appeared much the same except the side arms were absent.

In large motor neurons, extensive "roads" between the Nissl substance were occupied by microtubules, neurofilaments and mitochondria, (Wuerker and Palay, 1969). Neurofilaments in dendrites were arranged in bundles and were situated peripherally while axonal neurofilaments were evenly dispersed and microtubules were situated peripherally. Peters and Vaughn (1967), suggested that neurofilaments might be formed by a breakdown of the walls of microtubules into their constituent filaments. However, Wuerker and Palay (1969) pointed out that microtubules were 2 to 3 times the diameter of neurofilaments and the walls were twice as thick, making the unravelling hypothesis unweildy. Wuerker and Palay (1969), suggested that it might be possible for the fibrous protein to break into its component subunits and reassemble into a new configuration.

As to the function of microtubules and microfilaments, a review by Schmitt (1968) proposed the following: intracellular transport, intracellular support, and mechanochemical transduction and movement. Wuerker and Palay (1969) pointed out that in dendrites of anterior horn cells of the rat, neurofilaments were arranged in fascicles, unlike other large neurons. They suggested that perhaps the filaments were concerned with cellular movement. Since the neurofilaments were arranged in the "roads"

between the Nissl substance, "It is possible that the fascicles of neurofilaments might constitute a molecular motor for the transport of substances from the Nissl bodies where they are produced into the processes where they are needed or consumed. The longitudinal arrangement of the filaments in fascicles might provide a mechanism for directional transport in contrast to simple non-directed diffusion". They also proposed that the side arms of the neurofilaments may have possessed a homologous muscle ATPase activity.

Subsurface Cistern

Subsurface cisterns were first described by Rosenbluth (1962), as stacks of dense membranes in close apposition to the plasmalemma. A continuity between the stacks of cisternae and the endoplasmic reticulum of the neuron has been reported by Herndon (1963), Hartmann (1966), Nathaniel and Nathaniel (1966a) and Siegesmund (1968).

Siegesmund (1968), utilizing neural tissue from squirrels, cats, opossums, rats and humans described the subsurface cisterns as "true" organelles composed primarily of a pentalaminate membrane and found in close proximity (130Å) to the neuron plasmalemma and usually found in apposition to glial cell processes, mainly astrocytes. Herndon (1963), Hartmann (1966), Nathaniel and Nathaniel (1966a) and Rosenbluth (1962), suggested that this arrangement represented a functional relationship, involving a flux of materials in or out of the neuron.

Ribosomes were commonly found around the inner membrane and in some cases mitochondria were found in close juxtaposition to the pentalaminate membrane, replacing the endoplasmic reticulum in that area. Due to the close proximity of mitochondria to the subsurface cisterns, Rosenbluth

(1962) and Herndon (1963), have suggested that the mitochondria might be supplying high energy compounds to these membrane systems presumably involved in the transfer of metabolites into or out of the cell.

Subsurface cisternae were also found in sensory cells by Engstrom (1958) and Smith and Sjostrand (1961) in the flight muscle of the dragon fly. The studies indicated that the subsurface cisternae may have had a dual function. It was hypothesized that the enclosed channel might not have only served as a pathway for exchange of metabolites but also as an internal conductor of excitation.

Growth Cones

Growth cones have been described by light microscopists, Cajal (1890) and Harrison (1910). The ultrastructure of growth cones was first described by Bodian (1966) in embryonic monkey spinal cord, as being composed of a swollen bulb (0.5 micra) containing a number of large empty vesicles (400-600Å) situated on the tips of both growing axons and dendrites.

Del Cerro and Snider (1968), in the rat cerebellum, described the ultrastructural development of growth cones. The process began beneath the cytoplasmic membrane characterized by an accumulation of clear vesicles (1,100Å). A vesicle filled process then bulged into the neuropil and was designated as a "primary growth cone". The processes then elongated and cell organelles moved into the proximal portion of the process. In growing axons both synaptic and growth cone vesicles were observed to co-exist in the same ending. Dendrites of Purkinje cells displayed growth cones; the development of each spine seemed to be preceeded by a growth cone. As development proceeded the number of growth

cones decreased substantially and disappeared by the end of the third post-natal week. Tennyson (1970) described growth cones in rabbit dorsal root ganglia but unlike Bodian and Del Cerro and Snider, observed microtubules, neurofilaments and mitochondria in the growth cones of 11-12 day fetal rabbits. Other authors have published micrographs in which growth cones were observable but were not described, Voeller et al (1963) in the neocortex of cats, Candiollo and Filogamo (1966) in the neural tube of chick embryoes and in an abstract by Larramendi and Lemekey (1966) in mouse cerebellum. Growth cones have also been identified "in vitro" by numerous authors such as Yamada et al (1971) working with cultured chick dorsal root ganglia cells, described tips of growing axons consisting of a conical enlargement containing numerous vesicles, smooth endoplasmic reticulum, neurofilaments and microtubules. Varying numbers of long thin microspike processes were observed extending out of the growth cone. The authors suggested that vesicles arising in the Golgi complex were transported through the axon to accumulate in the growth cone. The vesicles then fused with the plasma membrane, thus serving to produce axonal elongation.

Observations - Neuron

The term "neuroblast" has been used throughout the text to describe the differentiating neuron until it reached the mature state. The term "blast" which was generally used to connotate a dividing cell population did not accurately describe the post-natal neuron of the rat, which was a non-dividing cell. However, for consistency with the literature the word neuroblast was used to describe the differentiating neuron population.

Neuroblasts, during the first post-natal week, were distinguished from other cellular elements by two main criteria (Fig. 1). The nuclear chromatin was relatively homogenous in appearance and lacked the peripheral condensation of chromatin seen in glial elements. The cytoplasm was paler in appearance than that observed in glial cells and contained few organelles, other than rudimentary granular endoplasmic reticulum. Similar observations were made by Caley and Maxwell (1968a) in developing rat cerebral cortex and by Radouco-Thomas et al (1971) in the rat cerebellum. The majority of the neuroblasts, present at birth possessed a round or ovoid nucleus, usually devoid of a nucleolus (Figs. 2 and 3). The cytoplasm of these primitive neuroblasts was characterized by paucity of cell organelles. Thus, the cytoplasm exhibited few scattered ribosomes, short segments of granular endoplasmic reticulum and few mitochondria. The Golgi apparatus, when present, was rudimentary.

The earliest observable axo-somatic synapses were present in very small numbers in the neonate animal. The majority of axo-somatic synapses present during the first post-natal week were distinctly

different from those present in the mature substantia gelatinosa. The "early" axo-somatic synapse was characterized by thin, symetrical thickenings on both pre- and post-synaptic membranes. In the bouton itself, vesicles were few in number and mitochondria were rarely observed (Fig. 3).

During the first post-natal week, early evidences of neuronal maturation began to appear in both the nucleus and cytoplasm. The nuclei of these cells lost their circular outline and demonstrated the beginnings of nuclear invagination (Fig. 4). Nucleoli, when present, were very compact in appearance. The nuclear envelope exhibited numerous connections with granular endoplasmic reticulum. Because of multiple communications between the nuclear envelope and the endoplasmic reticulum, circumscribed areas of cytoplasm became entrapped (Figs. 4 and 5).

Also present, at the site of nuclear envelope-endoplasmic reticulum communication, were numerous round "bodies" or "blebs" (Figs. 4, 5 and 8). Similar "bodies" were also observed inside of cisternae of granular endoplasmic reticulum, some distance from the nuclear membrane (Fig. 8).

The cytoplasmic features at this period comprised the presence of well defined organelles (Fig. 4). The channels of granular endoplasmic reticulum increased in length and revealed several sites of communication with the perinuclear space (Figs. 4, 5 and 8). Segments of granular endoplasmic reticulum located close to the cytoplasmic membrane exhibited, quite often, localized dilation, resembling immature subsurface cistern (Fig. 6). The walls bounding such a dilation or cistern were asymmetrical in their morphology. The wall adjacent to the cell membrane was devoid of ribosomes, while the opposite wall had ribosomes attached

to it. Mature subsurface cisterns were also present, but were few in number (Fig. 7).

The Golgi complexes were composed of short stacks of cisternae with few associated vesicles. In many instances the developing Golgi were adjacent to the nuclear membrane at the sites of granular endoplasmic reticulum connection to the nuclear membrane (Fig. 8).

Several neurons showed short, bulbous cytoplasmic processes which possessed a large number of clear vesicles often grouped together in a terminal expansion, resembling growth cones (Figs. 9 and 10). The proximal position of these (Fig. 10) processes generally contained microtubules. Structures resembling growth cones were also observed in the neuropil.

A significant feature of the neuroblasts during the second week of post-natal development was the remarkable irregularity of the nuclear outline resulting in a considerable increase in the nuclear surface area (Fig. 11). In addition there was a considerable increase in the number of the cytoplasmic organelles. The granular endoplasmic reticulum was randomly distributed throughout the cytoplasm. Few instances of communication between the perinuclear space and granular endoplasmic reticulum were encountered. The Golgi complexes were well defined and consisted of many stacks of smooth cisternae associated with considerable numbers of smooth and coated vesicles. There also appeared to be an increase in the number of Golgi. Lysosome-like dense bodies were seen for the first time in these developing neuroblasts (Figs. 11 and 12).

By the end of the second post-natal week, the majority of axosomatic synapses appeared to be morphologically mature. Such synapses were characterized by an increased post-synaptic thickness and bouton containing numerous vesicles. Mitochondria were also commonly observed

in the bouton (Fig. 12).

A singular feature observed during the second week of post-natal development of these neurons was the appearance of cisterns. Basically a cistern consisted of dilated granular reticulum enclosing channels lined by agranular reticulum. These cisternal complexes were located within the cell as well as at the periphery. The configuration of these complexes varied from a simple finger-like cytoplasmic projection into the cisternal space to a highly complex, concentric configuration of these cytoplasmic evaginations (Figs. 13 and 14 A,B,C,D,E). In some neurons these evaginations were so packed that the membranes of the adjacent processes became closely approximated to give rise to the appearance of aligned dense structures. These dense lines were formed by the fusion of smooth surfaced membranes limiting the channels with resultant elimination of the enclosed space (Figs. 14 F and G). The ribosomal attachment to the cisternal wall stopped abruptly at the point where cytoplasmic processes projected into the lumen of the enclosed space, demonstrating the continuity between granular and agranular reticulum.

Differentiation during the third post-natal week was restricted to two main areas. A greater percent of cells possessed complex nuclear infoldings. The cytoplasm of the cells was increased in amount and demonstrated greater growth and complexity of smooth endoplasmic reticulum which were located within the confines of a dilated cistern of granular endoplasmic reticulum. Short segments of granular endoplasmic reticulum were also found distributed at random in the cell. Golgi complexes, lysosome-like electron dense bodies and multivesicular bodies were well formed and present scattered through the cell. In short, a large proportion of cells demonstrated morphological features described

in the adult neuron of substantia gelatinosa of the rat by Nathaniel and Nathaniel (1966a).

Discussion - Neuron

The post-natal differentiation of the neurons in the substantia gelatinosa appeared complete by the fourth week, during which time there was an overall increase in the nuclear-cytoplasmic ratio; the number of cytoplasmic organelles and complexity of nuclear convolutions.

One of the most prominent features of the differentiating sensory neuroblast was the progressive increase in nuclear convolutions. This alteration in nuclear shape might represent an increase in the activity of the cell. Similar nuclear irregularities were manifest in other very active cells, such as Ehrlich and Yoshida tumor cells, (Wessel and Bernard, 1957), mammerary cancer of the rat, (Shultz, 1957), and the Harding-Passey melanoma of the mouse, (Nathaniel, Friedman and Rychuk, 1968 and Loader and Nathaniel, 1972).

Nuclear evaginations or "blebs", containing detached segments of material resembling nucleoplasm, observed in neuroblasts around one week of age, have also been reported by Pannese (1966) in chick embryo spinal ganglia. Nosal and Radouco-Thomas (1971) and Radouco-Thomas et al (1971) observed similar evaginations in rat Purkinje cells and suggested they may represent a transfer of nuclear material into the cytoplasm. Similar findings have been reported in growing oocytes by Schauer and Wurzlman (1969) who proposed that the extruded material might have acted as a local DNA template for RNA synthesis.

At the same stage in development as the nuclear evaginations were present, numerous connections were observed between the granular endoplasmic reticulum and the nuclear membrane. Similar connections between granular endoplasmic reticulum and nuclear membrane were reported

in other neuroblasts by Caley and Maxwell (1968a), Pannese (1968) and Nosal et al (1971). These numerous connections suggested a possible role for the nuclear membrane in the biogenesis of the granular endoplasmic reticulum. Endoplasmic reticulum which appeared to develop from the nuclear margin and extended towards the periphery, might have been involved in the production of the immature subsurface cisterns which were present by the end of the first week.

It was interesting to speculate that at the time when neuroblast activity was demonstrated by an increasing cytoplasmic volume and production of axons and dendrites, there were many direct connections, via the granular endoplasmic reticulum, between the nuclear membrane and the subsurface cisterns. An early establishment of such a network, capable of transporting possibly large amounts of metabolites into the cell, might have been required to sustain growth.

By one week post-natal, the Golgi apparatus consisted of several short cisternae with few associated vesicles. The Golgi apparatus was frequently observed in juxtaposition to the nuclear membrane at sites of granular endoplasmic reticulum connections to the nuclear membrane. Vesicles or "blebs" were observed inside the cisternae of the granular endoplasmic reticulum at these sites and also in cisterns farther away from the nucleus (Fig. 8). At no time was the Golgi observed in direct communication with nuclear membrane or were there signs of vesicles pinching off from the outer nuclear membrane. However, the proximity of developing Golgi to areas of the nuclear membrane, where vesicles were present within the endoplasmic reticulum-nuclear envelope junctions, might have represented a clue to the biogenesis of the Golgi apparatus. Evidence that the Golgi apparatus arose directly from the outer nuclear membrane by pinching off of vesicles and tubules has been reported by

authors such as Fawcett and McNutt (1969) in heart muscle and Weston et al (1972) in a variety of actively growing blood and tumour cells.

A secondary addition to the membrane system was the appearance of the agranular endoplasmic reticulum in direct continuation with the granular endoplasmic reticulum, during the third week. The whorls of smooth membranes were described in neurons of the posterior horn by Nathaniel and Nathaniel (1966a). Similar membrane systems have been described in the lateral geniculate nucleus of the cat by Morales and Duncan (1966), in cortical neurons by Rosenbluth (1962), and in medium-sized neurons of the rat substantia nigra by Gulley and Wood (1972). An apparent metabolic role has been elucidated for agranular endoplasmic reticulum. Hendelman (1969), utilizing thalium poisoning in cultered neurons, suggested that the agranular endoplasmic reticulum may be involved in fluid transport. Several of the membrane systems were observed in continuity with subsurface cisterns and may have been involved in storage or concentration of substances entering or leaving the cell. The late appearance of the agranular membrane system, at a time when the neuron appeared otherwise mature, may represent the final changeover from a cell mainly involved with growth, to a cell in a mature state.

Structures resembling the growth cones described by Bodian (1966) in monkey spinal cord and Del Cerro and Snider (1968) in rat cerebellum, were observed in direct continuity with the cell body during the period of active cell process formation. The completely vesicle filled expansions were observed both bulging from the plasmalemma and forming the terminal end of longer processes. Because of the undifferentiated state of the few processes observed exhibiting this feature, the identity of the processes or to wheter they were axonal or dendritic in nature, could not be determined. Other growth cones were ob-

served in the neuropil, with mitochondria present, but the site of origin of these processes might have been from other areas of the spinal cord. Tennyson (1970), described axonal growth cones containing vesicles, mitochondria, and segments of agranular endoplasmic reticulum, in rabbit dorsal root ganglia. Tennyson suggested that the reported variations in growth cone appearance might have been because of different states of maturation during development.

The only synaptic contacts examined during this study were of the axo-somatic type. The main reason for this discussion was the feeling that without utilizing specific techniques such as phosphotungstic acid, an exact determination of synaptogenesis was unattainable. The magnitude of such a study would have been beyond the general descriptive purpose of this thesis. However, this author realized the importance of such a study and would attempt it at a later date.

For the purpose of a very general description of synaptogenesis, the axo-somatic synapse was chosen because of ease in identification.

Although several axo-somatic synapses were observed at birth, it was not until one week post-natal when such synapses were encountered with regularity. During the second post-natal week there appeared to be a large increase in the number of axo-somatic synapses observed on any one individual cell. The axo-somatic synapses observed in both the neonate substantia gelatinosa and subsequently during the first post-natal week, differed in structure, from the mature axo-somatic synapse. Most of the synapses during this period were characterized by relatively thin, symmetrical, pre- and post-synaptic thickenings. Pre-synaptic vesicles were few in number and mitochondria were only rarely encountered.

During the second post-natal week, the synapses appeared morphologically mature. The mature axo-somatic synapses resembled Grey's Type II synapses, confirming the findings of Nathaniel and Nathaniel (1966b). The major alterations in synaptic appearance was an increased thickening of the post-synaptic membrane, an increase in the numbers of pre-synaptic vesicles and the presence of pre-synaptic mitochondria. These findings on synaptic development were in agreement with those of many other investigators as reviewed by Bunge et al (1967).

Whether or not the "early" synapses were physiologically active, remained unknown. A possible answer to this problem was reported by Bodian (1966), working with developing monkey spinal cord, who suggested that "onset of a particular function follows closely upon the minimal synaptic development essential for the purpose".

Summary - Neuron

1. Neuroblasts in the neonate substantia gelatinosa were characterized by an ovoid nucleus, scanty cytoplasm and rudimentary granular endoplasmic reticulum.

2. At the end of the first post-natal week, neuroblasts demonstrated the beginnings of nuclear invagination and numerous connections existed between the granular endoplasmic reticulum and the nuclear envelope. Cytoplasmic processes were few in number and some exhibited growth cones.

3. At two weeks post-natal, neuronal differentiation was much more advanced. Nuclear contour was more irregular and the cytoplasm contained a well developed granular endoplasmic reticulum, multiple Golgi complexes and numerous multivesicular and lysosomal-like bodies. Axo-somatic synapses were commonly present.

4. During the third post-natal week a greater number of neurons possessed highly irregularly shaped nuclei. Agranular endoplasmic reticulum "systems" appeared for the first time. Cells possessing the complex smooth membrane "systems" were considered to be mature neurons.

FIGURES - NEURON 1-14

Immersion Fixation	Perfusion Fixation
Figures 1-3	Figures 4-14

FIGURE 1. This micrograph represents the substantia gelatinosa at birth. Note the large amount of extracellular space present. The three neuroblasts (N) which are present, demonstrate ovoid nuclei with diffuse nuclear chromatin and scanty amounts of cytoplasm. A glioblast (G) characterized by the high overall electron density and thin rim of cytoplasm is also present.

X 7,660

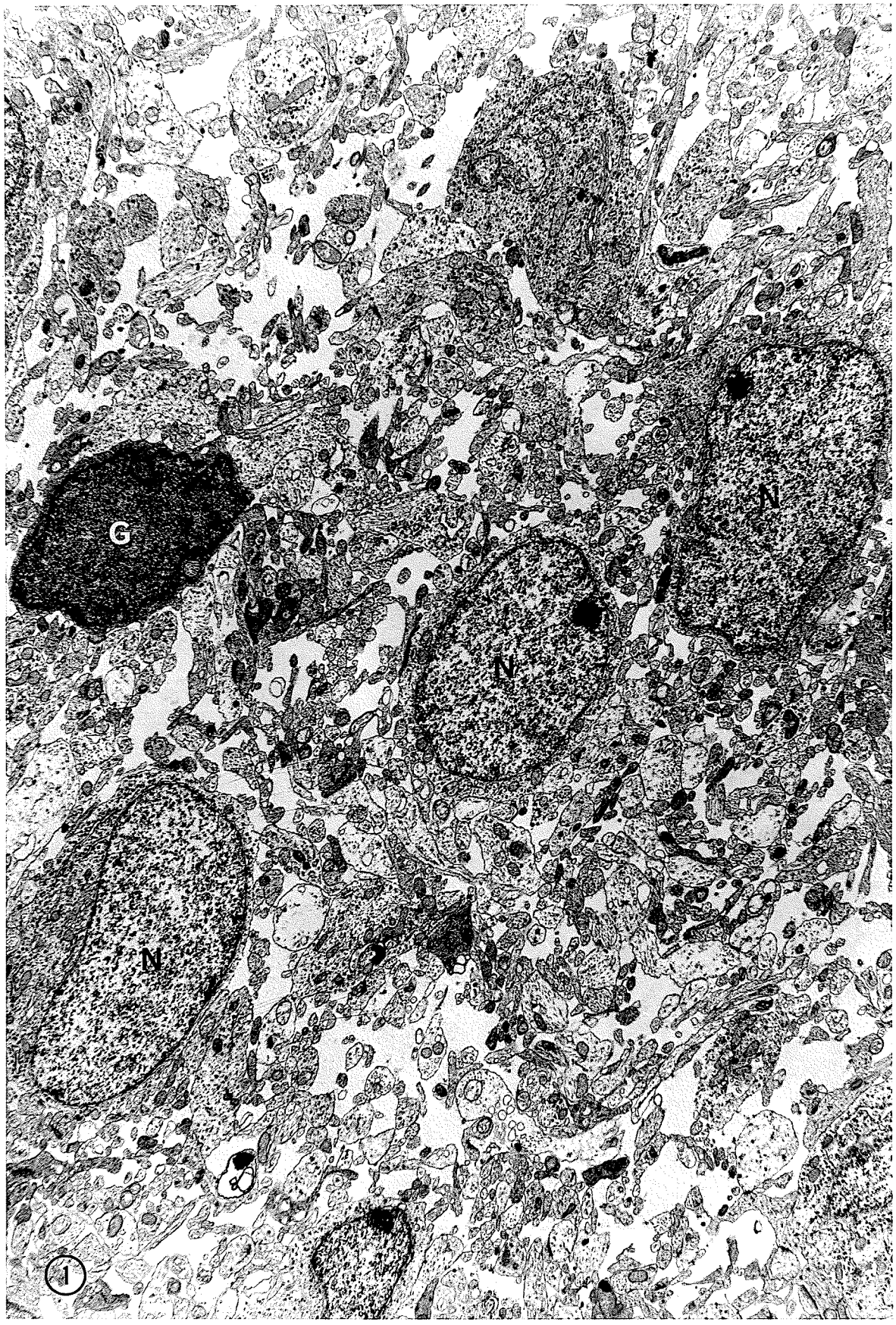

G
N
N
N
1

FIGURE 2. A neuroblast at birth which demonstrates an ovoid nucleus with a diffuse nuclear chromatin pattern. The thin rim of cytoplasm contains only scattered free ribosomes. Note the large amount of extracellular space present.

X 21,890

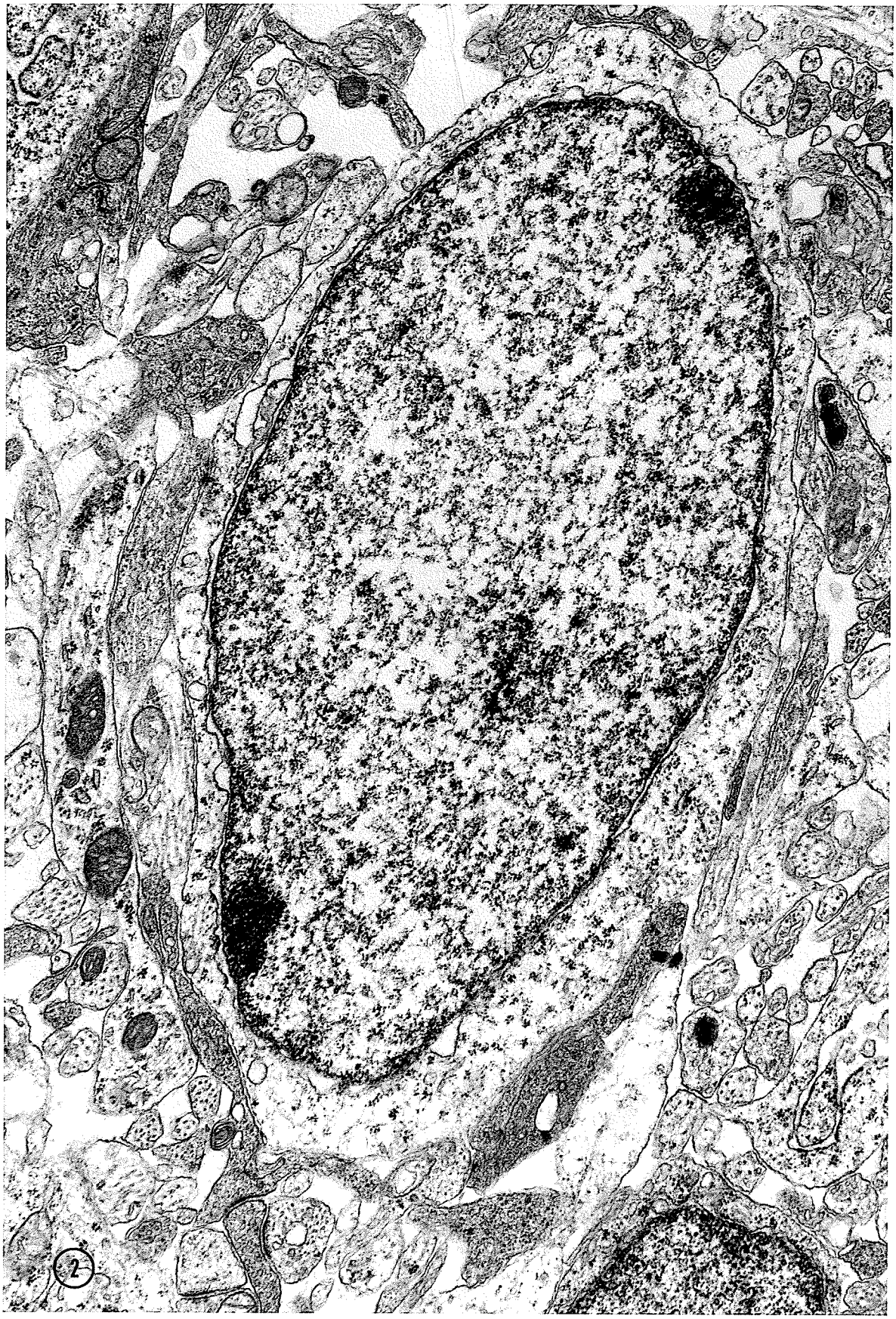
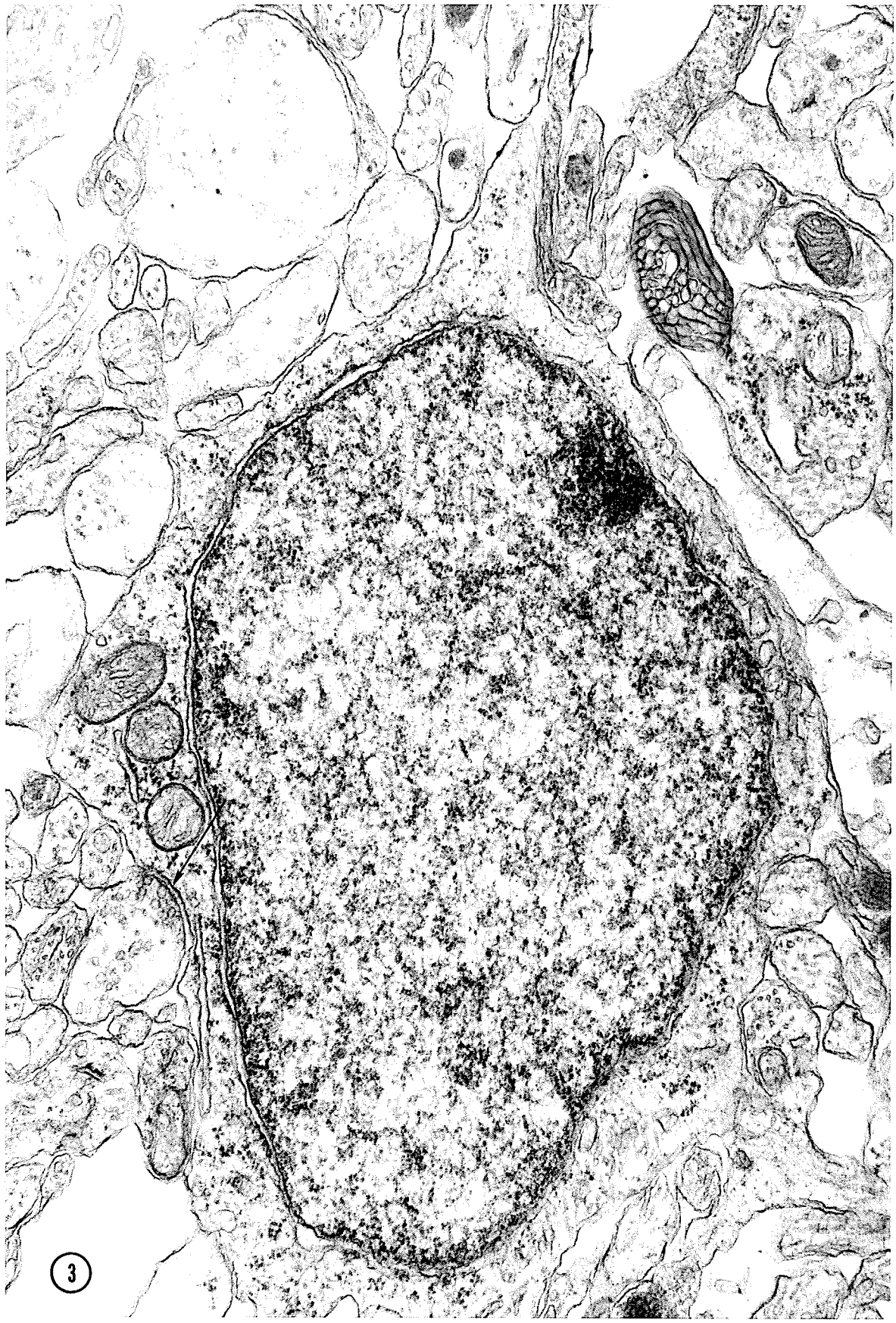

2

FIGURE 3. Neuroblast at birth with few cytoplasmic organelles. An axo-somatic synapse is present (arrow).

