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A QUALITATIVE AND QUANTITATIVE ULTRASTRUCTURAL STUDY OF GLIAL CELLS IN THE DEVELOPING VISUAL CORTEX OF THE RAT

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(i) This paper provides new information on the time course and fine structural features of glial cell differentiation, on the relative frequencies of glioblasts, astroblasts, astrocytes, oligodendrocytes and microglial cells, and on neuron: glia ratios in visual cortex of the rat between birth and maturity. The analyses were done on montages of electron micrographs of 75 μm wide strips extending the full depth of the cortex from animals 12 h and 4, 6, 8, 10, 12, 14, 20, 24, 90 and 180 days old (six montages from two or three animals at each age).

(ii) At birth, and up to 4 days, most non-neuronal cells are poorly differentiated, irregularly shaped cells with dark nuclei (*glioblasts*). A few at this stage and progressively larger numbers over the next few days, can be recognized as *astroblasts* by the presence of a distinctive form of granular reticulum (distended cisterns with a moderately electron dense content), and some also by their position in contact with the subpial or perivascular basal laminae. Astroblasts enlarge, develop processes and transform into *immature astrocytes*: their nuclei become paler, the granular reticulum is no longer distended, and glial filaments begin to accumulate. *Mature astrocytes* with pale nuclei, filaments and a low concentration of perikaryal organelles in a pale cytoplasmic matrix predominate at 24 days, and at 3–6 months 51 % of all glial cells are astrocytes.

(iii) Concentrations of glioblasts (at 0 and 4 days) and subsequently of cells of the astrocytic lineage are apparent in the most superficial and in the deepest cortical layers, and an additional small peak is seen at the level of layer IV in the adult animals. The superficial concentration is probably associated with the subpial glia limitans and the layer IV concentration with the high density of synapses in this region; several probable explanations are considered for the concentration in layer VI.

(iv) Processes of *radial glial cells* are apparent from birth to day 8 but not thereafter. No evidence was found for transformation of radial glia into astrocytes. A peak in phagocytic activity by immature microglial cells at days 6–8 suggests the possibility of loss of radial glial processes by degeneration rather than transformation.

(v) *Oligodendroblasts*, intermediate in morphology between glioblasts and light oligodendrocytes, appear suddenly in the deep cortex and subcortical white matter at day 6 and are rapidly replaced by *light oligodendrocytes*. These are large, organelle-rich cells with characteristically distended Golgi saccules, and are the only oligodendrocytes present during early myelination, which begins at day 10. Early in the 3rd postnatal week some light oligodendrocytes are replaced by *medium oligodendrocytes*, which are smaller and darker, with abundant orderly stacks of granular reticulum. *Dark oligodendrocytes* are first apparent at the end of the 3rd week, account for about one-third of all oligodendrocytes at day 24, predominate at day 40 and constitute 90 % of all oligodendrocytes at 3 and 6 months, at which time oligodendrocytes comprise 39 % of all cortical glial cells. We suggest that the progression from light to medium oligodendrocytes does not simply represent a diminution in the overall level of synthetic activity but that different components of the myelin sheath are being synthesized at the two stages.

(vi) *Microglia* are present from birth but are seen in significant numbers at days 6–10 and thereafter. Some are relatively mature in appearance, even in the youngest animals, and almost all are similar to the resting microglia of adult brain by day 16. At 3–6 months, 8 % of all cortical glial cells are identified as microglia and these cells are fairly evenly distributed throughout the cortical depth but are surprisingly and consistently poorly represented in layer VI. From day 6 to the end of the 2nd postnatal

week, cells with poorly differentiated cytoplasm (many free polyribosomes), but containing phagocytosed products of cell degeneration, are identified as *immature microglia*. However, it is possible that such cells do not mature into classical resting microglia but that they represent a different cell type.

(vii) The *neuron: glia ratio* is 4.54 at birth, rises to 5.09 at 4 days, and falls to approximately 2.5 at days 12–24. At 3–6 months the ratio is 2.13.

1. Introduction

The discovery of the neuroglia ('nerve glue') dates back to the middle of the 19th century and, although there were important antecedent publications, is generally attributed to Virchow (for historical reviews see Penfield 1932, Privat 1975, Sturrock 1975, Privat & Fulcrand 1977). Towards the end of the last century and in the early years of this, the development of selective metallic impregnation methods allowed the cellular nature of the neuroglia to be recognized, their characteristics to be investigated with the light microscope and the evidently heterogeneous neuroglial cell family to be subdivided into astrocytes, oligodendrocytes (together composing the macroglia), ependyma and microglia (see for example Cajal 1913, 1916, Hortega 1919, 1921, 1932).

The general morphological and cytological features of the different types of glial cell are now well known, and have been thoroughly described by light microscopy (see for example Glees 1955) and by electron microscopy (see reviews by Mugnaini & Walberg (1964), Vaughn & Peters (1971), Peters *et al.* (1976) and Wolff (1976)). In recent years too, much information has accumulated about their biochemical characteristics and their functions (Kuffler & Nicholls 1966, Hyden 1967, Watson 1974, Peters *et al.* 1976, Hertz 1977, Orkand 1977, Stewart & Rosenberg 1979, Henn & Henn 1980). As for their histogenesis, it is universally accepted that the macroglia, like the neurons of the central nervous system (c.n.s.), are of neuroectodermal origin, and there is considerable evidence, but not universal agreement (see for example Vaughn & Peters 1971, Oehmichen 1978) that the microglia, like the macrophages of other tissues, are of mesodermal origin, gaining access to the c.n.s. parenchyma from the meninges and from the walls of blood vessels (see for example Hortega 1932, Cammermeyer 1970, Peters *et al.* 1976, Ling 1981, Murabe & Sano 1982).

Although there has been considerable interest in gliogenesis over the last few years (e.g. see reviews by Privat (1975), Korr (1980) and Skoff (1980)), information on glial cell development is still deficient in many major respects. For example, although the recent immunocytochemical studies of Levitt *et al.* (1981) have shown that neuronal and astrocytic cell lines may already be distinct in the ventricular and subventricular zones at the height of cortical neurogenesis in monkey, general information on when or how the precursors of the neuronal and various glial cell lines separate and data concerning the morphological and biochemical changes accompanying the genesis and differentiation of mature glia from the earliest committed glial precursor cells are scanty. Furthermore, little is known about the effects of hormones or glial growth factors on these events. There is available a certain amount of information on the ultrastructural features of developing glial cells in the mammalian c.n.s., chiefly but not exclusively in white matter (see for example Meller *et al.* 1966, Caley & Maxwell 1968, Vaughn 1969, Vaughn & Peters 1971, Sturrock 1974, Privat 1975, Skoff *et al.* 1976*a*, *b*, Narang 1977, Privat & Fulcrand 1976, Imamoto & Leblond 1978, Imamoto *et al.* 1978). Much of this information, however, is qualitative, conflicting, and difficult to interpret.

The present study is part of our efforts to reach an understanding of the development of the visual cortex, and to provide background data for studies of cortical development in animals with inherited neurological disorders and in animals subjected experimentally to abnormal conditions that are known, or likely, to affect cortical development *in utero* and in the early postnatal period. Our approach has been to make a systematic and partially quantitative study of the postnatal development of glial cells in a single neocortical area, taking advantage of the fact that the cortex is immature at birth and the the proliferation, migration and maturation of its glial cell populations occur predominantly in the postnatal period.

Preliminary accounts of the principal findings have been published as abstracts (Luder *et al.* 1979, Pollard *et al.* 1979).

2. Material and methods

(*a*) *Animals*

Female albino rats were used. The following postnatal ages were examined: 12 h, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, 35, 40, 90 and 180 days. Two or three animals at each of the postnatal ages underlined were used for detailed analysis. Only animals whose body mass was within 15% of the mean mass for the litter or litters of a given age were included.

(*b*) *Tissue preparation*

Standard fixative mixtures and procedures, based on the methods of Peters (1970), were used for the entire series. The animals were anaesthetized with ether and perfused through a hypodermic needle in the left cardiac ventricle (animals younger than 16 days) or through a cannula tied into the ascending aorta (animals 16 days and older). In the 12 h to 14 day animals the calibre of the needles used and the rate of flow of fixative solutions were increased progressively over the period from a slow drip flow delivered in the newborn animals. The fixative solutions, at room temperature and delivered over a period of approximately 30 min with the aid of a flow inducer (Watson Marlow MHRE Mark III), comprised a dilute mixture (1% paraformaldehyde and 1.25% glutaraldehyde in 0.08 M cacodylate buffer at pH 7.3 ± 0.1) followed by a concentrated mixture (4% paraformaldehyde and 5% glutaraldehyde in the same buffer and at pH 7.3 ± 0.1). Neither heparin nor sodium nitrite was administered and the fixative solutions were not preceded by a buffered salt solution. Following the perfusion, the animals were left for approximately 5 h in plastic bags in the refrigerator, after which the heads were removed, immersed in concentrated fixative and stored in the refrigerator overnight.

The brains were removed from the skulls the following day and stored for about 2 h in refrigerated concentrated fixative. The occipital cortex and underlying hippocampus were then removed from the left hemisphere, immersed in chilled cacodylate buffer, and sectioned in the coronal plane at 100 μm in a Vibratome (Oxford Instruments). The hippocampus acted as a guide to the selection of a relatively consistent area of occipital cortex for analysis (primary visual cortex in older animals; presumptive visual cortex in younger animals). The Vibratome sections were post-fixed for 2 h in OsO_4 (2 g/100 ml) in 0.08 M cacodylate buffer, rinsed in buffer, stained for $1\frac{1}{2}$ h in aqueous uranyl acetate solution (1 g/100 ml), dehydrated through an ethanol series, passed through propylene oxide and embedded in a small amount of Araldite at the bottom of flat aluminium dishes.

Selected areas were cut from the slices and remounted on Araldite stubs for sectioning in the coronal plane. A block face including the full depth of the cortex was trimmed and semi-thin

sections (1–1½ μm) were cut with glass knives, stained with toluidine blue in borax solution (1 g/100 ml) and examined by light microscopy. To ensure that ultrathin sections for electron microscopy were cut as far as possible perpendicularly to the surface of the brain, semi-thin sections were cut at slightly varying orientations until optimal orientation, indicated by longitudinally sectioned pyramidal cell apical dendrites of maximum length parallel to the long axis of the section, was achieved. Ultrathin sections (silver to gold interference colours) were then cut and picked up on Robertson grids, which have parallel grid bars, 75 μm apart, oriented in only one direction. Care was taken to align the sections so that their long axes (and thus the apical dendrites of the pyramidal cells) were parallel to the grid bars.

The grids were stained with lead citrate for 20 min at room temperature and strips of cortex between two adjacent grid bars were photographed as a series of slightly overlapping fields at an instrumental magnification of ×3000 in a Philips 301 electron microscope. The negatives were printed at a final magnification of ×7500 and the prints were assembled into montages as described below. Just under 100 photographs were required to make a montage of the full cortical depth in the youngest animals and over 200 photographs in the older material. In all, 72 montages were prepared for analysis: six for each of the ages 12 h and 4, 6, 8, 10, 12, 14, 16, 20, 24, 90 and 180 days. The six montages per age were taken from either two or three different animals. Three additional montages were made from postnatal day 18 material, but these were not included in the material subjected to detailed analysis.

(*c*) *Analysis of the material*

Because of the unmanageable size of the final montages, the photographs were first assembled into units of three, each unit representing a horizontal strip of cortex between two grid bars (75 μm) and the units were stacked in sequence. The horizontal strips were subsequently matched and attached to adjacent strips with small pieces of adhesive tape, and the entire montage was assembled on a long laboratory bench for analysis.

Every cell with its nucleus present (both neuronal and non-neuronal) was classified, a note was made of its nuclear and cytoplasmic features, and its position was plotted on a scale map of the cortical strip on which the positions and depths of the cortical layers could be marked by reference to the adjacent stained semi-thin section(s). If the section was not oriented with apical dendrites of pyramidal cells parallel to the grid bars, the true depth below the surface of a given cell or landmark was calculated as the product of the apparent depth (measured along the grid and the cosine of the angle between the apical dendrites and the grid bar) (see § 4*a*(ii)).

Because of the considerable difficulties involved in classifying non-neuronal cells in the younger animals, the initial sequence of analysis of the montages was from mature to immature specimens. When the initial analysis had been completed, all the montages were re-examined at least once, starting with those from the youngest rats. The following classes of cells were recognized: neurons, glioblasts (subdivided when possible into astroblasts and oligodendroblasts), astrocytes (mature or immature), oligodendrocytes (light, medium or dark), microglia and pericytes. The criteria for classifying cells into one or other of these categories will be described in the Results (see § 3). Cells with cytological characteristics not fitting any of the categories used for classification (very rare) and cells whose classification could not be agreed between two or more investigators (rare in mature specimens, more common in the immature material) were placed in an additional category of 'unclassifiable glial cells'. The montages extended for a variable distance into the subcortical white matter. Because there is no sharp,

easily recognized transition between the cortical grey matter and the subcortical white, cells up to a depth of one photographic strip below the deepest neuron of layer VI (approximately 15 μm) were included in the survey, whereas more deeply situated non-neuronal cells were not. Cells partially obscured by grid bars were included in the survey provided that at least part of their nucleus and a sufficient amount of cytoplasm (on which to base an identification) were present.

Data from the montages of each age were pooled, and the relative proportions of each cell type were calculated. Histograms of cell number as a function of their vertical position (depth) in the cortex were prepared, and neuron:glia ratios were calculated for each age.

3. Results

(*a*) *Qualitative analysis*

(i) *Adult material* (3 *and* 6 *month old rats*)

There are no qualitative differences in the morphology and cytology of glial cells in the visual cortex of rats 90 and 180 days old, and the ultrastructural features of the various glial cell types are very similar to those previously described by many authors for glial cells in the cerebral cortex and other areas of the c.n.s. in adult mammals (Peters *et al.* 1976).

Astrocytes (figures 1–3†) are present at all levels in the cortex, but are more frequently encountered immediately below the surface (figures 1, 2) and in the deepest part of the cortex. Their nuclei are pale, with a fine granular nucleoplasm and a thin and generally rather even rim of heterochromatin immediately below the nuclear envelope; nucleoli, when present, are not prominent. Their cytoplasm is relatively electron lucent and their outlines are characteristically irregular: sections passing through the cell body commonly include part of a large process, and both the cell body and the processes are closely moulded to adjacent neuropil elements and commonly give rise to finger-like or sheet-like extensions such as those depicted by Špaček (1971), which penetrate between the surrounding elements. The most characteristic feature is the presence of filaments approximately 9 nm in diameter. These filaments are not especially common in rat cortical astrocytes (by comparison, for example, with fibrous astrocytes of the white matter), but when present they are grouped as small or large bundles, sometimes straight, sometimes wavy, disposed predominantly along the long axis of processes but with a variable orientation in the cell body (figure 2). Granular reticulum is sparse and represented by single short strands or cisterns, or small stacks of three or four cisterns; the density of ribosomes on the endoplasmic elements is high and large polysomal arrays are prominent. Additional non-membrane-bound polysome clusters are scattered throughout the cell body and large processes. Elements of the Golgi apparatus are generally small and associated with comparatively few vesicles. Microtubules are sparse, but prominent when present, owing to the low electron density of the surrounding cytoplasm. Mitochondria are numerous, often extremely elongate, and sometimes with a trefoil appearance. Spherical electron dense lysosome-like inclusions are fairly common, and multi-vesicular bodies are occasionally encountered. Glycogen granules were rarely present in our material.

No significant differences are found between astrocytes at different levels in the cortex, with the following conspicuous exception. Astrocytes contributing processes to the subpial glia limitans (figure 1), or lying close to the surface and likely to contribute such processes to the

† Figures 1–64 appear on plates 1–18.

glia limitans (figure 2), possess nuclei with a much darker appearance and with considerably more heterochromatin than other astrocytes. In addition, these superficial cortical astrocytes contain more prominent filament bundles, running both parallel and perpendicular to the pial surface, than more deeply situated astrocytes.

In all 12 montages constituting this group, there are a few (two to four per montage) immature astrocytes (termed thus because of their resemblance to cells seen in the early postnatal material). These cells commonly occur in pairs linked by puncta adherentia. Their nuclei are similar to the nuclei of mature astrocytes, and, like the latter, their contour is moulded to that of the surrounding elements and they give off fine sheet-like and broader processes, some of which abut nearby blood vessels. Their cytoplasm, however, is considerably richer in cytoplasmic organelles, with the exception that filaments are seldom observed. The granular reticulum and free ribosomes are prominent in the immature astrocytes and the former comprises numerous, short, wide cisterns which sometimes appear to contain a moderately electron dense flocculent material. Microtubules are relatively numerous and run in various directions. The Golgi elements are more prominent and are associated with more vesicles than in mature astrocytes.

Oligodendrocytes (figures 4–6) are present throughout the cortex, but are rare in layer I and most common in layers V and VI; no differences are seen between oligodendrocytes situated at different levels or between those immediately adjacent to neurons (satellite or perineuronal oligodendrocytes) and those lying singly or in clusters of two or three within fibre bundles (interfascicular oligodendrocytes). Oligodendrocytes were found to span a considerable range in terms of size and abundance of perikaryal organelles (compare figures 4 and 5), but most can be classified as dark and a few as medium oligodendrocytes (Mori & Leblond 1970). Most have relatively small, smooth surfaced cell bodies and smooth surfaced processes of generally small diameter (although occasionally larger processes stemming from a cell body are seen). The overall appearance of the cells is dark. The nucleus, often eccentrically situated in the larger cells (figure 4), contains much heterochromatin, with large clumps aggregated beneath the nuclear envelope, which is commonly slightly distended and studded with ribosomes along its cytoplasmic surface. The darkness of the cytoplasm is due in part to a high concentration of ribosomes and polysomal aggregates, and in part to a finely granular ground substance between the organelles. The granular reticulum comprises single cisterns of variable length, and small stacks of short cisterns. The lumens of the cisterns appear to be devoid of content and more electron lucent than the surrounding cytoplasm. The Golgi apparatus is generally prominent (figure 6); individual elements comprise three, four or more saccules which are commonly slightly distended and, like the granular endoplasmic cisterns and the perinuclear cistern, appear electron-lucent by comparison with the surrounding cytoplasm (figures 4, 6). Microtubules and multi-vesicular bodies are numerous and the former funnel from the cell body into the processes. Mitochondria are variable in diameter: large mitochondria are commonly present at the periphery of the cell body, but many others appear to be smaller than those in astrocytes. Lysosome-like dense bodies, lipofuscin bodies and centrioles (figure 6) are also present in some oligodendrocytes.

Microglia (figures 7, 8) are found in small numbers throughout the depth of the cortex, and there are no apparent ultrastructural differences between microglia situated at different levels. Some microglial cell bodies are rounded and 'fleshy' with centrally situated, rounded nuclei, but the cell bodies of most are smaller than those of macroglial cells, and often fusiform, narrowing gradually into the bases of large processes. The nuclei of such cells are generally oval or

Description of plates

Figures 1–64 are electron micrographs of thin sections, cut in the coronal plane, of the visual cortex of albino rats. Unless otherwise stated or evidently irrelevant, micrographs are mounted with the pial surface towards the top. Most of these micrographs are taken from the montages prepared for quantitative analyses.

List of abbreviations

A	astroblast	gr	granular endoplasmic reticulum
D	dendrite	l	lysosome-like dense body
G	glioblast	lf	lipofuscin body
N	neuron	m	mitochondrion
O	oligodendrocyte	mt	microtubule
P	pial cell	mvb	multivesicular body
ax	myelinated axon	n	nucleus
c	centriole	nu	nucleolus
db	large dense body	p	polyribosomes
f	astrocytic filaments	pr	process
ga	Golgi apparatus	v	blood vessel lumen
gc	growth cone		

Description of plate 1

Astrocytes in adult rats (3 months).

Figure 1. Superficially situated astrocyte forming part of the subpial glia limitans and extending long horizontal processes packed with filaments (f). Other organelles, which include small scattered cisterns of granular reticulum (gr), small components of the Golgi apparatus (ga), mitochondria (m) and a cluster of lysosome-like electron dense bodies of various sizes (l), are concentrated at opposite poles of the elongate nucleus. Neuropil of layer I lies below and to the left and the subarachnoid space above and to the right, separated from the astrocyte by a basal lamina (which is difficult to resolve at this low magnification), pial cells (P) and some pial collagen fibres (arrow). (Magn. × 7100.)

Figure 2. Astrocyte in layer II. This cell contains prominent bundles of filaments (f) running in various directions, which dominate the broad expanse of the cell body above the nucleus. Other organelles, including mitochondria, a very sparse granular reticulum, and Golgi elements, are concentrated in the perinuclear cytoplasm. Processes of this cell were traced to perivascular end feet and other, ascending processes probably contributed to the subpial glia limitans. (Magn. × 7100.)

Figure 3. Immature astrocyte (nucleus at n) of layer VI. The cytoplasmic organelles are tightly packed and include numerous free polyribosomes (p), a few granular reticulum elements and some microtubules, but no filaments. Parts of a neuron (N) and of a small oligodendrocyte (O) are shown. (Magn. × 10450.)

Description of plate 2

Oligodendrocytes and microglial cells in mature rats (40 days–6 months).

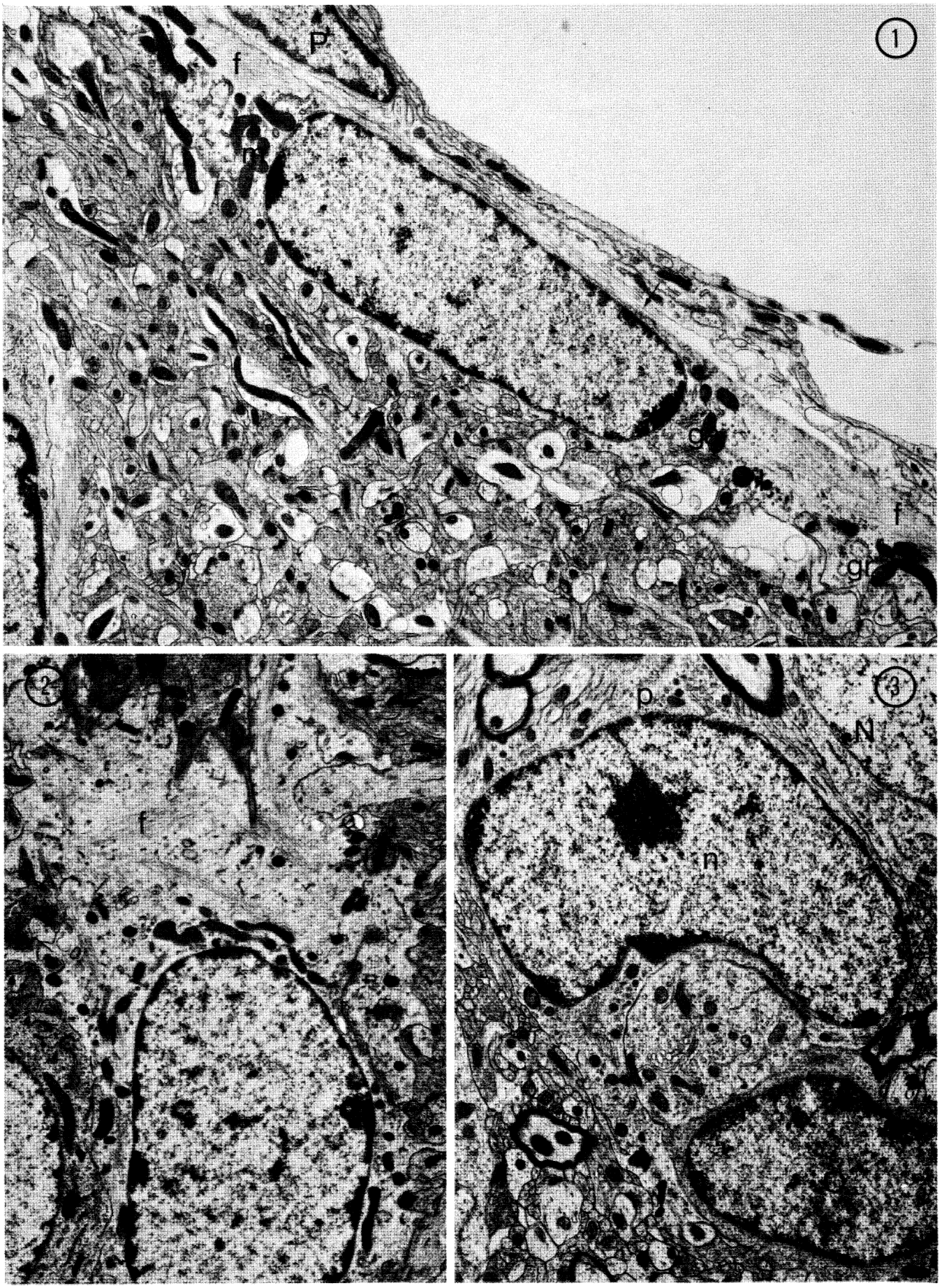
Figure 4. Large oligodendrocyte of layer VI. This cell has an eccentric nucleus, electron opaque cytoplasmic matrix and a rich complement of cytoplasmic organelles, including granular reticulum (gr), free polyribosomes, elements of the Golgi apparatus with distended saccules (ga), and microtubules (mt). The pattern of nuclear chromatin is that of a dark oligodendrocyte. Day 40. (Magn. × 11600.)

Figure 5. Small oligodendrocyte of layer VI. This cell has a centrally situated nucleus and a scanty cytoplasm containing free polyribosomes, a few granular reticulum cisterns and small Golgi elements. Cells such as this constitute a small number of adult oligodendrocyte types and probably support very little myelin. 6 months. (Magn. × 16150.)