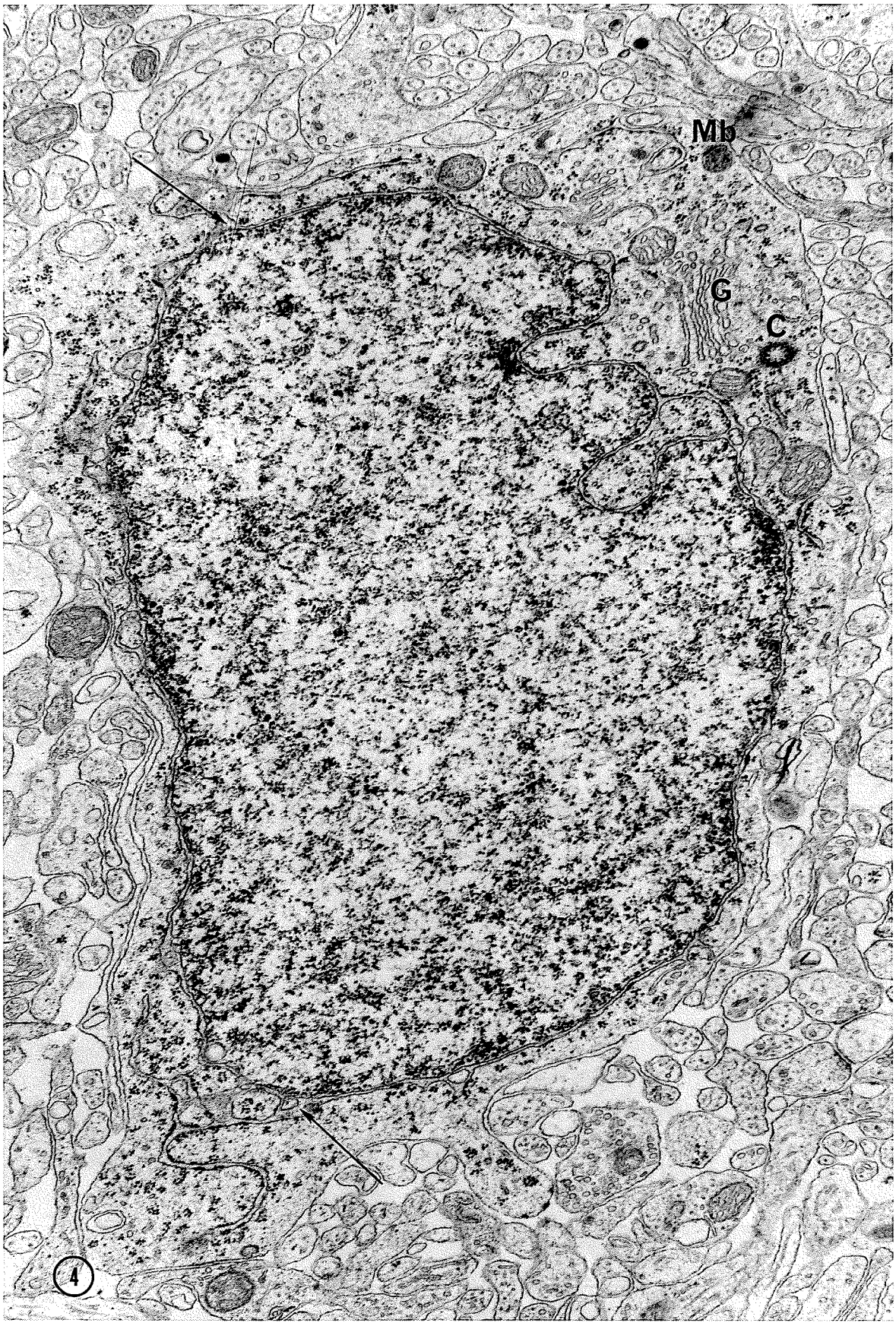
X 36,900



3

FIGURE 4. A neuroblast at one week of age. The nucleus demonstrates the early signs of nuclear invaginations. The granular endoplasmic reticulum is in communication with the nuclear envelope at many sites (arrows). A small Golgi apparatus (G), multivesicular body (Mb) and centriole (C), are also present.

X 27,520


Mb
G
C
4

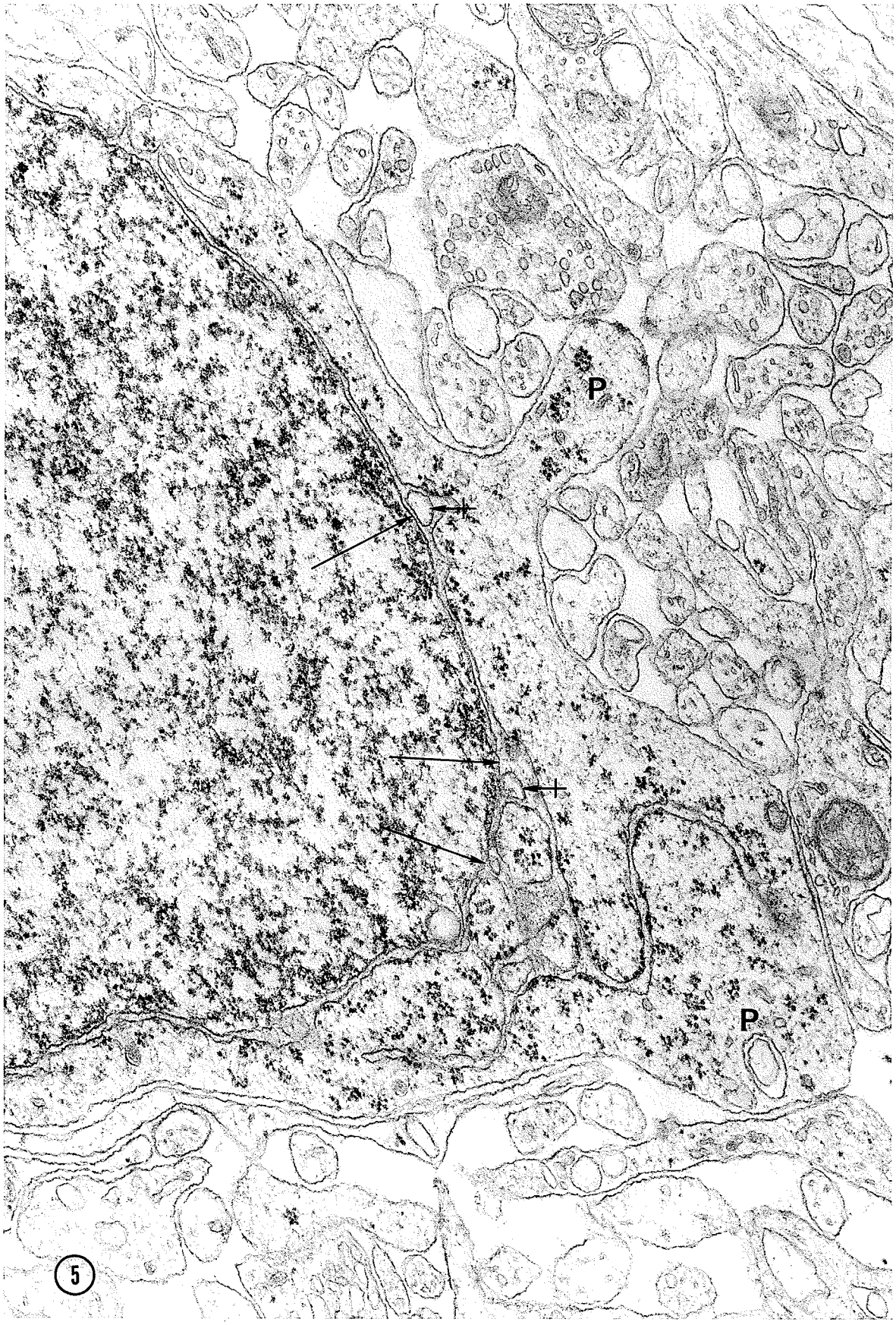
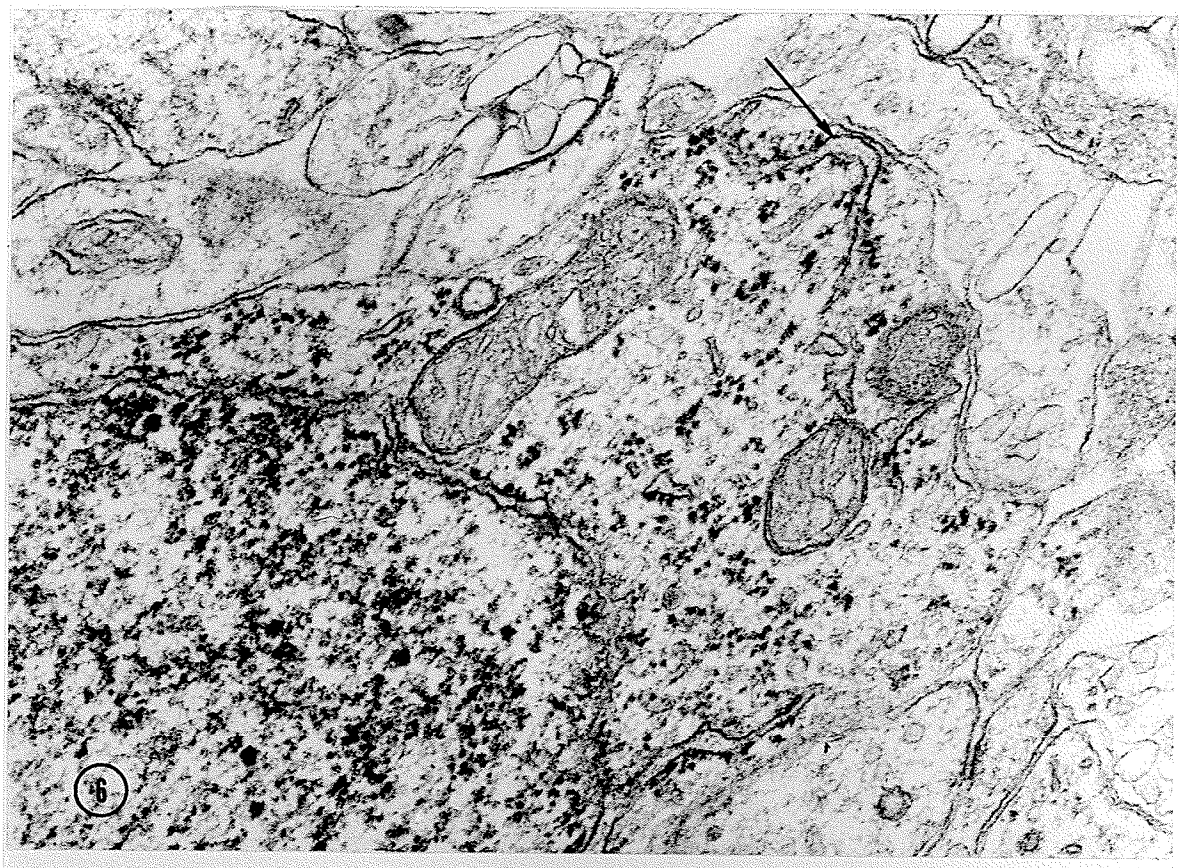

P
P
5

FIGURE 6. Portion of one week old neuroblast containing a primitive subsurface cistern. The side of the granular endoplasmic reticulum adjacent to the plasmalemma is devoid of ribosomes (arrow).
X 59,000

FIGURE 7. A mature subsurface cistern from a five week old animal is situated adjacent to an astrocytic process containing filaments (Fil). The subsurface cistern is in communication with the granular endoplasmic reticulum (arrow). A mitochondrion (M) was commonly found in juxtaposition to the subsurface cistern.
X 64,200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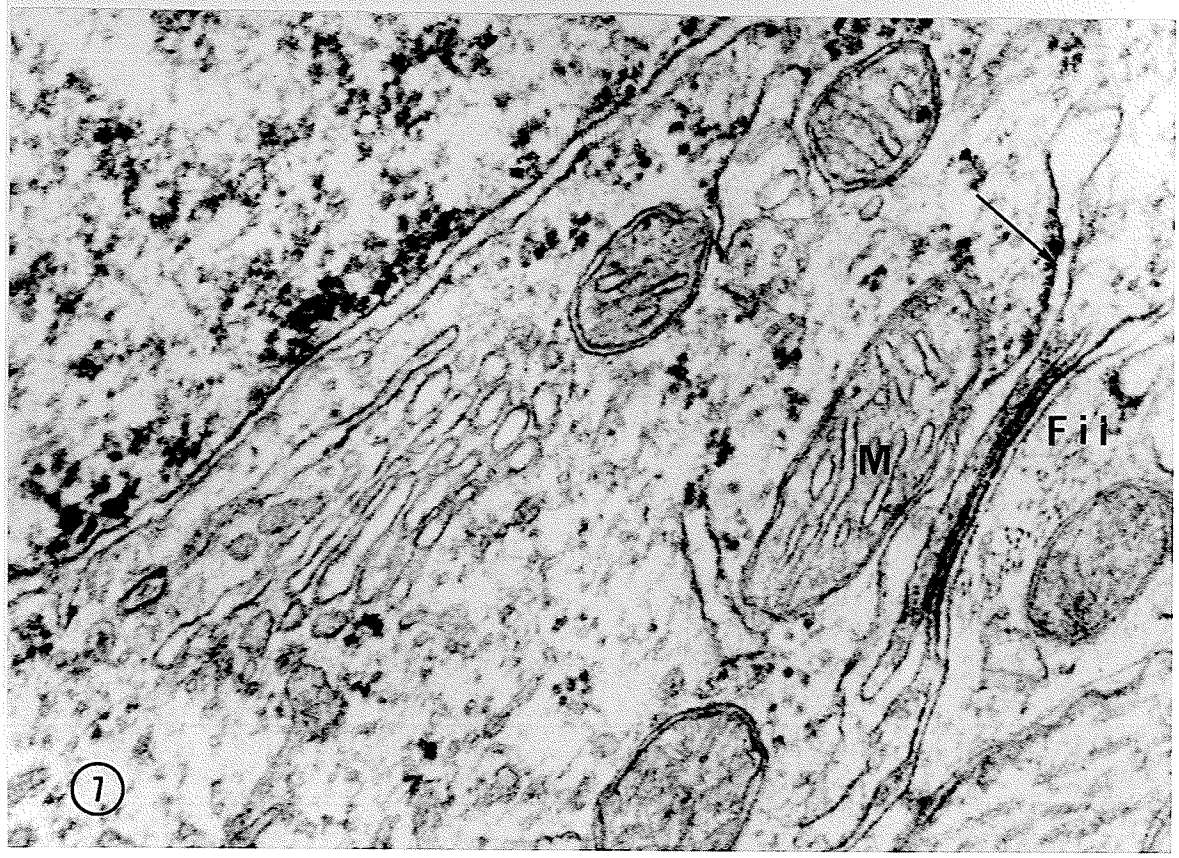

M
Fil
7

FIGURE 8. Multiple Golgi complexes (G) were commonly situated adjacent to the communications between the endoplasmic reticulum and the nuclear envelope by the end of the first post-natal week. Note the presence of "blebs" or vesicles in the endoplasmic reticulum-nuclear envelope junction (arrow) and in two isolated cisternae of endoplasmic reticulum (crossed arrows).

X 58,400

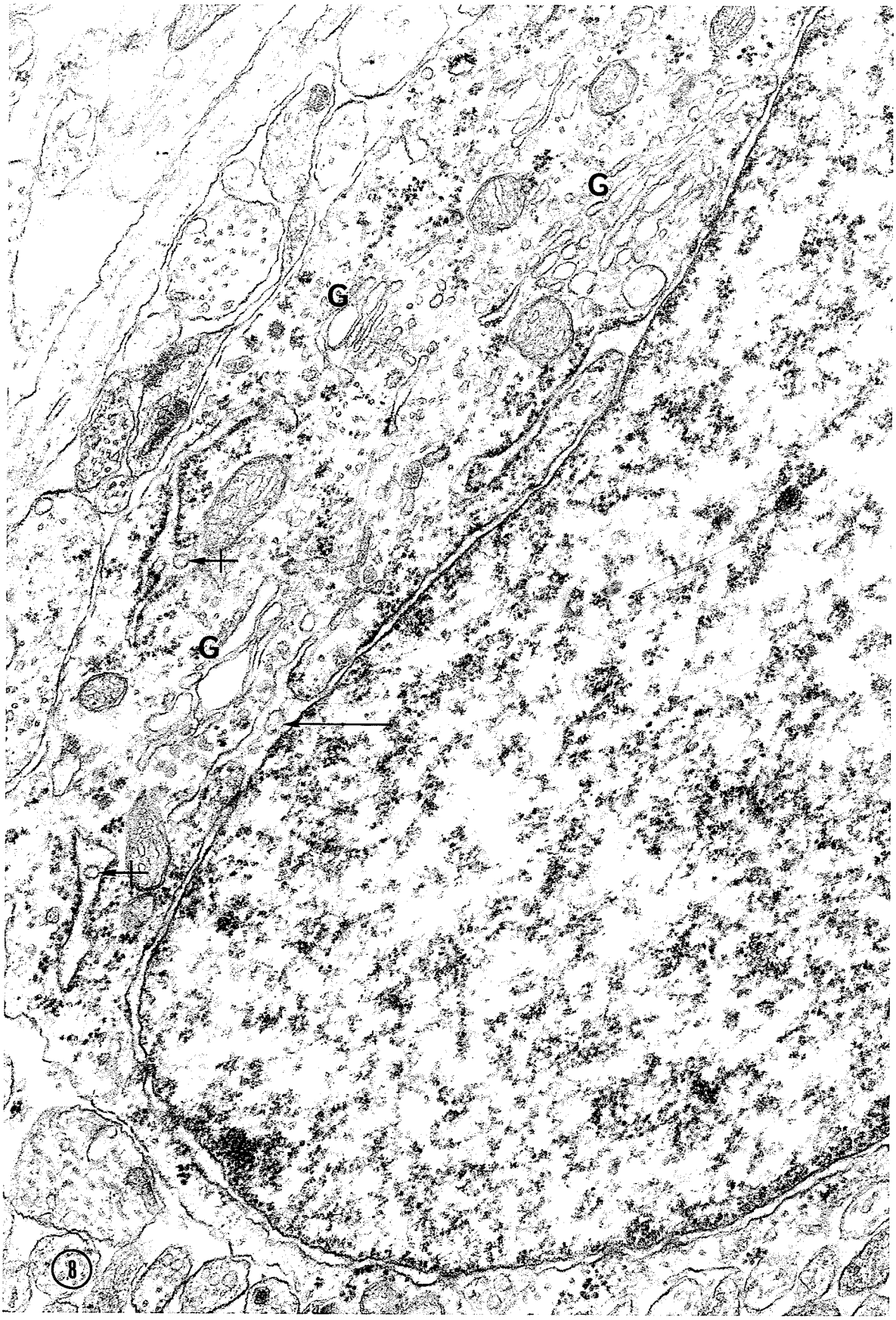
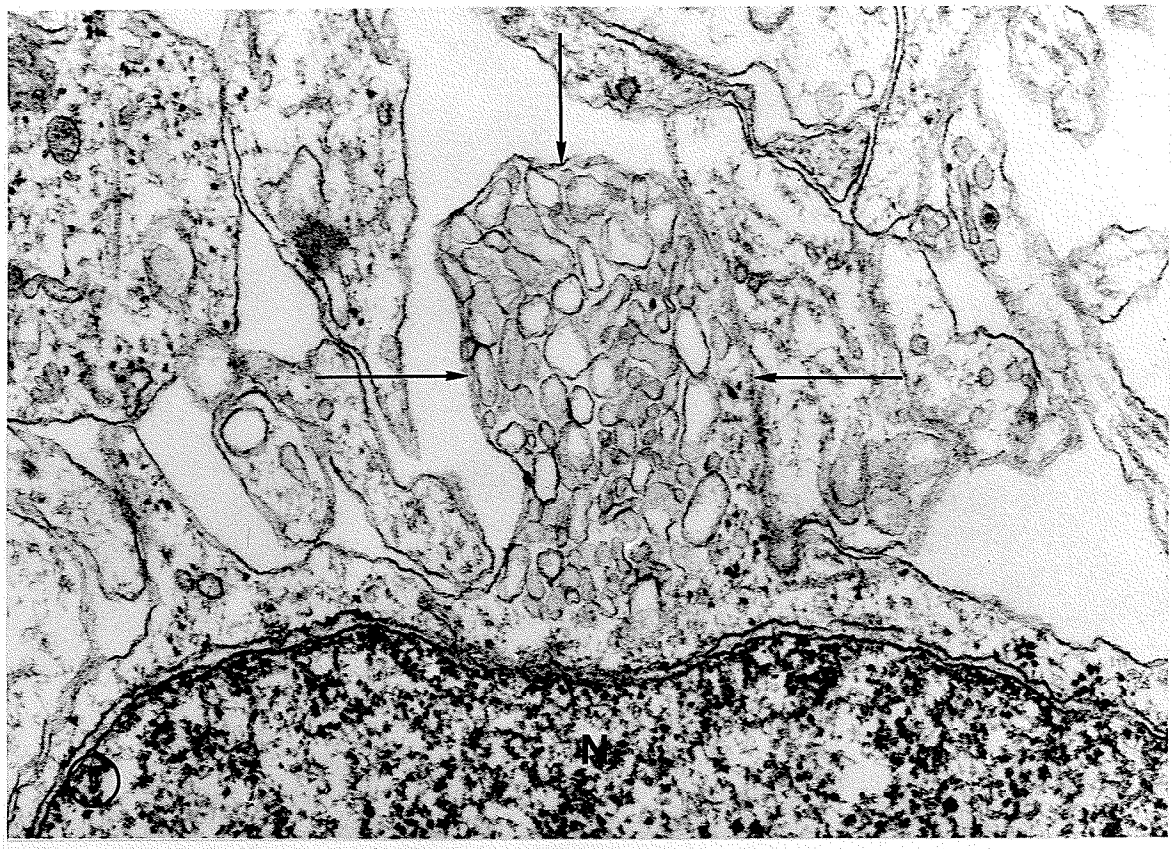

G
G
G
8

FIGURE 9. A growth cone (arrows), from a week old animal, containing large clear vesicles, may be observed protruding directly from the cell surface. The nucleus (N) is present at the bottom of the micrograph.
X 51,000

FIGURE 10. A growth cone, from a one week old animal, composed of large, clear, vesicles is present on a cytoplasmic process (arrow). Microtubules (Mt), short segments of agranular endoplasmic reticulum and a mitochondrion are also present in the growing cytoplasmic process. The nucleus (N) is situated to the right of the micrograph.
X 47,200


N

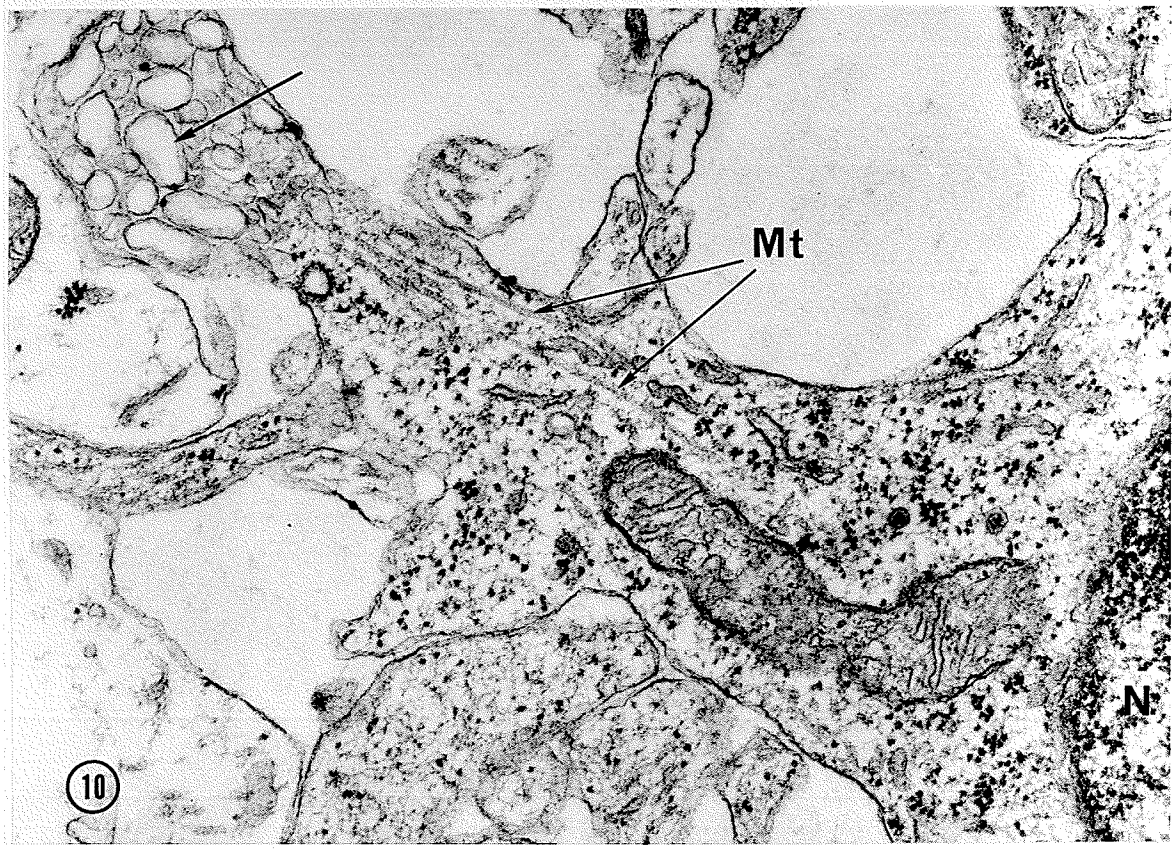

Mt
N
10

FIGURE 11. A portion of neuroblast from a two week old animal which demonstrates elaborate irregularities in nuclear contour.

X 59,400

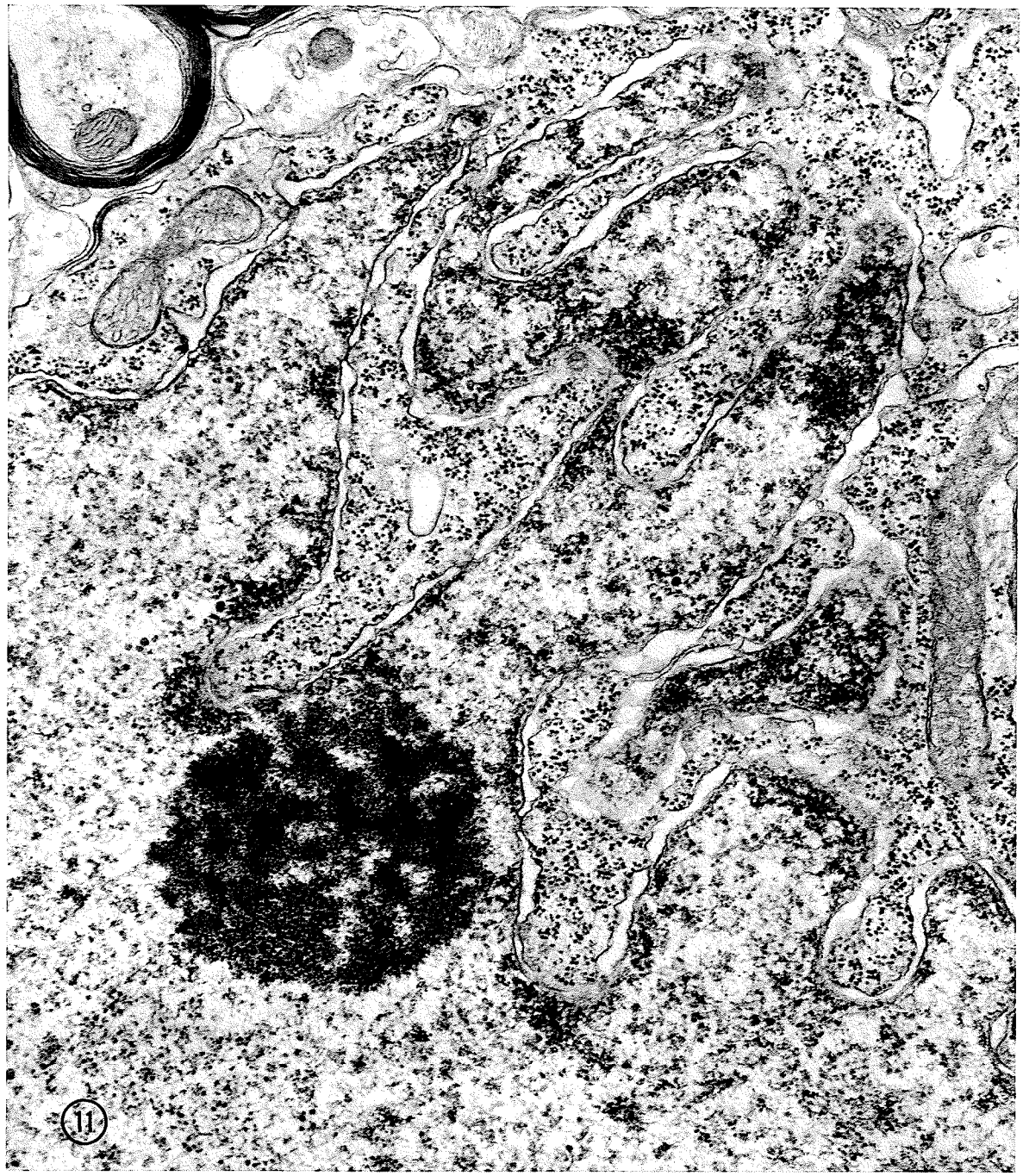

11

FIGURE 12. The cytoplasm of a neuroblast from a two week old animal containing well developed Golgi complexes (G), numerous mitochondria and lysosome-like bodies (B). The arrows demonstrate the numerous axo-somatic synapses present. Numerous coated vesicles (asterisk) may be observed in relation to the Golgi complex.