Figure 6. Part of the cytoplasm of a large dark oligodendrocyte of layer VI, displaying typical Golgi complexes, which consist of stacks of five to eight saccules, most of which are distended and electron lucent. Other organelles are microtubules, mitochondria, granular reticulum, polyribosomes, a multivesicular body (mvb) and a centriole (c). Day 40. (Magn. × 16450.)

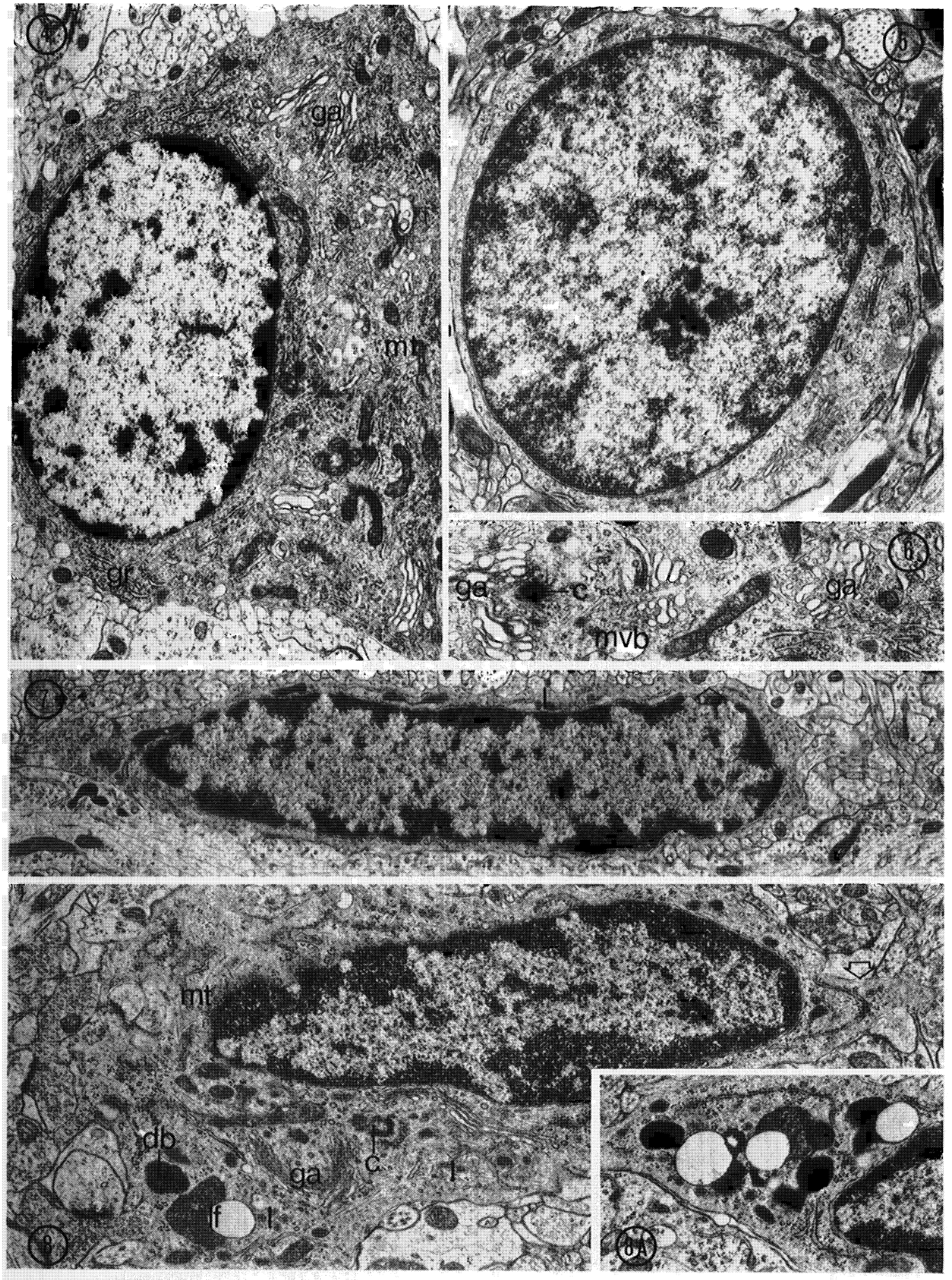
Figure 7. Microglial cell of layer IV. This small, elongate cell lies alongside the apical dendrite of a pyramidal cell (pia is to the left) and its moderately electron dense cytoplasm includes mitochondria (some barbell-shaped), free polyribosomes, single long cisterns of granular reticulum, and a few small lysosome-like dense bodies (l). Small, characteristic expansions of the extracellular space at the surface of the cell are indicated by an open arrow here and in figure 8. Note the characteristic large clumps of peripheral heterochromatin in the nucleus of this cell and those in figures 8 and 8A. 6 months. (Magn. × 11600.)

Figure 8. Microglial cell of layer IV with voluminous and organelle-rich cytoplasm. The organelles include single long cisterns of granular reticulum, microtubules (mt), prominent Golgi elements (ga), a centriole (c), numerous small, moderately electron dense lysosome-like bodies (l), some larger, more electron dense bodies (db), and a lipofuscin body (lf). Note here, and also in figures 7 and 8A, the fine granular filamentous material of the cytoplasmic matrix. 6 months. (Magn. × 15400.)

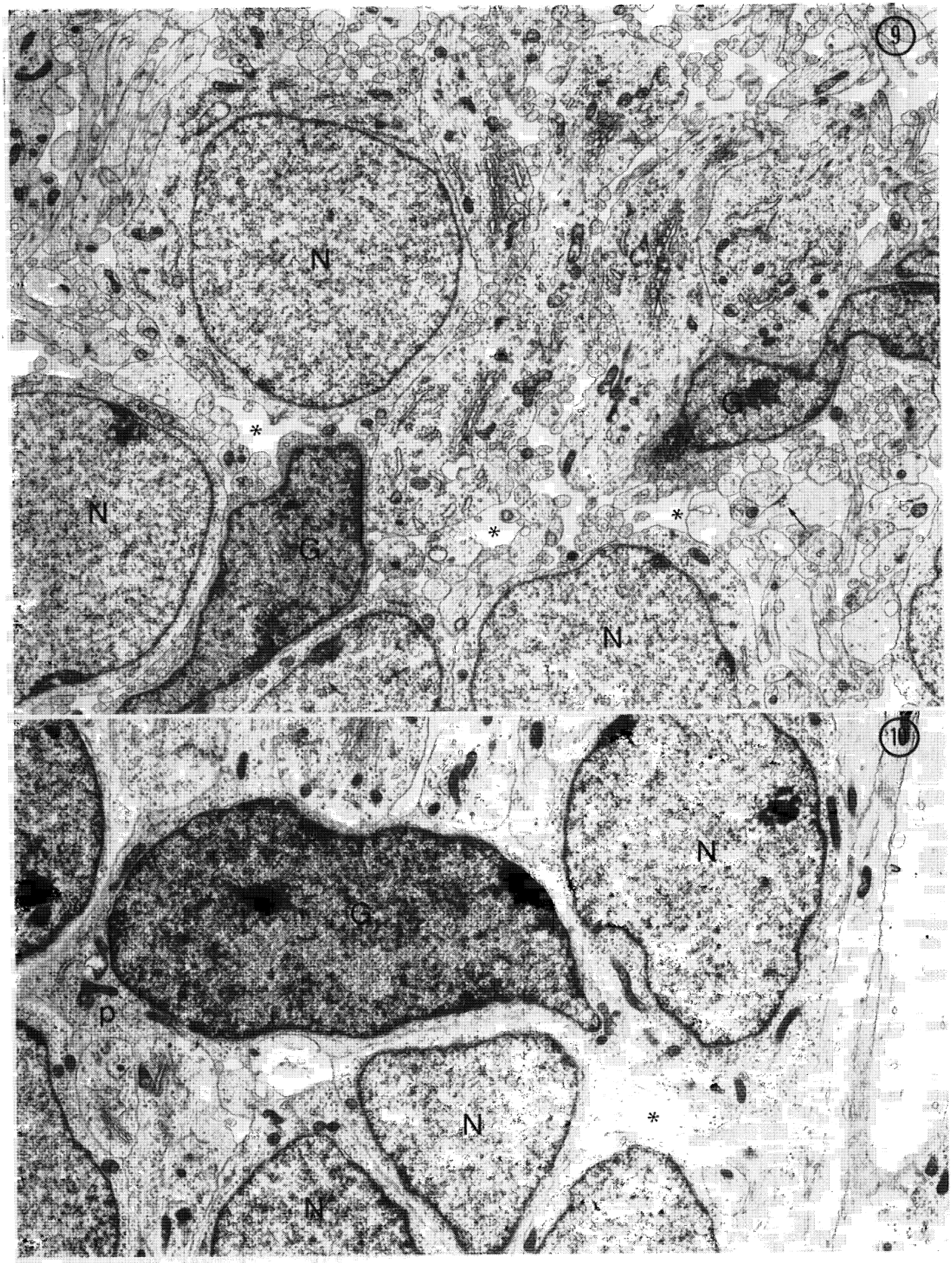


FIGURES 1–3. For description see opposite.

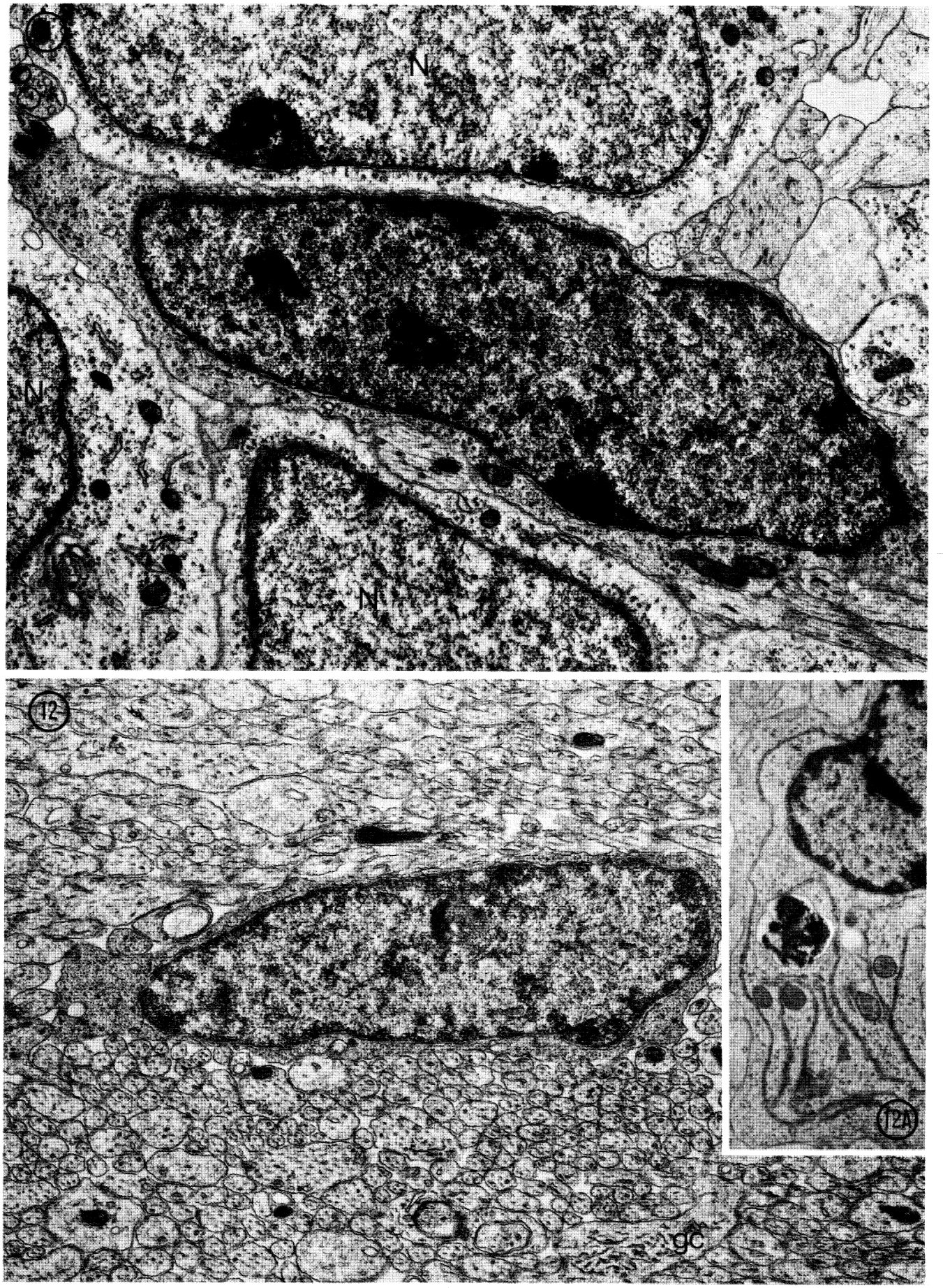
(*Facing p.* 62.)



FIGURES 4–8A. For description see page 63.



FIGURES 9 and 10. For description see page 63.



FIGURES 11–12A. For description see opposite.

more markedly elongate, and contain abundant heterochromatin clumped at the nuclear periphery but also distributed throughout the nucleus. The surface of the cell body and processes of microglial cells are commonly irregular and moulded to contiguous neuropil elements, but narrow finger-like and sheet-like expansions are not commonly observed. It is common to find areas of widened extracellular space at the surface of microglial cell bodies and processes (for example, at the arrowheads in figures 7 and 8), a feature perhaps related to the postulated migratory and phagocytic properties of these cells. The overall electron density of the cell is variable; most are darker than astrocytes but not as dark as dark oligodendrocytes, and some are as dark as the latter. Free polyribosomes, however, are not as numerous even in the darkest of these cells, as they are in oligodendrocytes. A characteristic cytoplasmic feature is the presence of long, stringy cisterns of granular reticulum (figures 8, 8A) meandering individually and sometimes tortuously through the cytoplasm and between other organelles such as small elements of the Golgi apparatus (figure 8), lysosome-like dense bodies, lipofuscin inclusions (figures 8, 8A), and mitochondria, which are occasionally barbell-shaped (figure 7).

Pericytes are defined as cells in the walls of capillaries external to the endothelium and completely enclosed by basal lamina. The number of such cells is small at all stages examined and pericytes are not included in the analysis presented here.

(ii) *Development of glial cells in the early postnatal period*

Postnatal days 0–4

During the first few days of postnatal life the cells of the cortex fall into two broad groups: neurons (including migrating cells) and non-neuronal cells (figures 9–16). The latter are for the most part fairly uniform in appearance and are referred to here as glioblasts, but a proportion can be distinguished as astroblasts, and according to our criteria (see below) some immature astrocytes may also be recognized. A very small number of microglial cells with long stringy cisterns of granular reticulum, numerous free polyribosomes, small lysosome-like dense bodies, and in some cases inclusion bodies indicative of recent phagocytic activity, are also present (figure 12A). The neurons (N, figures 9–11) can be distinguished from non-neuronal cells by their pale electron lucent nucleus which contains homogeneously dispersed nuclear

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Description of plate 3

Figures 9 and 10. Immature neurons (N) and glioblasts (G) in the cortical plate on day 0 (12 h postnatal). Figure 9 is from the deep cortical plate and figure 10 from a more superficial level. The glioblast nuclei are irregular in form and darker than the neuronal nuclei, and their cytoplasmic matrix is also more electron dense than that of the neurons. Cytoplasmic organelles of the glioblasts are minimal and are chiefly free polyribosomes (p), a few cisterns of granular reticulum, and some mitochondria. Asterisks indicate lacunae of the extracellular space and the arrow in figure 9 marks an immature synapse. (Figure 9 magn. × 6950; figure 10 magn. × 7500.)

Description of plate 4

Figures 11 and 12. Glioblasts in the cortical plate (figure 11) and in the subplate (figure 12) at day 0. The glioblast in the cortical plate lies among immature neurons (N); the other is situated among closely packed, parallel non-myelinated axons. Both glioblasts display an electron dense cytoplasmic matrix, in which free polyribosomes predominate, and similar dark nuclei; and the more highly differentiated cell in the cortical plate also contains mitochondria, microtubule and some granular reticulum. A growth-cone-like profile (gc) is seen among the non-myelinated axons in figure 12. (Figure 11 magn. × 11950; magn. × 14000.)

Figure 12A. Microglial cell of immature appearance but with cytoplasmic inclusions indicating recent phagocytic activity, in the cortical plate on day 0. (Magn. × 12650.)

chromatin and by a pale cytoplasm containing an assortment of organelles, the abundance of which depends on the state of maturity of the neuron.

Glioblasts are distinguished from immature neurons by their greater nuclear and cytoplasmic electron density and in addition by their frequently more irregular form (G, figures 9–11, 12A, 14, 15). Their nuclei display a homogeneously 'speckled' pattern with a rim of peripherally positioned heterochromatin which is thin but considerably more distinct than the peripheral heterochromatin of neurons at this stage. The shape of the nucleus is variable, ranging from round or oval to indented and elongate; a nucleolus is not usually observed. Some glioblasts contain only polyribosomes, a few cisterns of granular reticulum and mitochondria (figures 9–12). More highly differentiated cells, encountered more frequently at days 2 and 4 than at birth, display a more voluminous cytoplasm with cisterns of granular reticulum, Golgi bodies and numerous mitochondria; a high concentration of free polyribosomes is, however, still a feature of these slightly more differentiated cells (figures 13–16).

Among the population of glioblasts, some cells may be identified as astroblasts either because their cell body or a process is in contact with the glia limitans, or because of their distinctive cytoplasmic and nuclear appearance. Astroblasts are characterized by a distinctive form of granular reticulum, the cisterns of which are widened, studded with numerous ribosomes and filled with flocculent material. In some cells the distended cisterns and their finely granular electron dense content are extremely prominent (figures 14, 14A arrows). In others (figures 13–16) distension is barely perceptible, but the presence of an electron dense luminal content indicates that these glioblasts are very probably in the process of acquiring astroblast-like characteristics. In general, cells with, or in the process of acquiring, astroblast-like features display a somewhat more electron-dense cytoplasm than that of glioblasts, and their cytoplasmic organelles include Golgi complexes, rosettes of polyribosomes and dense bodies. Also the nuclei of these cells are generally paler than those of glioblasts an show some similarities to the nuclei of astrocytes. The number of astroblasts and immature astrocytes at this early stage of life is small compared to the number of glioblasts and they occur predominantly in the most superficial and in the deepest portions of the cortex.

Postnatal day 6

Astroblasts and immature astrocytes are the prevalent forms of non-neuronal cells at this stage of development (figures 17–24), although a few glioblasts are still present. Astroblasts resemble the corresponding cells observed in the previous ages, but at this stage very commonly give rise to prominent processes (figures 17, 20) and from these processes and from the cell bodies, narrower finger-like processes extend between adjacent elements of the developing neuropil (see for example figure 21). Also, at this stage their cytoplasm appears to be more voluminous and contains a greater abundance of organelles. Numerous widened cisterns of granular reticulum containing finely granular or flocculent electron-dense material are usually conspicuous throughout the cytoplasm (see especially figures 21–24) as are many rosettes of polyribosomes, mitochondria, dense bodies, and Golgi complexes, the latter sometimes associated with a few vesicles (ga, figures 18, 19, 21).

Also apparent at this stage of development are a very small number of immature oligodendrocytes (oligodendroblasts), the morphological characteristics of which will be described in the section of 8 day material (at which stage they are present in greater numbers), and microglia.

Most of the cells identified as microglial cells (figures 25–27) appear to be immature by comparison with their adult counterparts (immature microglia; figures 25, 27; see also figure 12A), but others are not very different from the mature microglia of adult animals (figure 26). Both types display stringy cisterns of granular reticulum, which frequently wind around the nucleus, Golgi complexes, microtubules and polymorphic dense bodies. The differences between immature and mature microglia are principally that the former contain large numbers of free polyribosomes and the latter very few; the mature cells contain much larger and more prominent Golgi complexes and a more prominent ring of peripheral heterochromatin. Microglial cells of both types at this early postnatal stage commonly display a large amount of cytoplasm sometimes filled with debris (figures 25, 27). Much of this accumulated debris appears to represent degenerating profiles of variable size, presumably engulfed from surrounding neuropil, in different stages of degradation.

Postnatal day 8

Some glioblasts and many astroblasts, the latter concentrated in the more superficial portion of the cortex, are still present. Like the astroblasts observed in younger animals, the nuclei of these cells are irregular in outline and contain clumps of heterochromatin (figures 28, 29). Several Golgi complexes, microtubules and a few elongate mitochondria are present in the cytoplasm, the overall density of which is generally greater than that of more mature astrocytes. Included in the cytoplasm are many rosettes of polyribosomes and cisterns of granular reticulum, which at this age are only slightly widened but are still conspicuously studded with numerous ribosomes and contain a flocculent material. The general cell outline at day 8 is slightly more regular than is characteristic of mature astrocytes.

No mature oligodendrocytes are seen at this age, but a few cells designated as immature oligodendrocytes or oligodendroblasts, on account of their morphology and ultrastructural differences from more mature cells of this line, are present (figures 32–34). Apart from a slight peripheral accumulation, chromatin is evenly dispersed in the nucleoplasm and the even pattern is only broken by small aggregates of heterochromatin. Oligodendroblasts appear more electron dense than astroblasts and contain more polyribosomes, mitochondria and numerous microtubules, which run in all directions through the cytoplasm. Cisterns and/or tubules of granular reticulum are abundant and generally narrower than those observed in astroblasts. Golgi elements composed of small stacks of short and slightly distended saccules are also present. The cisterns of both the Golgi apparatus and the granular endoplasmic reticulum lack electron dense content and stand out against the background of darker cytoplasm. At this stage of development, oligodendroblasts are almost exclusively present in the lower third of the cortical grey matter, with many found among the bundles of unmyelinated axons present in the deepest parts of the cortex.

Finally , immature microglia occur sporadically throughout the montages surveyed at this age, and their morphology is similar to that of the cells described in the day 6 material.

Postnatal days 10–14

It is during this period of development that most glial cells acquire adult characteristics. At day 10, a few glioblasts and astroblasts are scattered throughout the cortical thickness, but such cells become progressively more sparse during the following days and are virtually absent by postnatal day 14.

Description of plate 5

Differentiation of glioblasts at 2–4 days postnatal.

Figure 13. A well differentiated glioblast in deep cortical plate at postnatal day 2. The cytoplasm of this cell contains abundant mitochondria, scattered cisterns of granular reticulum and Golgi elements (note the juxtaposition of granular and agranular cisterns at the *cis* face of the Golgi elements (Rothman 1981): double-headed arrows). A few of the granular cisterns appear to be widened and to contain traces of a flocculent material (arrows), suggesting that this cell may be, or is likely to become, an astroblast. (Magn. × 20500.)

Figure 14. Postnatal day 4. A well differentiated glioblast displaying an irregular nucleus and cytoplasm filled with polyribosomes and other organelles, and, in the upper right corner, a portion of an astroblast (A) within which are short, swollen cisterns of granular reticulum containing an electron dense material. The inset shows a cell with astroblast-like characteristics at day 0 (12 h postnatal). (Magn. × 8750; inset magn. × 11600.)

Figures 15 and 16. Postnatal day 4. Putative astroblasts in layer V. All three cells have in their cytoplasm a few cisterns of granular reticulum containing electron dense material (arrows) although none of the cisterns are swollen in these cells. Figure 16 is orientated with the pia to the left. (Figure 15 magn. × 10900; figure 16 magn. × 9500.)

Description of plate 6

Astroblasts at postnatal day 6.

Figure 17. Astroblast in layer VI. This cell possesses an irregular nucleus and gives rise to several processes, one of which extends for some distance among numerous unmyelinated axons. Here, and in the remaining figures on this plate, examples of cisterns of granular reticulum with an electron dense content are indicated by arrows. (Magn. × 8550.)

Figures 18 and 19. Astroblasts in layer V. The nuclei of these cells are irregular in shape and their perinuclear cytoplasm contains a granular reticulum, free polyribosomes, microtubules, mitochondria and elements of the Golgi apparatus (ga). An obliquely cut centriole (c) is seen in figure 18 and a transversely cut one in figure 20. (Figure 18 magn. × 12050; figure 19 magn. × 11600.)

Figure 20. Astroblast of layer IV. This cell gives rise to a prominent process, rich in organelles. At the top of the figure this large process gives off one branch which continues vertically at the extreme right and another which passes horizontally at the left. (Magn. × 11500.)

Figure 21. Perinuclear cytoplasm of a layer IV astroblast, in which distension of the cisterns of granular reticulum is particularly marked. A process (indicated by open arrows) extends from the perikaryon of this cell and penetrates between elements of the surrounding neuropil. (Magn. × 24600.)

Figures 22–24. Cytoplasmic characteristics of astroblasts in layer III. Both the perinuclear cytoplasm shown in figures 22 and 23, and the cytoplasm of the large process in figure 24, which lies immediately below a large dendrite (D), display numerous moderately to markedly widened cisterns of granular reticulum containing electron dense material, elements of the Golgi apparatus and microtubules. (Figure 22 magn. × 11600; figure 23, magn. × 20500; figure 24 magn. × 13300.)

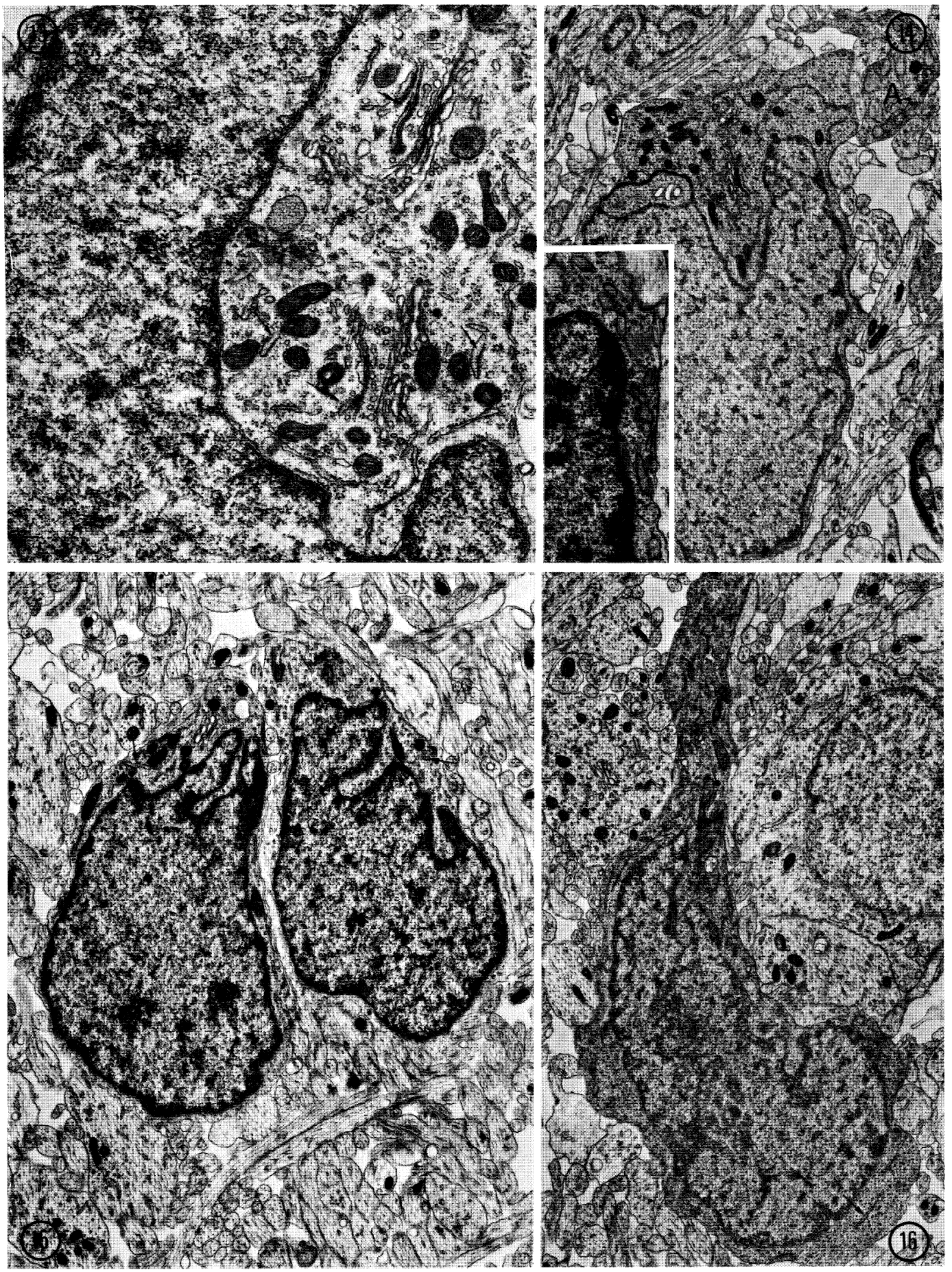
Description of plate 7

Microglia at postnatal day 6.

Figure 25. This immature microglial cell in layer V has a dark nucleus and its cytoplasm contains large numbers of polyribosomes, stringy cisterns of granular reticulum and large inclusion bodies of apparently phagocytosed and partially digested debris. (Magn. × 11950.)

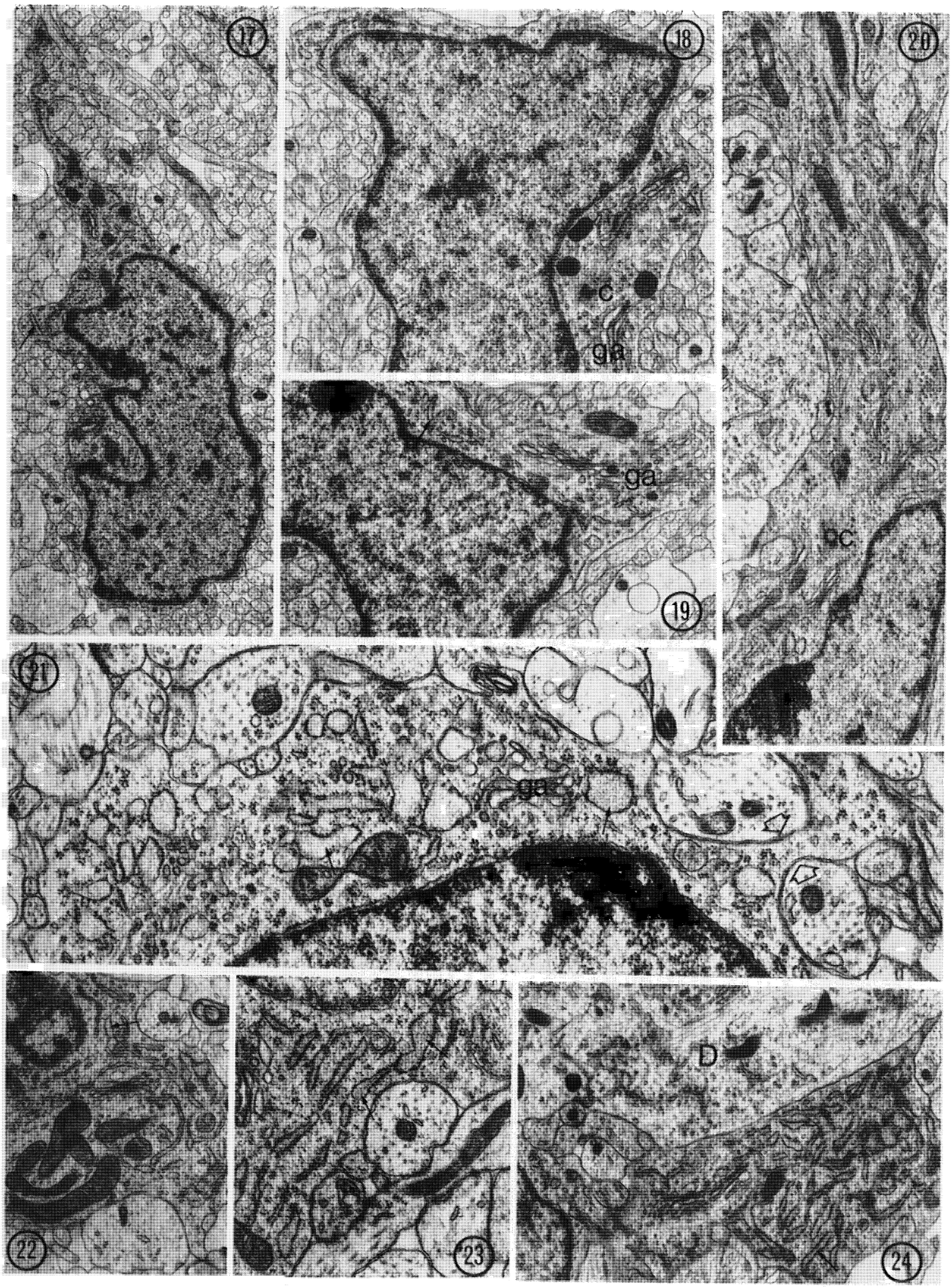
Figure 26. Microglial cell of layer VI with a more mature appearance. Its organelle-rich cytoplasm contains some barbell-shaped mitochondria, prominent Golgi complexes and lysosome-like dense bodies of variable size and electron opacity (for example, compare 11, 12, and 13). (Magn. × 15500.)

Figure 27. An immature microglial cell (or possibly parts of two adjacent such cells) in layer III. The cytoplasm is packed with large numbers of large inclusion bodies resembling phagocytosed and partially digested debris, and numerous smaller lysosome-like dense bodies. (Magn. × 9300.)

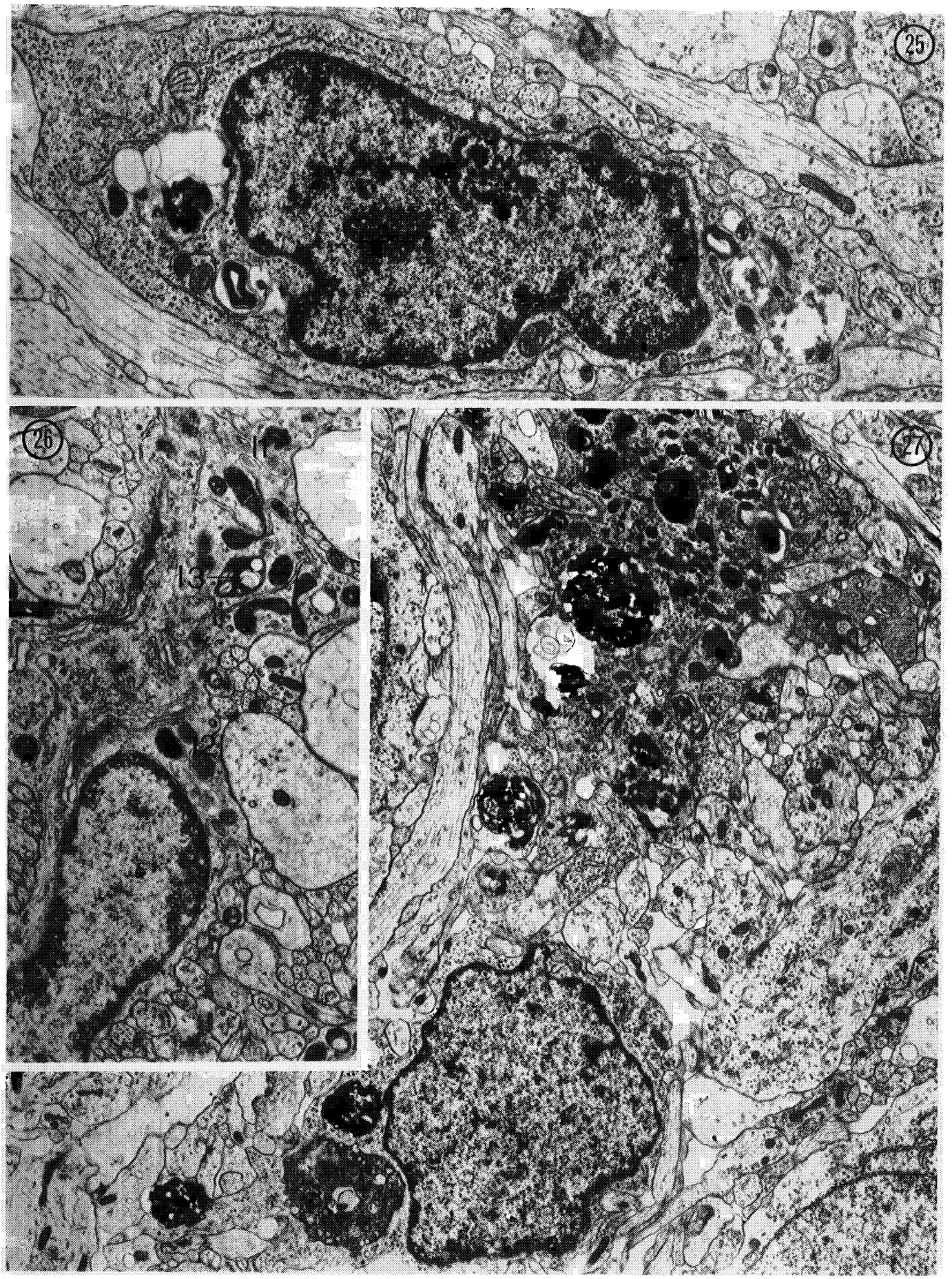


FIGURES 13–16. For description see opposite.

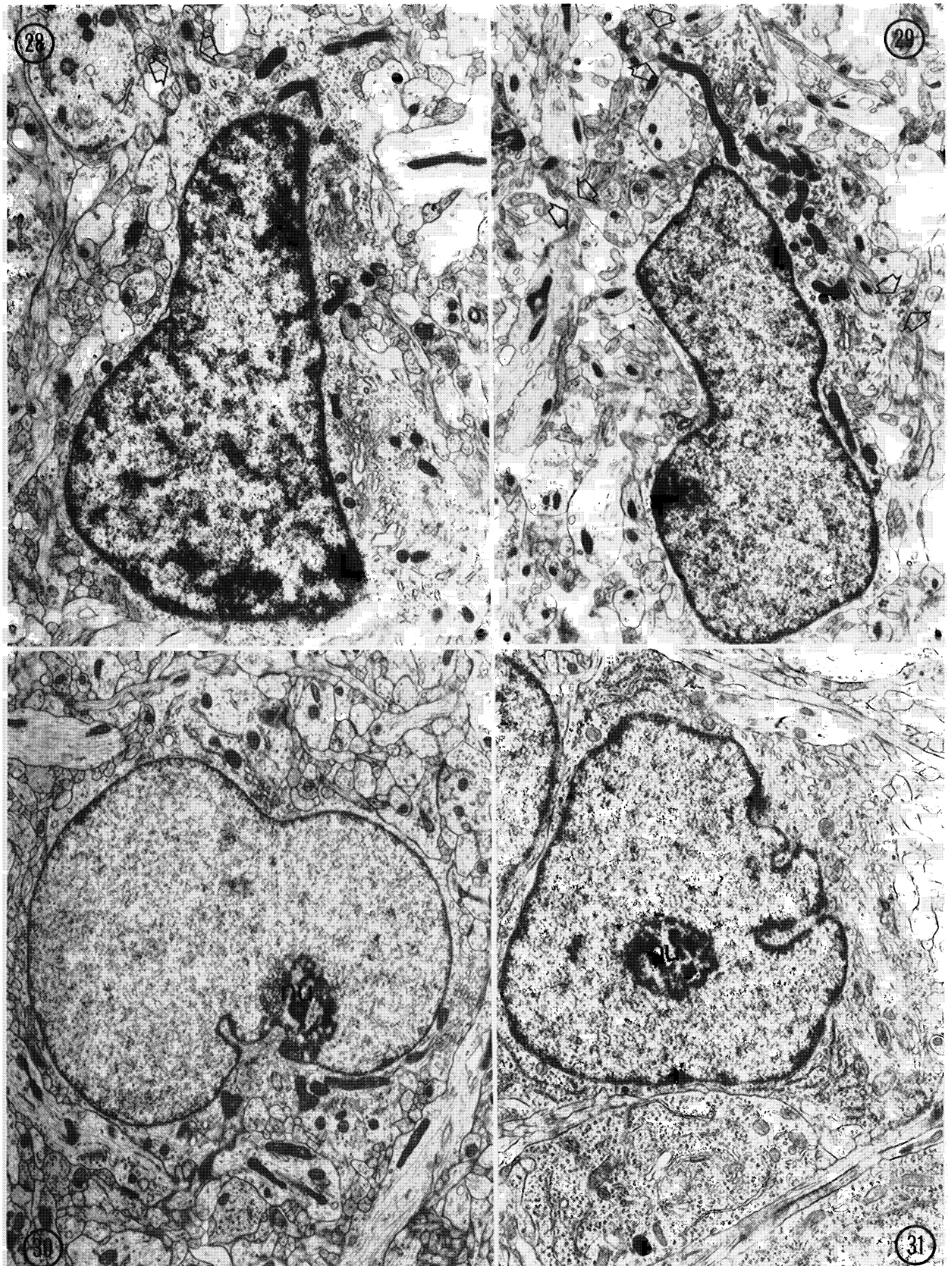
(*Facing p.* 66)



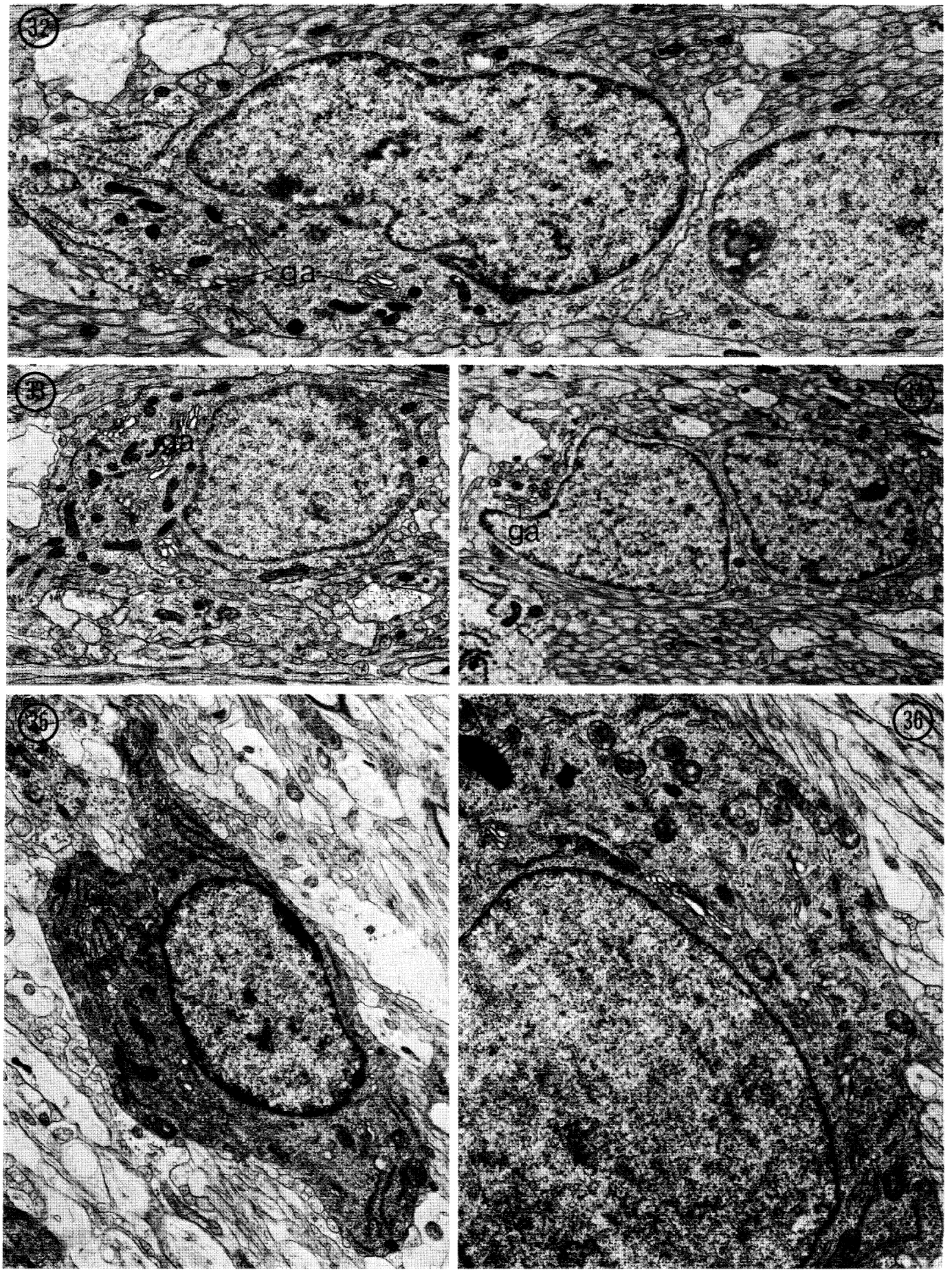
FIGURES 17–24. For description see page 66.



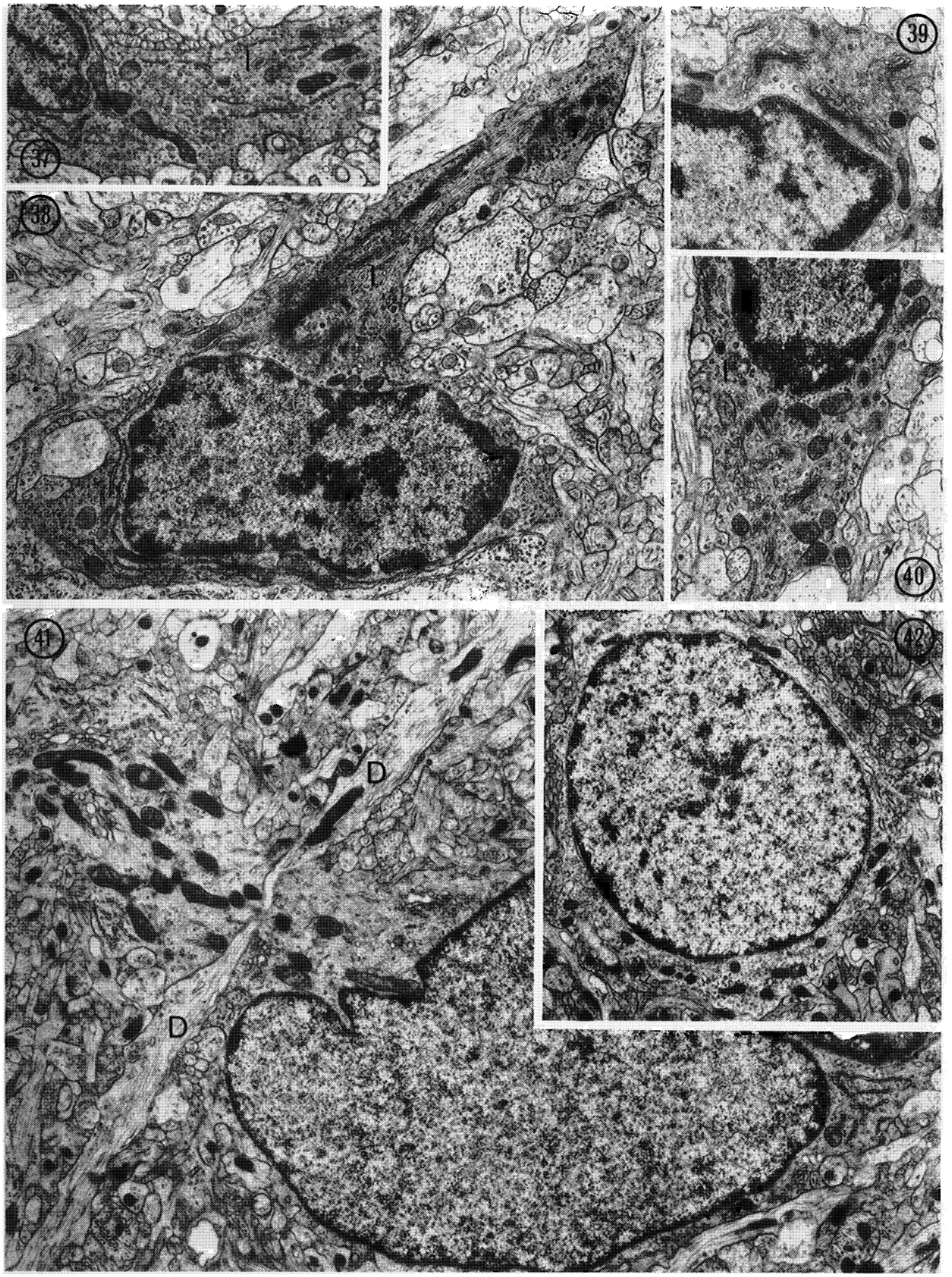
FIGURES 25–27. For description see page 66.



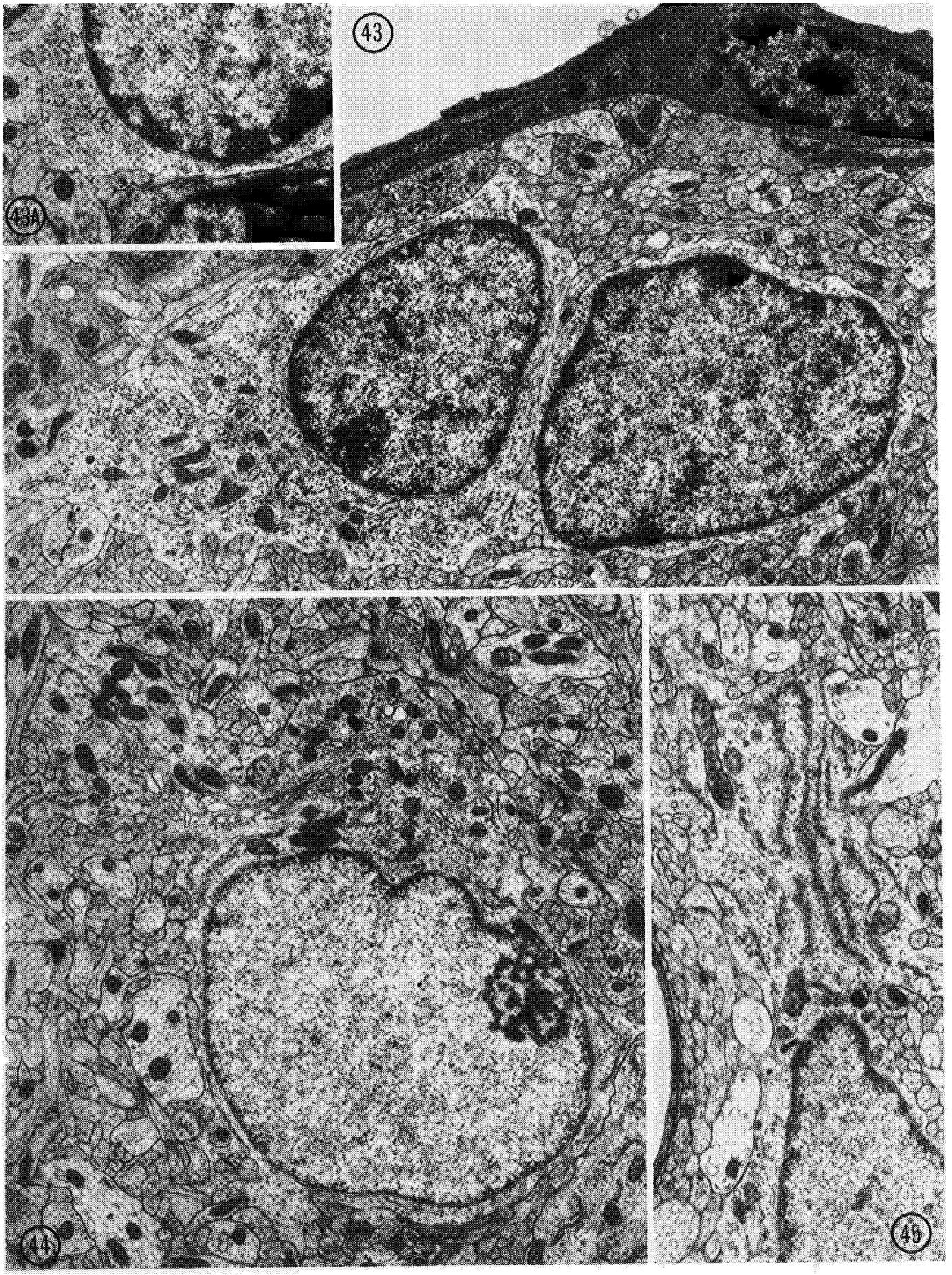
FIGURES 28–31. For description see facing plate 10.