X 33,860

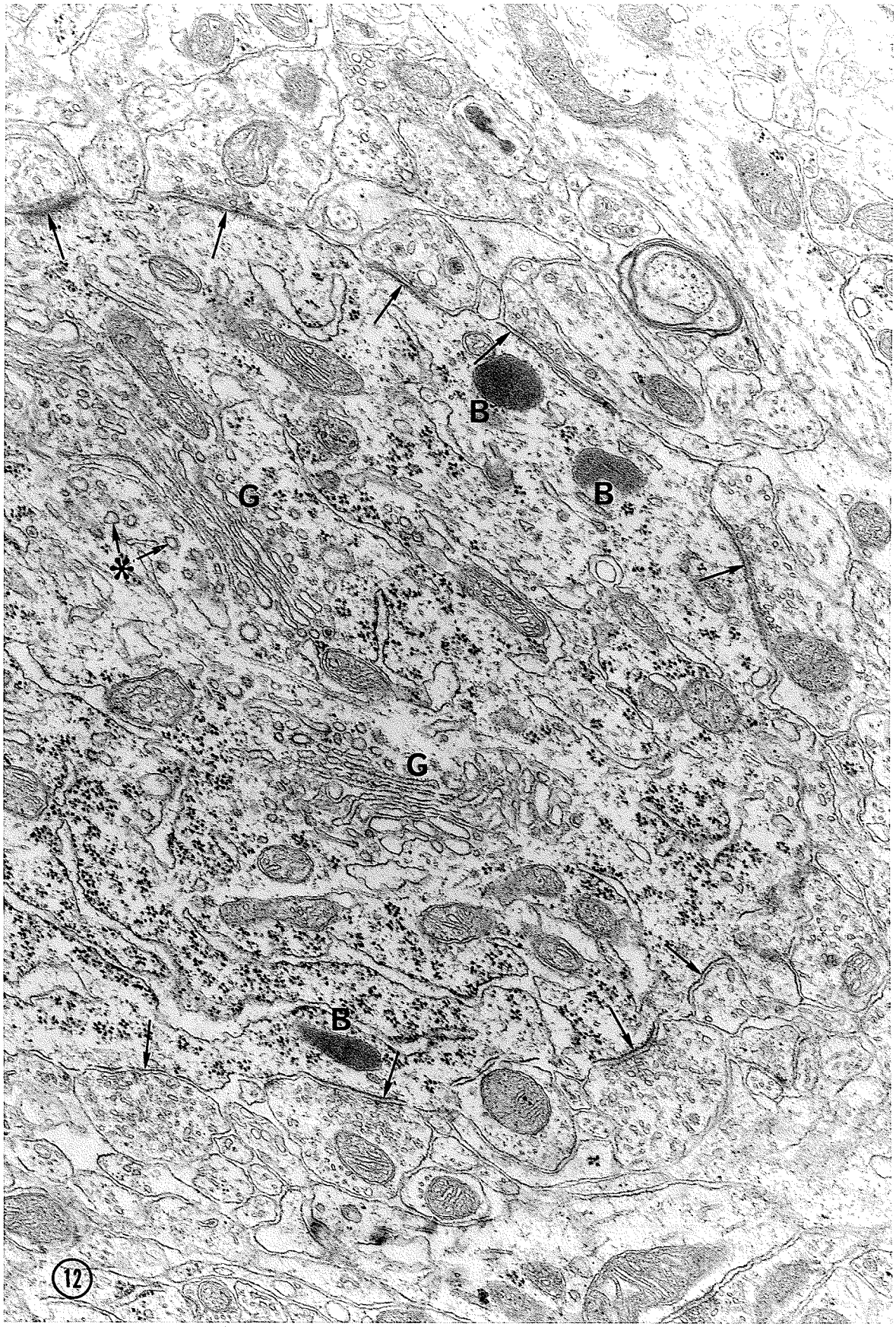

B
B
G
G
B
12

FIGURE 13. This cell from a three week old animal is designated as a mature neuron because of the presence of agranular membrane systems (arrows). Other mature characteristics include the disorderly array of the granular endoplasmic reticulum, multiple Golgi complexes, lysosomal-like bodies, multivesicular bodies, numerous mitochondria and several axo-somatic synapses.

X 28,100

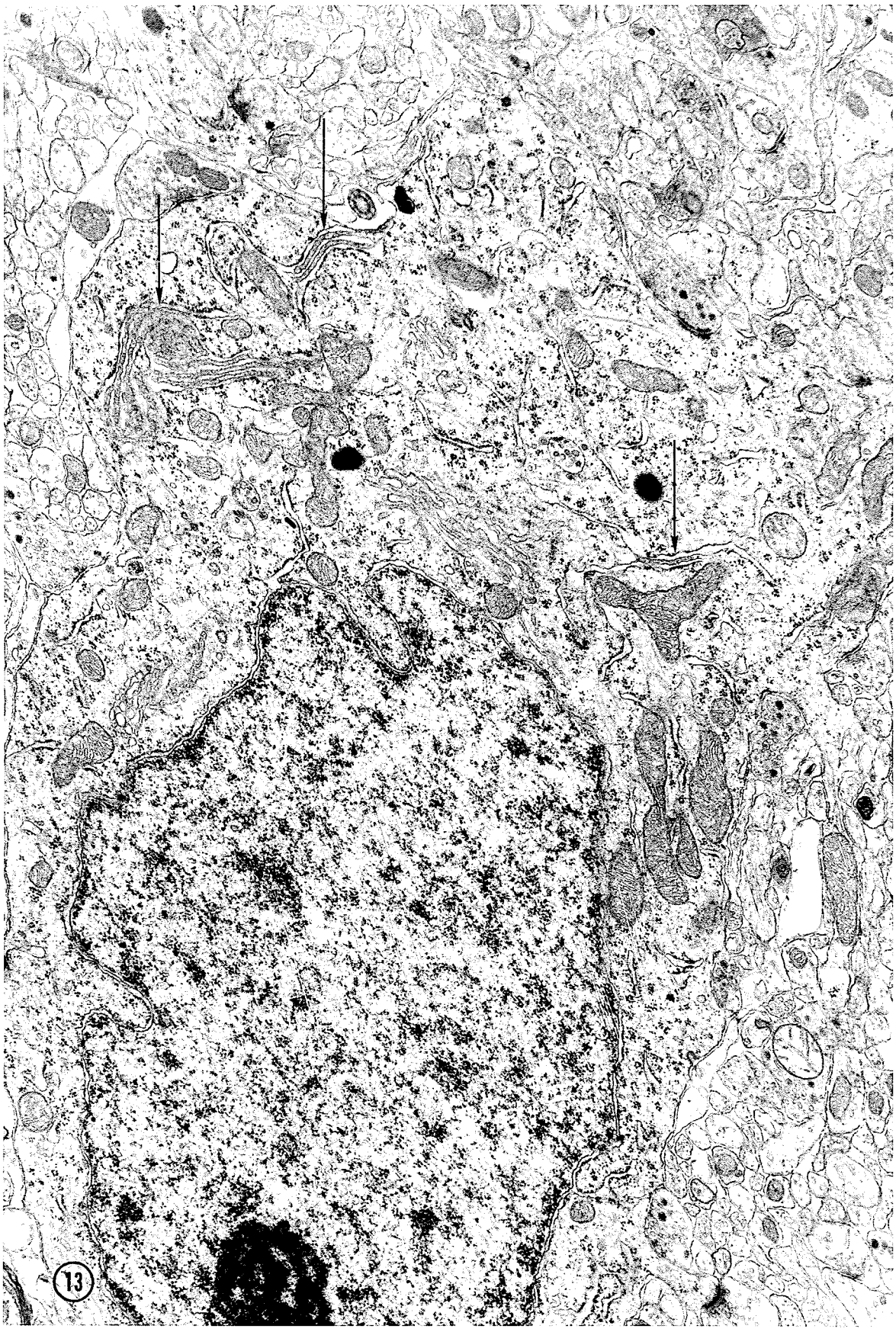

13

FIGURE 14. This series of micrographs illustrates the possible developmental pattern of the agranular membrane systems.

14-A. A single agranular membrane enclosed within a larger cistern of granular endoplasmic reticulum.

X 41,040

14-B. Similar to 14-A with two agranular membranes present.

X 58,500

14-C. Similar to 14-A with three agranular membranes present.

X 45,960

14-D. Numerous agranular membranes enclosed within a granular cistern.

X 35,910

14-E. A whorl of agranular membranes enclosed within a large granular cistern. Note the proximity and direct communication to the subsurface cistern (arrow).

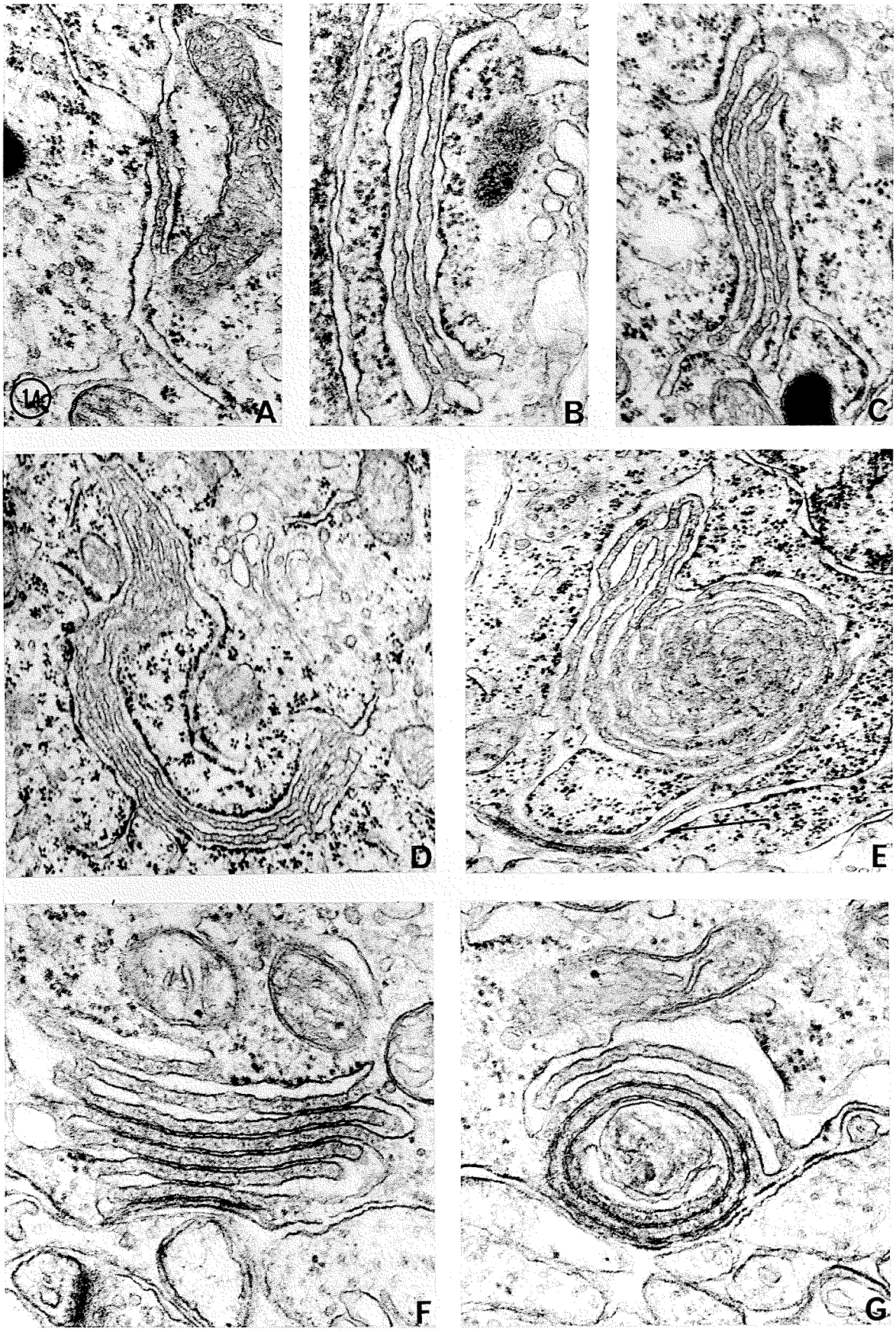
X 38,200

14-F. The dense lines formed by the approximation and fusion of adjacent agranular membranes are demonstrated in this micrograph.

X 64,700

14-G. A whorl of agranular membranes which is similar to 14-E but with dense lines present.

X 64,700


14
A
B
C
D
E
F
G

Review of the Literature - Blood Vessel

Many studies have attempted to describe the post-natal growth of blood vessels of the central nervous system, utilizing quantitative methods, Craigie (1925), Petren (1938), Alsen and Petren (1939), Gyllenstein (1959), and Sakla (1965). The main finding was an intense period of vascularization during the first post-natal month, after which time there was little change.

Klosovskii (1963), suggested that brain capillaries developed as solid cords of cells sprouting from existing blood vessels. They subsequently joined with efferent vessels, at which time a lumen appeared in the cord, completing the circuit. Pessacq (1971), described cords of cells branching from patent vessels in human cerebral cortex. Caley and Maxwell (1970), examined the cerebral cortex of the rat and suggested that most of the vessels developed during the first ten days, as solid cords of mesodermal cells, containing a non-patent lumen. Caley and Maxwell concurred with Strong (1961) and suggested that the lumen became patent due to internal pressure and blood flow became established within a short period of time, when connection was attained with another patent vessel.

In general the capillaries of the central nervous system, with the exception of the hypothalamus, possessed a continuous layer of endothelial cells. The vessels were completely enveloped by a basal lamina, which split to envelop the discontinuous layer of pericytes. Pinocytotic vesicles were sparce and adjacent plasma membranes were fused over extensive areas.

The endothelial cell cytoplasm has been described as being relative-

ly thick in immature blood vessels, becoming attenuated as development proceeded (Donahue and Pappas, 1961, Caley and Maxwell, 1970, and Phelps, 1972). A conflict existed as to the rate at which the attenuation took place. Caley and Maxwell (1970) suggested the attenuation was fairly rapid, while Phelps (1972) reported a gradual attenuation.

Donahue (1964), described presence of large numbers of pinocytotic vesicles and "flaps" or "flanges" of cytoplasm projecting into the lumen of immature blood vessels. Utilizing thorium dioxide injections into the blood stream of rabbits of varying ages, the cytoplasmic projections were observed curving over and coalesing with the plasma membrane, forming large vesicles of thorium dioxide in the cytoplasm. The tracer was also taken up by "conventional" pinocytosis, resulting in small vesicles containing thorium dioxide. Cytoplasmic projections, large and small vesicles appeared to decrease as the vessel matured. Donahue suggested that the formation of large vesicles by the cytoplasmic projections and very active pinocytosis, represented a mechanism for the increased uptake of metabolites and was related to the needs of the actively growing central nervous system.

The presence of the basal lamina around the endothelial cells, both pre- and post-nataly, was confirmed by Donahue and Pappas (1961), and Caley and Maxwell (1970), in the cerebral cortex of the rat, Phelps (1972) in the rat spinal cord and Weschler (1965) and Delorme (1968) in the avian brain. Donahue and Pappas (1961) and Caley and Maxwell (1970), noted an increase in basal lamina thickness with increasing age. Caley and Maxwell (1970), suggested a relationship between the thickness of the basal lamina and the astrocytic investment of the vessel. When astrocytic end feet were applied to the vessel wall, the basal lamina was thicker at that site, than in other areas where the basal lamina was

in communication with the extracellular space.

Junctional complexes between endothelial cells, regarded as "tight junctions" by Brightman and Reese (1969), were described as long tortuous structures, with fusion of adjacent cell membranes. Such junctional complexes have been observed between endothelial cells, even in the most primative vessels by Donahue and Pappas (1961), Caley and Maxwell (1970), Phelps (1972) and Hannah and Nathaniel (1972).

Pericytes, (cells imbedded in the basal lamina but not forming part of the luminal wall), have been described to be present with the earliest identifiable vessels by Donahue and Pappas (1961), Donahue (1964), Delorme et al (1968), Caley and Maxwell (1970), Phelps (1972) and Hannah and Nathaniel (1972). At no time were cell junctions observed in relation to the pericytes.

The rate of astrocytic investment of the blood vessels, demonstrated a different time course, not only between different species of animals but between different regions of the central nervous system in the same species. For example, in the rat, Caley and Maxwell (1970), reported the astrocytic investment complete with junctional complexes by 21 days postnatal, in the cerebral cortex. However, Phelps (1972), stated that investment was complete before birth in the anterior grey horn of the spinal cord, with no mention of junctional complexes.

The blood-brain barrier has been known to exist for many years, dating back to the work of Goldman (1908), protecting the nervous system from absorbing excess electrolytes and colloids, by maintaining a steady balance. The barrier also prevented the passage of large molecules, including toxic substances, from entering the nervous system.

In terms of the blood-brain barrier in developing animals, it has been demonstrated for a variety of substances by authors such as Himwich

and Himwich (1955), Dobbing and Sands (1963), Vernadakis and Woodbury (1965) and Luciano (1968), that the passage across the barrier decreases with increasing age.

Dobbing (1968) suggested that the increased permeability in the blood-brain barrier in the developing brain may have merely been a reflection of the increased metabolite requirements during the "growth spurt", (period of maximum growth by weight of the brain). Therefore, the controlling factor on incoming metabolites may be either the increased demand for metabolites during the growth spurt or the blood-brain barrier itself.

The actual anatomical site of the blood-brain barrier remained controversial. Dempsey and Wislocki (1955), suggested the basal lamina of the capillary, while De Robertis and Gershenfeld (1961), Gray (1964) and Delorme et al (1968), suggested the perivascular end feet as the site of the barrier. Perhaps the most convincing possibility for the site was suggested by Reese and Karnovsky (1967) and Brightman and Reese (1969), to be the tight junctions between the endothelial cells. However, it appeared that one or more of the above mentioned structures may have acted as a barrier to different substances and permability may have varied in different areas of the central nervous system, as suggested by Luciano (1968).

Observations - Blood Vessel

Sections of one-half micron thickness, of the posterior horn, were examined with the optical microscope, to determine the number of blood vessel profiles at different ages. Although not enough specimens were examined for a statistical analysis, there appeared to be a large increase in the number of blood vessel profiles from one week to three weeks of age, after which time, the number of vessel profiles exhibited only a slight increase, up to the six week period examined (Figs. 15 and 16).

Many blood vessels present in the posterior grey horn of the spinal cord at birth appeared to be non-patent. Non-patent blood vessels were identified by the presence of a slit-like lumen, which was present in both immersion, (Fig. 17), and perfusion fixed tissue, (Fig. 18). A basal lamina and astrocytic end-feet were also a constant feature.

The nuclei of endothelial cells of non-patent vessels were irregular in outline, containing large amounts of clumped chromatin. The cytoplasm which surrounded the slit-like lumen was relatively thick, contained many free ribosomes and exhibited a high electron density. Many vesicles were present which appeared to be pinocytotic in origin, with vesicles opening to both the lumen and the basal lamina surfaces, (Fig. 19). Pseudopod-like projections of the cytoplasm, also extended into the lumen. The basal lamina was present throughout post-natal development. During the first week, segments of the endothelial wall not covered by glial processes and in continuation with the extracellular space, possessed a basal lamina which appeared as a thin irregular band of flocculent material. However, when astrocytic processes were in juxtaposition to the cytoplasmic membrane, the basal lamina appeared more defined and much thicker, although not as uniform in thickness as

in mature vessels,(Figs. 17 and 18). Astrocytic investment of the blood vessels was completed by the end of the first week of post-natal development.

Several vessels, during the first two weeks, were observed with an ovoid lumen, possessing an attenuated endothelial wall, yet retaining the electron dense appearance of the cytoplasm. Pinocytotic vesicles, pseudopod-like projections and a basal lamina of non-uniform thickness, characteristic of less advanced vessels were also present. These vessels were present in both immersion, (Fig. 20) and perfused specimens, (Fig. 21).

By the end of the second post-natal week, most vessels appeared mature with a patent ovoid lumen. The nuclei were less dense and conformed to the contour of the lumen. The cytoplasm was markedly attenuated with reduced electron density. Pseudopod-like projections were reduced in size to structures resembling microvilli. Zonulae occludents (tight junctions) were observed between endothelial cells in all stages of development studied. The junctions were very long and tortuous in appearance, (Figs. 22A and 22B).

Although astrocytic investment of the blood vessels was complete by one week of age, it was not until the end of the second week that filaments were present with regularity in the perivascular astrocytic end-feet. The presence of filaments coincided with the appearance of junctional complexes between the astrocytic end-feet resembling the "gap junctions" described by Brightman and Reese (1969), Figs. 23A, 23B and 24). The basal lamina was uniform in thickness, completely investing the periphery of the vessel.

The pericytes were small ovoid cells of relatively high electron density, completely invested by a basal lamina which was continuous with

the basal lamina enclosing the endothelium, (Figs. 20 and 25). Although the cytoplasm adjacent to the nucleus was relatively thick, the cytoplasmic processes became much thinner as they surrounded the vessel wall. The high density of the cytoplasm made description of the organelle pattern difficult, however, it closely resembled the pattern of the endothelial cell. Nuclear shape was varied from flattened to irregular in contour with no apparent relationship to the age of the cell. At no time were cell contacts, of any kind, observed involving the pericytes. Astrocytic processes were commonly observed adjacent to the external surface of most pericytes (Fig. 25), but areas of some pericytes were devoid of astrocytic processes and were adjacent to other neuropil components such as unmyelinated axons, (Fig. 20).

Although centrioles were observed in several endothelial cells only one mitotic figure was actually observed at two weeks post-natal. The mitotic figure was present as part of a larger vessel and was attached to adjacent endothelial cells, (Fig. 26).

Discussion - Blood Vessel

A relationship between the morphology of the developmental process of blood vessels and the blood-brain barrier remained obscure. The main alteration in blood vessel morphology, during post-natal development, was a decrease in the number of pinocytotic vesicles and reduction of pseudopod projections to the status of microvilli, which concurred with the findings of Donahue (1964). The pseudopod-like projections observed in this study resembled the "flaps" or "flanges" reported by Donahue (1964), who utilized thorium dioxide injections to demonstrate vesicle forming capacity of the projections. Donahue also demonstrated that "normal" pinocytosis was also active in the immature vessel, representing a dual system for the uptake of metabollites. Such activity in the endothelial cell was present at a time when neuron and glial elements were rapidly growing, thus suggesting a rapid method of transporting needed metabolites when few vessels were patent during the first post-natal week. Large amounts of extracellular space were also present during this time and have been demonstrated to contain polysaccarides by Caley and Maxwell (1970) in rat cerebral cortex. The large amount of extracellular spaces may have possibly provided a rapid route for the diffusion of the incoming metabolites, to the actively growing cells of the nervous system.

As development proceeded into the second post-natal week, the extracellular space was greatly reduced and patent vessels rapidly increased in numbers, attaining a mature appearance. It was possible that the increased vascularity negated the need for large scale pinocytosis, allowing for a more complete or discriminatory blood-brain barrier.

An alteration in the blood-brain barrier has been described by a number of authors, who have demonstrated that a variety of substances entered the neonatal brain at a greater rate than in the adult: chloride and inulin, (Vernadakis and Woodbury, 1965), glutamic acid, (Himwich and Himwich, 1955), cholesterol, (Dobbing and Sands, 1963) and sodium (Luciano, 1968).

The possibility also existed, that the active pinocytosis in young blood vessels may merely have been meeting the metabolic needs of the endothelial cell itself and not solely involved with metabolite transport to other cells. The subsequent decrease in vesicles may then have represented a decrease in metabolic need of the endothelial cell as it attained a steady state.

The alteration in the nuclear shape from highly irregular in contour, to a regular contour, during development, may have resulted from one or a combination of mechanisms. A decline in activity of the endothelial cell as it approached maturity may have produced such a change. It was also possible that a relationship existed between the change in nuclear shape and patency of the lumen. Increased lumen pressure may have produced flattening of the nucleus, causing it to conform to the round or ovoid shape of the lumen, since the nuclear shape in patent vessels was fairly regular in outline. Plasticity of endothelial cell nuclei has been demonstrated in muscle by Majno et al (1969).

Some patent vessels observed during the first two post-natal weeks, with blood elements present in immersion specimens, possessed a relatively attenuated vessel wall, irregular basal lamina, pseudopod projections and a larger number of pinocytotic vesicles than found in the mature vessel. Caley and Maxwell (1970) suggested that blood vessels became patent within hours, when efferent flow became established. It

was interesting to speculate, that the patent vessels, maintaining some immature characteristics, observed in this study may have represented newly opened vessels. If such was the case, differentiation of the endothelial cell continued after vessel patency.

The presence of an endothelial cell undergoing mitosis at two weeks of age confirmed that blood vessels were continually growing up to that time.

The incomplete investment of the vessel wall by astrocytic end-feet until the end of the first post-natal week in the dorsal horn observed in this study, differed from the findings of Phelps (1972), in the ventral horn of the rat cervical spinal cord, who reported astrocytic investment complete before birth. Phelps (1972) generalized his findings in the ventral horn, to include the entire spinal cord, which did not agree with the findings of this study. The conflict in findings may have represented a difference in the rate of astrocytic development between the dorsal and ventral horns. Variations in astrocyte vascular relationships have been noted elsewhere; Caley and Maxwell (1970) reported that astrocytic investment of vessels in rat cerebral cortex was not complete until 21 days of age.

Even though astrocytic investment was complete by the end of the first week, filaments and junctional complexes did not appear with regularity until the end of the second week, after the blood-brain barrier was supposedly complete. Due to their late appearance, it seemed that neither the filaments nor junctional complexes played any major role in the blood-brain barrier.

Summary - Blood Vessel

1. The non-patent blood vessel, in both immersion and perfusion specimens possessed a slit-like lumen.

2. The nuclei of developing endothelial cells were irregular in contour and were dense with clumped chromatin.

3. The cytoplasm of developing endothelial cells was characterized by a high concentration of free ribosomes, pinocytotic vesicles and numerous psuedopod projections.

4. As the blood vessels approached maturity, the endothelial cell cytoplasm became progressively thinner with a subsequent loss of psuedopod projections and pinocytotic vesicles. The endothelial cell nuclei became less dense and conformed in shape to the contour of the lumen. By three weeks of age all blood vessels appeared mature.

5. The basal lamina was present throughout post-natal development. The basal lamina which was irregular in thickness early in development, attained a uniform thickness in the mature vessel.

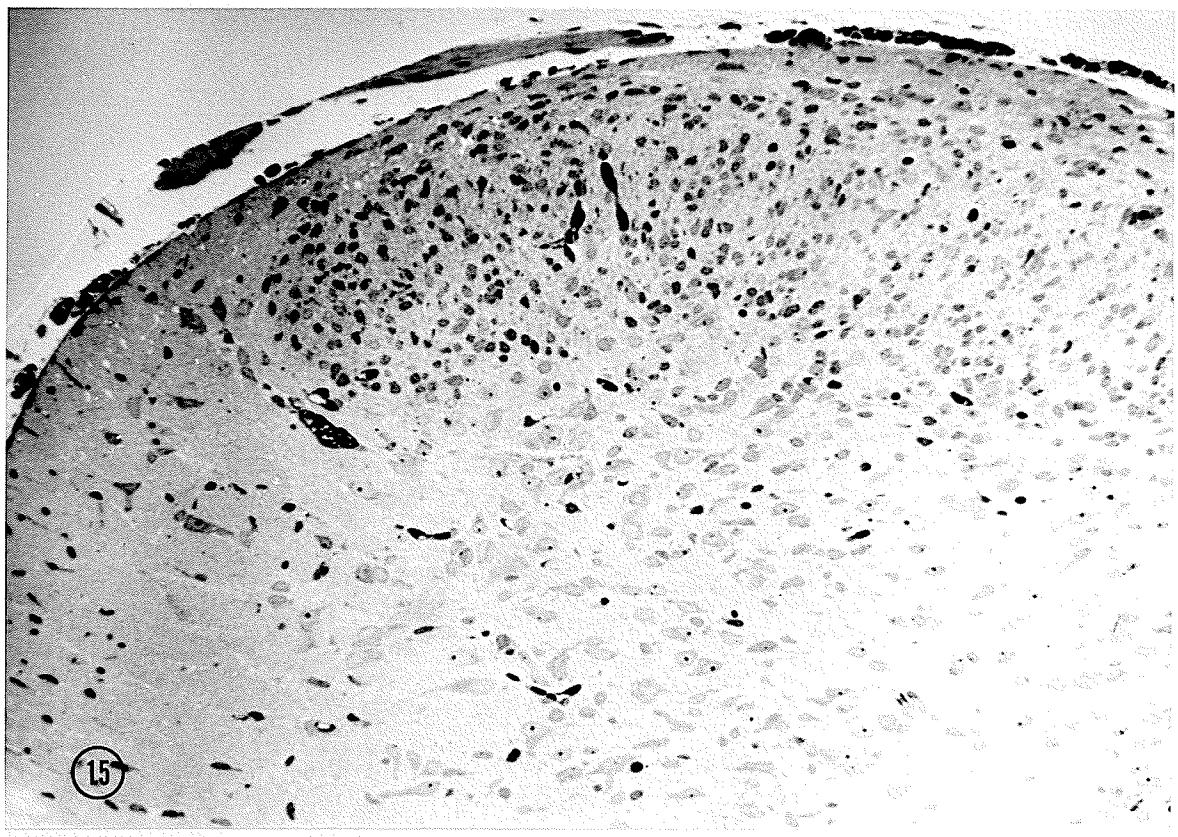
6. The astrocytic end-feet investment of the vessel wall was complete by one week post-natal. The presence of filaments within the perivascular end-feet and junctional complexes between adjacent end-feet were not complete until three weeks of age.

7. Pericytes were present at all stages of post-natal development.

FIGURES - BLOOD VESSEL 15 - 26

FIGURE 15. Photo micrograph of the posterior horn of the spinal cord at birth. Patent blood vessel profiles are few in number.
X 180

FIGURE 16. Photo micrograph of the posterior horn of the spinal cord at six weeks post-natal. Compare the large number of blood vessel profiles present in this micrograph, with those present in Fig. 15.
X 180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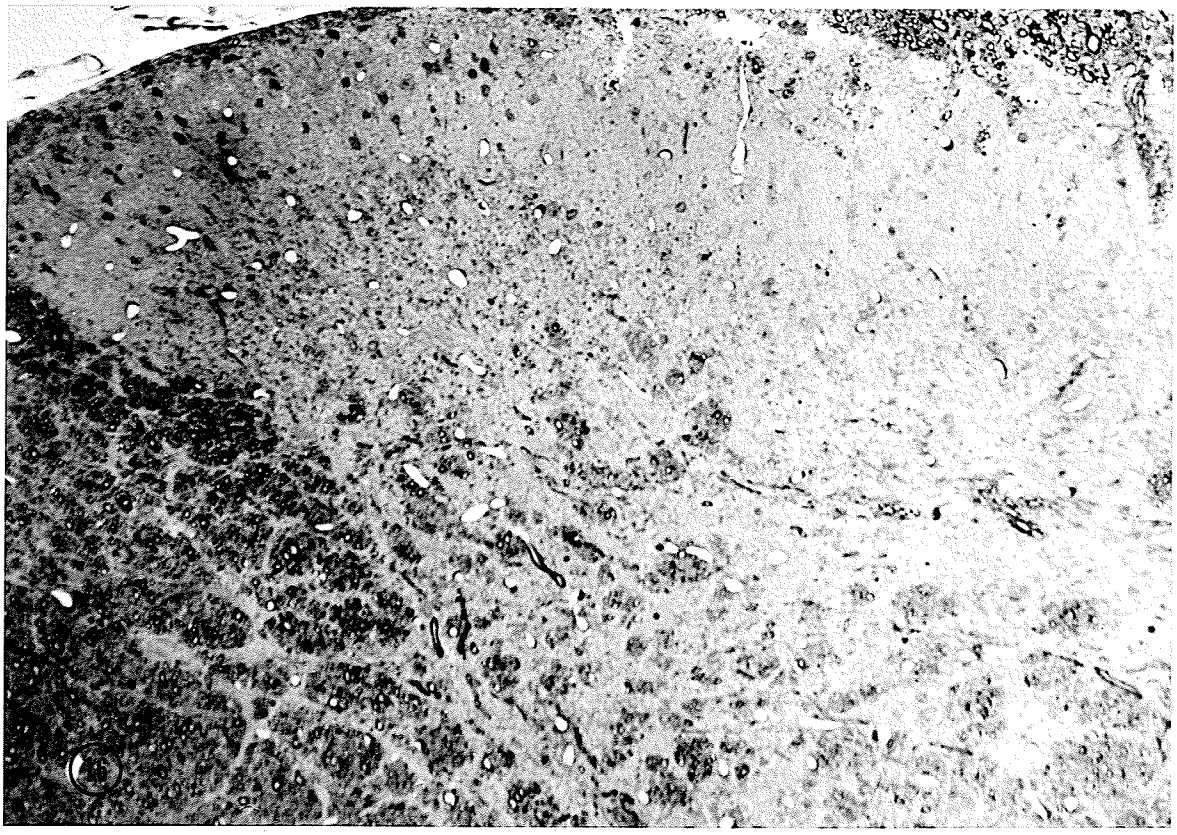

1.6

FIGURE 17. A non-patent blood vessel with a slit-like lumen from a neonate animal (immersion fixation). The slit-like lumen made blood vessels such as this, unidentifiable as such with the light microscope. Note the areas (arrows) where astrocytic end-feet are absent and the basal lamina is composed of thin flocculent material. In other areas (crossed arrows) where astrocytic end-feet enclose the vessel wall, the basal lamina is thicker.

X 31,350

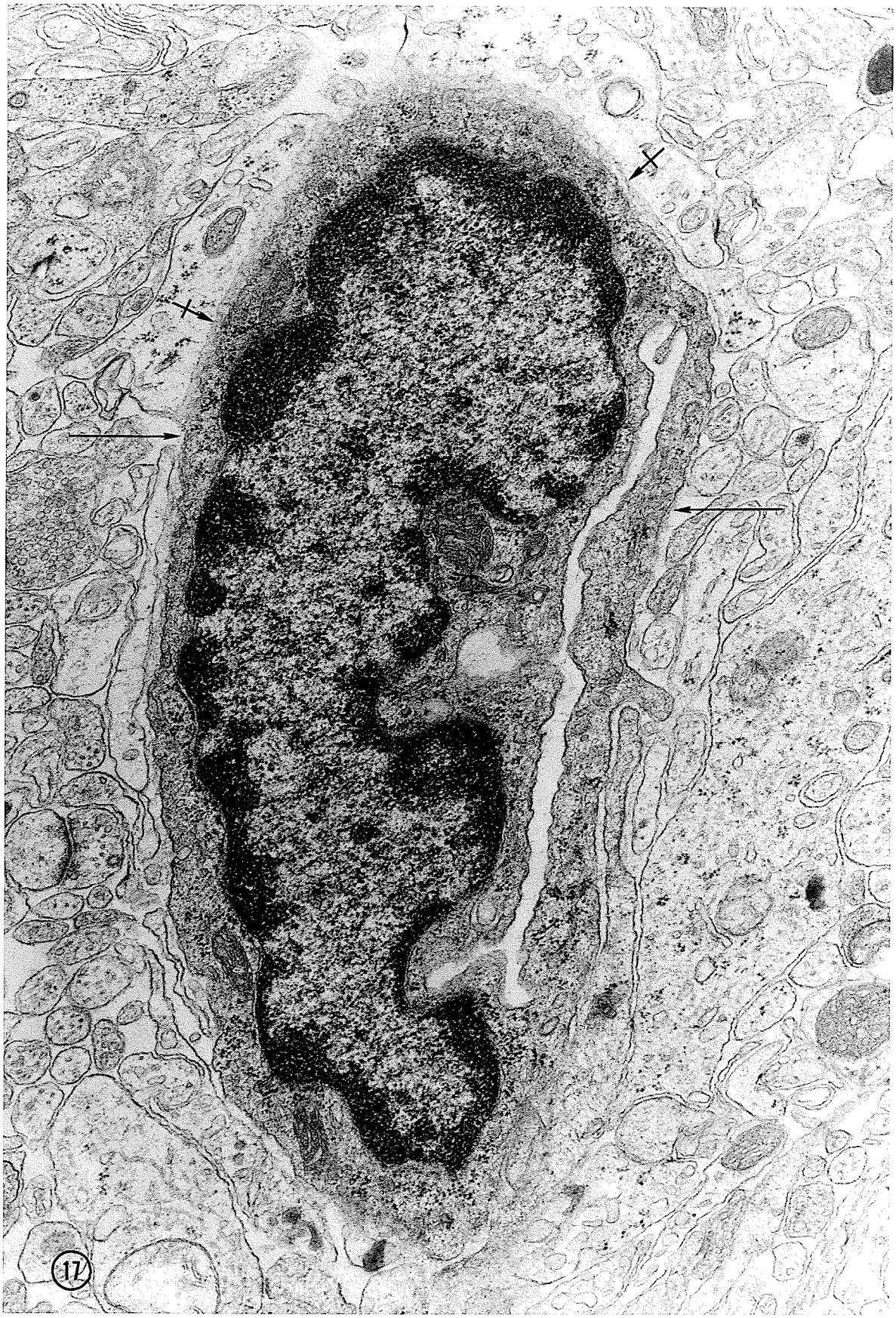

17

FIGURE 18. A non-patent blood vessel from the spinal cord of a three day post-natal animal (perfusion fixation). The endothelial cell is composed of an irregularly shaped nucleus and relatively thick cytoplasmic walls enclosing the slit-like lumen. The basal lamina is non-uniform in thickness.

X 20,240

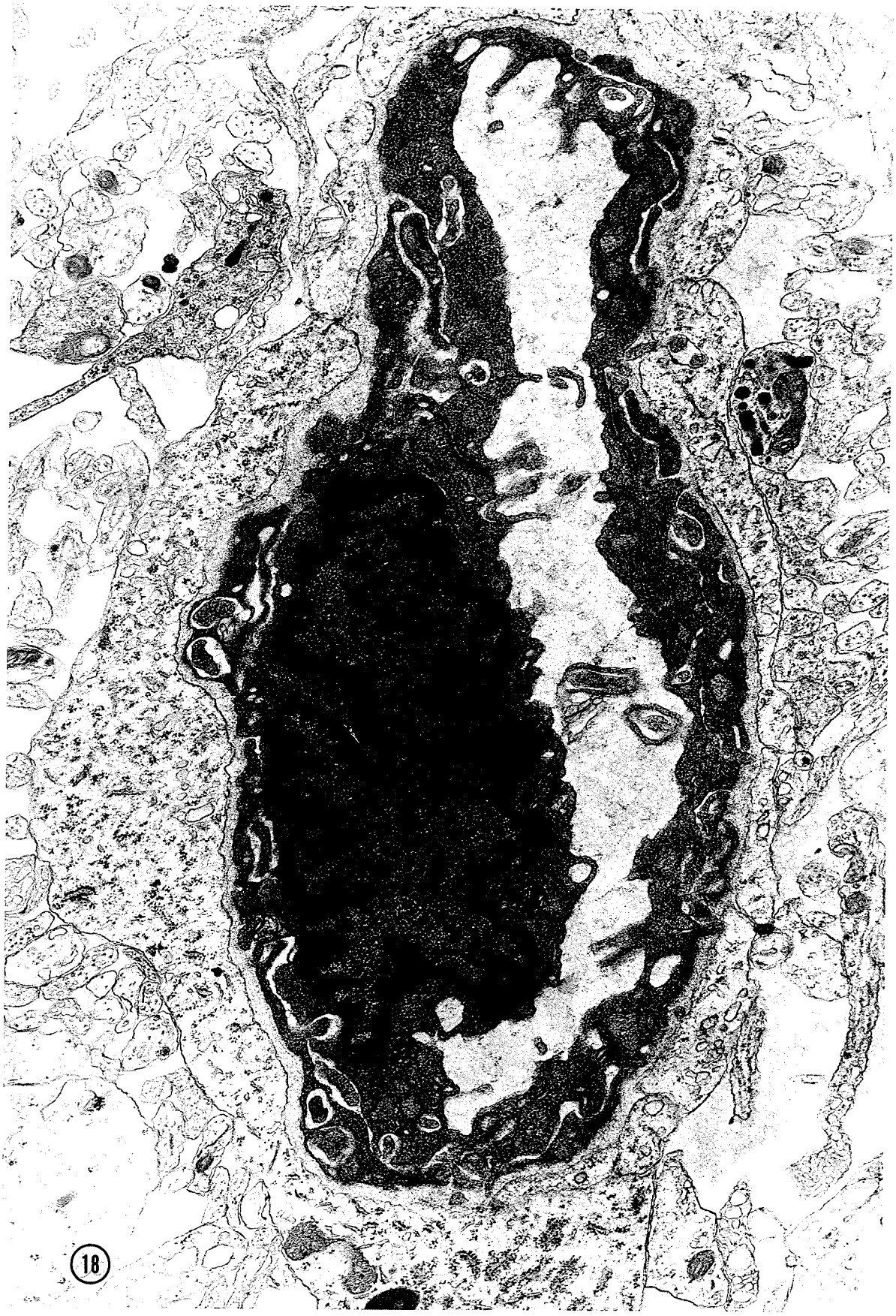

18

FIGURE 19. An immature blood vessel, at three days post-natal (immersion fixation) which demonstrates pseudopod-like projections (arrows). Pinocytotic vesicles open to both the lumen surface (crossed arrows) and to the basal lamina surface (double crossed arrows). A pericyte process (P) is enclosed by a basal lamina on both sides. Astrocytic end-feet (As) surround the vessel.

X 32,830

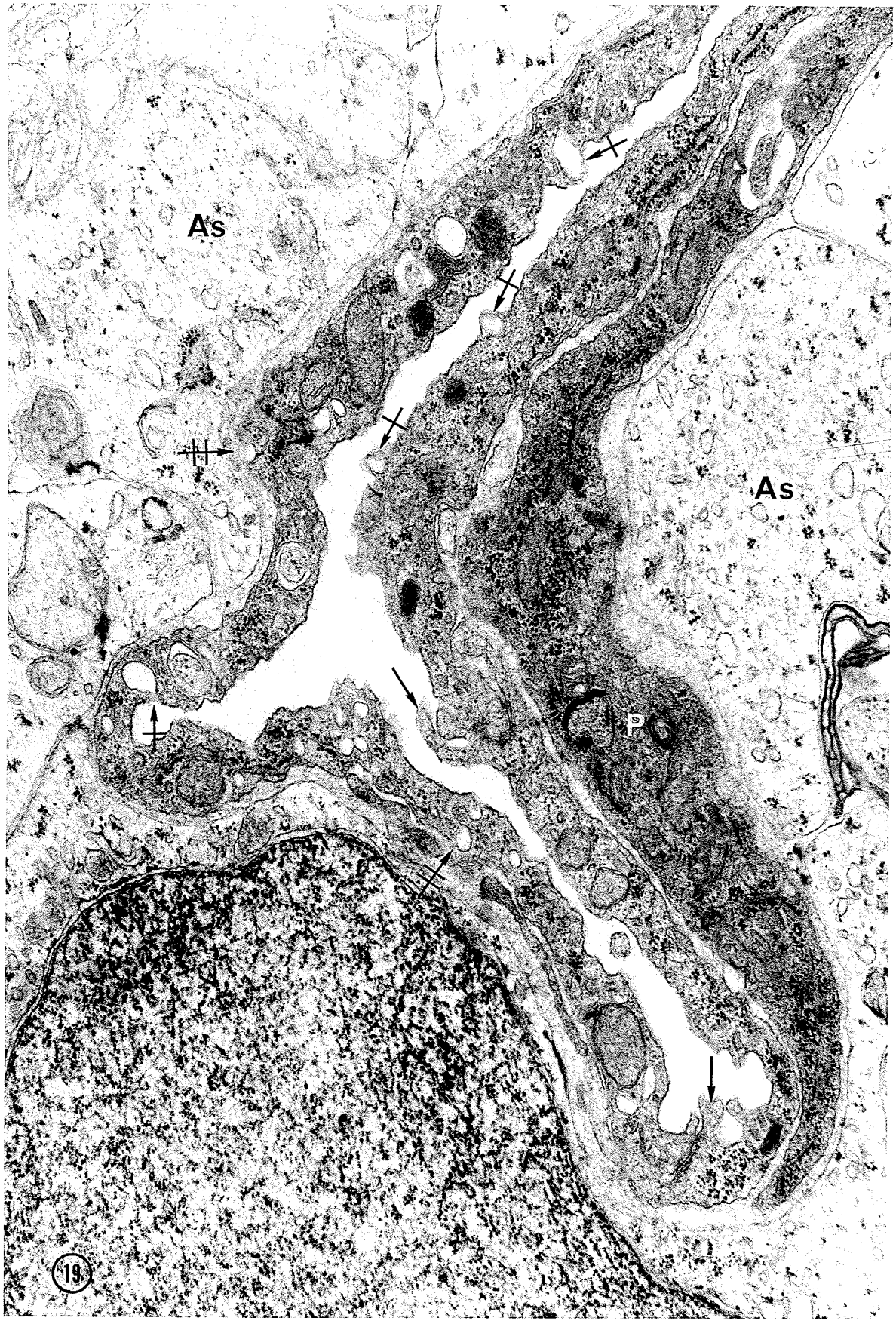

As
As
P
19

FIGURE 20. Immature patent blood vessel from a three day post-natal animal (immersion fixation). Although the cytoplasm is relatively attenuated, numerous pinocytotic vesicles and a pseudopod-like projection are present. Note the pinocytotic vesicle which is open to the basal lamina surface (arrow). A pericyte (P) situated adjacent to the endothelial cell is completely enclosed within the basal lamina. At the portion of pericyte devoid of astrocyte end-feet (crossed arrow), the basal lamina is very thin. The high electron density of the cytoplasm obscures most of the organelles. The nucleus is electron dense with clumped chromatin and is irregular in outline.
X 31,800

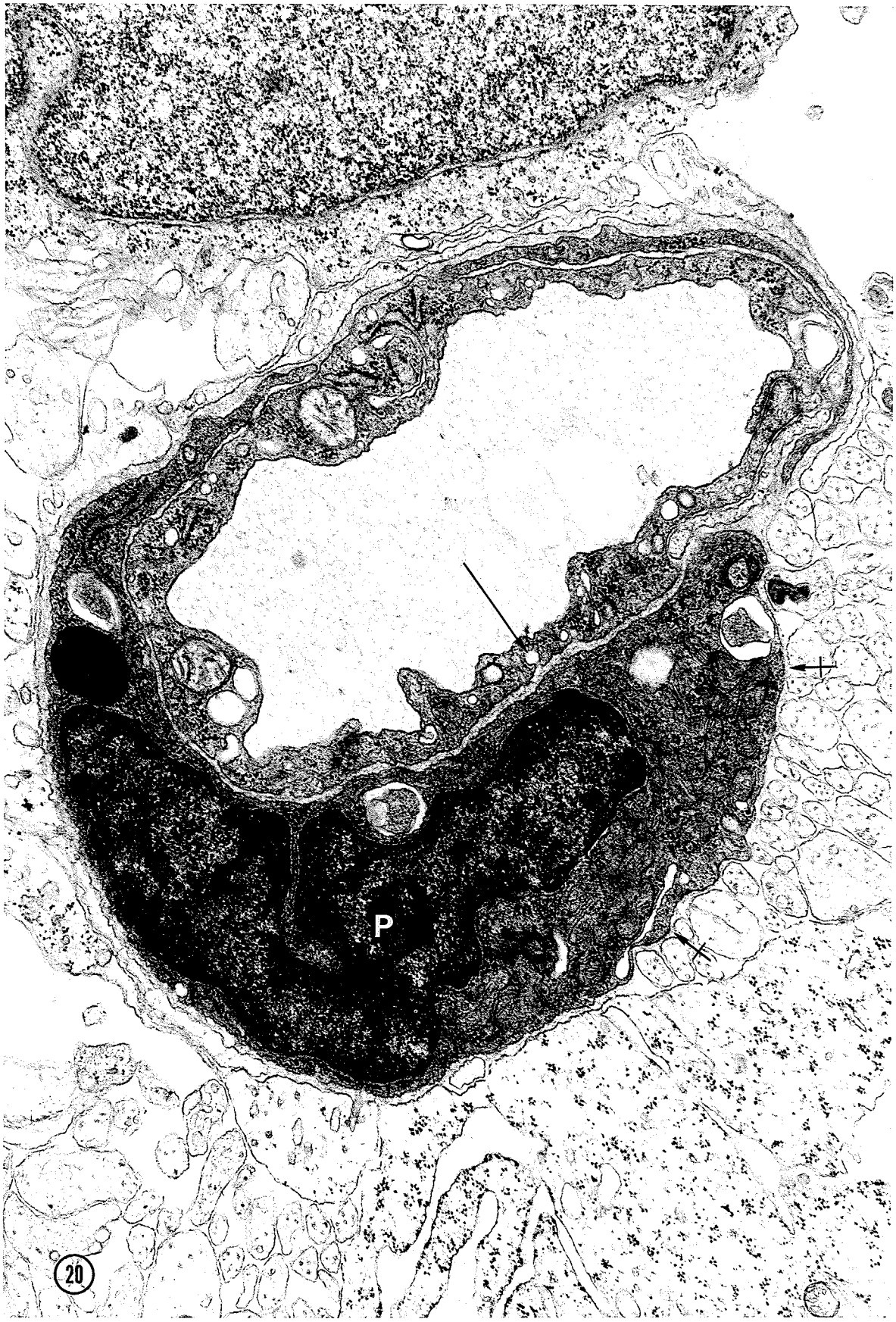
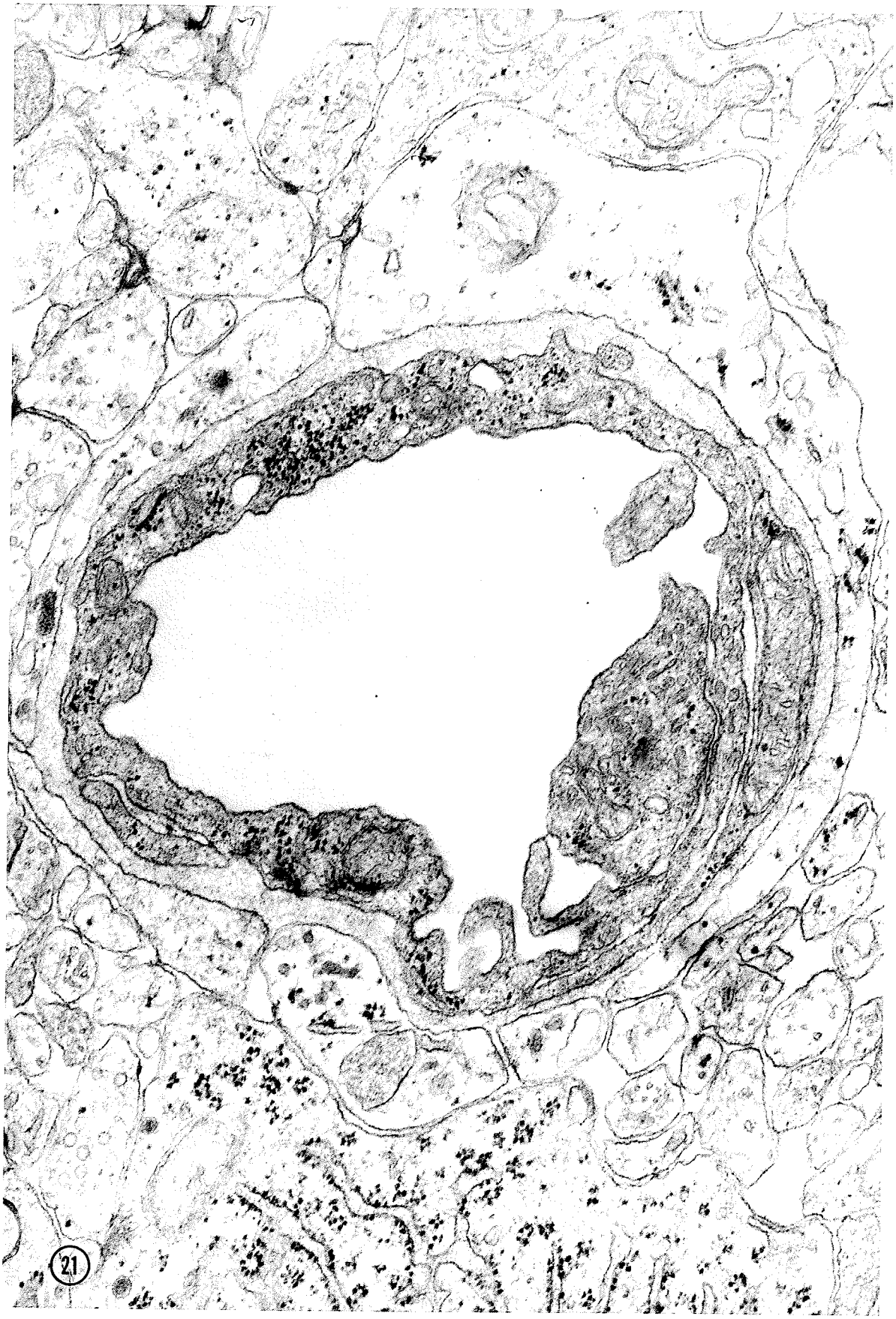

P
20

FIGURE 21. An immature patent blood vessel from a one week post-natal animal (perfusion fixation). Pinocytotic vesicles and pseudopod-like projections are present (compare with Fig. 20).

X 53,350

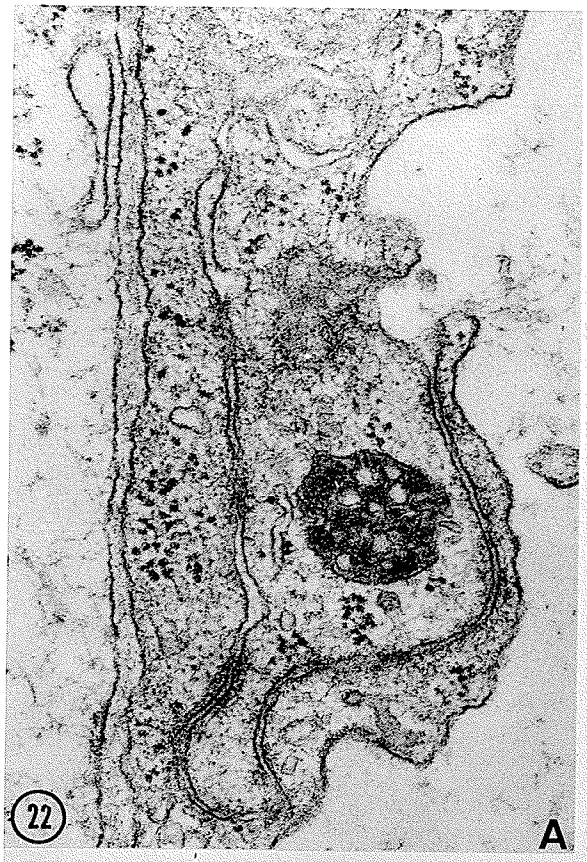

21

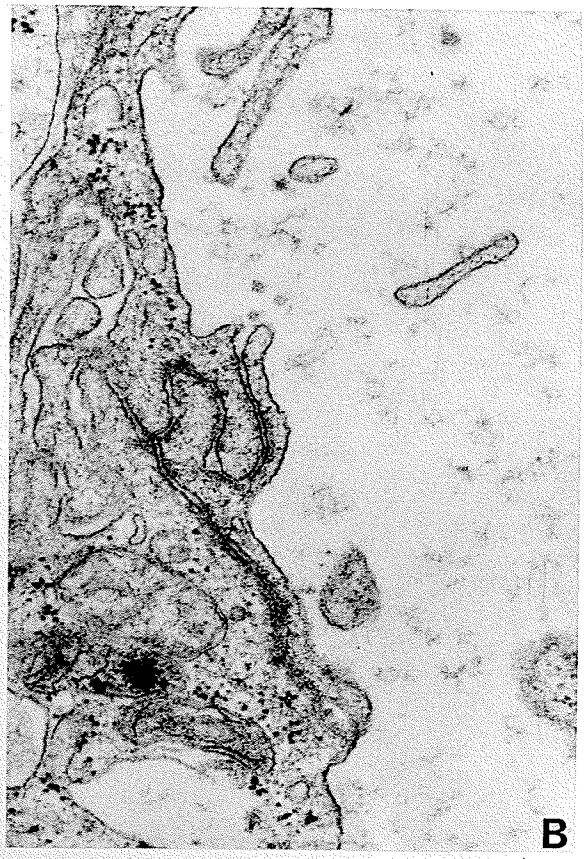
FIGURES 22 A and B. A zonula occludens or tight junction may be observed joining two adjacent endothelial cells. Note the extreme length and tortuosity of these junctions.

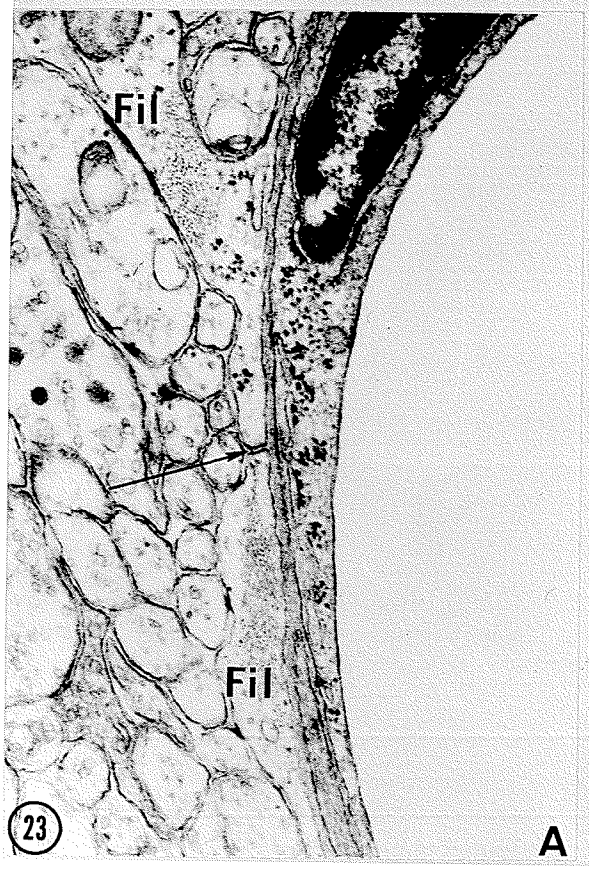
A - X 54,360 B - X 42,000

FIGURES 23 A and B. Zonula adhaerens or gap junction (arrow) between adjacent astrocytic end-feet. Note the presence of filaments (Fil) in the astrocytic end-feet in Fig. 23A.