FIGURES 32–36. For description see facing plate 10.



FIGURES 37–42. For description see opposite.



FIGURES 43–45. For description see facing plate 10.

Description of plate 8

Figures 28 and 29. Astroblasts at postnatal day 8. These two cells both give off processes that penetrate between adjacent neuropil elements (between open arrows), and both display granular reticulum with mostly undistended cisterns. Figure 28, layer V (magn. × 11400); figure 29, layer II (magn. × 8750).

Figures 30 and 31. Immature astrocytes at postnatal day 12. Both cells have an irregular outline, and give rise to small and large processes. The nuclei of both are irregular in shape, contain nucleoli (nu) and display a homogeneous 'speckled' chromatin pattern, with a thin rim of clumped heterochromatin beneath the nuclear envelope. In both, the cytoplasmic matrix is pale, granular reticulum is composed of undistended cisterns, and there are Golgi elements and microtubules Figure 30, layer II (magn. × 9600); figure 31, layer IV (magn. × 10150).

Description of plate 9

Figures 32–34. Oligodendroblasts in layer VI at postnatal day 8. These cells are moderately electron dense and their cytoplasm includes numerous polyribosomes and variable amounts of granular reticulum. Prominent in these cells are elements of the Golgi apparatus with distended saccules (ga). (Figure 32 magn. × 11400; figures 33 and 34 magn. × 7400.)

Figures 35 and 36. Postnatal day 12; light oligodendrocytes of layer VI. These cells have rounded, rather pale nuclei and a voluminous cytoplasm filled with mitochondria, free polyribosomes, narrow cisterns of granular reticulum (aggregated in several small stacks in figure 35), microtubules, and Golgi complexes with distended saccules. (Figure 35 magn. × 8250; figure 36 magn. × 13100.)

Description of plate 10

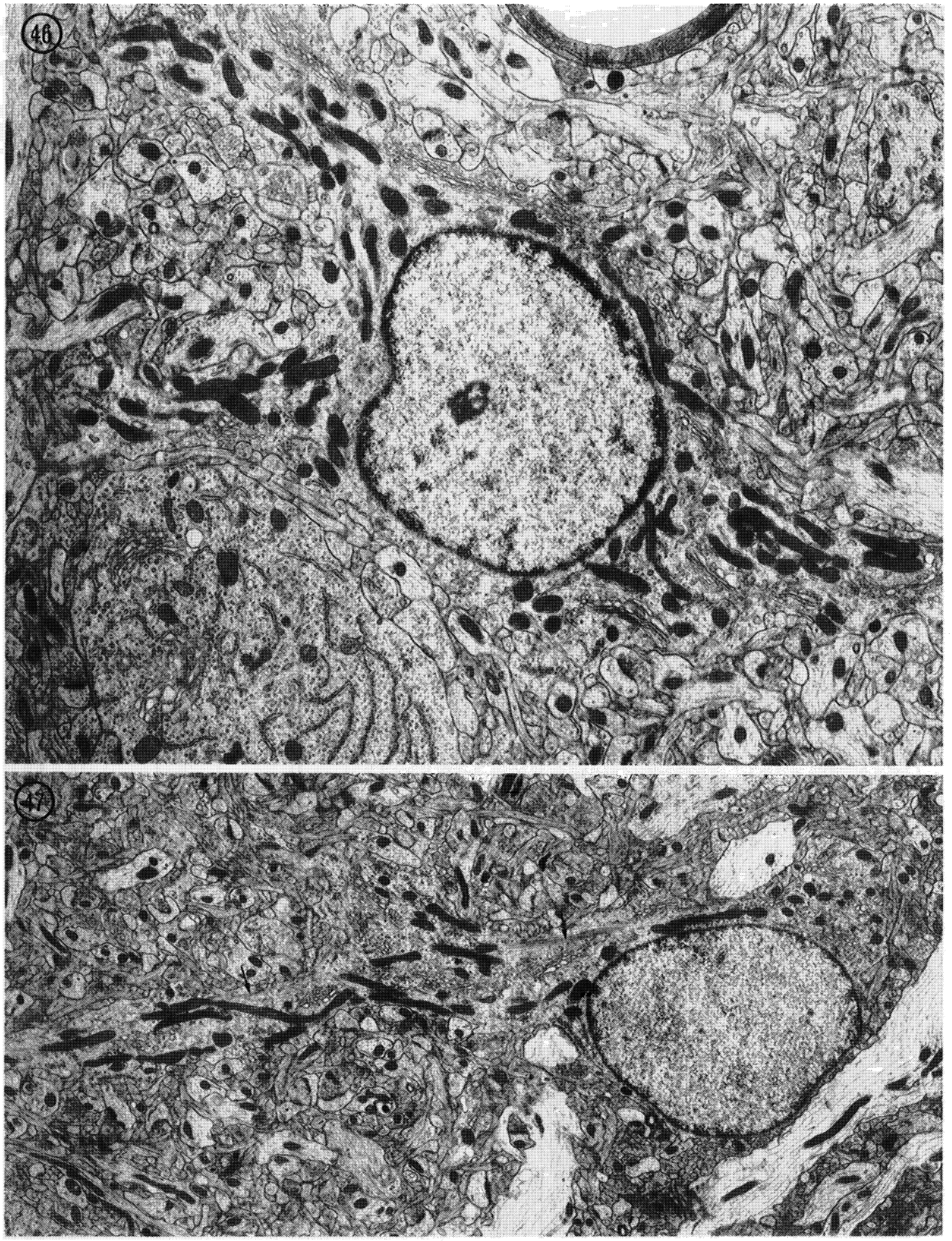
Figures 37–40. Microglia at postnatal days 8 and 12. These four cells span a considerable spectrum with respect to the shape of the cell body and their cytoplasmic organelles, but all show the characteristic large blocks of peripheral nuclear chromatin and the small, lysosome-like bodies with moderately electron dense content (l) that are one of the hallmarks of this cell type. The cells in figures 37 and 38 also show the characteristic stringy cisterns of granular reticulum and those in figures 38–40, Golgi elements with tightly packed and only occasionally distended saccules. Figure 37, postnatal day 12, layer V (magn. × 11600); figure 38, postnatal day 12, layer III (magn. × 11600); figure 39, postnatal day 8, layer III (magn. × 15300); figure 40, postnatal day 12, layer III (magn. × 13850).

Figures 41 and 42. Immature astrocytes at day 14. The cell in figure 41 has a dark cytoplasmic matrix and gives off a broad process towards the top left of the picture, into which a longitudinally sectioned dendrite (D) is impressed. The cell in figure 42 is less mature, has few microtubules and displays an irregular periphery as a result of the many small protrusions that extend between adjacent neuropil elements. Figure 41, layer II (magn. × 11400); figure 42, layer V (magn. × 9500).

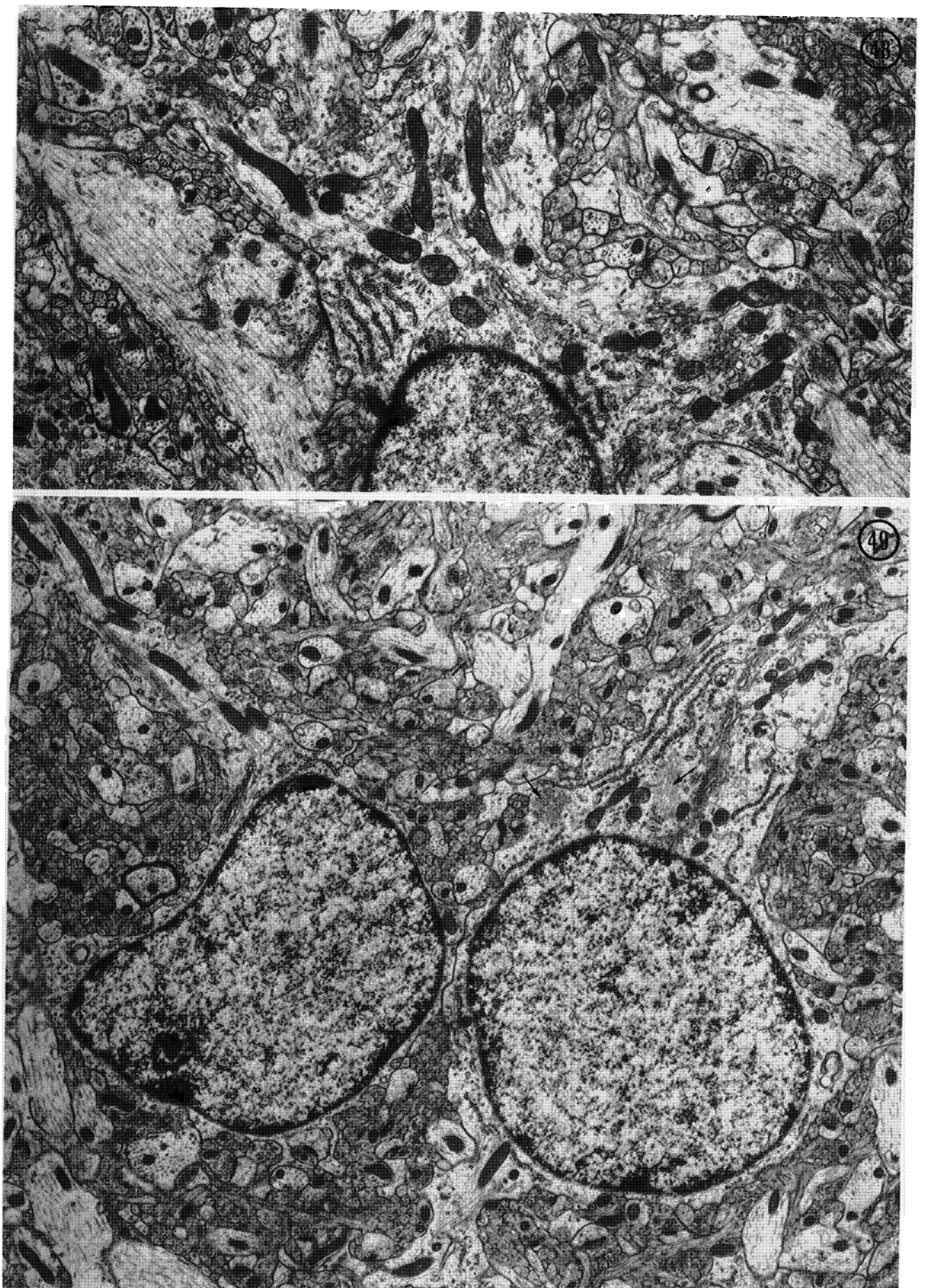
Description of plates 11 and 12

Figures 43–47. Immature astrocytes at postnatal day 16. These figures illustrate the extensive range of nuclear and cytoplasmic features observed at this time of rapid change. Figure 43 shows a pair of extremely immature, small astrocytes with relatively dark nuclei and a pale cytoplasm in which free ribosomes predominate. These cells and the small cell in figure 43A have cisterns of granular reticulum that are distended, with an electron dense content, a characteristic of astroblasts. Figures 44 and 45 show more mature astrocytes with paler nuclei, and a richer, more voluminous cytoplasm. Both cells still contain free polyribosomes (e.g. in the region of the perikaryal evagination at the bottom of figure 44) but granular reticulum is well developed with long parallel narrow cisterns bearing tightly packed polyribosome arrays in figure 45. Figures 46 and 47 show two large astrocytes, each with several large processes leaving the cell body. Bundles of filaments are seen in the cell body and one of the processes of the cell in figure 47 (arrows). Note the absence of large extracellular spaces at this age and the mature appearance of the neuropil.

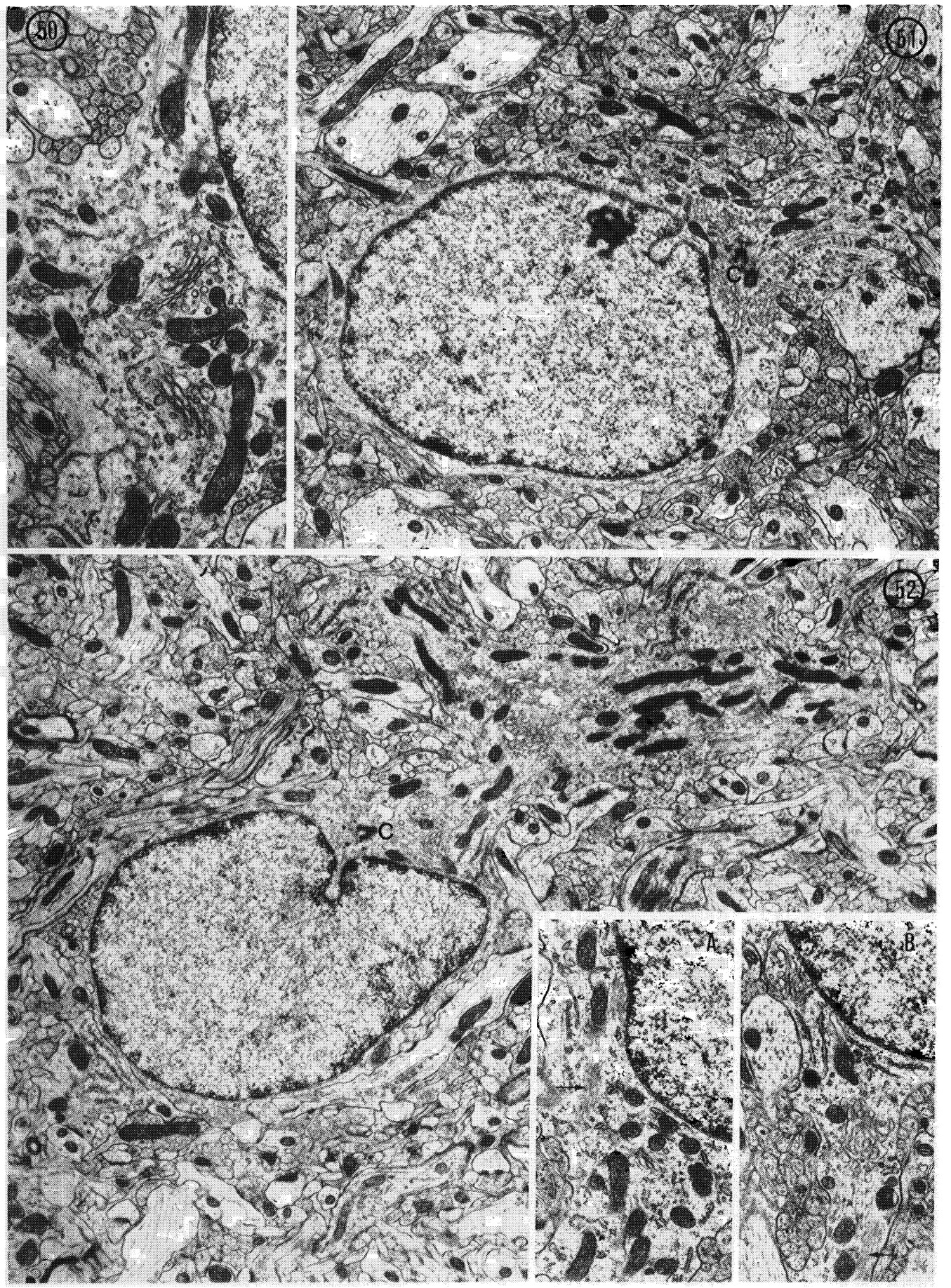
Figure 43, layer IV (magn. × 11400); figure 43A (magn. × 13950); figure 44, layer VI (magn. × 10750); figure 45, layer I (magn. × 11600); figure 46, layer II (magn. × 6650); figure 47, layer III (magn. × 10250).



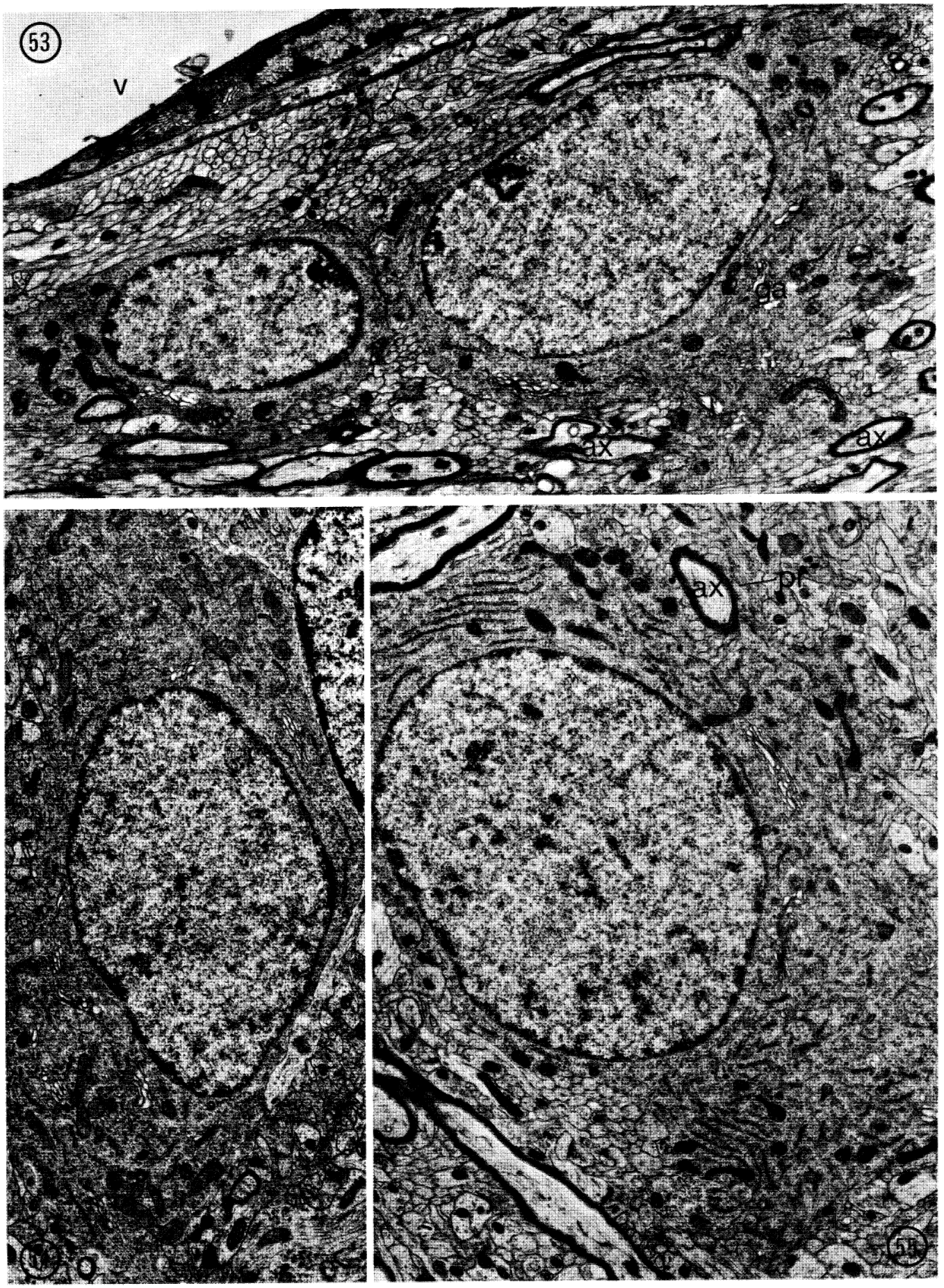
FIGURES 46 AND 47. For description see facing plate 10.



FIGURES 48 AND 49. For description see page 67.



FIGURES 50–52. For description see page 67.



FIGURES 53–55. For description see opposite.

Immature astrocytes (figures 30, 31, 41, 42) tend to be concentrated in the cortical areas adjacent to the pia and the subcortical white matter, but are similar in appearance whatever their position. They display numerous elements of the Golgi apparatus and microtubules. Their mitochondria are generally smaller and more round than in mature astrocytes, although some elongate forms are present. Centrioles are occasionally seen. The nuclei of the immature astrocytes are darker than those of mature astrocytes and display a thin rim of heterochromatin immediately below the nuclear envelope.

There is an increase in the number of oligodendrocytes at these stages of development (figures 35 and 36, 12 days; figure 53, 14 days) and all are of the light variety (Mori & Leblond 1970). They are large cells with large, smooth surfaced, relatively electron lucent nuclei (compared with nuclei of medium and dark oligodendrocytes). The granular reticulum is most commonly in the form of irregularly dispersed short single cisterns (figures 36, 53), although occasional stacks of longer cisterns are present (see for example figure 35). Golgi complexes with short, characteristically distended saccules are common, as are mitochondria, microtubules running in various directions, and abundant polyribosomes. The cell outline generally conforms to the surrounding neuropil and commonly the cell body is slightly invaginated by groups of axons.

The number of microglial cells is still small at this stage and these cells occur singly and scattered throughout the cortical thickness. Some cells are very irregular in shape and many display the entire range of adult characteristics (figures 37, 38, 40). Polyribosomes and lysosome-like bodies of various sizes and degrees of electron opacity are prominent organelles in the cytoplasm, as are stringy cisterns of granular reticulum and Golgi bodies composed of

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Description of plates 13 and 14

Figures 48–52. Maturing astrocytes at postnatal days 18–20. Most of these cells display mature nuclear features and many mature cytoplasmic features, including in most cases the presence of bundles of filaments, which are particularly well shown at the arrows in figure 49 and in insets A and B of figure 52. All the cells contain some free polyribosomes, but granular reticulum and membrane-attached polyribosomes predominate. Cytoplasmic organelles are less densely packed in the perikaryon than in more immature astrocytes. The cytoplasmic matrix of these cells is electron lucent, with the exception of the cell in figure 52, which otherwise clearly resembles figure 51 (even to the extent of showing a centriole adjacent to a nuclear indentation and opposite the origin of a large process). The cytoplasmic matrix of the 'dark' immature astrocyte in figure 52 resembles that of the cell in figure 46; the significance of cells with these features is not understood. Figure 48, day 18, layer II (magn. × 11600); figure 49, day 20, layer I (magn. × 10900); figure 50, day 18, layer I (magn. × 13950); figure 51, day 20, layer II (magn. × 10900); figure 52, day 20, layer V (magn. × 10900); insets A and B, day 20, layer II (magn. × 9600).

Description of plate 15

Light oligodendrocytes at postnatal days 14–24.

Figure 53. Postnatal day 14; light oligodendrocytes in deep layer VI. These cells extend processes towards myelinating axons (ax) and contain Golgi complexes (ga) with electron lucent saccules. A blood vessel (lumen at v) is seen in the top left corner. (Magn. × 9200.)

Figure 54. Postnatal day 20; light oligodendrocyte of layer V. This cell displays a pale nucleus with small clumps of heterochromatin. Polyribosomes, Golgi complexes with distended saccules, mitochondria, microtubules and cisterns of granular reticulum are scattered in the perinuclear cytoplasm. (Magn. × 10900.)

Figure 55. Postnatal day 24; large light oligodendrocyte of layer VI. This cell has a process (pr) which runs towards and may be associated with a myelinated axon (ax). Note the typical Golgi elements and the numerous microtubules, and that some of the granular reticulum is in the form of stacks of parallel cisterns. (Magn. × 10450.)

stacks of short saccules (figures 38–40). Some microglia at these ages are abnormally large, contain debris, and resemble those illustrated in figures 25 and 27 (6 day postnatal material).

Postnatal days 16–24

Astrocytes. This period is marked by transformation of most atrocytes from the immature to mature form (figures 43–52). At the beginning of the period most of the cells present, while better differentiated than most of those seen at days 8–12, are still immature, and with a few exceptions (see for example figure 50), devoid of filaments. Immature cells are particularly evident in the more superficial cortical layers, where they commonly occur in pairs. During the early part of this period and in the few days preceding it, there are clear signs that the cells are engaged in the formation of processes. This activity is manifested by an extremely irregular cell body outline, and the presence of numerous finger-like or sheet-like protrusions from cell bodies and large processes.