A - X 63,840 B - X 41,000


22
A


B


Fil
Fil
23
A

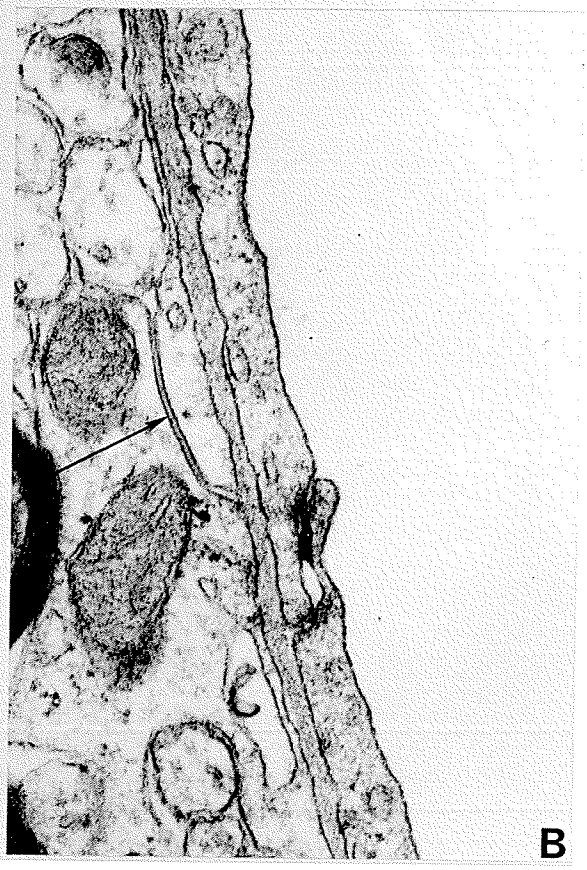

B

FIGURE 24. Mature blood vessel from six week post-natal animal (perfusion fixation) demonstrates an attenuated vessel wall and flattened nucleus. The basal lamina (Bl) is well defined and uniform in thickness. Astrocytic end-feet with filaments present are joined by a junctional complex (arrow). Pseudopod-like projections are absent and pinocytotic vesicles are few in number.

X 28,730

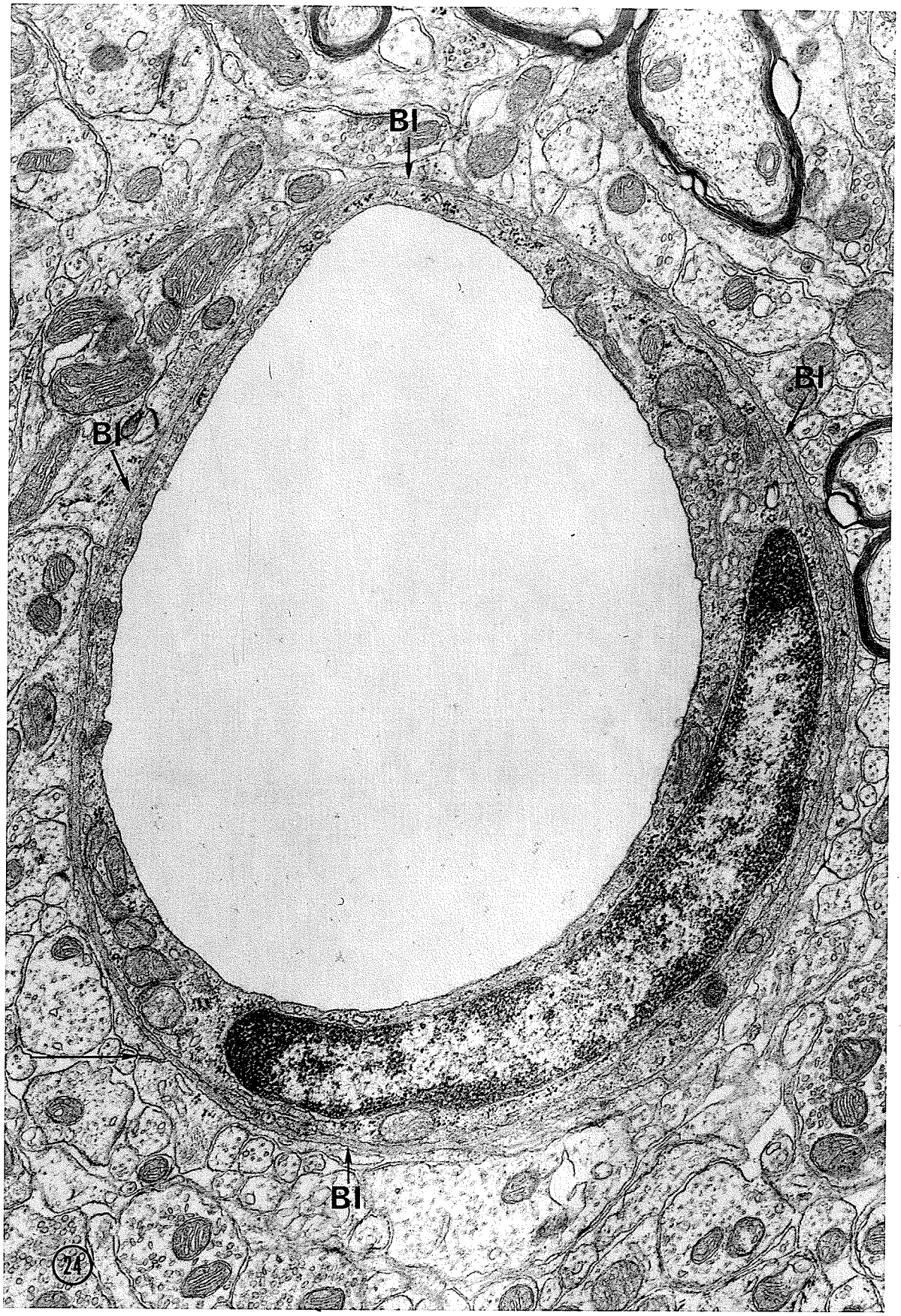

BI
BI
BI
BI
24

FIGURE 25. A pericyte (P) at five weeks post-natal (perfusion fixation) may be observed wrapped around the mature vessel wall and is completely enclosed by the basal lamina (compare with pericyte in Fig. 20). The endothelial cell cytoplasm resembles that of the endothelial cell present in Fig. 24.

X 41,320

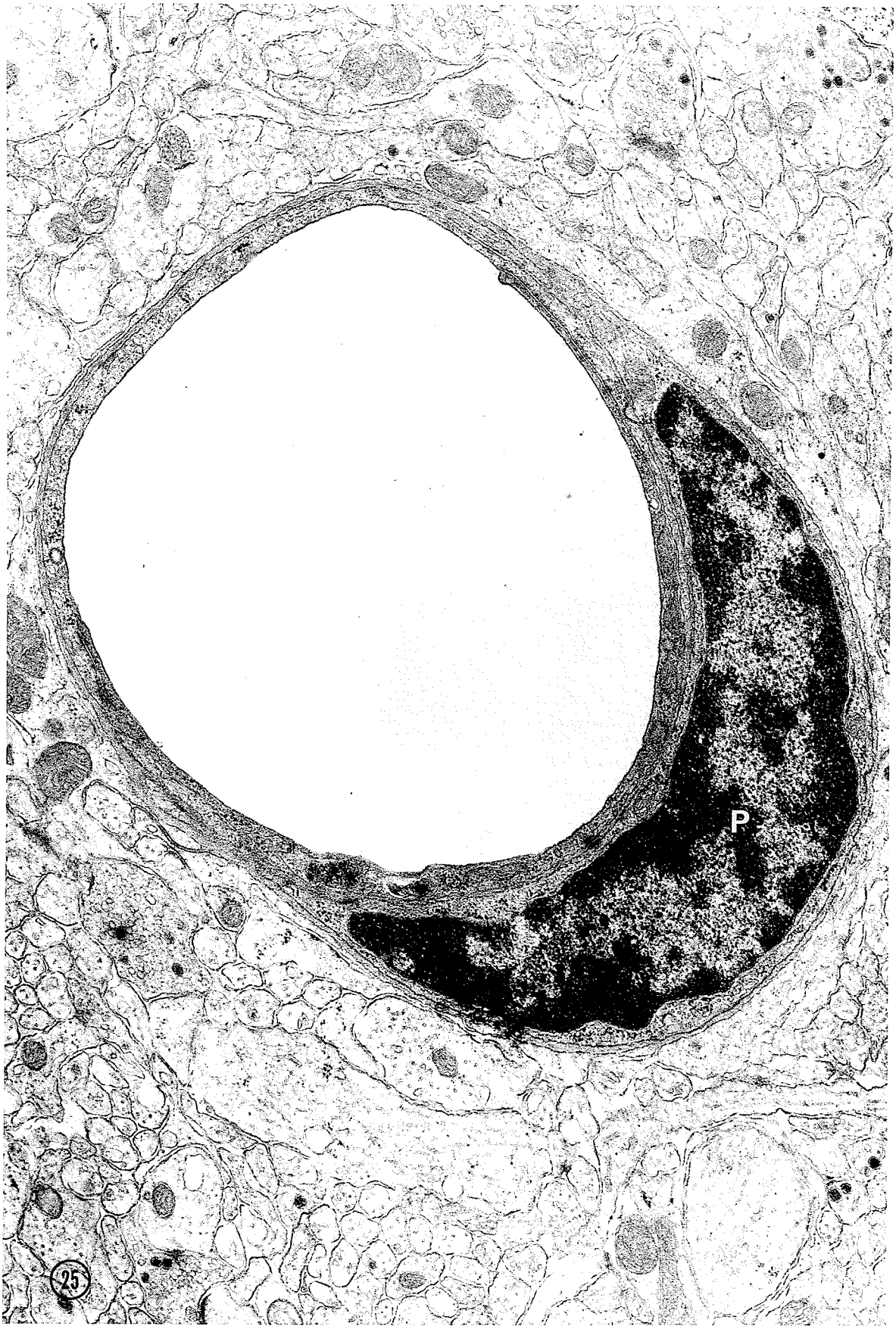
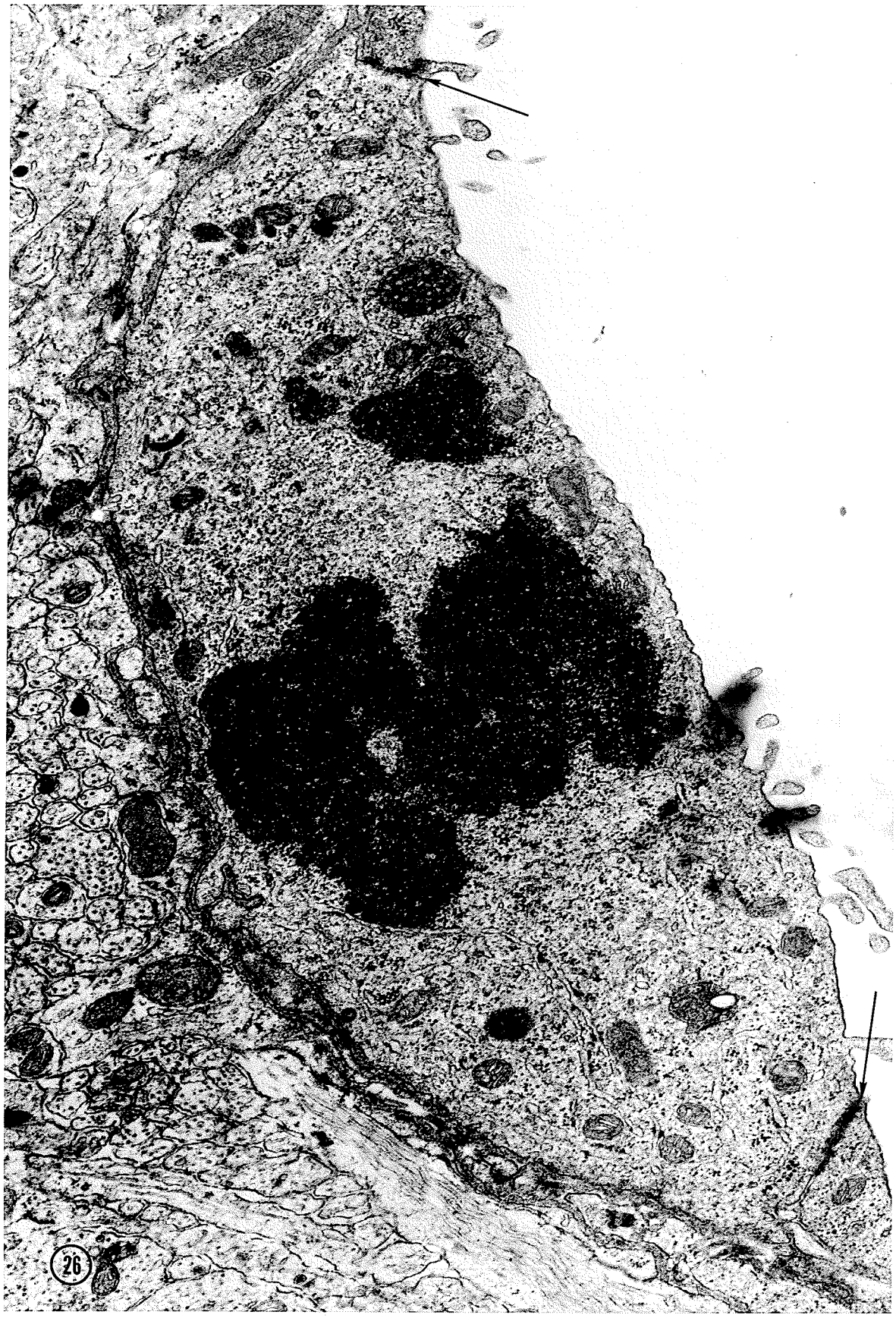

P
25

FIGURE 26. An endothelial cell which is undergoing mitosis in a two week old animal. A zonula occludens or tight junction is present at either end of the cell (arrows).

X 31,800


26

Introduction - Glial Cell Development

The description of glial cell development in the region of the substantia gelatinosa was reported and discussed in the following section. The topic of glial cell development was divided into the following sub-topics: glioblast development, astrocyte development and oligodendrocyte development. Each sub-topic included the appropriate observations and discussion. The micrographs were compiled into one section and were located at the end of the section.

The terminology utilized in this section relied upon cell identification. Those cells which demonstrated characteristics of a mature cell line were classified as either young astrocytes or young oligodendrocytes. The bivalent cells from which young astrocytes and young oligodendrocytes differentiated, were grouped into the classification of "glioblast".

Review of Literature - Gliogenesis

Gliogenesis, at the ultrastructural level, has been described by very few authors. The authors agreed on the presence of a primitive "blast" cell capable of differentiating into either oliodendroglia or astroglia.

Fujita (1963) described gliogenesis in the chick embryo cerebrum and stated that glioblasts differentiated from matrix cells, synonomous with the "spongioblasts" reported by Smart (1961) and Smart and Leblond (1961). Fujita and Fujita (1964) stated that the "glioblast" may potentially differentiate into either oliodendroglia or astroglia.

The glial cell precursor of the rat cerebral cortex was described by Caley and Maxwell (1968b) as a "spongioblast" which possessed cytoplasmic processes containing free ribosomes, many mitochondria, a prominent

Golgi complex and scanty granular endoplasmic reticulum. The nucleus contained peripherally clumped chromatin.

Glioblasts in the kitten optic nerve, described by Blunt et al (1972) had some features in common with the spongioblast described by Caley and Maxwell (1968b). The nucleus contained dispersed chromatin with very slight condensations at the nuclear membrane and a prominent nucleoli were frequently present. The cytoplasm contained numerous lysosomes, prominent Golgi apparatus, mitochondria of varying matrix densities, narrow cisternae of granular endoplasmic reticulum, a filamentous material containing microtubules, many free ribosomes and glycogen particles.

In contrast to the findings of Blunt et al (1972), Vaughn (1969) working with rat optic nerve described different glioblast morphology which he described in varying stages. Vaughn termed the first identifiable glial cell, a "small glioblast" which differentiated from the multipotential matrix cell first described by Fugita (1963). The small glioblast possessed a round or oval nucleus with large amounts of peripherally clumped chromatin. The cytoplasm contained long narrow cisternae of granular endoplasmic reticulum and numerous ribosomes. The small glioblast subsequently differentiated into a "large glioblast", which was larger and characterized by an alteration of the granular endoplasmic reticulum into a few short cisternae. The large glioblast differentiated into "large glial precursors", possessing prominent nucleoli, and more abundant cytoplasmic organelles, which then differentiated into either young astrocytes or young oligodendrocytes. Vaughn and Peters (1971) considered the "small glioblast a pivotal cell in neuroglial development" and suggested that this cell may remain in the cell cycle or differentiate

into macroglia.

Observations and Discussion - Glioblast

Due to the wide range of cells that were classified as glioblasts the following section was composed of both observations and discussion for reasons of clarity.

Glial elements exclusive of the cells identifiable as either the astrocyte or oligodendrocyte cell line were described as glioblasts. Discrimination between glioblasts as to their possible potential was outside the scope of this thesis. The scattering of glioblasts in the posterior horn made identification difficult. Even in the optic nerve which contained no neuron cell bodies there was disagreement in the classification of glioblasts.

The cells designated as glioblasts in this study varied in both size and shape. This unidentifiable cell population probably represented the full range of precursors to the oligodendrocyte and astrocyte cell lines. At birth a small number of cells were observed which resembled the earliest glioblast reported by Fujita and Fujita (1964), which were derived directly from the matrix cell. Such cells possessed a round to ovoid nucleus with a dense nuclear chromatin pattern. The cytoplasm was scanty and formed a thin rim around the nucleus. Numerous ribosomes and several mitochondria were the principal constituents of the cytoplasm. Endoplasmic reticulum and Golgi were rarely observed (Figs. 27 and 28). The possiblility existed that this cell type was the parent cell of all other glial elements present, as none were observed after the first post-natal week.

The remaining glioblasts were grouped into a large heterogeneous

collection of cells, which this author was unable to classify as either astrocytic or oligodendrocytic in appearance (Fig. 29). During the second post-natal week this group of cells had all but disappeared. The observed decline in the numbers of glioblasts coincided with the increase in identifiable macroglia.

Vaughn (1969) and Vaughn and Peters (1971), working with the rat optic nerve, described the oligodendrocyte and astrocyte precursors in several stages. However, Blunt et al (1972) working with cat optic nerve were unable to discriminate between the early glioblasts.

Review of the Literature - Astrocyte

The ultrastructure of mature astrocytes has been reported by many authors such as Schultz et al (1957), De Robertis and Gershenfeld (1961), Mugnaini and Walberg (1964), Maxwell and Kruger (1965), Palay (1966) Wendell-Smith et al (1966), Kruger and Maxwell (1967), Mori and Leblond (1969a), and Vaughn and Peters (1971). Fibrous astrocytes occurring in white matter are characterized by an irregularly shaped nucleus, with minimal clumping of chromatin. A nucleolus was occasionally present. Cytoplasmic organelles were sparse, except for the presence of numerous fibrils 80-90Å in diameter. The granular endoplasmic reticulum consisted of short cisternae. The Golgi apparatus which consisted of a few small stacks of cisternae was usually located in an area of the cell where the cytoplasm was greatest in volume. Microtubules were few in number and mixed with the fibrils. Lysosomes and glycogen particles were also present. Fibrous and protoplasmic astrocytes have been observed in the posterior horn of the rat spinal cord (Nathaniel and Nathaniel, 1966b). Fibrous astrocytes were reported in the cat substantia gelatinosa by

Ralston (1965).

Protoplasmic astrocytes occurring in grey matter demonstrated much the same appearance as fibrous astrocytes except for a fewer number of fibrils present in the cytoplasm, irregular cytoplasmic projections and a usually round or oval nucleus.

The differentiating astrocyte has been described by the following authors: Caley and Maxwell (1968b), Vaughn (1969) and Blunt et al (1972). It was the concensus of these authors, that the developing astrocytes, displayed a decrease in the matrix density of the cytoplasm with increasing age. Filaments, which characterized the astrocytic cell, increased in numbers during differentiation.

Vaughn and Peters (1971) suggested a relationship between the increasing numbers of filaments and a concommitent decline in the numbers of microtubules; with microtubules possibly giving rise to the filaments.

Both Vaughn (1969) and Blunt et al (1972) described the granular endoplasmic reticulum, consisting of short segments of wide cisternae containing a dense flocculent material, which was present from an early stage, throughout development. Vaughn (1969), however, described a young astrocyte which was composed of short narrow segments of granular endoplasmic reticulum, but lacking the flocculent material of more mature endoplasmic reticulum. Blunt et al (1972), observed a gradual increase in the density of the flocculent material present in the wide cisternae.

Vaughn (1969), described the mitochondria as being "longer, plumper, and less dense" than in other cell types present and suggested that mitochondrial appearance may be a criteria in distinguishing the identity of developing astrocytes.

Electron dense bodies were observed during all stages of development

by both Vaughn (1969) and Blunt et al (1972). Blunt and co-workers also observed a decrease in the numbers of electron dense bodies as differentiation proceeded.

The nuclei of developing astrocytes were described as possessing relatively dispersed chromatin, with a thin rim of condensed chromatin on the nuclear membrane. Blunt et al (1972), however, described an earlier astrocyte, containing large amounts of clumped nuclear chromatin.

Observations - Astrocyte

The earliest form of an identifiable astrocyte was present in the neonatal rat. The young astrocyte was readily identifiable from cells of the oliodendrocyte line by a less dense appearance of both nuclei and cytoplasm (Figs. 30 and 31). The cytoplasm of the young astrocyte demonstrated many processes which extended into the neuropil. Many cytoplasmic processes were observed forming perivascular end-feet around blood vessels, which was one criteria in the identification of the young astrocyte (Fig. 32). The cytoplasmic density of young astrocytes was variable. While some cells possessed a low density characteristic of mature astrocytes (Figs. 32 and 33), other young astrocytes maintained a slightly more dense appearance (Figs. 30, 31 and 34). It should be noted that even the most electron dense young astrocyte was lighter in appearance than the young oligodendrocyte.

The granular endoplasmic reticulum of young astrocytes resembled that of mature astrocytes and was a primary identifying feature of young astrocytic cells. Short segments of wide cisternae were scattered throughout the cytoplasm. The ribosomes on the cisternae were unevenly distributed, resulting in areas that were devoid of ribosomes. The free

ribosomes existed mainly in rosettes (Fig. 35). The Golgi apparatus appeared to be well developed and was present in multiple arrays (Fig. 35). Electron dense bodies were present in relatively large numbers when compared to those present in the mature cell (Figs. 32, 34 and 35). Glycogen particles were very difficult to identify (see Discussion). Astrocytic filaments, which were characteristic of mature astrocytes, were rarely observed in young astrocytes and when present, they were few and scattered (Figs. 33 and 35).

The nuclei of young astrocytes were usually irregular in contour, with a relatively homogeneous nuclear chromatin pattern. However, most young astrocytes did display a very thin rim of condensed chromatin along the nuclear envelope. A very small number of astrocytes observed, demonstrated marked clumping of the nuclear chromatin throughout the nucleus (Fig. 32).

As development proceeded, there was a concomitant decrease in the cytoplasmic density (Compare Fig. 32 with Fig. 37). The relative numbers of electron dense bodies also appeared to decrease. The most prominent cytoplasmic alteration was an increase in the number of filaments.

Most astrocytes, by the end of the second week post-natal, possessed small bundles of filaments, which were readily observed in the perivascular end-feet (Fig. 36). As was noted in the description of blood vessel development, junctional complexes resembling gap junctions, first appeared at this stage, between adjacent astrocytic end-feet (Figs. 23A and 23B).

By six weeks post-natal, most astrocytes appeared to be mature. Large aggregations of filaments were present in most cells. Such large quantities of filaments resulted in most of the cells being designated

as fibrous astrocytes. Due to the considerable areas of cytoplasm containing filaments, the cytoplasmic organelles were very difficult to observe.

The granular endoplasmic reticulum of the mature astrocyte closely resembled that of the young astrocyte, but appeared to be decreased in quantity (Fig. 37). The Golgi were small in size when compared to that in young astrocytes (Fig. 37). Both mitochondria and electron dense bodies appeared to be reduced in number.

Junctional complexes resembling gap junctions, which were observed between adjacent perivascular end-feet at two weeks post-natal, appeared to increase in number by six weeks post-natal.

By the fifth post-natal week, junctional complexes were also observed between astrocytic processes, exclusive of the end-feet (Fig. 38).

The nuclei of mature astrocytes tended to be regular in outline and the nuclear chromatin appeared to be more coarse in appearance than the young astrocyte.

Discussion - Astrocyte

Peters et al (1970) reviewed the possible functional roles of astrocytes. Functions attributed to the astrocytes included structural support, repair of brain damage, blood brain barrier, and isolation of receptive surfaces. Other than a discussion of the blood-brain barrier, which is discussed with blood vessel development, the other possible functional roles are beyond the scope of this thesis.

Marked clumping of nuclear chromatin in "early" astrocytes as described by Blunt et al (1972) was not observed in this study. However, a few astrocytes were observed with minor condensations of nuclear chromatin; whether these few cells represented an earlier stage in de-

velopment was unknown. It also seemed fruitless to compare amounts of nuclear chromatin clumping with the work of Blunt et al (1972) because of the exclusive use of glutaraldehyde in that study which reportedly produced different effects on nuclear chromatin than other fixatives. The chromatin appearance of astrocytes in this study closely resembled that reported by Vaughn (1969) who utilized a mixture of glutaraldehyde and formaldehyde.

A decrease in the overall density of the maturing astrocyte was in agreement with the findings of Caley and Maxwell (1968b), Vaughn (1969) and Blunt et al (1972).

Unlike the reports of Vaughn (1969) and Blunt (1972) who have chosen to describe the developing astrocyte in two distinct stages, only one such stage can be identified in this study, which generally resembles the second or late stage of the above authors. It was possible that post-natal astrogliogenesis was more advanced in the spinal cord than in the optic nerve.

The apparent decrease in the numbers of electron dense bodies during the maturation process were in agreement with the findings of Blunt et al (1972). If indeed the dense bodies were lysosomal in nature it would have been interesting to have examined why young astrocytes required such a multiplicity of these structures. It was interesting to note that astrocytes reported to be active in the repair of brain damage also contained a large number of lysosomal-like bodies. Perhaps the presence of many electron dense bodies was inherent in active astrocytes whether they were young astrocytes in the process of growing or mature astrocytes in the process of repair.

There also appeared to be a decrease in the amount of Golgi present.

If the electron dense bodies were lysosomes, such a decrease in Golgi activity may have accounted for the aforementioned decrease in the numbers of electron dense bodies. According to the findings of Novikoff (1964), the Golgi complex was implicated in the formation of lysosomes.

The overall increase in the numbers of filaments observed in differentiating astrocytes was in agreement with the findings of Caley and Maxwell (1968), Vaughn (1969) and Blunt et al (1972). The presence of many fibrous astrocytes in the substantia gelatinosa of the rat was reported by Nathaniel and Nathaniel (1966a) and in the cat by Ralston (1965).

Glycogen particles in the astrocyte cytoplasm were difficult to detect. In very few instances was the glycogen particle size large enough to allow discrimination from the ribosomes present. It was therefore difficult to utilize the presence of glycogen particles as a criteria in the identification of astrocytes.

Review of the Literature - Oligodendrocyte

The existence of many types of oligodendrocytes was first recorded by light microscopists del Rio-Hortega (1928), Kryspin-Exner (1943), Cammermeyer (1960), Smart and Leblond (1961). However, the presence of such a variety has been a subject of controversy among electron microscopists.

De Robertis and Gerschenfeld (1961), Malmfors (1963), Magnaini and Walberg (1964), Schultz (1964), Bodian (1964), Wendell-Smith et al (1966) Stensaas and Stensaas (1968) and King (1968), have all described oligodendrocytes as being smaller and denser than astrocytes and possessing few processes. The nucleus was round or oval, exhibiting aggregations of nuclear chromatin. The cytoplasm was rich in organelles, with a

prominent Golgi apparatus and numerous microtubules. However, Kruger and Maxwell (1966), and Caley and Maxwell (1968b) described oligodendrocytes having varying nuclear and cytoplasmic densities. Mori and Leblond (1970), also identified three classes of oligodendrocytes in the corpus callosum of young rats. All three classes had some common features; numerous ribosomes and microtubules and a variable number of non-branching fine processes of uniform diameter. Class I, light oligodendrocytes were large cells with a pale nucleus, large nucleolus and many free ribosomes. Class II, medium shade oligodendrocytes, were smaller cells with a moderately dense nucleus and cytoplasm, with well developed organelles. Class III were even smaller cells with a very dense nucleus and cytoplasm, a prominent Golgi and lameller bodies.

Mori and Leblond, proposed a line of cell differentiation, proceeding from Class I (light) to Class III (dark) oligodendrocytes. Similar findings were reported in the rat cerebral cortex by Mori and Hama (1971).

The pattern of differentiation of the oligodendroglia described by Caley and Maxwell (1968b), Vaughn (1969) and Blunt et al (1972) are in agreement on several points. The overall size of the cell increased with age, maintaining the cytoplasmic density characteristic of oligodendrocytes. There was also an overall increase in the numbers of ribosomes, mitochondria, microtubules, granular endoplasmic reticulum and especially the prominent Golgi apparatus, as differentiation proceeded.

Blunt et al (1972) noted an increase in the amount of clumping of nuclear chromatin as differentiation proceeded. However, Vaughn (1969) described an "active oligodendrocyte" which was less dense than more mature forms, containing evenly dispersed nuclear chromatin and a prom-

inent nucleoli. Active oligodendrocytes were reported to be present during the time of active myelination. Vaughn suggested that the dispersed chromatin pattern may have represented an increase in cellular activity related to RNA synthesis during the process of myelination. Oligodendrocytes in stages of differentiation, before and after myelination, contained aggregated chromatin.

The development of cytoplasmic processes was described by Caley and Maxwell (1968b), as long thin processes extending into the neuropil and contacting axons, while Vaughn (1969) in a more detailed description, observed both, large processes, giving rise to more slender projections and slender processes arising directly from the perikarya.

Observations - Oligodendrocyte

The earliest form of identifiable oligodendrocyte was present by three days post-natal. The young oligodendrocyte was identifiable as such by its resemblance to the mature oligodendrocyte. The young oligodendrocyte exhibited prominent Golgi complexes, numerous microtubules, narrow cisternae of granular endoplasmic reticulum and an overall density of the cytoplasm, characteristic of mature oligodendrocytes (Fig. 39).

The perineuronal position of most young oligodendrocytes was also a guide in determining their identity. This author realized that astrocytes were also situated in juxtaposition to neurons, however, the distinct differences between the two cell lines made discrimination relatively simple (Figs. 34 and 40).

Even though the young oligodendrocyte possessed some mature characteristics, they were distinctly different from the mature cell in several aspects. The relatively dense cytoplasm formed numerous processes.

The processes varied in shape from a single broad process giving rise to several more slender processes, to single slender processes arising directly from the cell surface (Fig. 41). Although the processes extended into the neuropil, none were observed directly wrapping axons. Only one axon in the process of myelination was observed adjacent to a young oligodendrocyte. Even though the connection was not continuous, the proximity of the axon to the oligodendrocyte and similarity of the process ensheathing the axon to that of the cytoplasm of the oligodendrocyte was suggestive of an oligodendrocyte process wrapping an axon (Figs. 42 and 43).

Prominent multiple Golgi complexes were observed (Figs. 41,42,44 and 45). The granular endoplasmic reticulum which was distinctly different from the short wide cisternae characteristic of astrocytes, was composed of long narrow cisternae in a fairly organized pattern (Figs. 41,42,44 and 45). Microtubules were present throughout the cytoplasm but were especially abundant in the cytoplasmic processes (Fig. 39). Electron dense bodies were regularly observed in the cytoplasm (Figs. 44 and 45).

The nuclei of young oligodendrocytes was generally ovoid but some cells exhibited slight irregularities in outline. The most prominent feature of the nucleus was the dispersed appearance of the nuclear chromatin. However, some young oligodendrocytes possessed slight accumulations of clumped chromatin at the nuclear margin. Even with the slight chromatin clumping in some cells,they were far more homogeneous in appearance than mature oligodendrocytes. Prominent nucleoli were commonly present.

During the second and third post-natal weeks an increasing number

of oligodendrocytes appeared to be mature. The differentiation of the young oligodendrocyte to a mature state was demonstrated by two major morphological alterations. The numerous cytoplasmic projections characteristic of the young oligodendrocyte were relatively scarce in the mature cell, resulting in a rounded appearance of the cell (Fig. 46). The nucleus exhibited marked chromatin clumping which was especially dense at the nuclear margin (Figs. 46 and 47). The pattern of cytoplasmic organelles showed no appreciable change (Fig. 47).

Discussion - Oligodendrocyte

The earliest identifiable oligodendrocytes were present by three days post-natal but were very few in number in comparison to the number of young astrocytes present. During the following ten days there appeared to be a large increase in the number of young oligodendrocytes.

The young oligodendrocytes described in this study possessed dispersed nuclear chromatin pattern and a large amount of organelle rich cytoplasm forming both broad and slender processes. This stage in oligodendrocytic development closely resembled the active oligodendrocyte described by Vaughn (1969) in the rat optic nerve, by day five post-natal. Vaughn (1969), noted that the active oligodendrocytes were most common during the period of intense myelination and observed many connections between oligodendrocyte processes and developing myelin sheaths. Due to the paucity of myelinated fibers in the substantia gelatinosa, identification of oligodendrocytes by that criteria, was very difficult. Only one young oligodendrocyte was observed to be possibly in the process of myelination.

The diffuse nature of the nuclear chromatin in the young oligoden-

drocyte was suggestive of a very active cell as was also noted in the growing neuroblast in this study. It was also reported by Vaughn (1969), that the dispersed chromatin pattern may have represented an increased cellular activity related to an increase in RNA synthesis during the period of intense myelination. It was interesting to compare the young oligodendrocyte with the light oligodendrocyte reported in the rat corpus callosum by Mori and Leblond (1970) and in the rat cerebrum by Mori and Hama (1971). The light oligodendrocyte was reported to undergo mitotic division in the mature animal and it should be expected that there would be some features in common with the young growing oligodendrocyte which was also engaged in active protein synthesis. The most striking similarity was the diffuse appearance of the nuclear chromatin. Both cell "types" exhibited numerous cytoplasmic processes containing many microtubules. However, cytoplasmic organelles appeared greatly reduced in number and complexity in the light oligodendrocyte when compared to those of the young oligodendrocytes of this study. This author was unable to either confirm or disprove the existence of separate classes of oligodendrocytes. Only very slight variations were present by the end of the six week period studied in the supposedly mature oligodendrocytes. A more complete study of mature animals which was beyond the scope of this developmental study may answer this question.

As the young oligodendrocyte progressed to the mature state there was a subsequent loss of many of its cytoplasmic processes, thus resulting in a more rounded appearance. The nuclear chromatin became progressively more clumped in appearance. This final pattern of development was in agreement with the findings of Caley and Maxwell (1968b), Vaughn (1969), Vaughn and Peters (1971) and Blunt et al (1972). The rounding

off of cytoplasmic processes and clumped nuclear chromatin suggested a generalized decrease in cellular activity.

Microglia

The existence of a "microglia" cell remained a controversial subject. Vaughn and Peters (1968) and Vaughn (1969) suggested the existence of a third glial cell type in adult rat optic nerves, which had the potential to enter the cell cycle, differentiate into either oligodendrocytes or astrocytes, or become phagocytic. The cell was characterized by a round or elongated nucleus with clumps of chromatin adjacent to the nuclear membrane. The cytoplasmic density was in between that observed in either oligodendrocytes or astrocytes. The granular endoplasmic reticulum consisted of "a few long and stringy cisternae". Microtubules were few in numbers while dense laminar bodies and lipid droplets were commonly present in greater numbers than other glial cells. Vaughn and Peters (1968) stated that the "small glioblast" present in large numbers during early development, closely resembled the third glial cell type described above. The description of Vaughn's "small glioblast" closely resembled the cell described as a "spongioblast" by Caley and Maxwell (1968b) in developing rat cerebral cortex. Mori and Leblond (1969b), described a microglial cell in the corpus callosum of the adult rat, resembling that described by Vaughn. The major disagreement in the literature, appeared to be the embryonic origin of this cell type, whether it was mesodermal, entering the Central Nervous System with developing blood vessels, or neuroectodermal existing from the beginning as a primitive glial element.

During the course of this study, no cell was observed which resembled the microglial cell reported by the previously mentioned authors.

Summary - Neuroglia

1. A number of glioblasts, which represented the precursors of the oligodendrocyte and astrocyte cell lines were present during the first post-natal week.

2. Young astrocytes were characterized by relatively low nuclear and cytoplasmic densities and numerous cytoplasmic processes forming perivascular end-feet. The cytoplasm contained short, wide cisternae of granular endoplasmic reticulum, well developed Golgi, numerous electron dense bodies and a few scattered filaments.

3. As the young astrocytes approached maturity, there appeared to be a decrease in cytoplasmic density and a reduction in the number of electron dense bodies. Astrocytic filaments increased in numbers with the advancing age of the cell.

4. Young oligodendrocytes were characterized by an overall high electron density. The nucleus contained a dispersed nuclear chromatin pattern with only a thin rim of condensed chromatin at the nuclear envelope. The organelle rich cytoplasm formed both broad and slender processes.

5. As young oligodendrocytes approached maturity there was a loss of many of the cytoplasmic processes and the nuclear chromatin became progressively more clumped in appearance.

6. Both astrocytes and oligodendrocytes appeared mature by six weeks post-natal.

FIGURES - NEUROGLIA 27-47

Immersion Fixation	Perfusion Fixation
Figures 27	Figures 36
28	37
29	38
30	41
31	42
32	43
33	44
34	45
35	46
39	47
40	

FIGURE 27. A glioblast from neonate substantia gelatinosa, exhibiting a round nucleus containing condensed chromatin and a thin rim of cytoplasm containing few organelles.
X 27,215

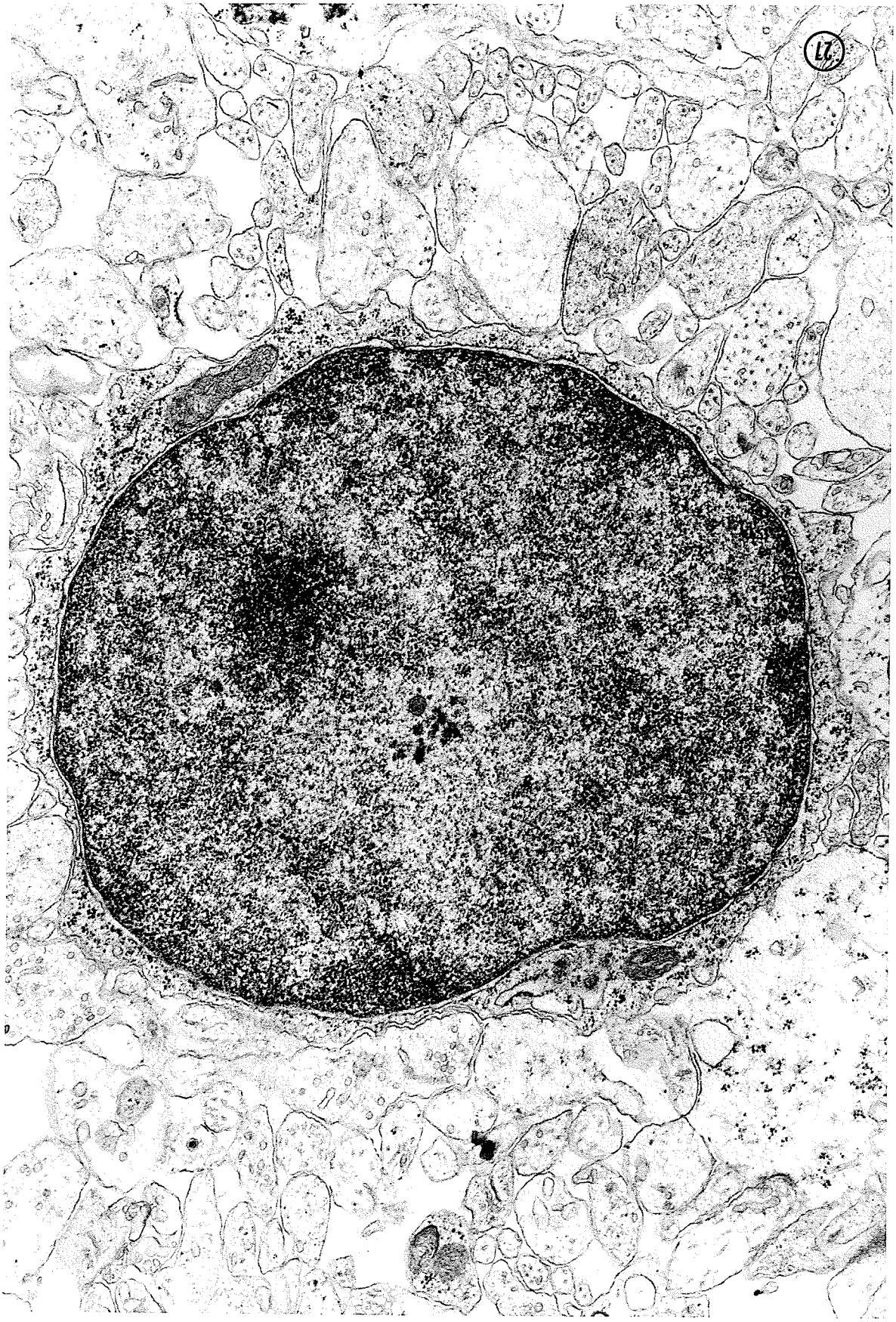

27

FIGURE 28. A glioblast from neonate substantia gelatinosa, possessing an ovoid nucleus with condensed chromatin. The cytoplasm is scarce, containing free ribosomes and several mitochondria.
X 28,880

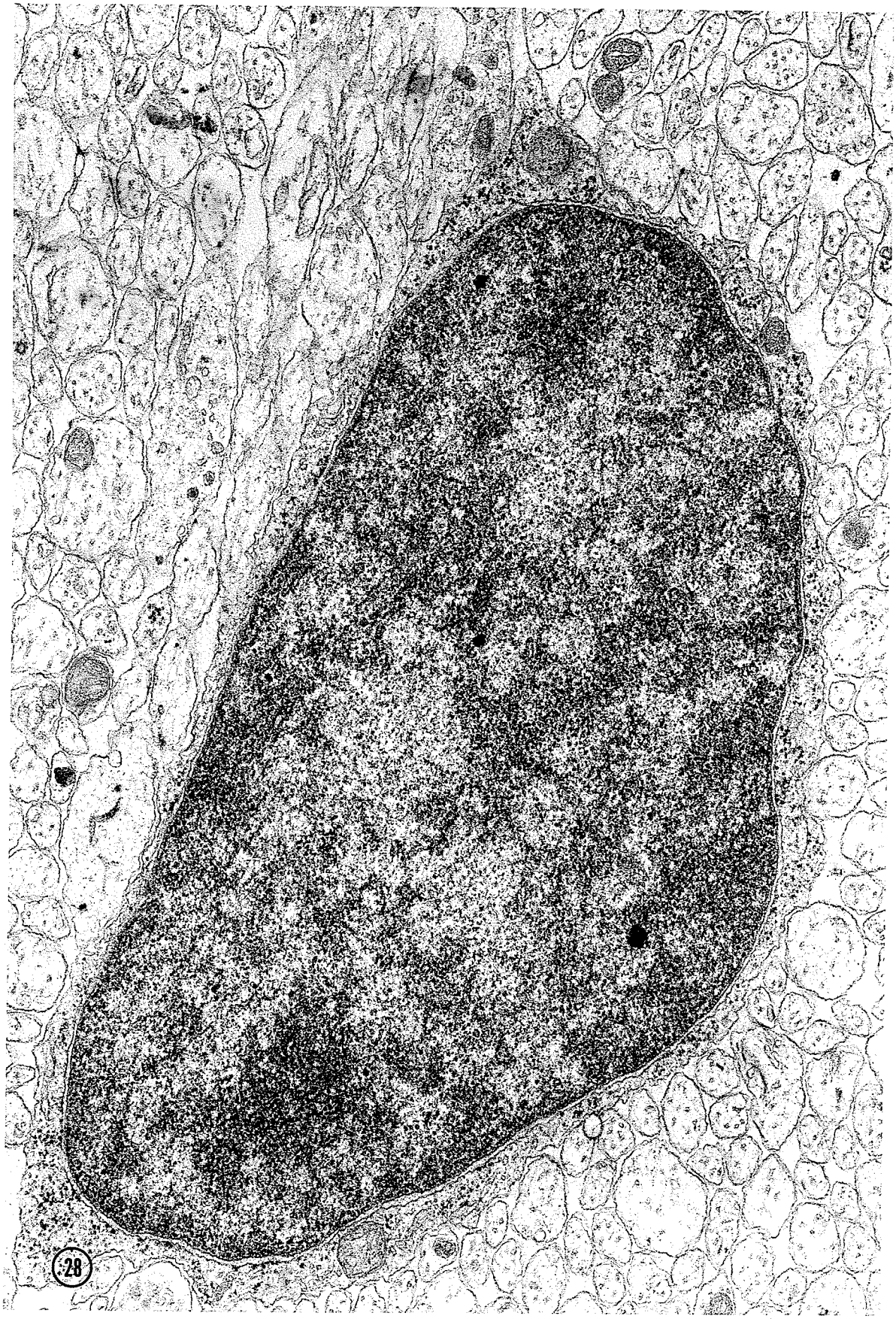

28

FIGURE 29. A glioblast from a three day post-natal animal, exhibiting slight irregularities in nuclear contour. The cytoplasm contains several cisternae of granular endoplasmic reticulum, free ribosomes and several mitochondria.

X 40,120

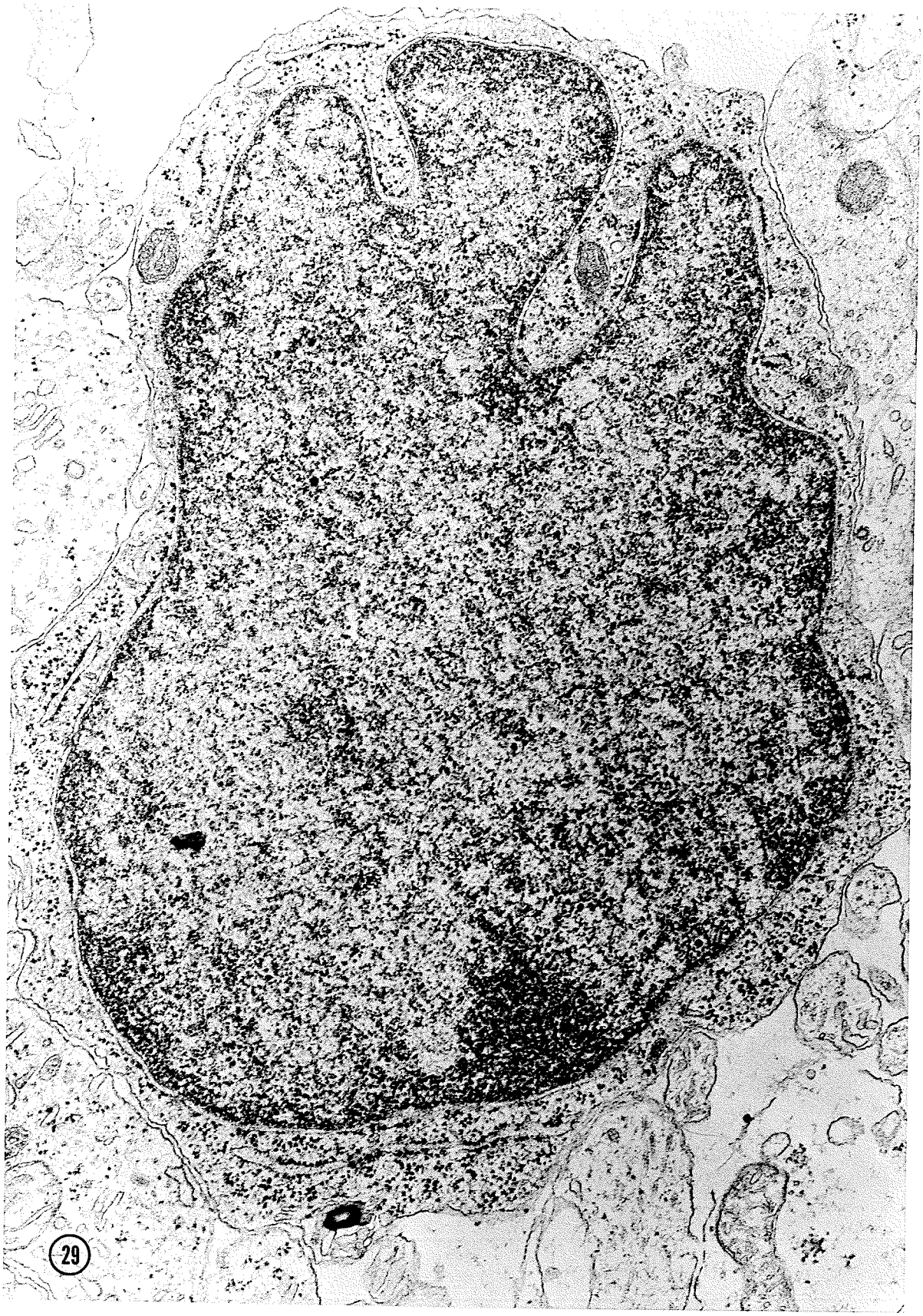

29

FIGURE 30. A low power micrograph demonstrating a young astrocyte (As) and a young oligodendrocyte (Ol) from a three day old animal. Note the distinct difference in overall electron density between the two cell types. The young oligodendrocyte possesses a greater electron density of both nucleus and cytoplasm than that of the young astrocyte. The short wide cisternae of granular endoplasmic reticulum which characterize the astrocyte are strikingly different from the long narrow cisternae of the oligodendrocyte.

X 16,360

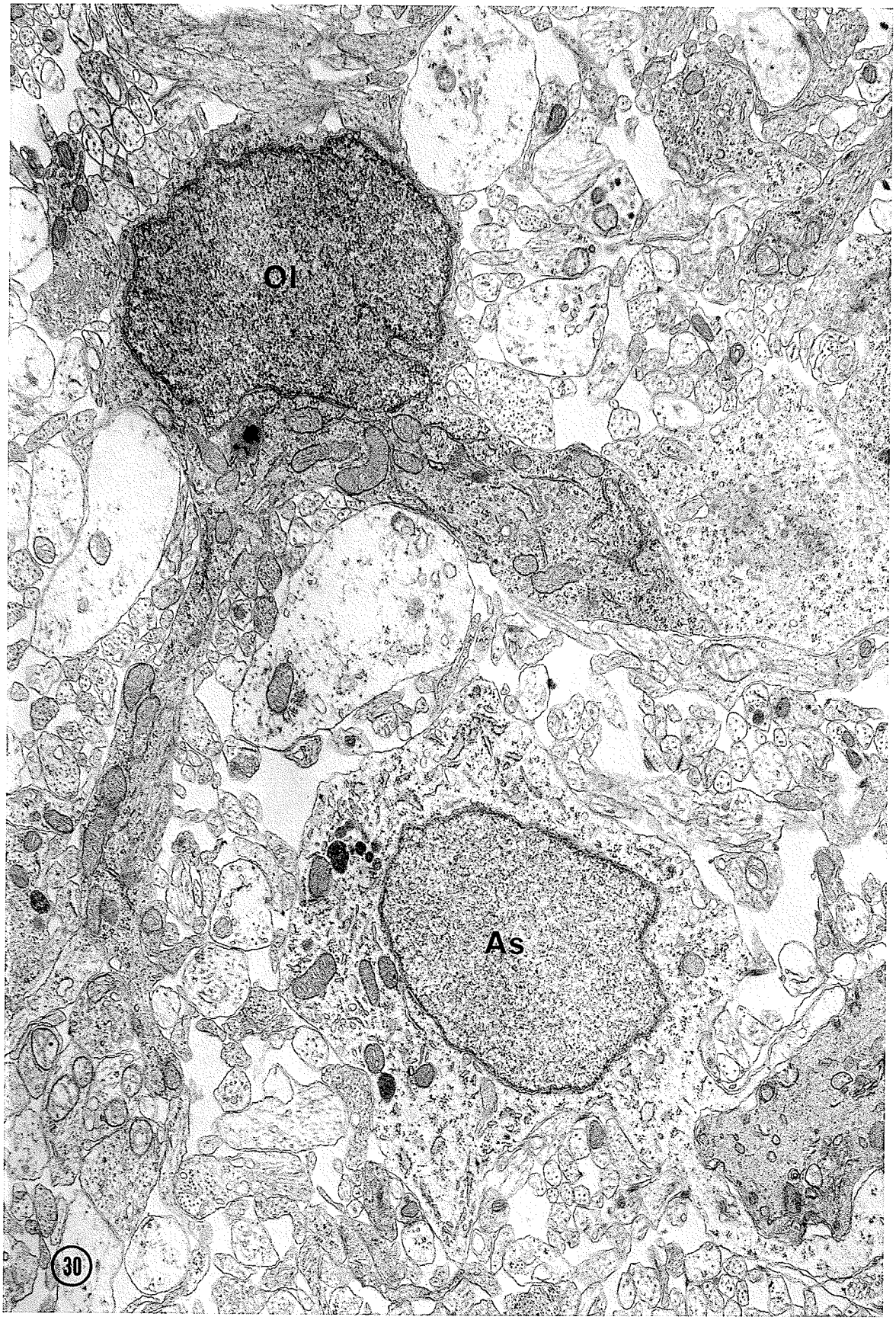

Ol
As
30

FIGURE 31. A low power micrograph from a three day old animal which was similar to Fig. 30. A young astrocyte (As) and a young oligodendrocyte (Ol) are situated adjacent to each other.

X 20,109

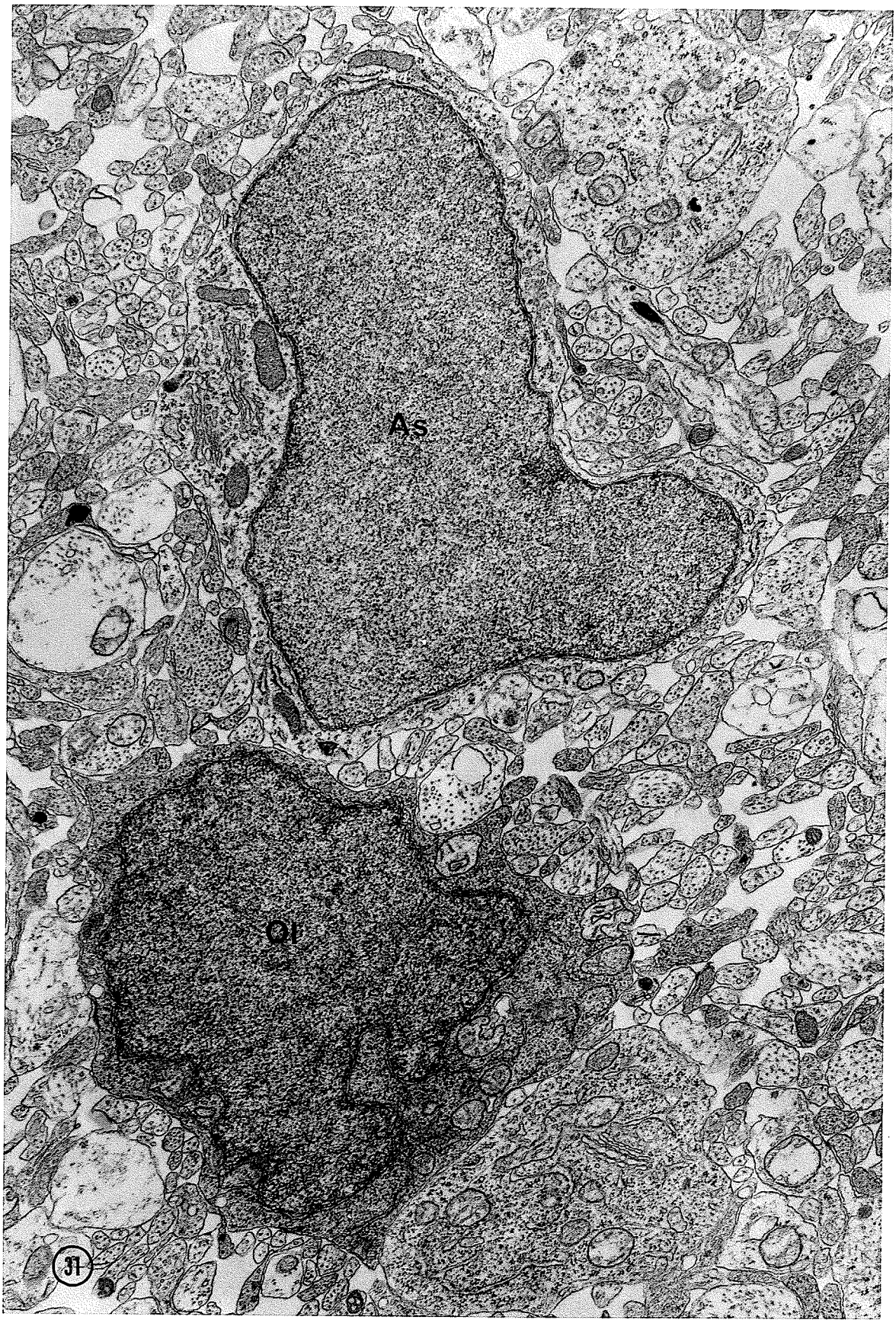

As
Ol
J1

FIGURE 32. A young astrocyte (As 1) with cytoplasmic processes surrounding a primitive blood vessel (V) was noted in a neonate animal. The nucleus contains a relatively homogeneous nuclear chromatin pattern. The cytoplasm contains the short wide cisternae of granular endoplasmic reticulum characteristic of mature astrocytes. Mitochondria are numerous. An electron dense body is also present. A possible young astrocyte (As 2) resembles As 1 with the exception of the increased amount of nuclear chromatin clumping. Portions of neuroblasts (N) are also present in this micrograph.