Immature astrocytes are characterized above all by the richness and close packing of organelles in their perikaryal cytoplasm and in the cytoplasm at the bases of their larger processes. Other distinctive features are a cytoplasmic matrix that is more electron opaque than at earlier stages, and the presence of prominent, often peripherally situated nucleoli. Conspicuous among the concentrated perikaryal organelles are: mitochondria and free polyribosomes; numerous elements of the Golgi apparatus, the saccules of which may be distended, and which are associated with prominent clusters of peripheral vesicles; and irregularly dispersed cisterns or tubules of granular reticulum which are seldom aggregated into stacks. In some immature astrocytes, particularly at day 16, the cisterns of granular reticulum may be distended and display a luminal content of moderately electron dense material (figures 43, 43A), a characteristic feature of the astroblasts of earlier developmental stages, but less commonly found in immature astrocytes and not seen at all in mature astrocytes. Microtubules are commonly present in these cells and are occasionally extremely conspicuous, particularly near centrioles (figures 45, 51, 52).

At the other extreme are cells in which all or almost all of the characteristics of the mature cortical astrocyte are seen (see for example figure 48, 18 days, and figures 49 and 52A, B, 20 days). Briefly, these are (1) an irregular outline of the cell body owing to the origins of large processes and of smaller finger-like or sheet-like expansions, (2) pale, rounded nuclei with finely granular nucleoplasm and a thin and inconspicuous rim of heterochromatin beneath the nuclear envelope, (3) a comparatively electron lucent cytoplasmic matrix with a low packing density of cytoplasmic organelles and the presence of *ca.* 9 nm filaments usually in the form of bundles of large numbers of parallel filaments lying parallel to the long axis of the processes and in variable orientations in the cell body, (4) comparatively few free polyribosomes and granular reticulum in the form of undistended cisterns of variable length, commonly arranged in stacks of three or four parallel cisterns, and invariably studded with a high density of ribosomes in polysomal configurations, (5) elements of the Golgi apparatus associated with comparatively few vesicles, and in general with undistended constituent saccules. It is a consistent observation that astrocytes in contact with the sub-pial or perivascular limiting membranes are among the most mature cells present at a given postnatal stage.

Oligodendrocytes. Both medium and light oligodendrocytes are present at days 16–18, with the latter predominating. Light oligodendrocytes at this time are commonly observed in close association with, or extending processes towards, myelinating axons. By day 20, dark

oligodendrocytes are also present, but the light and medium varieties predominate. By day 20, approximately one-third of the oligodendrocytes are dark and very similar to those of adult animals.

There is recognizable in the material covering this period, not only a temporal gradation, but gradations also in size and cytological features between the light, medium and dark varieties (figures 53–60 and especially figure 61), which reinforce the view of previous authors (Mori & Leblond 1970, Paterson *et al.* 1973, Privat & Fulcrand 1978) that the three types represent a developmental continuum, with the dark oligodendrocyte being the mature cell in a quiescent state at the completion of myelination. The cell bodies and nuclei of light oligodendrocytes are largest, those of dark oligodendrocytes smallest. There is also a gradation in chromatin content, the greatest amount being present in dark and the least in light oligodendrocytes. Light and medium oligodendrocytes commonly contain large, nucleolonemal nucleoli (figures 56, 57), sometimes associated with clumps of heterochromatin, whereas nucleoli of dark cells, when observed, are small and with a condensed appearance.

Differences in cytoplasmic electron opacity between the three varieties rest in part on differences in the concentration of cytoplasmic organelles (ribosomes and polysome clusters, while abundant in light oligodendrocytes, are often more tightly packed in medium and dark oligodendrocytes), but relate chiefly to the greater amounts of an electron dense amorphous material in the cytoplasmic matrix of the dark oligodendrocytes. Light oligodendrocytes, in addition to ribosomes, contain significant amounts of granular reticulum, commonly dispersed as individual or small clusters of cisterns, and seldom as massive aggregates, and microtubules running in all directions. In medium oligodendrocytes, granular reticulum is very prominent and is often in the form of large aggregates of parallel cisterns (figures 56, 60). In the dark stage, stacks of granular reticulum are present and often prominent, but are not as large as those seen in the medium oligodendrocytes, and microtubules are less irregularly dispersed and funnel in an orderly manner into the bases of the processes.

Elements of the Golgi apparatus are prominent in all three varieties and characterized at all stages by slightly distended saccules, the luminal content of which appears electron lucent by comparison with the surrounding cytoplasm. Mitochondria, multivesicular bodies and microtubules are common at all stages.

Microglia. The numbers of recognizable microglia increase considerably in the period 16–20 days, to approach adult values. Most have a mature appearance, and long 'strings' of granular reticulum are common (see for example figure 62). However, the Golgi complexes of many microglial cells at this stage are strikingly prominent and apparently more complex than older animals (figures 63, 64). Also, their content of small, primary-lysosome-like bodies (figures 62, 64) is greater and that of the larger, more electron dense lysosome-like bodies is smaller, than for most microglial cells in older animals. The characteristic expansions of the extracellular space around the cell bodies and processes of these cells are seen throughout this period (arrowheads in figures 61–64). Evidence of phagocytic activity is seen in a few of these cells.

Postnatal days 28–40

There are no obvious differences in the morphology of glial cells at these stages of development and in the adult animals, with the exception that immature astrocytes are more common than in older animals.

(*b*) *Quantitative analysis*

Quantitative data on the frequency and distribution of glial cell types, on the distribution of neurons, and on the neuron:glia ratios in the adult animals and in the various postnatal stages, are summarized in figures 65 and 66 and in table 1.

The following points should be emphasized.

(1) At postnatal days 0 and 4, it was difficult to subdivide the cells of the glial lineage. Thus, although some astroblasts and immature astrocytes could be recognized as such (see § 3 (ii)), all glial types have been grouped together under the heading 'glioblasts' for the purpose of constructing the histograms at these stages.

(2) The accumulation of glial cells immediately below the pial surface is conspicuous at both day 0 and day 4 and there is also a clear concentration of glial cells in the deepest part of the cortical plate, the latter probably representing a wave front of cells migrating into the cortical plate at this stage. Concentrations of astroblasts/astrocytes in the most superficial and deepest parts of the cortical plate remain evident at 6, 8 and 10 days, but, from day 16 on, a more even distribution of astrocytes is seen, with smaller concentrations in layers I and VI and lower IV/upper V.

(3) Although microglia are recognizable in the immediate postnatal period, at which time glioblasts are still identifiable, the numbers of each are relatively small and microglia and glioblasts are tabulated together under the latter heading in figure 65 (0 and 4 days). In subsequent developmental stages, microglia occur throughout the cortical plate, but there is a curious tendency for microglia to be absent from the deepest stratum of cortex adjacent to the white matter, and a suggestion that they are also poorly represented at approximately mid-cortical level.

(4) The concentration of oligodendrocytes in the deep cortical layers in the early postnatal period is very striking, and only at day 16 and subsequently are any oligodendrocytes detected above the deepest fourth of the cortical plate. Even in the adult, the number of oligodendrocytes is very small in layers I and II, and maximal in layer VI.

(5) The relative proportions of the three major glial cell types are notably consistent between the two adult groups. Thus the relative percentages of astrocytes, oligodendrocytes and microglia in the 6 month rats are 50, 40 and 8%, and in the 3 month rats are 53, 38 and 8%.

Description of plate 16

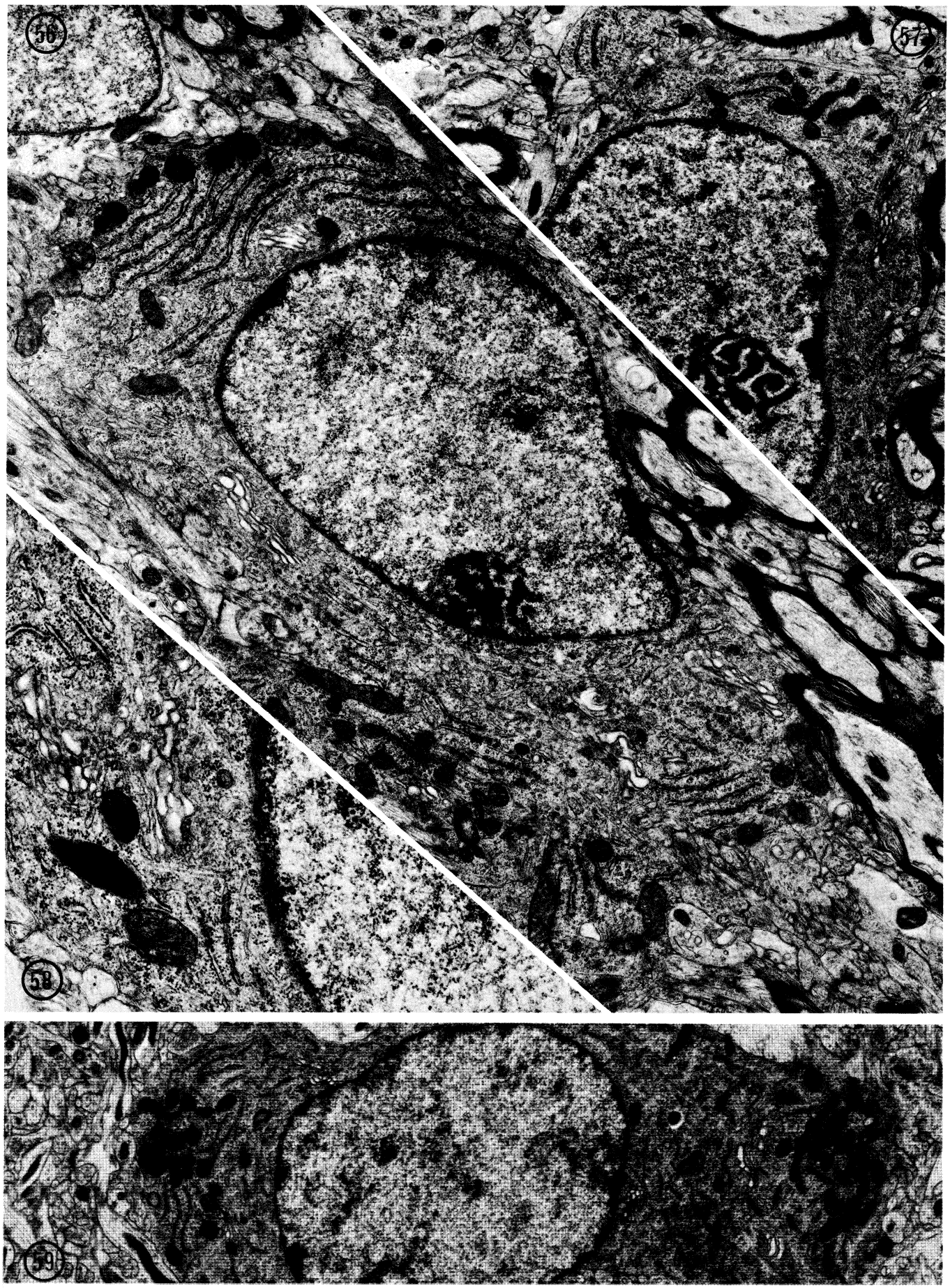
Medium oligodendrocytes at postnatal days 16–24.

Figure 56. Postnatal day 20; medium oligodendrocytes of layer VI. This cell is closely associated with numerous myelinated axons. Its cytoplasm contains large aggregates of granular reticulum, numerous free polyribosomes and microtubules, Golgi complexes with smaller saccules and mitochondria concentrated at the periphery of the cell body. (Magn. × 11600.)

Figure 57. Postnatal day 24; part of a smaller medium oligodendrocyte in layer VI. The nucleus of the cell contains a prominent nucleolus and peripherally dispersed clumps of heterochromatin. (Magn. × 10450.)

Figure 58. Postnatal day 16; part of a medium oligodendrocyte in layer V, showing the characteristic Golgi complexes, with electron lucent widened saccules in the perinuclear cytoplasm. (Magn. × 20500.)

Figure 59. Postnatal day 20; medium oligodendrocyte of layer III. Large peripheral accumulations of mitochondria are present at opposite poles of the elongated cell body. (Magn. × 9300.)



FIGURES 56–59. For description see opposite.

(*Facing p.* 70)

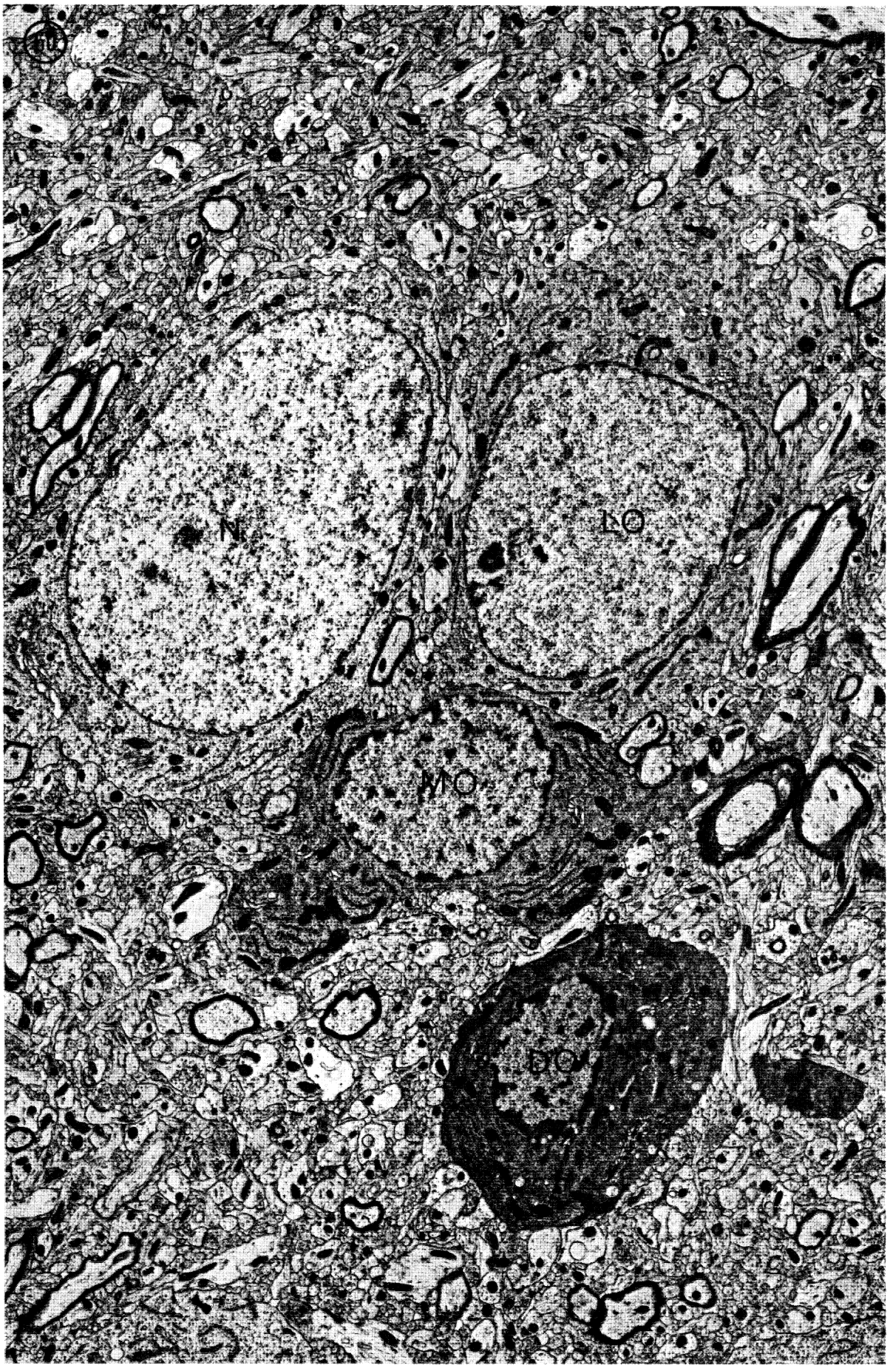
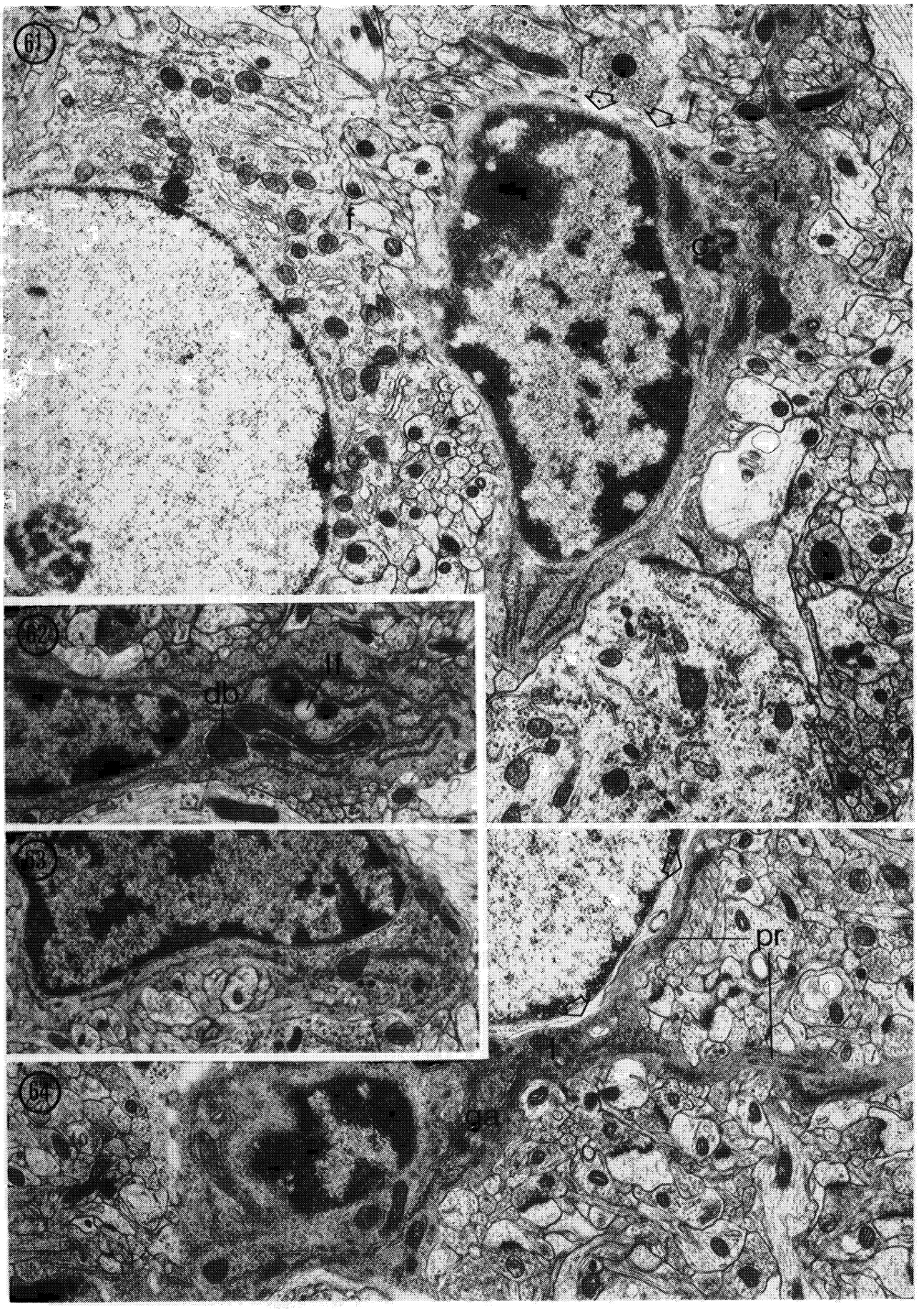


FIGURE 60. For description see opposite.

Description of plate 17

Figure 60. Postnatal day 24; a light, a medium and a dark oligodendrocyte (LO, MO, DO) and a neuron (N) layer V. Note the gradations in size and in cytoplasmic and nuclear characteristics from the light to the dark oligodendrocyte. (Magn. × 7100.)



FIGURES 61–64. For description see opposite.

TABLE 1. RELATIVE PERCENTAGES OF DIFFERENT GLIAL TYPES AND NEURON:GLIA RATIOS

age/day	0	4	6	8	10	12	14	16	20	24	90	180
relative percentage of												
glioblasts	100	100	16	14	0	0	0	0	0	0	0	0
astrocytes (and blasts)			80	71	86	86	78	67	70	61	53	50
oligodencrocytes	0	0	4	4	5	5	9	17	15	25	38	40
microglia	0†	0†	0†	0†	4	5	9	11	12	11	8	8
unclassifiable cells	0‡	0‡	0‡	11	5	4	4	5	3	3	1	2
neuron:glia ratio	4.54	5.09	4.46	4.56	4.07	2.60	2.36	2.22	2.44	2.30	2.13	

† The few microglia identified at days 0–8 were grouped with glioblasts.
‡ Unclassifiable cells at these early ages were grouped with glioblasts.

4. DISCUSSION

(*a*) *General comments on the techniques, experimental design and problems of interpretation*

(i) *Experimental design*

Our approach in this study of glial cell development in the cerebral cortex was to classify, count and plot the position of every nucleated soma present in a series of montages of electron micrographs of unobstructed strips of cortex 75 μm wide and extending from the pial surface into the subcortical white matter. These analyses were done on six montages from two or three animals at each of twelve postnatal ages (12 h and 4, 6, 8, 10, 12, 14, 16, 20, 24, 90 and 180 days), each montage representing an area of approximately 100000 μm², and the observations on this material were supplemented by qualitative observations on a few additional montages and numerous individual electron micrographs from animals 2, 18, 28, 35 and 40 days old. These were costly and time consuming procedures. Nevertheless, we believe that this approach has provided us with a description of the fine structure, distribution, and relative proportions (in a cortical strip of fixed width) of developing glial cells that is based on an adequate amount

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DESCRIPTION OF PLATE 18

Microglial cells at postnatal days 16–20.

FIGURE 61. Postnatal day 16; large microglial cell in layer V. This cell has a dark nucleus with large clumps of heterochromatin, and a cytoplasm containing stringy cisterns of granular reticulum, Golgi elements, mitochondria, small lysosome-like bodies with moderated electron dense cores (l) and a pair of centrioles (c). Note the presence of widened extracellular spaces at the surface of this cell (open arrows), and the long process extending towards the top of the figure. The cell to the left is an immature astrocyte and contains small bundles of filaments (f). (Magn. × 11600.)

FIGURE 62. Postnatal day 16; elongate microglial cell of layer V. This cell has stringy cisterns of granular reticulum, a lipofuscin inclusion (lf), and large dense body (db) which may be a phagocytic or autophagic vacuole. (Magn. × 11600.)

FIGURE 63. Postnatal day 16; small microglial cell of layer IV. Note the large number of small lysosome-like bodies present in the cytoplasm. (Magn. × 10900.)

FIGURE 64. Postnatal day 20; large microglial cell of layer III. This irregularly shaped cell gives rise to several narrow, branching processes (pr). Note the characteristic Golgi complexes and associated small lysosome-like dense bodies (l) and the widened extracellular spaces surrounding the cell (open arrows). (Magn. × 11600.)

of material and that covers, without significant gaps, the period over which maturation occurs. Furthermore, we consider that this approach, coupled with the precautions taken to control for possible inter-observer and inter-animal variation (see § 4*a*(v)), has served to reduce to a minimum bias in the selection of cells for analysis.

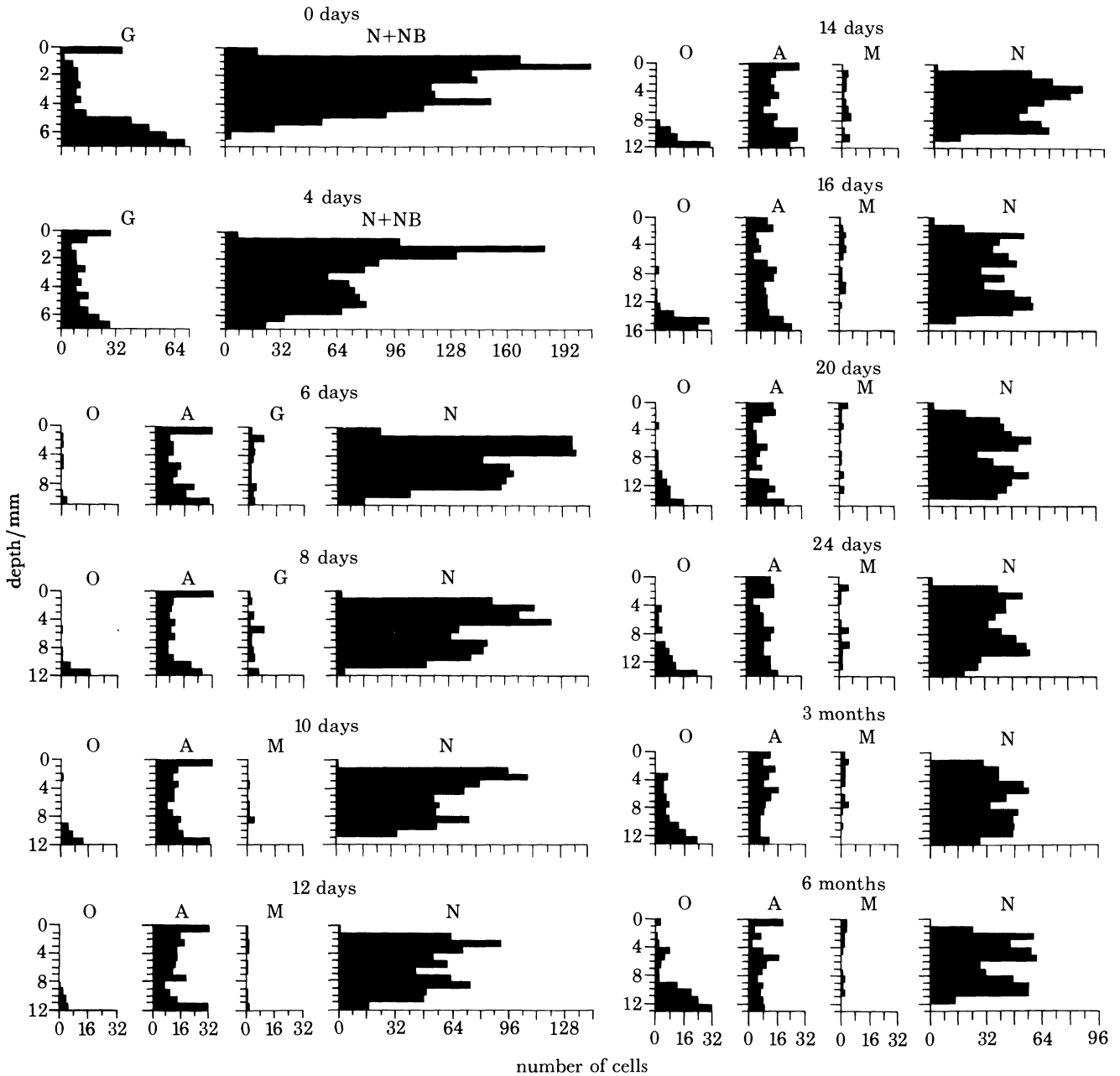

FIGURE 65. Histograms illustrating the distribution of neurons and glia throughout the depth of the visual cortex of rats of various postnatal ages. The data represent the total number of cells of each category identified in 6 montages at every age examined. *Key*: A, astrocytes; G, glioblasts; M, microglia; N, neurons; NB, neuroblasts; O, oligodendrocytes.