X 22,900

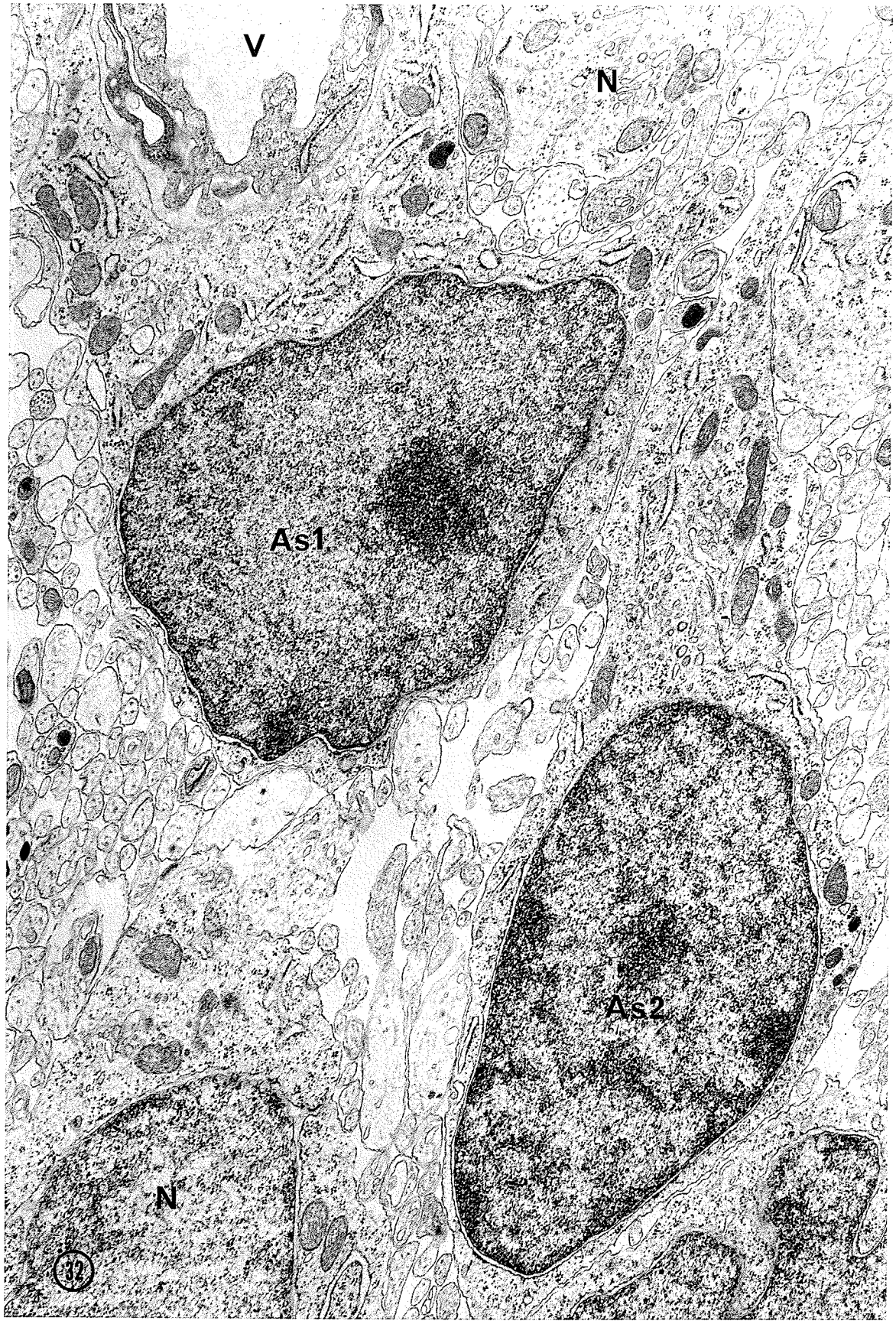

V
N
As1
As2
N
32

FIGURE 33. A young astrocyte from a neonate animal demonstrating a slightly higher electron density than more mature astrocytes. The nucleus possesses a diffuse nuclear chromatin pattern with only a slight condensation at the nuclear envelope. The endoplasmic reticulum is composed of short wide cisternae. Some filaments (arrow) are present in the cytoplasmic process.

X 27,355

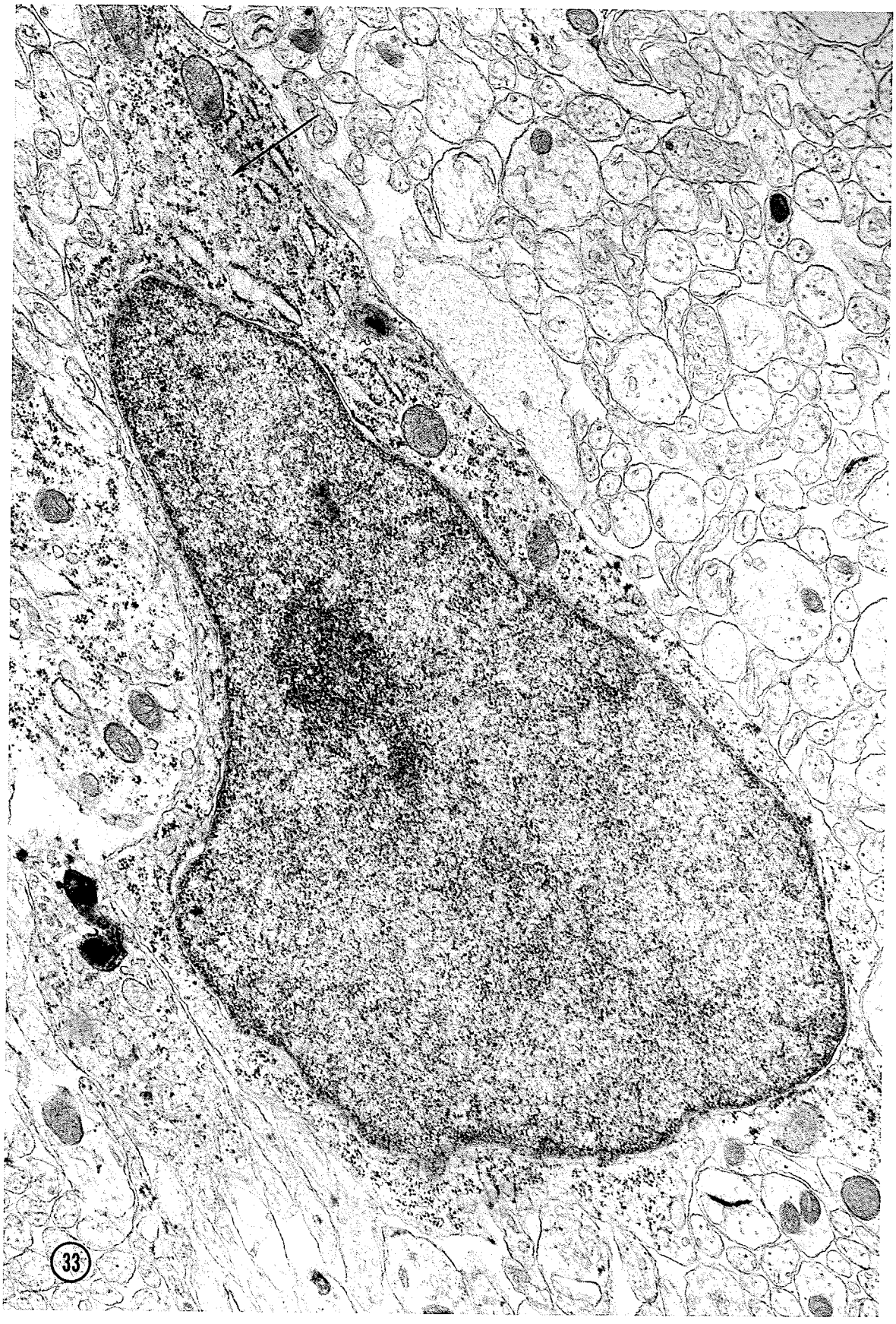

33

FIGURE 34. A young astrocyte at three days post-natal, with a lighter appearing cytoplasm than the young astrocytes shown in Figs. 32 and 33. Golgi complexes and numerous electron dense bodies are present. Astrocytes were commonly observed in juxtaposition to neurons (N) as is demonstrated by this micrograph.

X 23,120

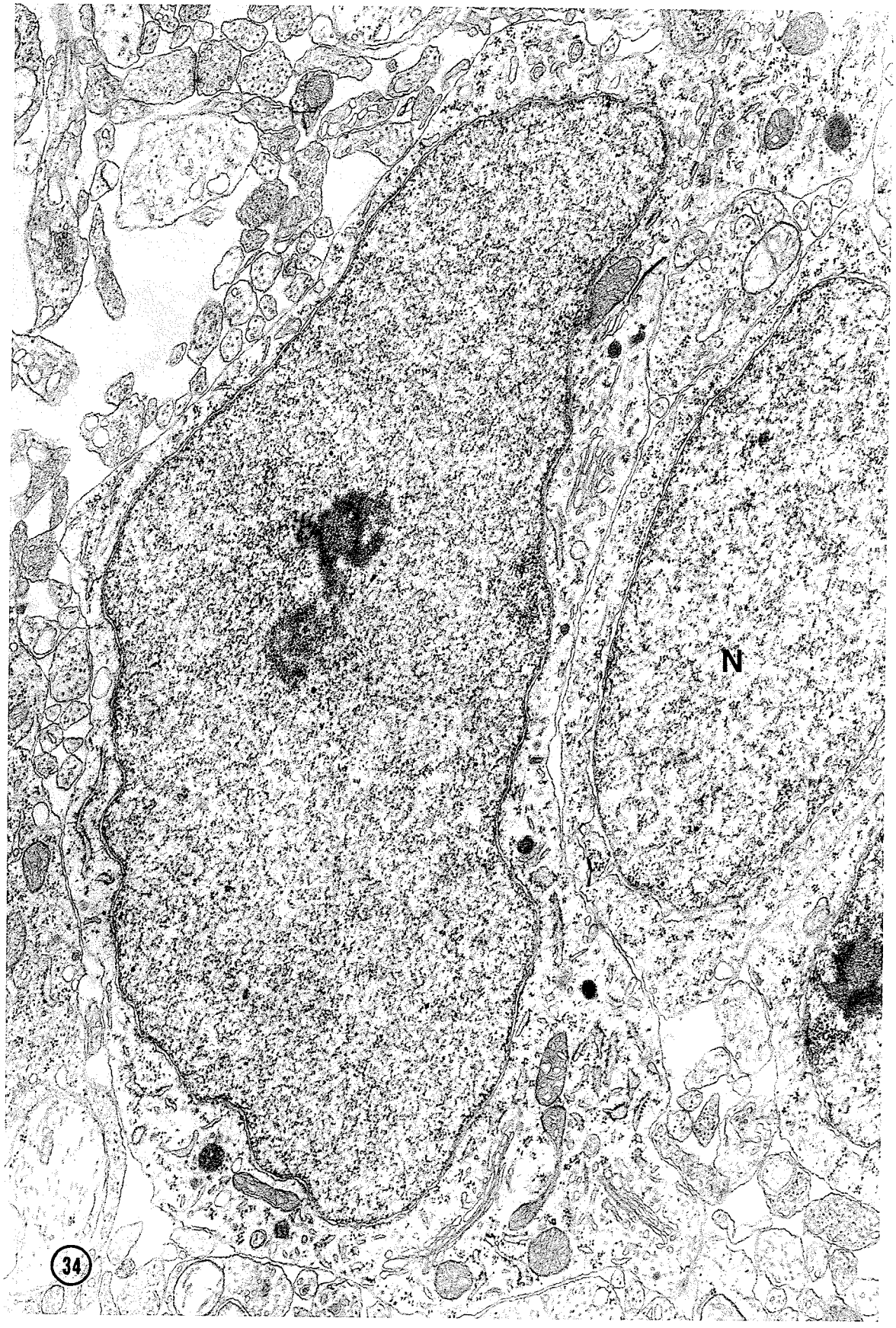

N
34

FIGURE 35. A micrograph demonstrating the pattern of organelles in an astrocyte (As) at three days post-natal. Mitochondria are numerous. The Golgi complexes appear well developed. Ribsosomes are unevenly attached to the cisternae of endoplasmic reticulum. A few scattered microtubules (Mt) are also present.

X 27,350

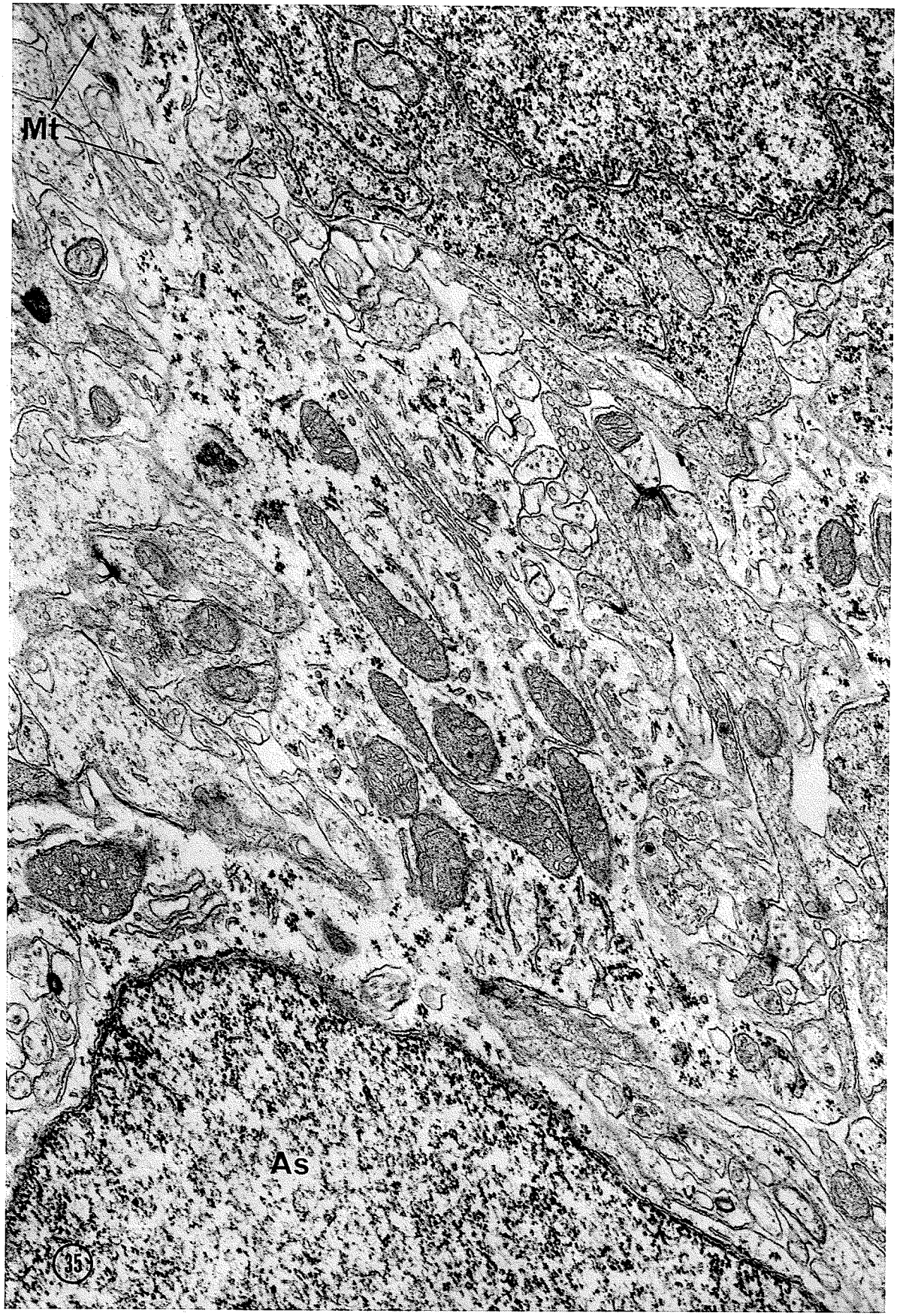

Mt
As
35

FIGURE 36. An astrocyte at two weeks post-natal, demonstrates a small bundle of filaments (arrow) in the cytoplasm. A portion of the astrocyte cytoplasm is forming a perivascular end-foot on the adjacent blood vessel (V).

X 17,310

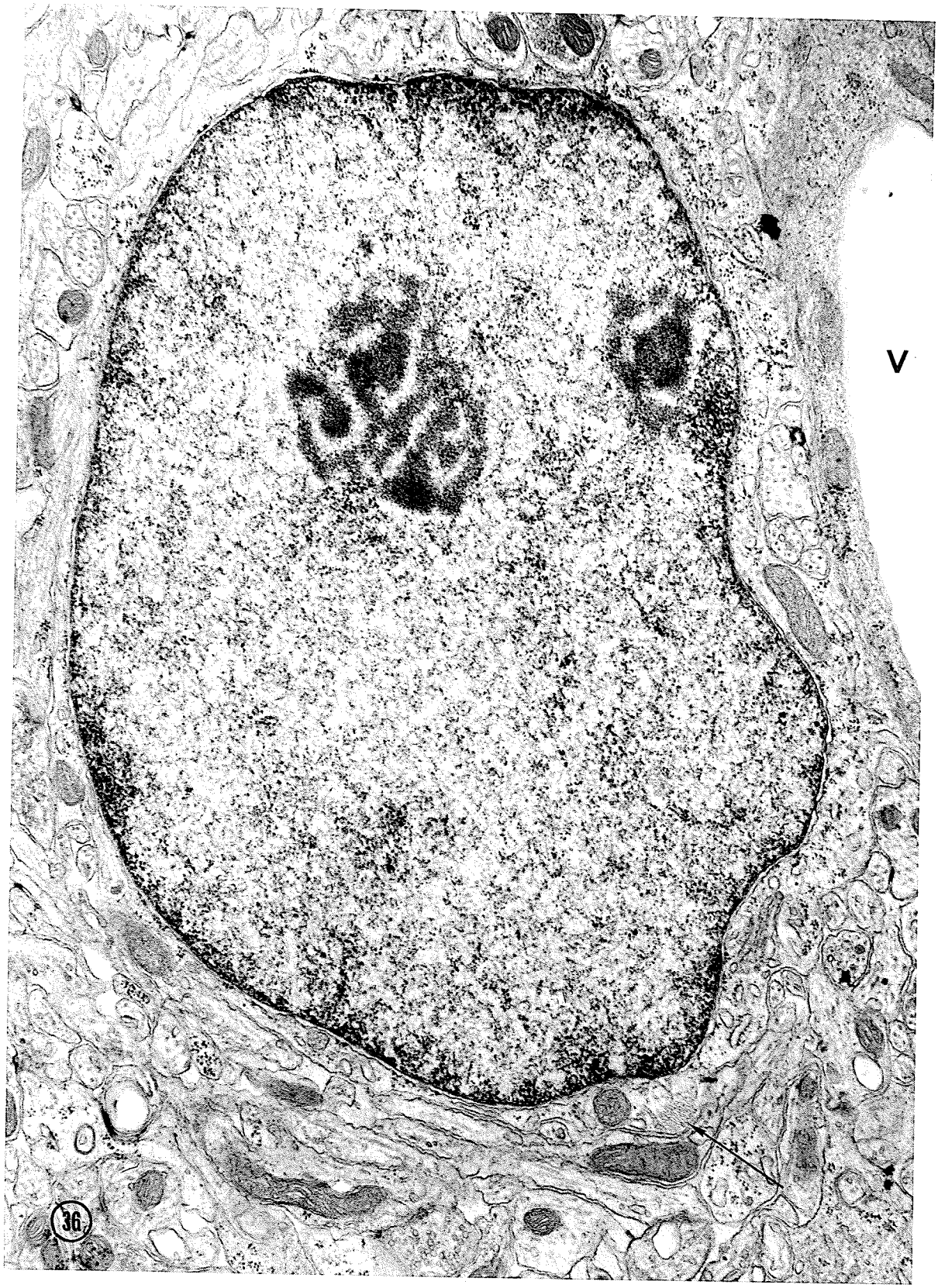

V
36

FIGURE 37. A mature astrocyte from a six week old animal contains numerous filaments (arrows). A few scattered short segments of endoplasmic reticulum and a small Golgi complex are present. The nuclear chromatin appears coarse when compared to the chromatin pattern of the young astrocyte.

X 25,900

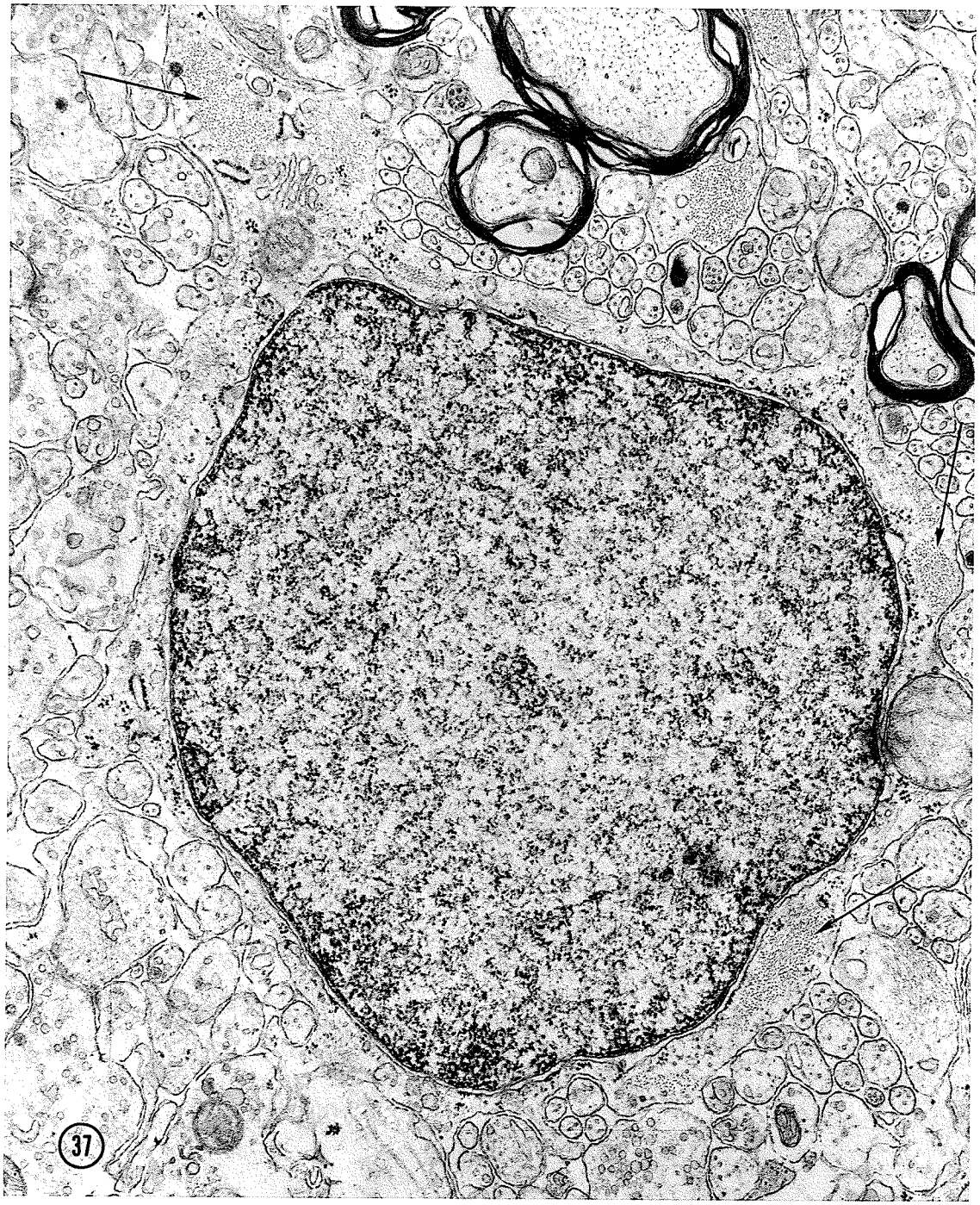

37

FIGURE 38. This micrograph from a five week old animal demonstrates the type of junctional complex (arrows) occurring between adjacent astrocytic processes, exclusive of the junctional complexes present between adjacent perivascular end-feet. Note the large number of filaments which are present. The astrocyte nucleus (N) is present at the bottom of the micrograph.

X 50,970

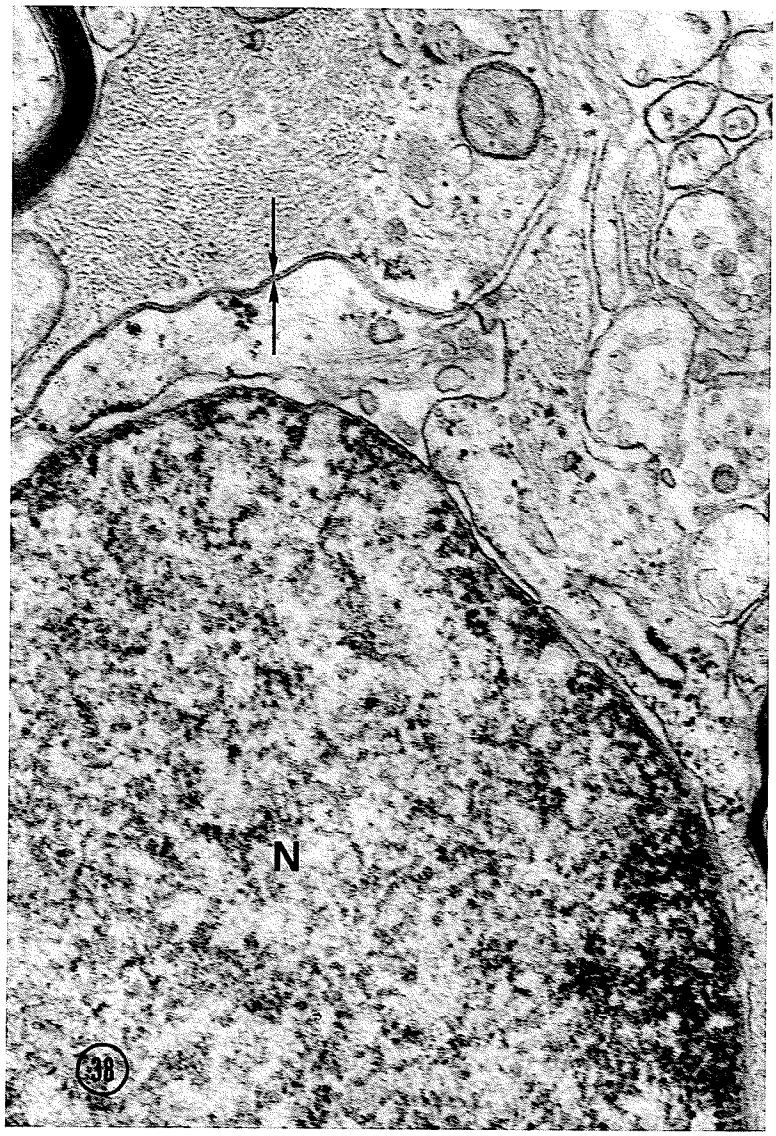

N
38

FIGURE 39. A young oligodendrocyte from a three day post-natal animal demonstrating two broad processes containing numerous microtubules. The electron dense nucleus contains diffuse chromatin with a thin rim of condensed chromatin at the nuclear envelope and a prominent nucleolus. X 24,280.

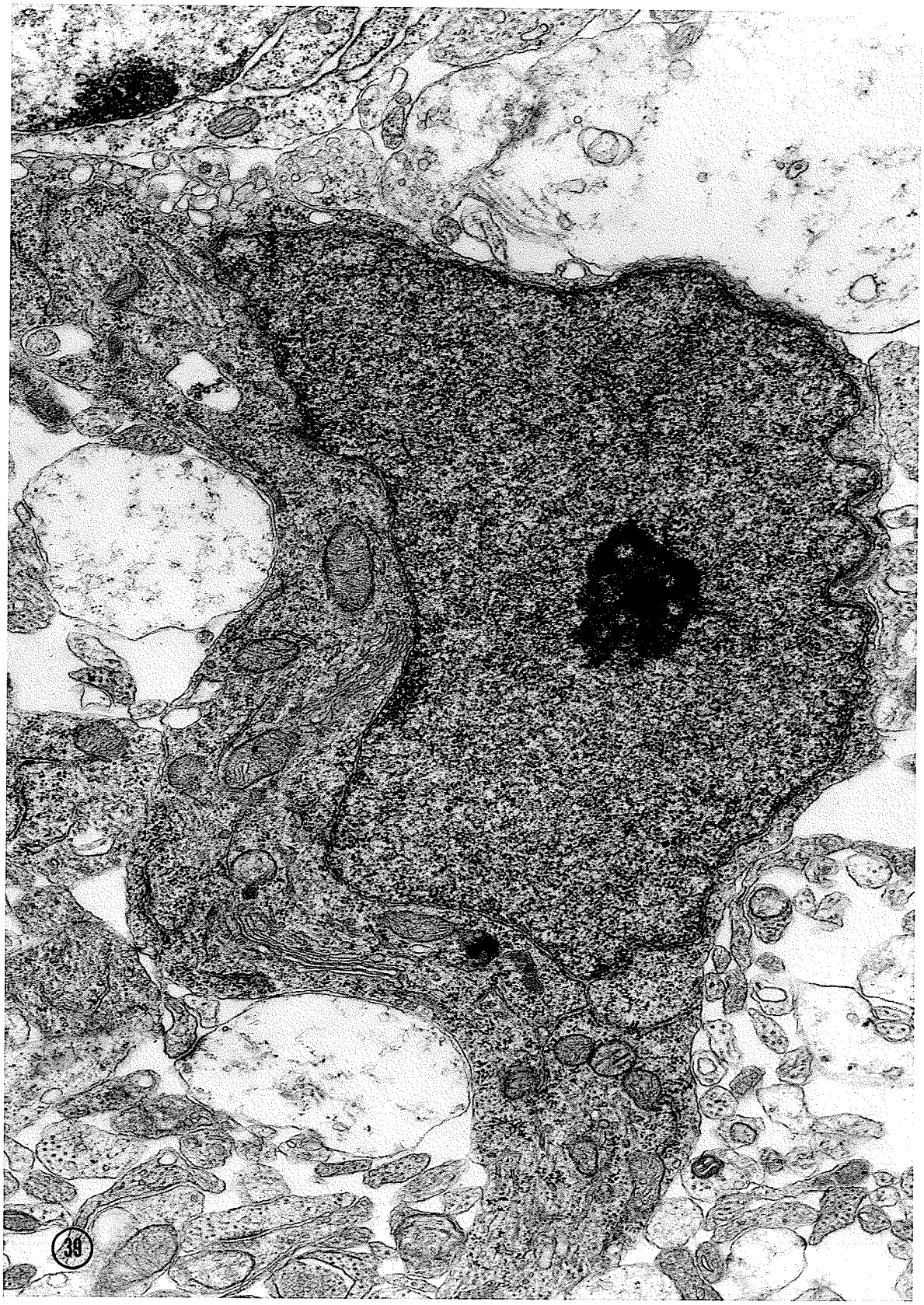

39

FIGURE 40. A young perineuronal oligodendrocyte (Ol) is adjacent to a neuroblast (N) at three days post-natal. Note the difference in overall electron density between the two cell types.