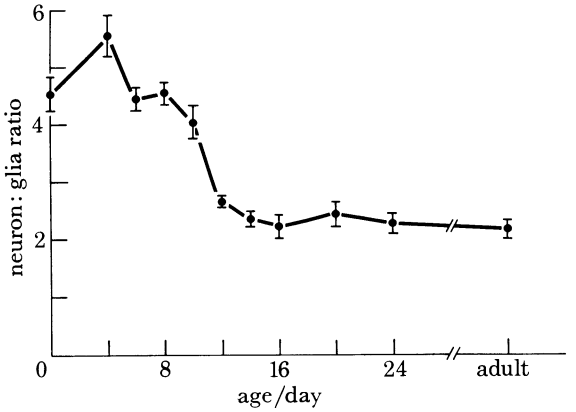

FIGURE 66. Plot of the neuron: glia ratio as a function of age in the visual cortex of the rat. Shown are mean ratios and standard errors of the mean derived from six montages at every age examined between 12 h (0 days) and 24 days after birth. The adult point represents the combined mean for the 3 and 6 month groups (i.e. 12 montages in all).

(ii) *Plotting cell position*

To position cells at the correct depth below the pial surface, and to make it possible to compare directly the plots of cell positions in different montages, the ideal requirement is that every montage be based upon sections cut perpendicularly to the pial surface and mounted on the grid with the radial axis of the cortex parallel with the long edges of the grid aperture. The first of these requirements was relatively easy to achieve by carefully adjusting the orientation of the block face before thin-sectioning until semi-thin sections cut from the block showed maximal lengths of longitudinally cut pyramidal cell apical dendrites. The second was achieved to a great extent by careful picking-up of sections and rejection of any not satisfactorily orientated, but there were still a few montages in which the apical dendrites were inclined at an angle of a few degrees to the long axis of the grid aperture. In such cases the positions of cells in the plots of the montages were calculated by measuring the apparent depth of the cell below the surface along a line parallel with the grid bar and multiplying this depth by the cosine of the angle between the apical dendrites and the grid bars.

(iii) *Defining the grey–white boundary*

The interface between cortex and subcortical white matter, apparently sharply demarcated at the level of light microscopy, it indistinct at the level of electron microscopy. We therefore adopted the arbitrary practice of including as cortical components all glial cells situated within the width of a single photographic strip (approximately 15 μm) below the most deeply situated neuron of layer VI. One of the problems associated with adopting this operational definition of the grey–white boundary is that surprisingly large numbers of neurons are present within subcortical white matter, both in the developing and in the mature brain (see for example Das & Kreutzberg 1968, Rickmann *et al.* 1977, Kostovic & Rakic 1980). Kostovic & Rakic (1980) for example, estimate that in the 2 month old monkey there may be approximately one million such cells (interstitial cells) in each hemisphere below the visual cortex alone. Clearly, the presence of interstitial cells in the subcortical white matter and the inevitable tendency to identify some of the most superficially situated of these as layer VI neurons would result in the grey–white boundary being set at an unduly deep level in such cases and would thus bias the

counts in favour of oligodendrocytes, which predominate in the white matter, and the neuron: glia ratio in favour of the glial cells.

(iv) *Controls for inter-observer and inter-animal variation*

Two other problems and potential sources of error in gathering quantitative data based on poorly defined criteria for cell identification (see § 4*a* (v) below) are variations between different observers analysing the same material, and variation in the results derived from different animals of the same age. In fact, neither inter-observer nor inter-animal variation was very great. Cells in every montage were classified independently by at least two observers; when one observer could not decide on the classification, or there was disagreement on classification between observers, all observers conferred and if there was still disagreement the cell was listed as 'unclassifiable'. Variation between animals of the same age was small (probably because we confined our observations to animals whose body mass was within 15 % of the mean mass for the litter). The relative frequencies of different cell types calculated for different animals of the same age were invariably within 10 % of one another, and we take this as adequate justification of the use of pooled data from more than one animal at each developmental stage.

(v) *Criteria for cell identification*

In the earliest stages examined, the principal distinction drawn was between cells of the neuronal lineage and those of the glial lineages. This was based to a considerable extent on differences in the overall electron density of nuclei and cytoplasm (glial cells, especially their nuclei, are more electron dense), and to a lesser extent on shape (glial cells more irregular) and position (glial cells abut pial surface and blood vessels). The cells identified as glioblasts correspond in most respects to the glioblasts or glial precursor cells of the developing or early postnatal rat optic nerve (Vaughn 1969, Skoff 1975) and to the cells of the subependymal plate in young rats (Privat & Leblond 1972, Ling *et al.* 1973, Privat 1975). We assume that the glioblasts in early postnatal rat cerebral cortex are mitotically active cells, but out observations provide no evidence on this point; nor do they allow any comment to be made or the question of whether the glioblasts are multi-potential glial precursors or are a heterogeneous population of precursor cells committed to specific lineages but not distinguishable as such on morphological grounds alone.

Astroblasts were identified chiefly on the basis of their distinctive granular reticulum (widened cisterns with a flocculent electron dense content), which Vaughn & Peters (1967, 1971) and Vaughn (1969), have previously shown to be characteristic of developing astrocytes. Other features of astroblasts were a cytoplasm generally richer and darker than that of glioblasts and a nucleus that was generally paler. These cytological identification criteria were reinforced for many of these cells by their position in contact with the basal laminae at the surface of the brain or around cortical blood vessels. We should add that, although others have included as astroblasts cells containing conspicuous numbers of glial filaments (see for example Caley & Maxwell 1968, Privat & Fulcrand 1977), we designated such cells immature astrocytes (see below).

Although astroblasts as defined here are relatively well differentiated cells, it is probable that they are mitotically active (as implied by the -blast suffix) for there is evidence that such cells do divide (Skoff 1980), that formation of astrocytes by cell division occurs within the cortex at this time (Skoff 1980) and that well differentiated cells of the astrocytic lineage retain their

ability to divide well into adult life in this species (Hommes & Leblond 1967, Kaplan & Hinds 1980).

We designated as immature astrocytes cells similar in many respects to astroblasts, but lacking distended cisternae of granular reticulum. An electron dense luminal content was generally present in the least mature of the immature astrocytes, but not in the more mature cells. Immature astrocytes spanned a very considerable spectrum in terms of their size and ultrastructure since mature astrocytes were classified as such only if they showed the full range of defining characteristics (see § 3*a*(ii)). Only the more mature cells of this category contained conspicuous numbers of glial filaments.

In the oligodendrocyte lineage, we had great difficulty in identifying and defining oligodendroblasts (or immature oligodendrocytes). Others have experienced similar difficulties and have reported the sudden appearance of oligodendrocytic cells without obvious precursor cells. We recognized cells as oligodendroblasts chiefly at days 6–8 in the deep cortex and subcortical white matter and they were more electron dense than astroblasts, with more microtubules and more polyribosomes than astroblasts, with granular reticulum elements lacking electron dense content and with small Golgi complexes characterized by distended saccules. We think it likely that these cells are mitotically active but that the oligodendrocytes to which they give rise are essentially post-mitotic cells (Imamoto *et al.* 1978, Privat & Fulcrand 1977, Skoff 1980; but see Kaplan & Hinds 1980). The criteria that we used to identify light, medium and dark oligodendrocytes were those of Mori & Leblond (1970; see also Mori & Hama 1971, Privat 1975, Privat & Fulcrand 1977).

Criteria for the identification of microglia were essentially those of Mori & Leblond (1969), Vaughn & Peters (1971) and Blakemore (1975). However, in the early postnatal material (days 6–14) there were cells with rather different nuclear and cytoplasmic characteristics, which nevertheless appeared to be involved in phagocytic activity. These cells had a more even distribution of nuclear chromatin and a paler, less organelle-rich cytoplasm (with a high proportion of free polyribosomes) than mature microglial cells. These 'immature' microglia will be discussed below (see § 4*b*(iv)).

(*b*) *Developmental history of cortical glial cells*

(i) *Some general considerations*

At the time of birth, in rats, the genesis of neurons constituting the cerebral cortex is largely complete (a minor exception concerns non-pyramidal cells of layer IV, which continue to be generated well into adult life (Kaplan 1981; cf. Ichikawa & Hirata 1982)) and most of the neurons have migrated into the cortical plate, although a few complete their migration only during the first few postnatal days (Berry & Rogers 1965, Lund & Mustari 1977). The study of neuronal differentiation in the postnatal period is thus relatively straightforward (Parnavelas & Lieberman 1979). Gliogenesis, on the other hand, begins early in the period of neurogenesis (Skoff 1980) but is far from complete at birth (Mareš & Brückner 1978, Korr 1980, Sturrock 1982), and, in interpreting the present observations, it has to be borne in mind that undifferentiated, or poorly differentiated cells may continue to migrate into the cortical plate in considerable numbers after birth (Ichikawa & Hirata 1982), that glial precursor cells in the cortex, and even more highly differentiated glial cells may undergo mitotic division *in situ*, (see for example Privat 1975, Mareš & Brückner 1978) and that there may be a considerable delay

between the genesis and final differentiation of some glial cells (Schmechel & Rakic 1979, Skoff 1980, Choi 1981). A further consideration in interpreting the present observations is the evidence, strong but not wholly convincing at present, that radial glia, which at very early developmental stages span the telencephalic wall from the ventricular region, where their cell bodies are located, to the external limiting membrane of the brain, and which appear to provide mechanical guide lines for migrating neurons in cerebral cortex (Rakic 1972, 1981), cerebellar cortex (Rakic 1971, Swarz & Oster-Granite 1978) and probably other regions of the c.n.s. as well (Levitt & Rakic 1980), may transform at later developmental stages into astrocytes (Cajal 1911, Choi & Lapham 1978, 1980, Schmechel & Rakic 1979, Levitt & Rakic 1980, Choi 1981). So far as this consideration is concerned, the processes of radial glial cells could be readily identified in the early material (0–8 days postnatal) but not thereafter. These observations are compatible with the transformation into astrocytes thought to occur by many authors, but are equally compatible with the possibility that the disappearance of radial glial elements is the result of their degeneration. The latter possibility seems to us more likely than the former. First, we were unable to identify transitional forms between radial glia and ordinary astrocytes. Secondly, indications of phagocytic activity by immature microglial cells were particularly evident at days 6–8. Together, these observations suggest that disappearance of radial glial cells may occur as a result of degeneration and phagocytosis around the end of the first postnatal week. The evidence is, however, indirect and equivocal and the phagocytic activity seen at this time could as well have been directed towards degenerating neural elements (see for example Heumann *et al.* 1978).

(ii) *Astrocytes*

Whether or not some astrocytes are the products of transformation of radial glial cells (see above), it is probable that most develop from cells that begin to migrate into the cortex several days before birth (prenatal day 13; Wolff & Rickmann 1977) and continue to do so and/or to divide *in situ*, for up to 2 weeks (Ichikawa & Hirata 1982) thereafter, and which, rapidly over the first 3 weeks and more slowly subsequently, differentiate into fully mature astrocytes.

Their precursors are glioblasts, small cells with dark, sometimes irregularly shaped nuclei and a scanty cytoplasm dominated by free polyribosomes. The first stage of their differentiation detectable by electron microscopy appears to be enlargement and considerable enrichment of the cytoplasmic organelles, and most notably the development of a characteristic granular reticulum. These cells enlarge, grow processes, show increasing enrichment of organelles (Golgi elements, microtubules, granular reticulum) and lightening of the nuclear chromatin, and during the 2nd postnatal week the granular reticulum, while still abundant, ceases to be swollen and tends to lose its electron dense content, and the first cytoplasmic filaments begin to appear in what are now recognizably immature astrocytes. During the 3rd postnatal week, the immature astrocytes enlarge further, the concentration of cytoplasmic organelles (with the exception of filaments) falls in the perikaryal region and many of these astrocytes acquire the full range of morphological features that mark them as mature. Others continue to mature more slowly. The general pace of astrocytic differentiation in rat cerebral cortex is compatible with that seen in the optic nerve, where it takes around 3 weeks from the time of final cell division to the differentiation of mature fibrous astrocytes (Skoff *et al.* 1976*b*), and the ultrastructural characteristics of differentiation described in this study are generally comparable with those established in previous studies (see especially Vaughn & Peters 1967, 1971, Vaughn 1969).

(iii) *Oligodendrocytes*

Our interpretation of the sequential development of oligodendrocytes is entirely consonant with the previous conclusions of Vaughn (1969), Mori & Leblond (1970), Privat (1975), Skoff *et al.* (1976*a*, *b*) and Imamoto *et al.* (1978). Oligodendroblasts were first recognized in the deep cortex and subcortical white matter at day 6, some 4 days before the initiation of myelination, which in our material commenced on day 10 and not, as previously reported by Jacobson (1963), on day 14. They give way to light (Mori & Leblond 1970) or active (Vaughn 1969) oligodendrocytes, which are the only oligodendrocytic type present in the first 2 weeks, and which are large cells with an organelle-rich cytoplasm in which granular reticulum with electron lucent cisterns and Golgi complexes with distended saccules predominate. Later, in the 3rd postnatal week, medium oligodendrocytes appear, which are smaller and darker, with more orderly and larger granular reticulum aggregates than in light oligodendrocytes, but with the same type of Golgi complex with distended saccules. Finally, dark oligodendrocytes appear towards the end of the 3rd week, represent one-third of all oligodendrocytes at day 24, predominate by day 40 and constitute over 90% of the mature oligodendrocytes seen at 3 and 6 months.

In other regions of the c.n.s., where large-diameter myelinated fibres are numerous (e.g. spinal cord white matter), larger, paler oligodendrocytes with many of the characteristics of medium oligodendrocytes are found even in adult animals (Blakemore 1978). However, there is evidence that the larger, paler forms are associated with large-diameter fibres and support the largest amounts of myelin, whereas the typical dark oligodendrocyte supports comparatively little myelin (Blakemore 1978), and, since the myelinated fibres of the cerebral cortex and subcortical white matter in the rat are all small-diameter fibres, the predominance of dark oligodendrocytes is not surprising.

It is widely accepted and obvious from the close temporal correlation between active myelination in the cortex and the presence of light and medium oligodendrocytes that these cells are engaged in myelination. It also seems to be generally agreed that the progression from light to dark oligodendrocyte correlates with progressive diminution in myelinating activity, dark oligodendrocytes being quiescent with respect to the bulk formation of myelin and active only in the maintenance of the sheath and turnover of its constituent molecules. However, while medium oligodendrocytes are certainly intermediate in morphology between light and dark oligodendrocytes, and become less numerous towards the end of the period of rapid myelination, they do not have the appearance of inactive or involuting cells; indeed their Golgi complexes are as prominent as in light cells and their granular endoplasmic reticulum is as abundant, although with a distinct tendency to form large aggregates with long, orderly stacks of parallel cisterns rather than the individual dispersed cisterns and small strands seen most commonly in light oligodendrocytes. It may be, therefore, that the differences between light and medium oligodendrocytes reflect not only (or not chiefly) differences in the overall level of perikaryal synthetic activity, but differences in the materials being synthesized at each stage. Perhaps the light oligodendrocytes are engaged primarily in the rapid synthesis of plasma membrane constituents and in the formation of the myelin membranes, whereas the medium oligodendrocytes are concerned more with synthesis of special membrane constituents that are inserted into the myelin membranes after the basic myelin framework has been constructed. It is known, for example, that myelin-specific proteins are synthesized and inserted into myelin

at significantly different rates, and by separate routes (Braun *et al.* 1980), that proteolipid protein is produced more abundantly at the pre-compaction stage and basic protein at the stage of compaction (see for example Tennekoon *et al.* 1977) and that the myelin-associated glycoproteins synthesized during the period of myelin compaction and maturion are of lower molecular mass than those synthesized earlier in myelination (Quarles 1980). Testing this hypothesis will, however, require careful correlation between morphological data on the one hand and biochemical and immunohistochemical studies of oligodendrocytes and myelination on the other.

(iv) *Microglia*

The nature, origin and life history of the microglial cells of mammalian c.n.s. remain remarkably uncertain. For 50 years, the relations between 'resting' microglia in c.n.s. parenchyma, and perivascular cells, free subarachnoid cells and epiplexus cells, and between these cell types and the mononuclear phagocytes that appear in large numbers in pathological or injured c.n.s. tissue have been the subject of intensive investigation but lasting controversy. These problems, which extend well beyond the question of whether the microglia are of mesodermal (Hortega 1932) or ectodermal origin (Rydberg 1932), and the vast amount of experimental literature bearing upon them have been well reviewed by Oehmichen (1978; see other reviews by Privat 1975, Peters *et al.* 1976, Privat & Fulcrand 1977, Kitamura 1980, Ling 1981, Murabe & Sano 1982).

The observations that we have made on microglia in developing visual cortex, and the interpretations of these observations, are, unfortunately, well rooted in this long tradition of uncertainty. Although microglial cells, some evidently engaged in phagocytic activity, were seen at birth and subsequently, only at days 6–10 did their numbers become significant. In the early postnatal period, these microglia appeared to fall into two classes: (1) cells with cytoplasmic and nuclear features essentially identical to those of mature microglial cells and (2) cells that we have designated 'immature microglia'. These cells possessed nuclei with less prominent peripheral chromatin patches and a cytoplasm dominated by free polyribosomes but also containing some long, 'stringy' cisterns of granular reticulum.

Some cells were observed with intermediate cytoplasmic features, which would tend to suggest the transformation of the immature into the mature type of microglial cell, and the immature type of cell was no longer present by the 3rd postnatal week. However, in most cases, the cells could be classified either as microglia (with essentially mature features) or as immature microglial cells. Both types were apparently engaged in phagocytic activity and, although we have insufficient data to make a firm statement on the matter, it appeared as though the immature cells were more involved in this activity than the mature cells at around days 6–8. During the 3rd postnatal week, as in the adult, the microglial cells (now all of the mature variety) spanned a considerable range in terms of both size and cytological characteristics. The two extremes were represented by (1) cells with voluminous, organelle-rich perikaryal cytoplasm and several processes stemming from the cell body; and (2) smaller cells, generally fusiform, with a scanty cytoplasm much poorer in organelles than the first variety, with many free polyribosomes and some stringy cisterns of granular reticulum, but a less prominent Golgi apparatus and very few lysosome-like dense bodies, particularly of the larger varieties. The nuclear characteristics of all microglial cells during and after the 3rd postnatal week were similar, irrespective of the amount of perikaryal cytoplasm and its organelle content.

These observations are open to a variety of interpretations. It is possible that the microglial population is essentially homogeneous but displays a wide spectrum of cell size and cytological differentiation, and that the immature microglia of the early postnatal period differentiate into the mature forms (cf. Murabe & Sano 1982). It is, however, possible that there are two different types of microglial cell in the mature brain, and that (some of) the immature cells of the early postnatal period differentiate into the smaller, less highly differentiated type of microglial cell seen in mature cortex. Another possibility, and the one that we tend to favour at present, is that the cells that we have classed as immature microglial cells are in fact cells of a different lineage. Their cytological and nuclear features are similar to those of glioblasts and astroblasts, and the criteria by which we recognized them as members of the microglial cell family (namely stringy cisterns of granular reticulum, small lysosome-like dense bodies, phagocytic activity) are not absolute criteria. It may be, therefore, that these cells are in fact cells of the macroglial lineage that participate in phagocytosis at a developmental stage at which the demand may be considerable, owing to cell death, and the supply of microglial and/or other phagocytic elements inadequate. If this is the correct interpretation of our observations, it would follow that microglia appear fairly abruptly in the early postnatal cortex in a relatively mature state. Such a conclusion would tend to favour the view that they develop elsewhere and migrate into the c.n.s. parenchyma as already well differentiated cells. However, no evidence of such migration was found in our study, and evidence from several different lines of investigation tends to suggest, albeit not conclusively, that the resting microglial cell is not a cell that has migrated into the c.n.s. parenchyma from the blood (Oehmichen 1978, Kitamura 1982, Murabe & Sano 1980).

(*c*) *Distribution and frequency of glial cells in the developing visual cortex*

Histograms of cell numbers as a function of cortical depth show that the density of *astrocytes* throughout development is higher in the area immediately below the pial surface and in the deepest part of the cortex. These increased concentrations are also present, although not as conspicuous as in young animals, in the adults, with an additional peak occurring at the level of layer IV. The peak present in the superficial cortex is likely to be due to astrocytes forming the glia limitans. The study of Wolff & Rickmann (1977) shows that a small number of astrocytes assume a superficial position in the cerebral cortex of the rat as early as day 13 of gestation. As for the peak present in the area adjacent to the white matter (deep layer VI) in young animals it may be due to migrating astroblasts and astrocytes. The slightly higher density of astrocytes in this area in adult rats may be due to a variety of factors. Astrocytes have been observed to accompany and even ensheath individual axons or bundles of axons in the deeper layers of the cortex and in the subcortical white matter (Wolff 1978), and there is evidence that cooperative interactions occur between astrocytes and oligodendrocytes in the process of myelination (Blakemore 1978). Another reason for the presence of larger numbers of astrocytes in layer VI could be related to removal of degenerating myelin during development and, perhaps, in later life: the studies of Vaughn & Pease (1970) and of Privat & Fulcrand (1976) have shown astrocytes in rat optic nerve to contain axonal and myelin debris. As for the higher concentration of astrocytes in the region that corresponds to layer IV of adult animals, it may be due to the large number of synapses present in that area (Wolff 1978). It has been reported that the disposition of astrocytes is favoured in regions of high synaptic density (Peters *et al.* 1976, Wolff 1976, 1978).

Nearly all *oligodendrocytes* are present in layer VI throughout postnatal development and the largest number of oligodendrocytes is also found here in adult animals, presumably owing to the concentration of myelinated fibres in this region. The present data indicate that oligodendrocytes increase in number with age and that their movement towards the upper layers continues up to the adult stages (3 and 6 months).

Microglial cells are present from birth but first appear in significant numbers in the developing visual cortex between days 6 and 10 and are evenly distributed throughout the cortex at all ages. Their number increases markedly between days 10 and 16, at which time the adult value is reached. This corresponds precisely with the time that Mareš & Brückner (1978) observed an increase in the incorporation of [^{3}H]thymidine by mesodermal elements (endothelial cells and pericytes were included in the counts) in the cerebral cortex of young rats (but see §4*b*(iv)).

Reports of the frequency and distribution of glial cells and of the neuron:glia ratio in the developing and mature cerebral cortex are sparse (Ramon-Moliner 1961, Brizzee *et al.* 1964; see Wolff 1976 for additional references). Direct comparisons between previous reports and the present findings (51% astrocytes, 39% oligodendrocytes, 8% microglia) are not straightforward. Recently Leibnitz *et al.* (1982) reported values of 37% for astrocytes, 46% for oligodendrocytes and 17% for microglia in the adult rat visual cortex, but their data are based on light microscopy of silver-impregnated tissue. In two other studies, both employing electron microscopy, which also yielded relatively high percentages of oligodendrocytes, Mori & Hama (1971) and Mori (1972) restricted their counts of glial cells to cortical layer VI of 60–80 g rats (32% astrocytes, 56% oligodendrocytes, 11% microglia) while Ling & Leblond (1973) included pericytes in their estimates of glial cell types in the dorsolateral cortex of rats between 22 days and 5 months (36% astrocytes, 43% oligodendrocytes, 21% microglia). The relative percentages of glial cells reported by Sturrock (1978) for the indusium griseum of 140 day old rats are closer to our findings (57% astrocytes, 21% oligodendrocytes, 22% microglia) but were based on counts in semi-thin sections, and it is probably not appropriate in any case to compare indusium griseum with neocortical grey matter. Our figures are, however, closely comparable with those of Vaughn & Peters (1974) in the auditory cortex of 3 month old rats: using electron microscopy, these authors reported the presence of 51% astrocytes, 31% oligodendrocytes and 18% microglia. The relative percentage of astrocytes reported by Vaughan & Peters (1974) is identical to that found by us in the visual cortex but the values for oligodendrocytes and microglia are at variance. The difference may be accounted for, at least in part, by differences in the cortical areas examined. Furthermore, the discrepancy in the percentages of microglial cells could reflect varying pathological conditions of the animals used in the respective studies (see Peters *et al.* 1976).

Mori (1972) estimated the neuron:glia ratio to be 2 in layer VI of the cerebral cortex of the rat. In a study of the visual cortex of rats reared in an enriched environment, Diamond *et al.* (1966) reported a neuron:glia ratio of 2.6. The value of 2.13 reported here for the visual cortex of adult rats is comparable with the ratios reported in previous studies.

(*d*) *Concluding remarks*

This study has provided new information on the time course and fine structural features of glial cell differentiation, on the distribution and relative frequencies (in cortical strips of constant width) of different glial cell types, and on neuron:glia ratios in the rat visual cortex during postnatal development. Achieving reliable cell type identification on the basis of ultrastructural

criteria alone was a major problem, and the uncertainties that this introduces into the results must be acknowledged. There is no doubt that more reliable data will be forthcoming when less subjective criteria than those used here can be applied to the identification of cell types in electron micrographs. The combination of electron microscopy with enzyme histochemistry, immunocytochemistry or other cell type-specific labelling techniques offers the best current approach to this problem, and a number of useful labels are already available. Microglia, for example, can be marked by the presence of high levels of 5′-nucleotidase, nucleosidediphosphatase and thiamine pyrophosphatase in their plasma membrane (Kreutzberg & Barron 1978, Murabe & Sano 1982). Astrocytes, immature astrocytes and even very early astrocytic precursor cells in the ventricular zone can be marked by their content of glial fibrillary acidic protein (GFAP) (see for example Choi & Lapham 1980, Levitt *et al.* 1981) or vimentin (Dahl *et al.* 1981, Schnitzer *et al.* 1981, Bignami *et al.* 1982). Oligodendrocytes appear to offer a particularly wide range of potentially useful surface and internal markers, such as the surface antigens galactocerebroside (Raff *et al.* 1979), an antigen or antigens that can be revealed by experimental allergic encephalomyelitis serum (Bonnaud-Toulze *et al.* 1981), the Wolfgram proteins and carbonic anhydrase II of their cytoplasm (Roussel *et al.* 1978, Ghandour *et al.* 1980) and the myelin basic protein and myelin-associated glycoprotein, which these cells contain in greatest concentrations during the period of active myelin formation (Sternberger *et al.* 1978, 1979). Despite these problems of cell identification, we believe that these findings represent a useful contribution to knowledge of glial cell development, and that they provide an essential background for future studies designed to investigate the effects on glial cell development, and/or neuron:glia ratios, of genetic mutations or of experimental manipulations such as exposure of very young animals to enriched or impoverished environments, to toxins, or to abnormal hormonal or nitritional conditions.

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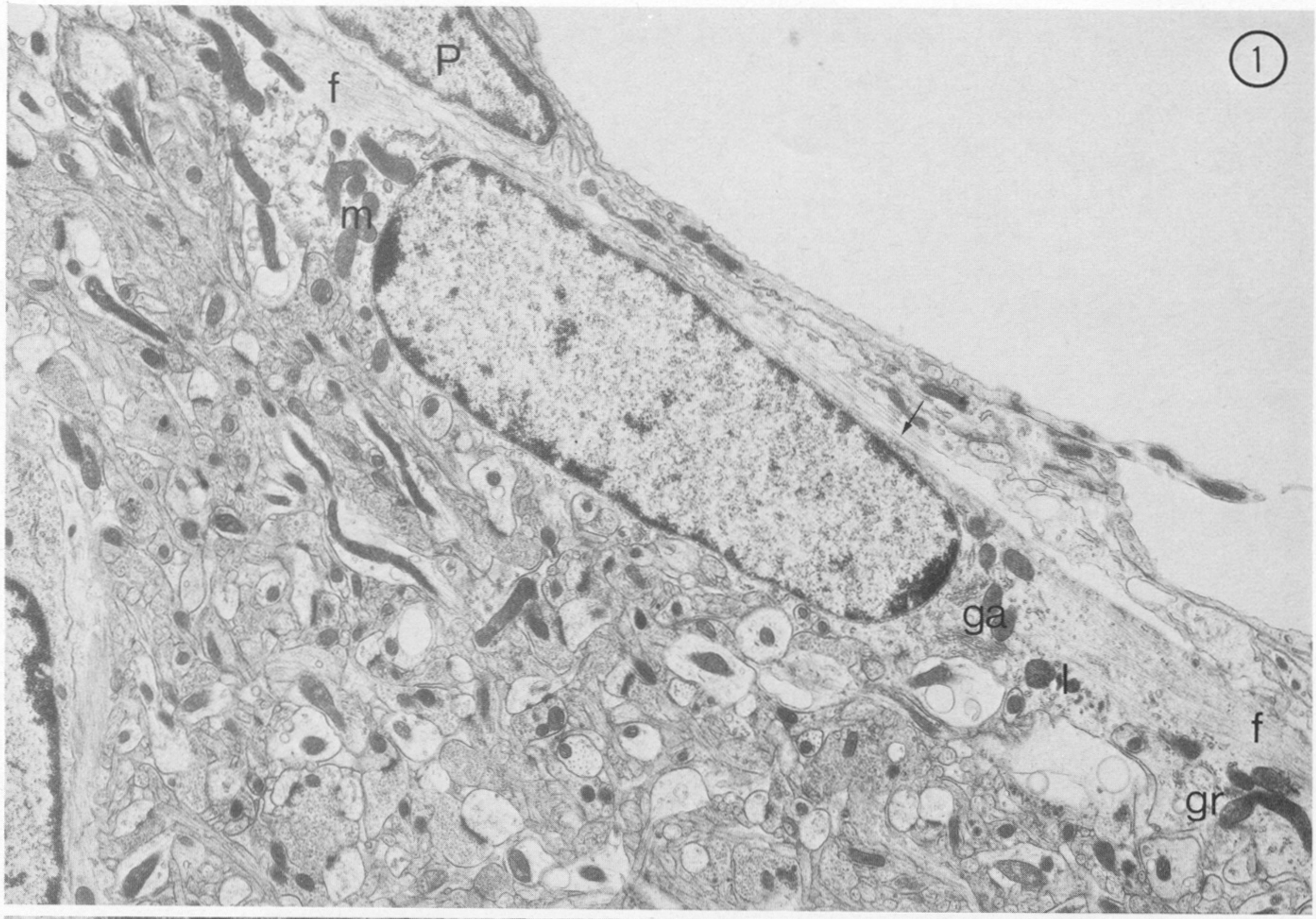
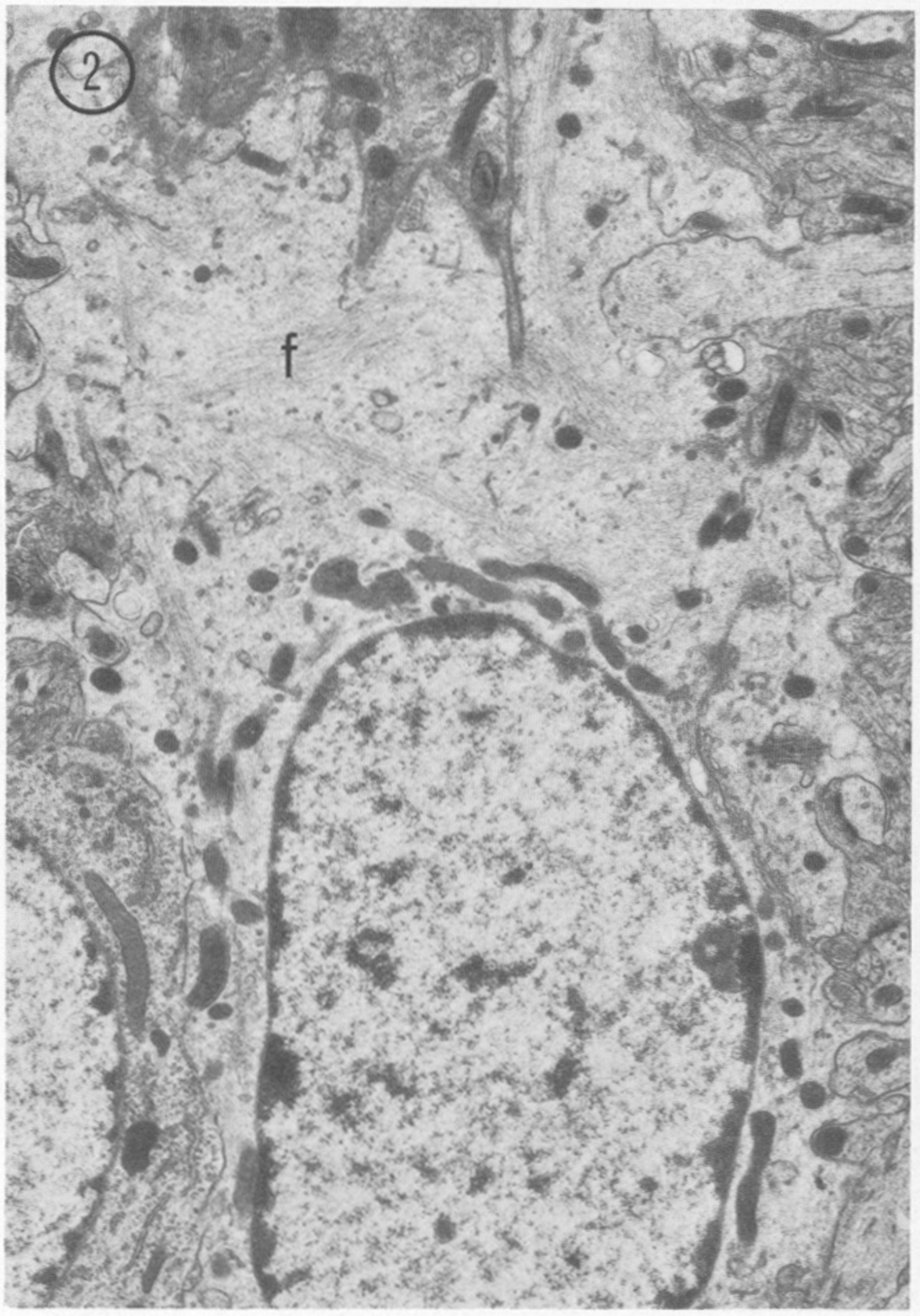
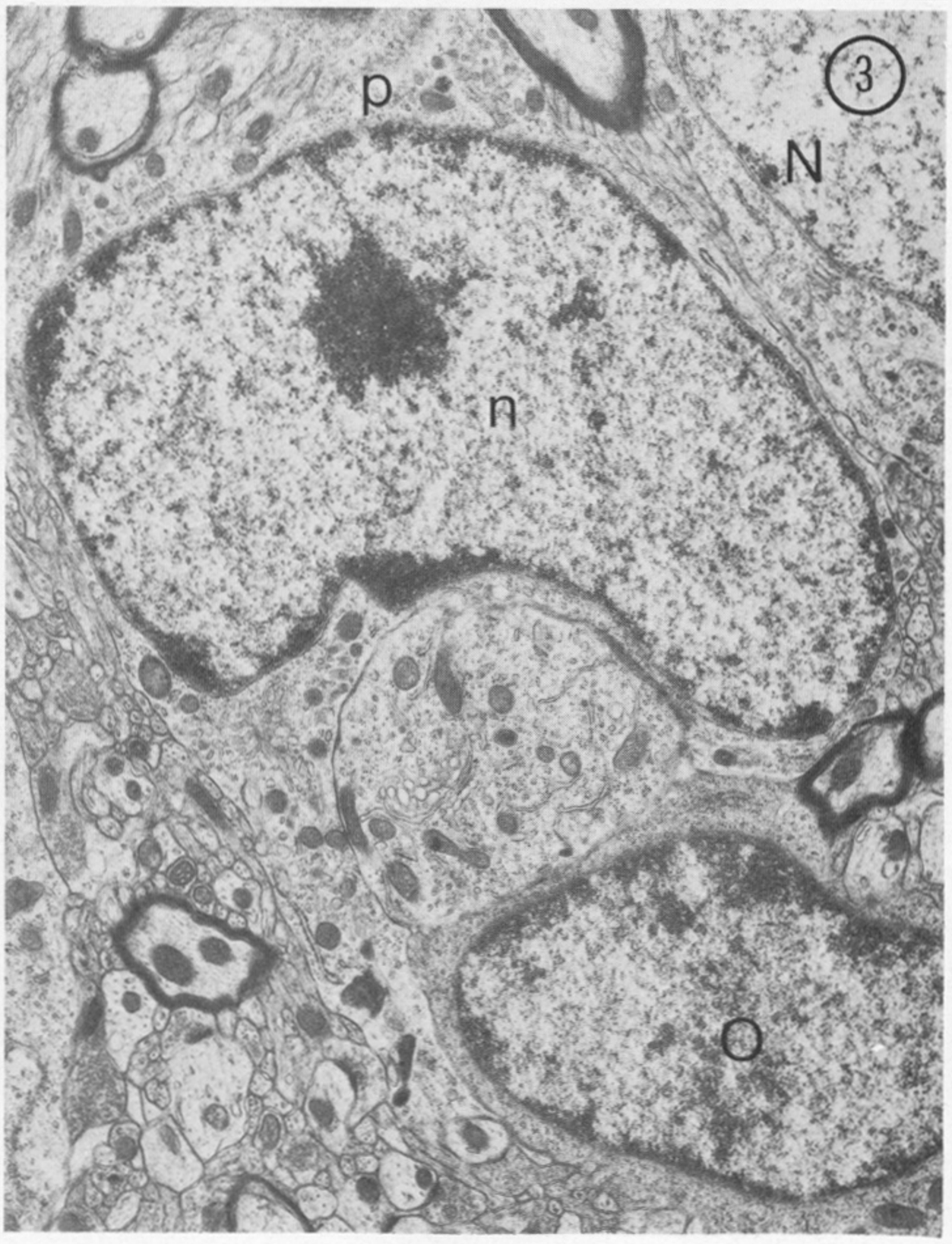
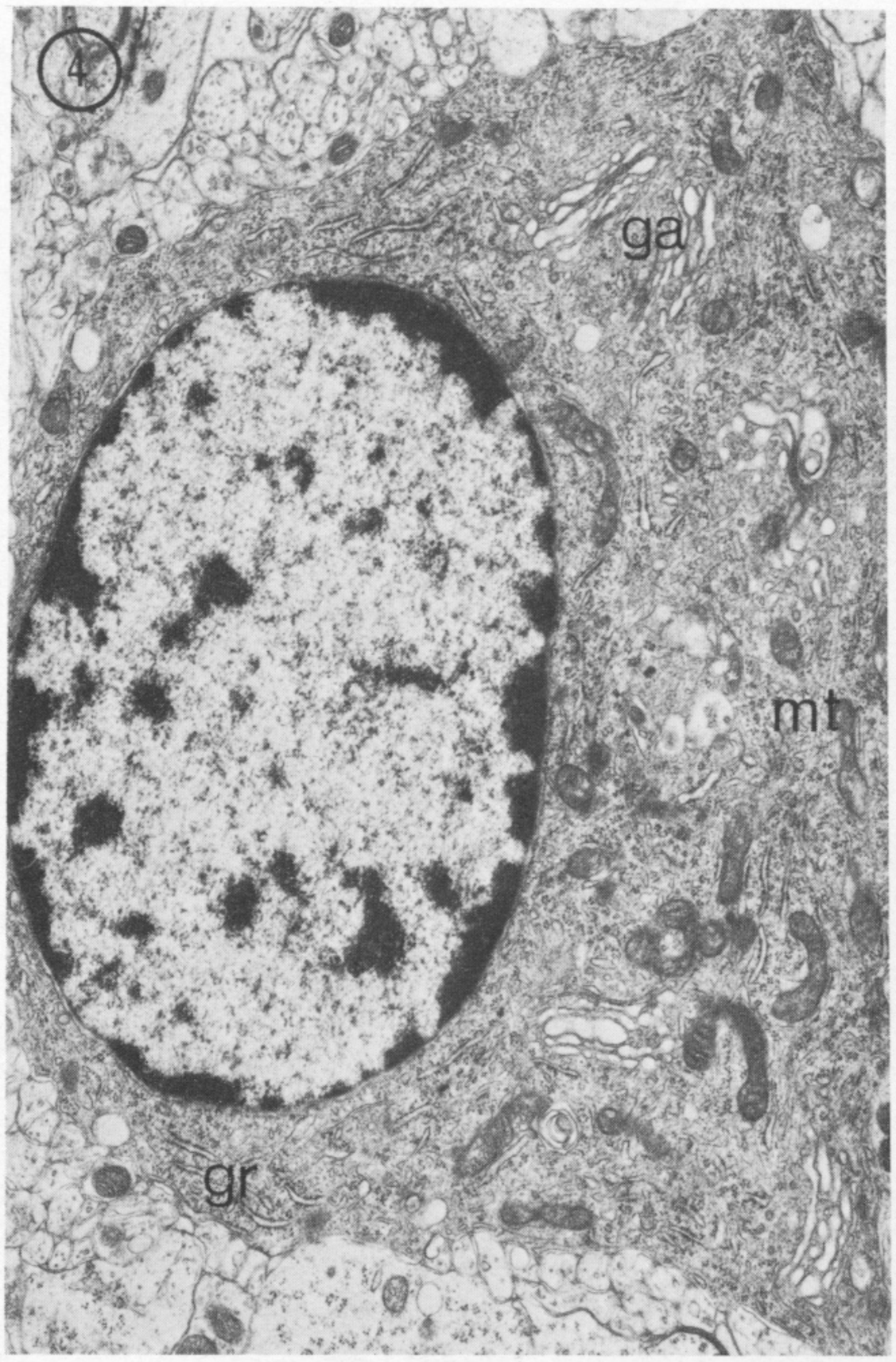
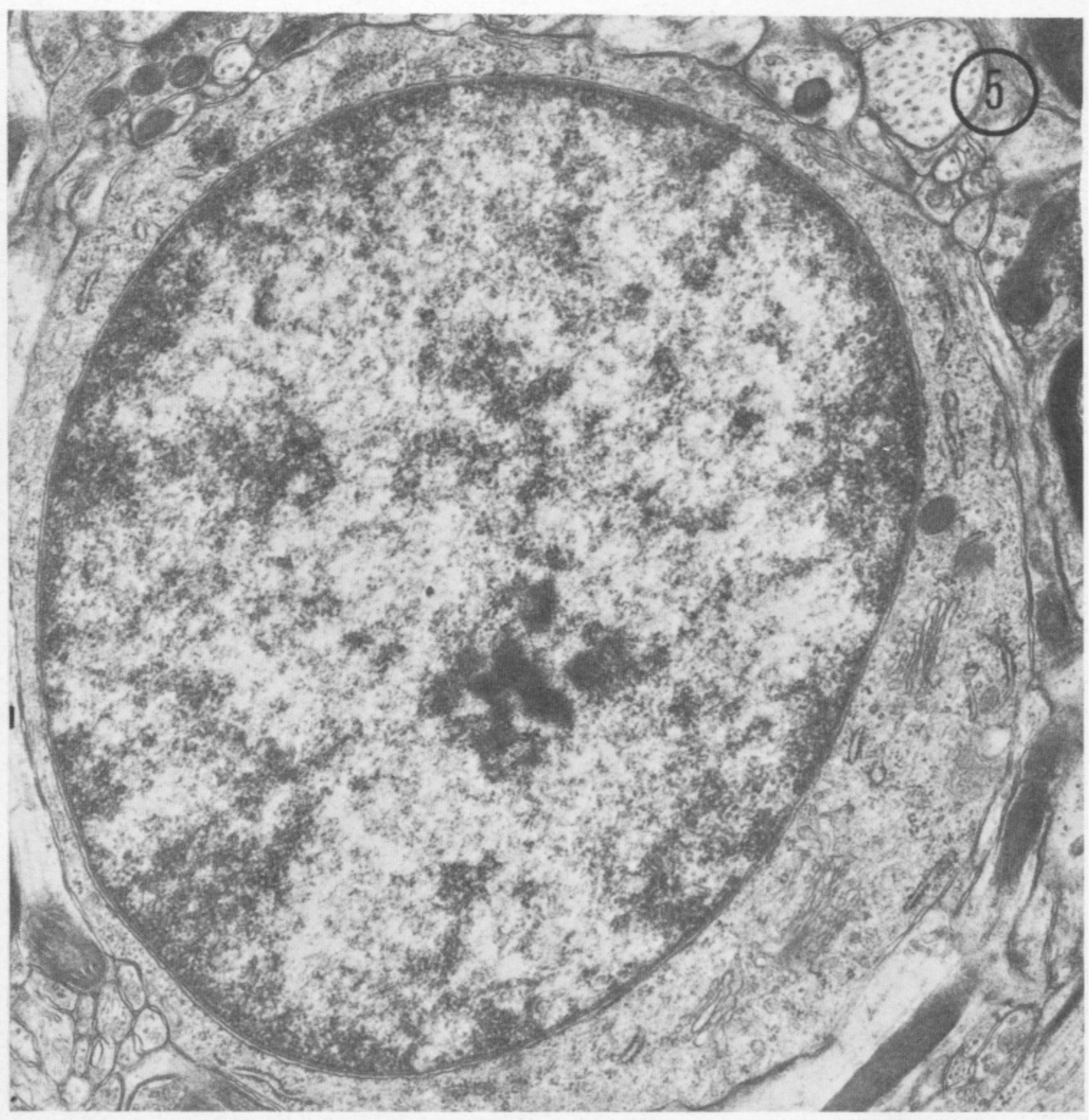


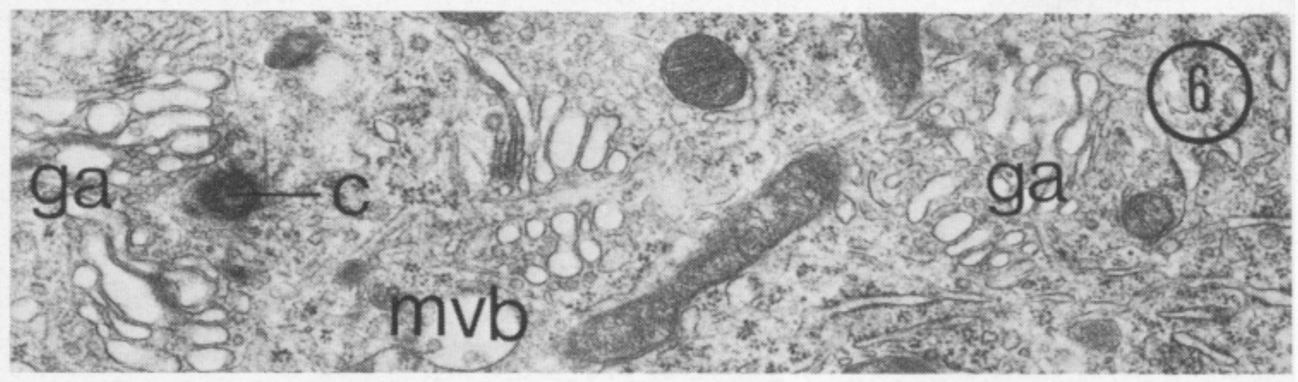


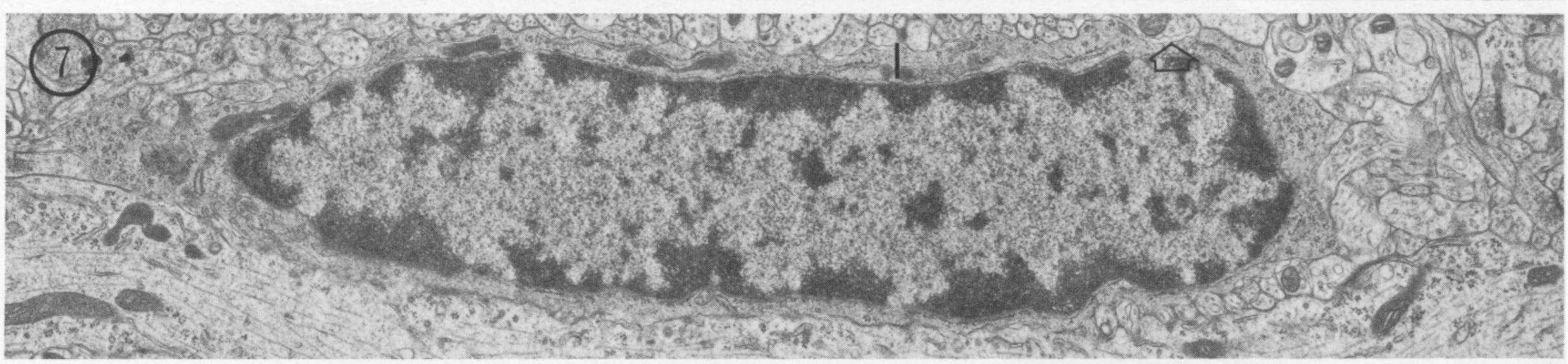


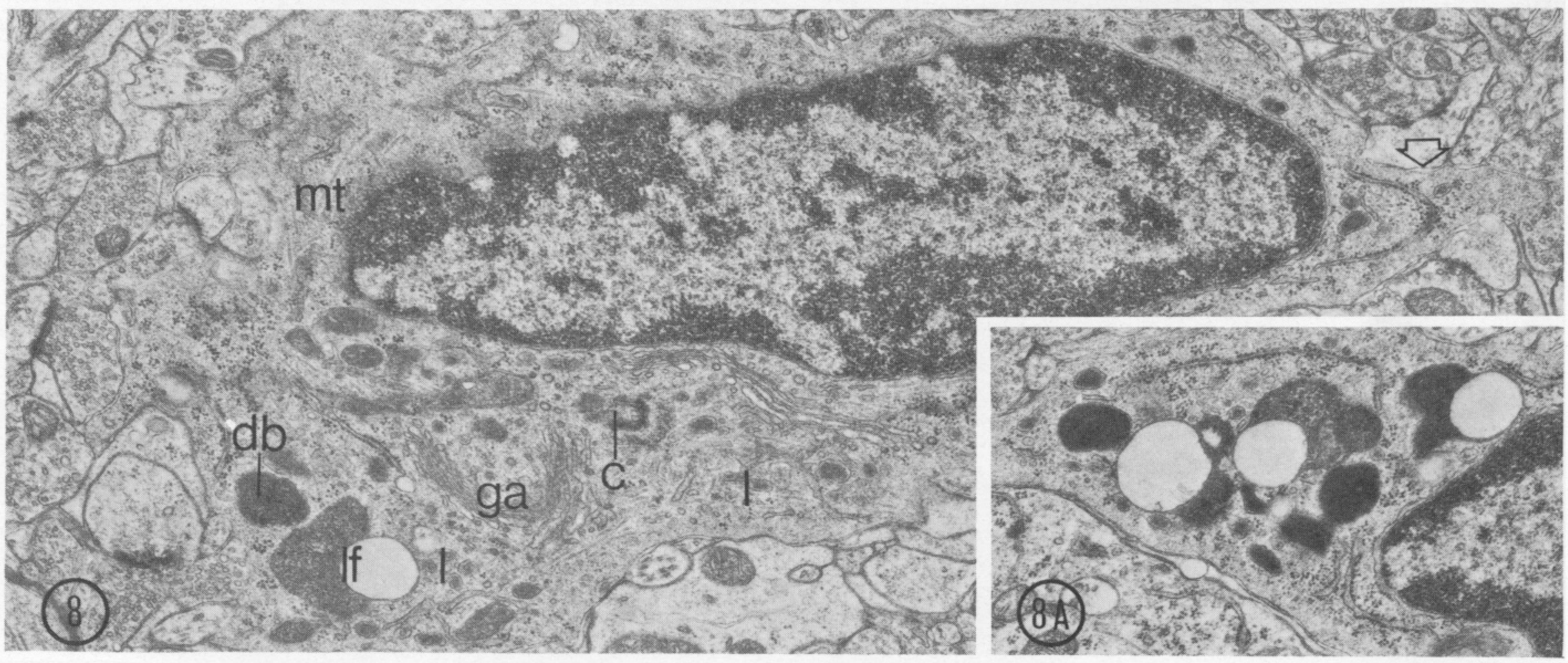
FIGURES 1–3. For description see opposite.