X 24,620

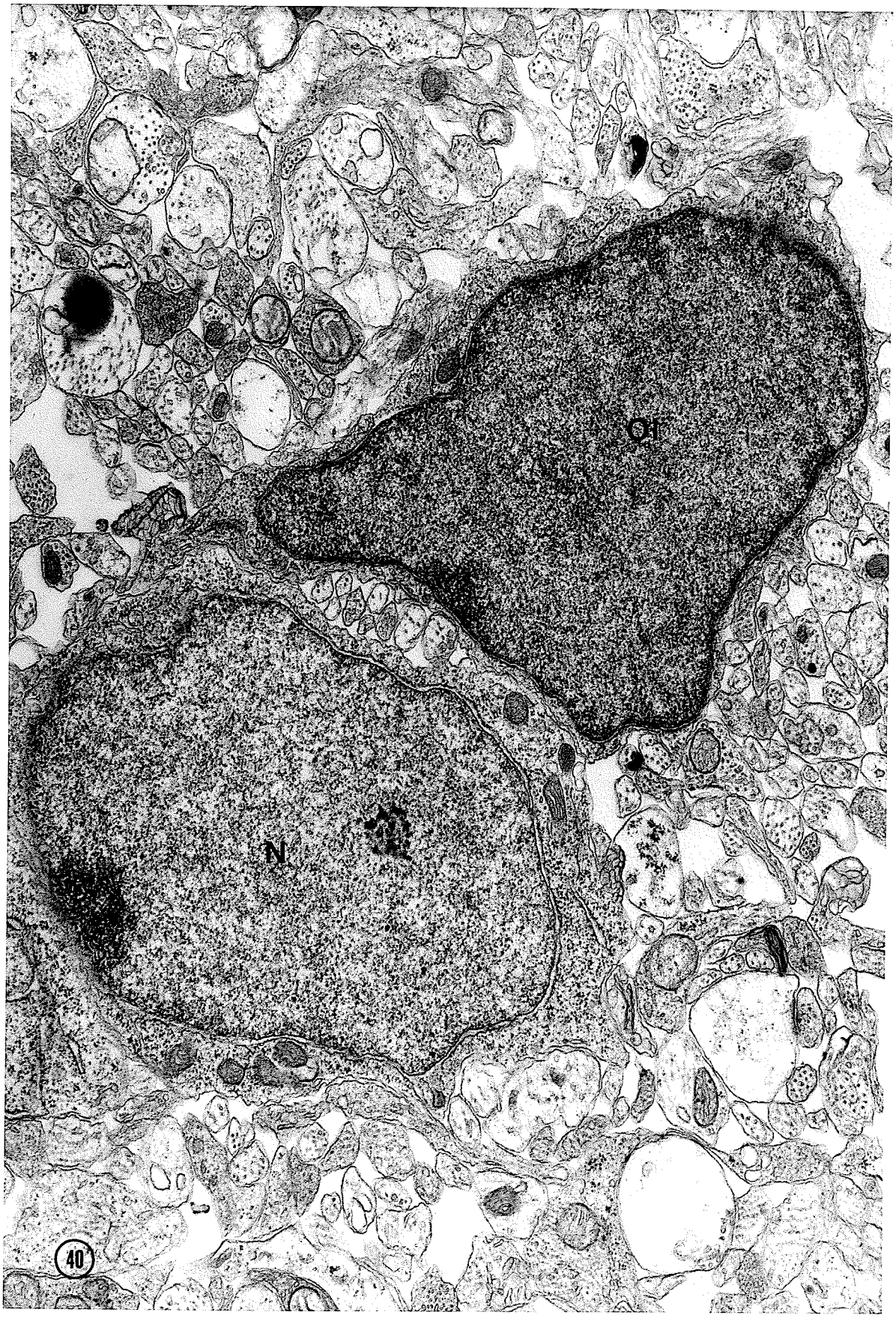

Ol
N
40

FIGURE 41. A young perineuronal oligodendrocyte from a one week old animal exhibiting numerous broad and slender cytoplasmic processes. The cytoplasm contains a well developed granular endoplasmic reticulum, multiple Golgi complexes, numerous free ribosomes and mitochondria. A neuroblast (N) is present at the top of the micrograph.
X 23,960

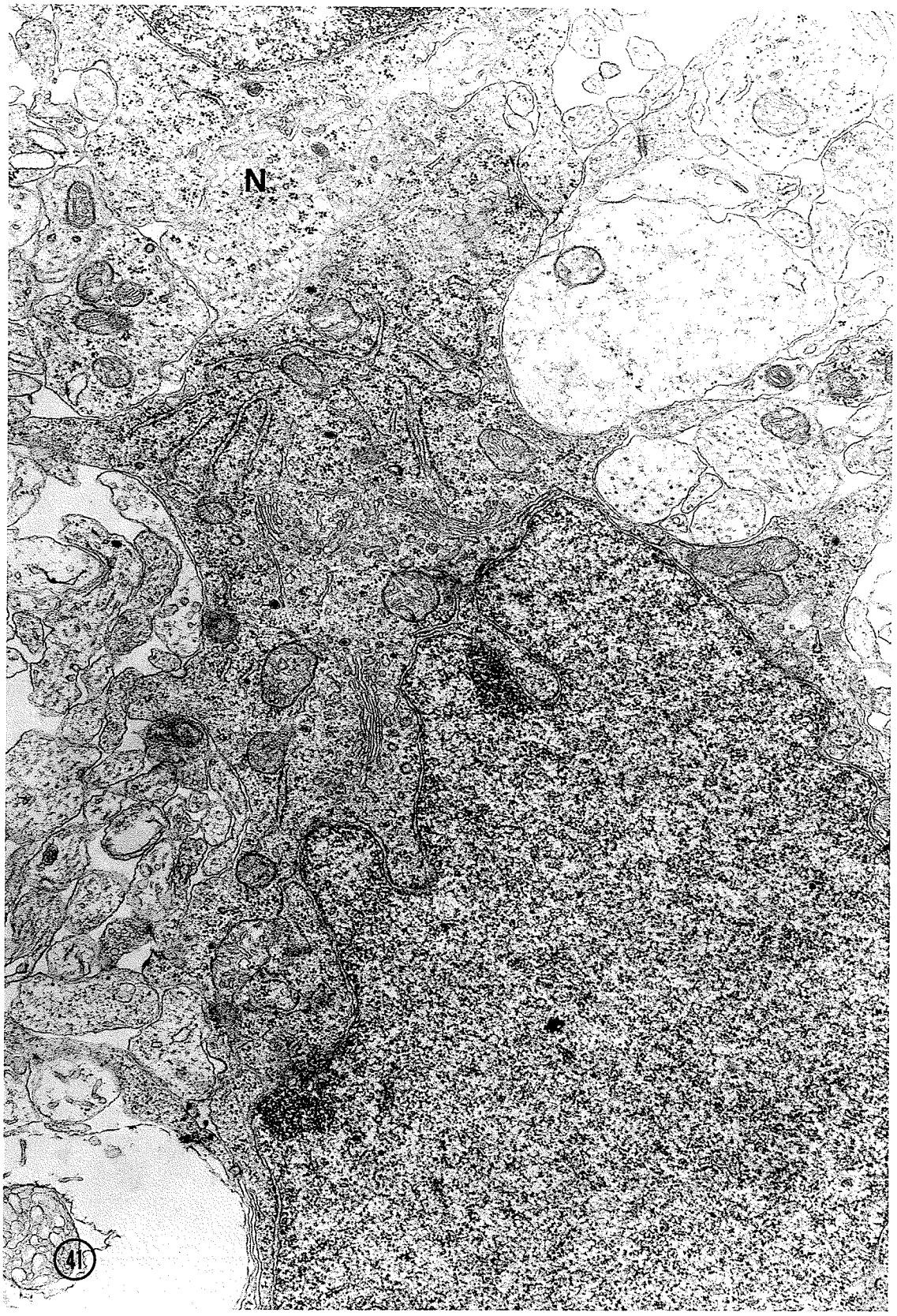

N
41

FIGURE 42. A young oligodendrocyte at one week post-natal, with one broad process (P). The cytoplasm contains prominent Golgi complexes. A possible site of axonal wrapping is present (arrow).

X 27,490

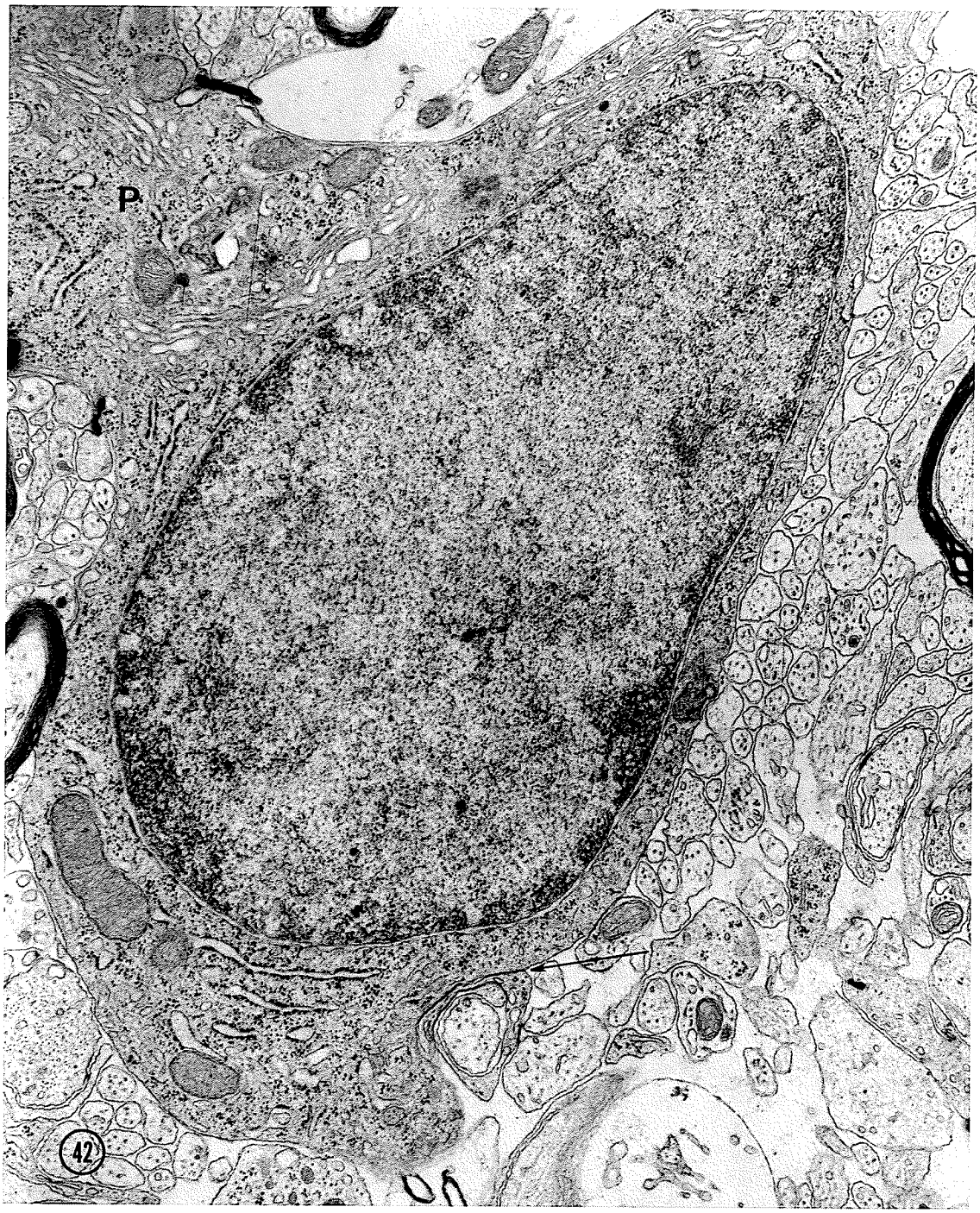

P
42

FIGURE 43. Higher magnification of Fig. 42 which demonstrates a possible site of axonal (Ax) wrapping by the adjacent oligodendrocyte. Although there is no direct connection. The cytoplasm of the ensheathing process resembles that of the oligodendrocyte.

X 65,250

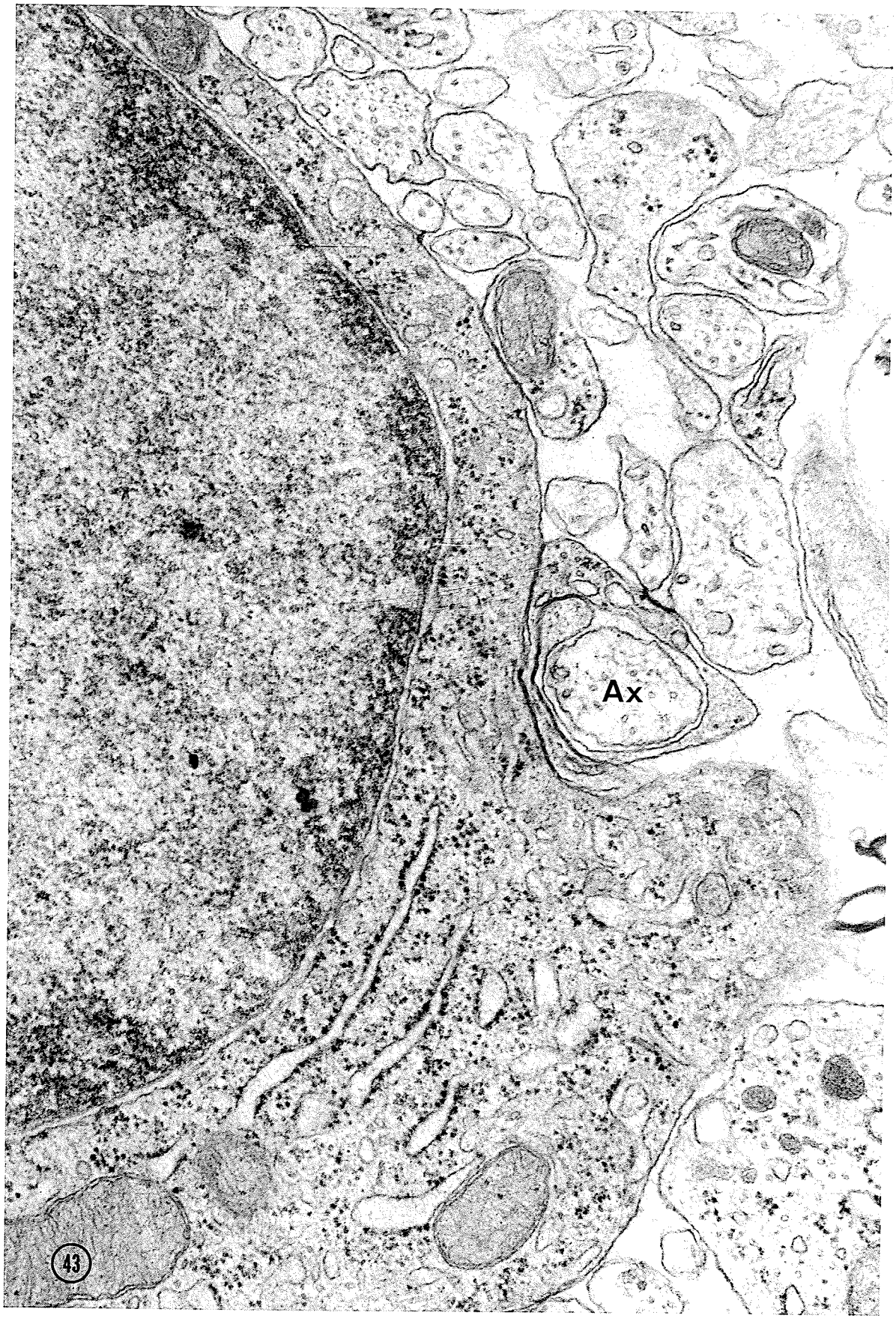

Ax
43

FIGURE 44. A young perineuronal oligodendrocyte at two weeks post-natal which contains a well developed granular endoplasmic reticulum and multiple Golgi complexes. Numerous electron dense bodies are also present. Many free ribosomes were a constant cytoplasmic feature. A neuroblast (N) is present at the top of the micrograph.

X 17,100

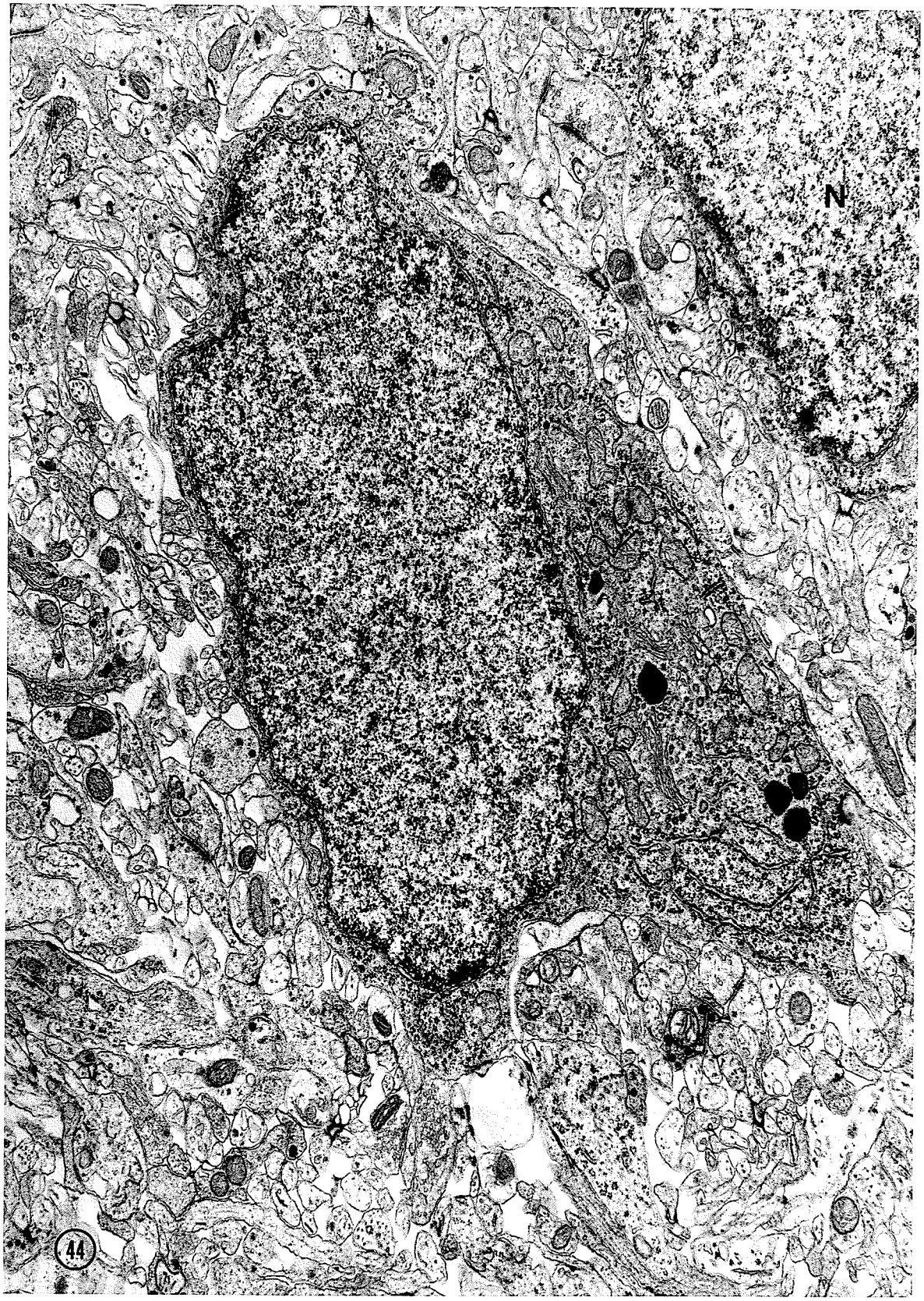

N
A
N
44

FIGURE 45. A micrograph of a young oligodendrocyte from a one week old animal. Note the presence of large quantities of free ribosomes, a well developed granular endoplasmic reticulum and multiple Golgi complexes. Electron dense bodies are also present.

X 20,670

45

FIGURE 46. A mature oligodendrocyte from a five week old animal exhibiting clumping of nuclear chromatin. The cytoplasm presents a rounded appearance when compared to a young oligodendrocyte. Note the watery nature of the astrocytic processes (As) containing filaments. A portion of neuronal (N) cytoplasm is present in the lower aspect of the micrograph.

X 26,160

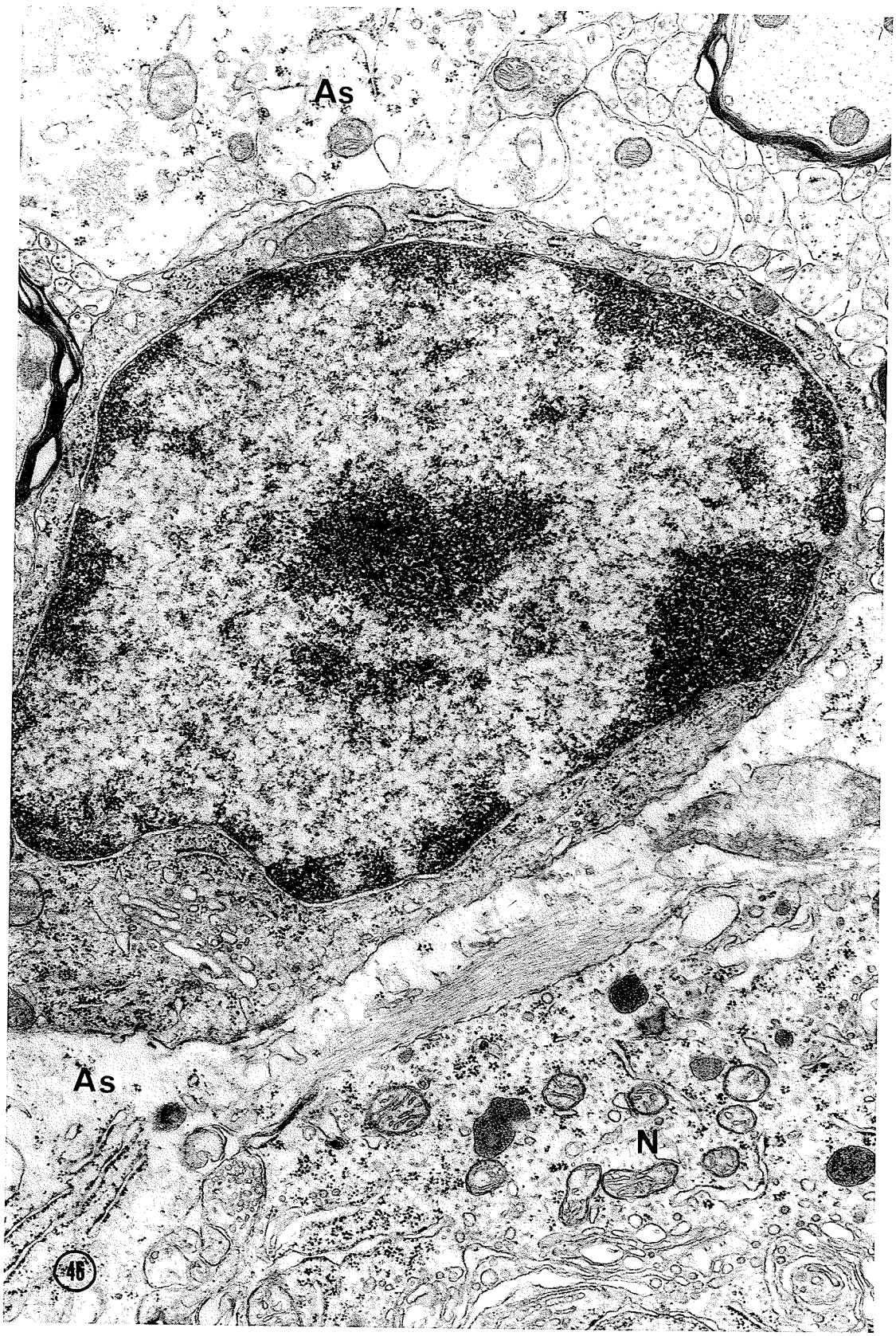
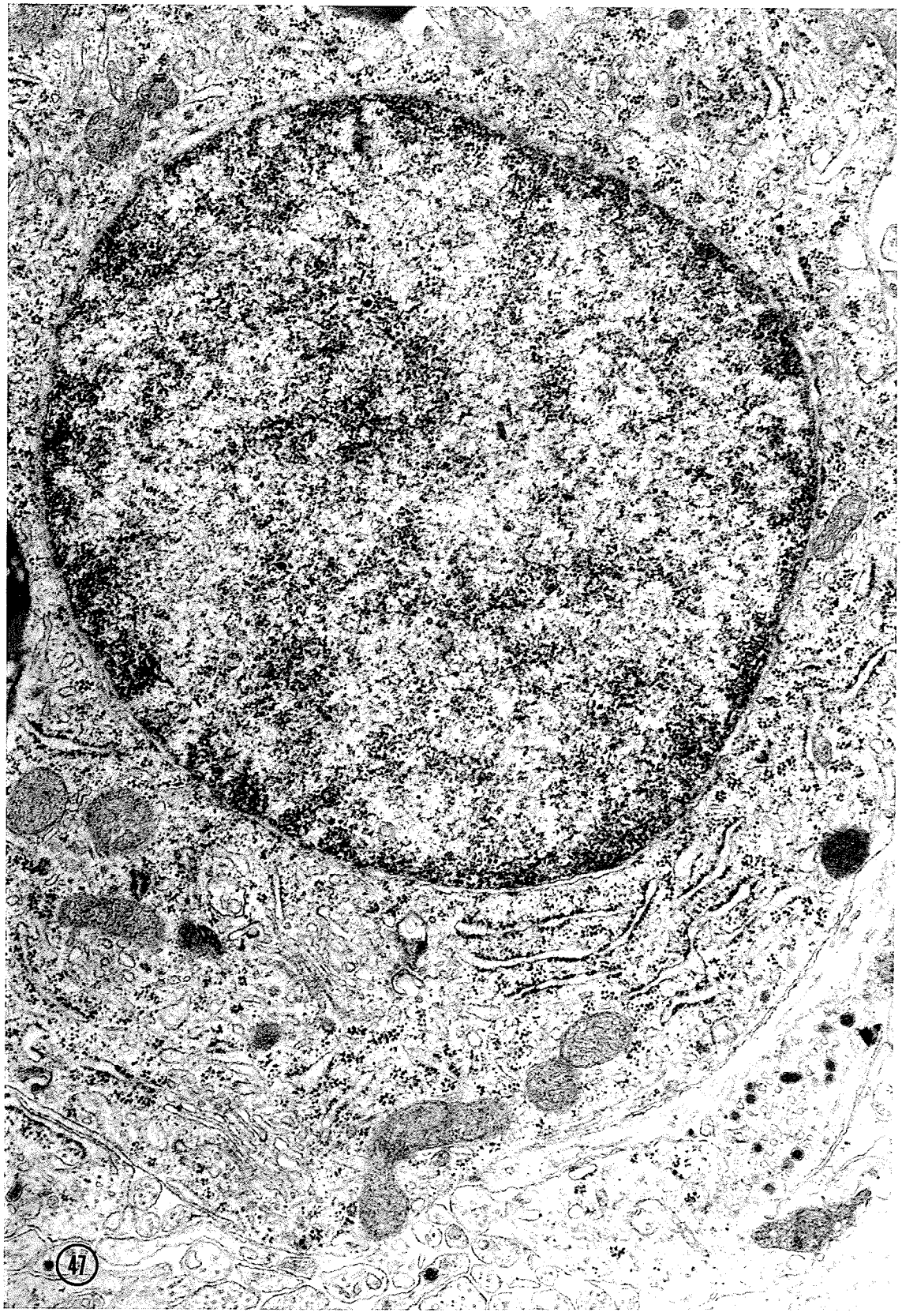

As
As
N
46

FIGURE 47. A mature oligodendrocyte from a six week old animal. The oligodendrocyte possesses more cytoplasm than the oligodendrocyte in Fig. 46. The round nucleus contains condensed chromatin. The cytoplasm contains prominent Golgi complexes, scattered granular endoplasmic reticulum, mitochondria and large quantities of free ribosomes. A few electron dense bodies are also present.

X 32,830


47

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