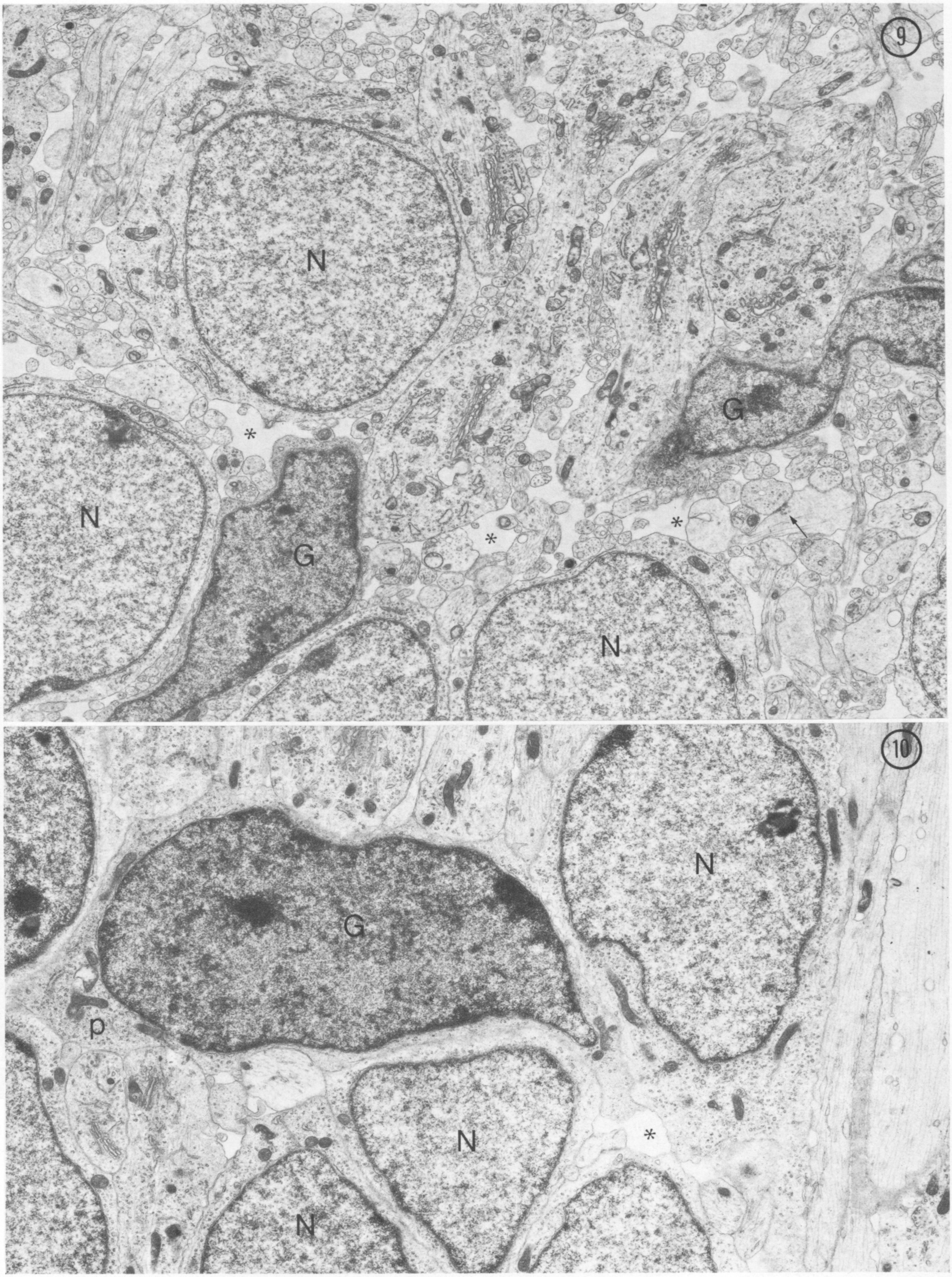




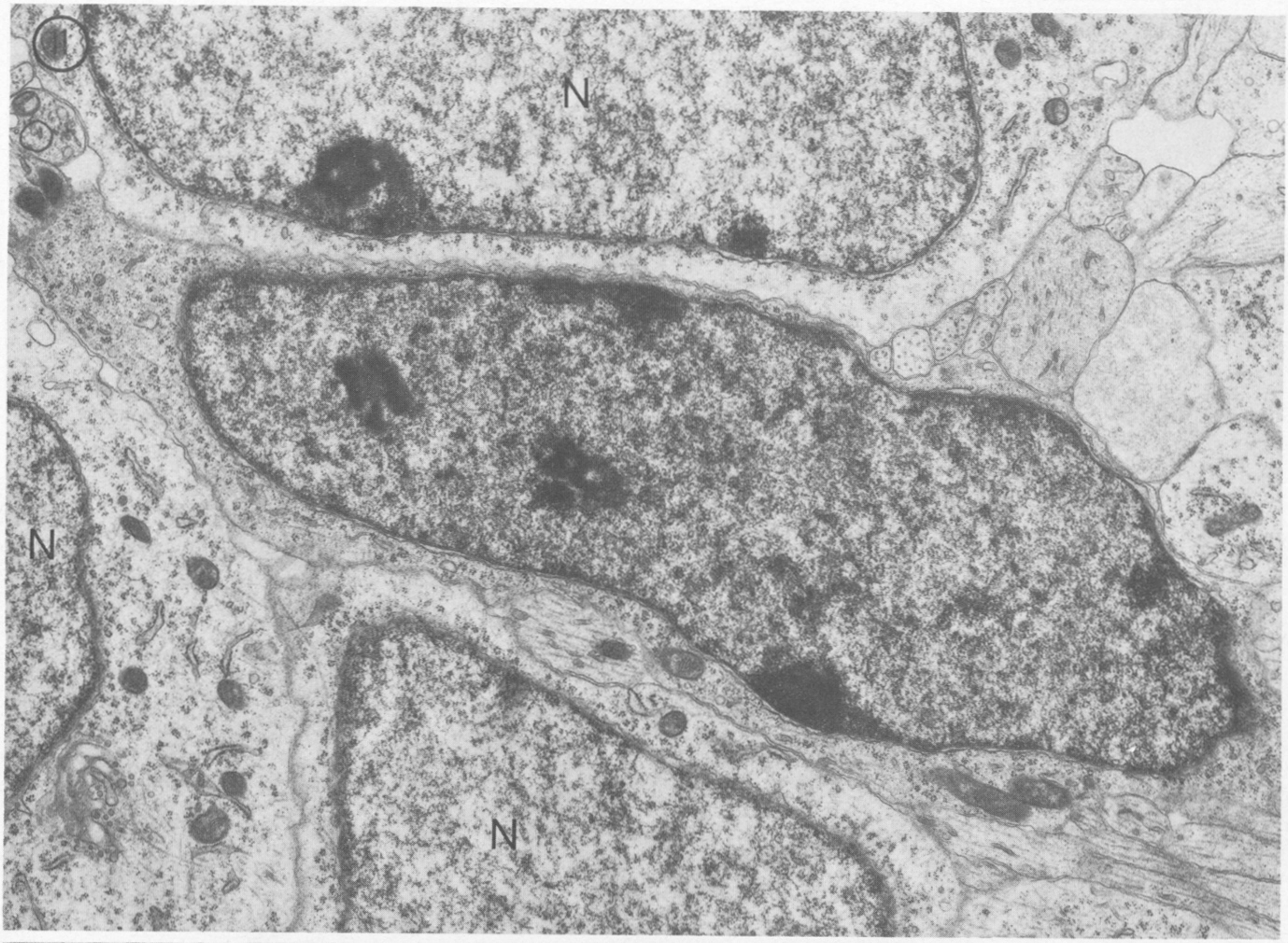


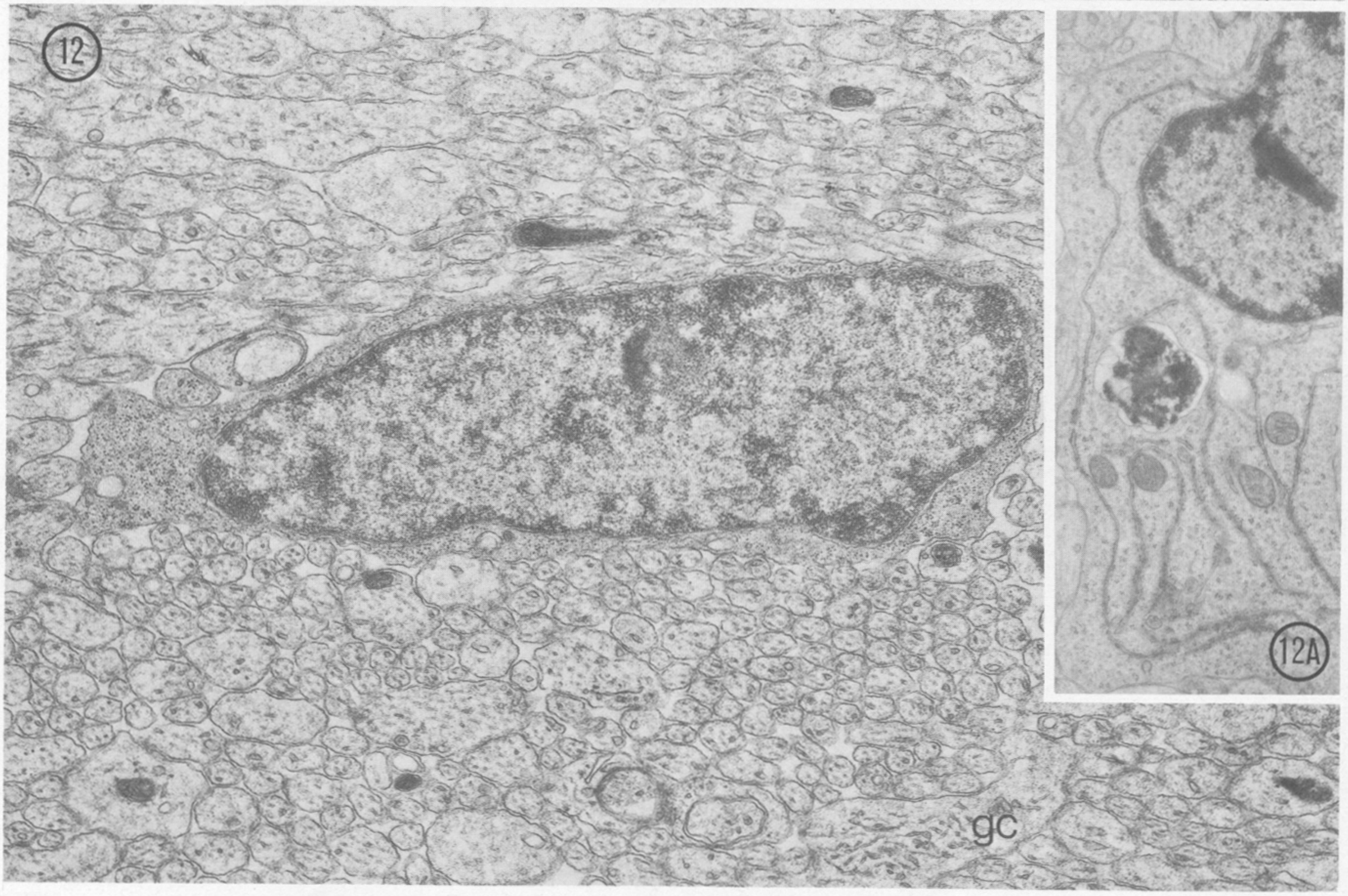


FIGURES 4–8A. For description see page 63.

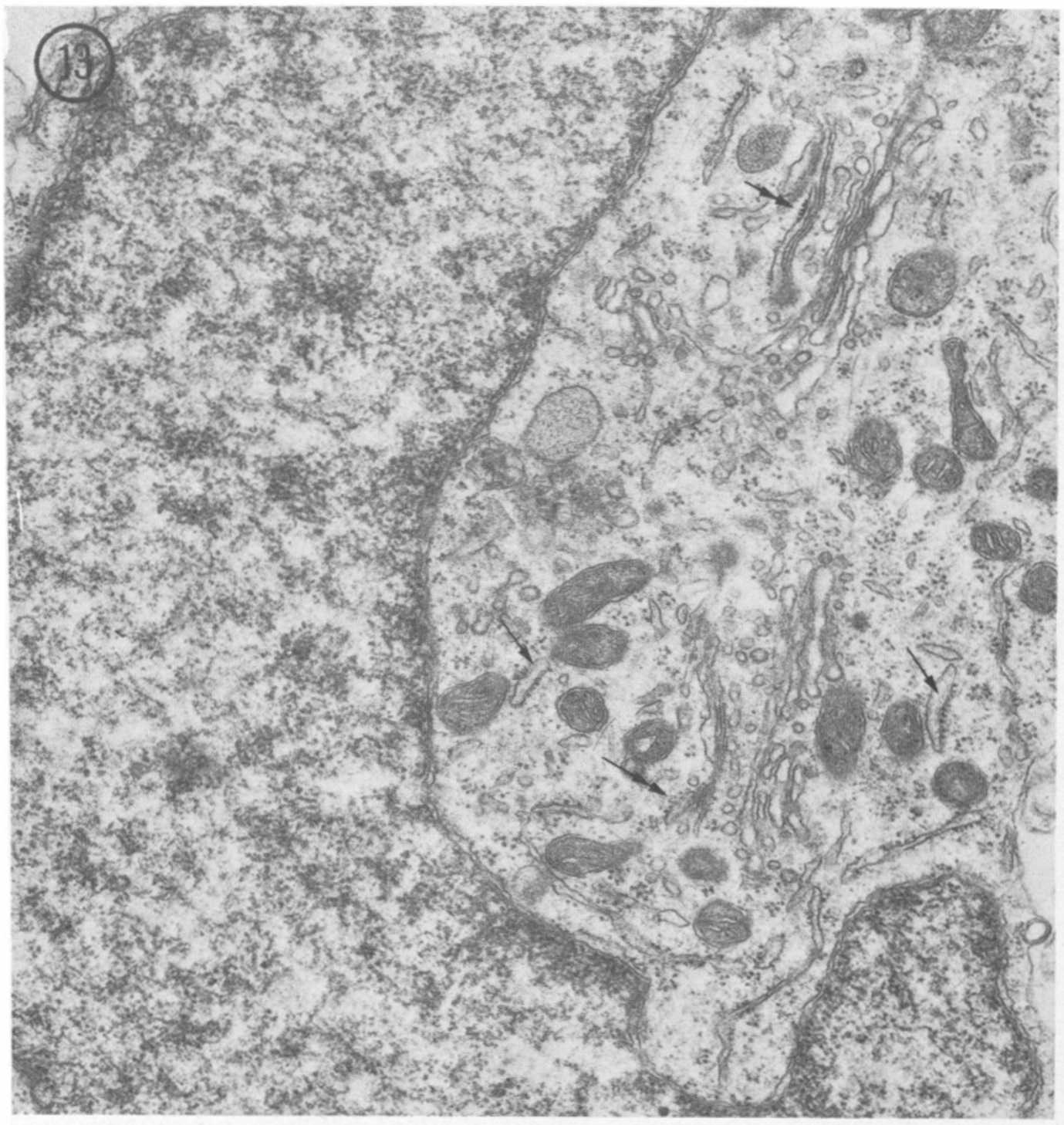


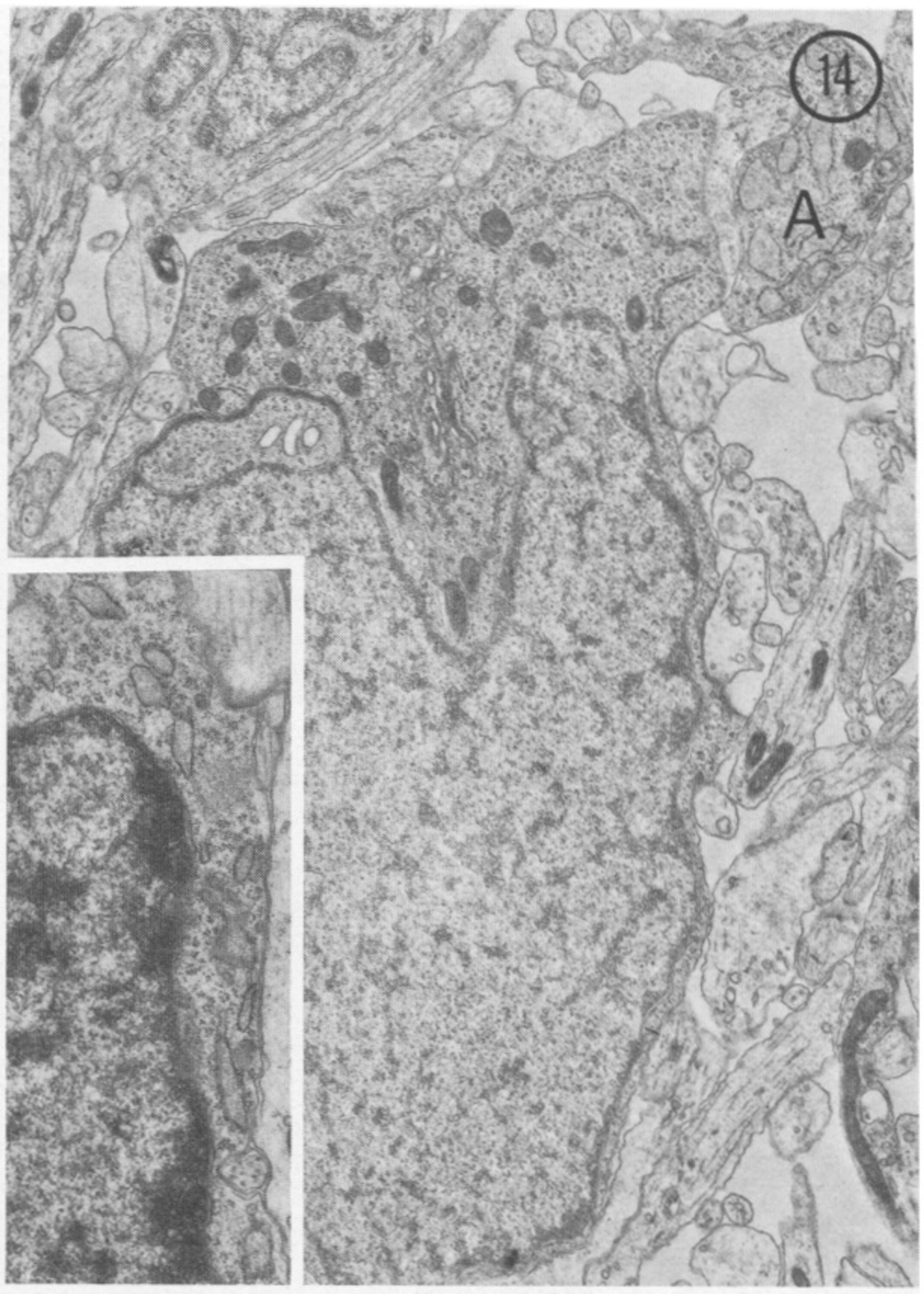
FIGURES 9 and 10. For description see page **63.**

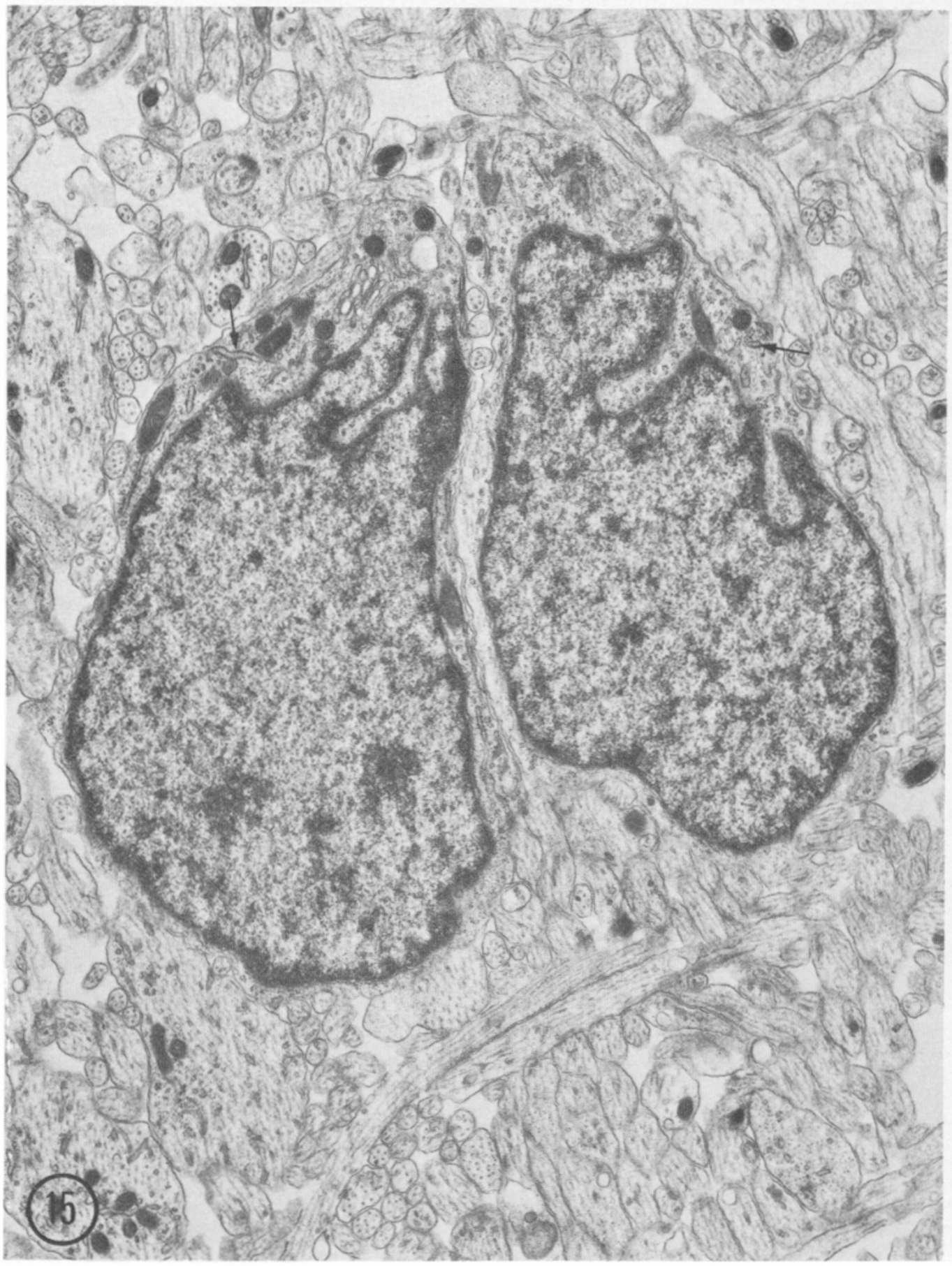


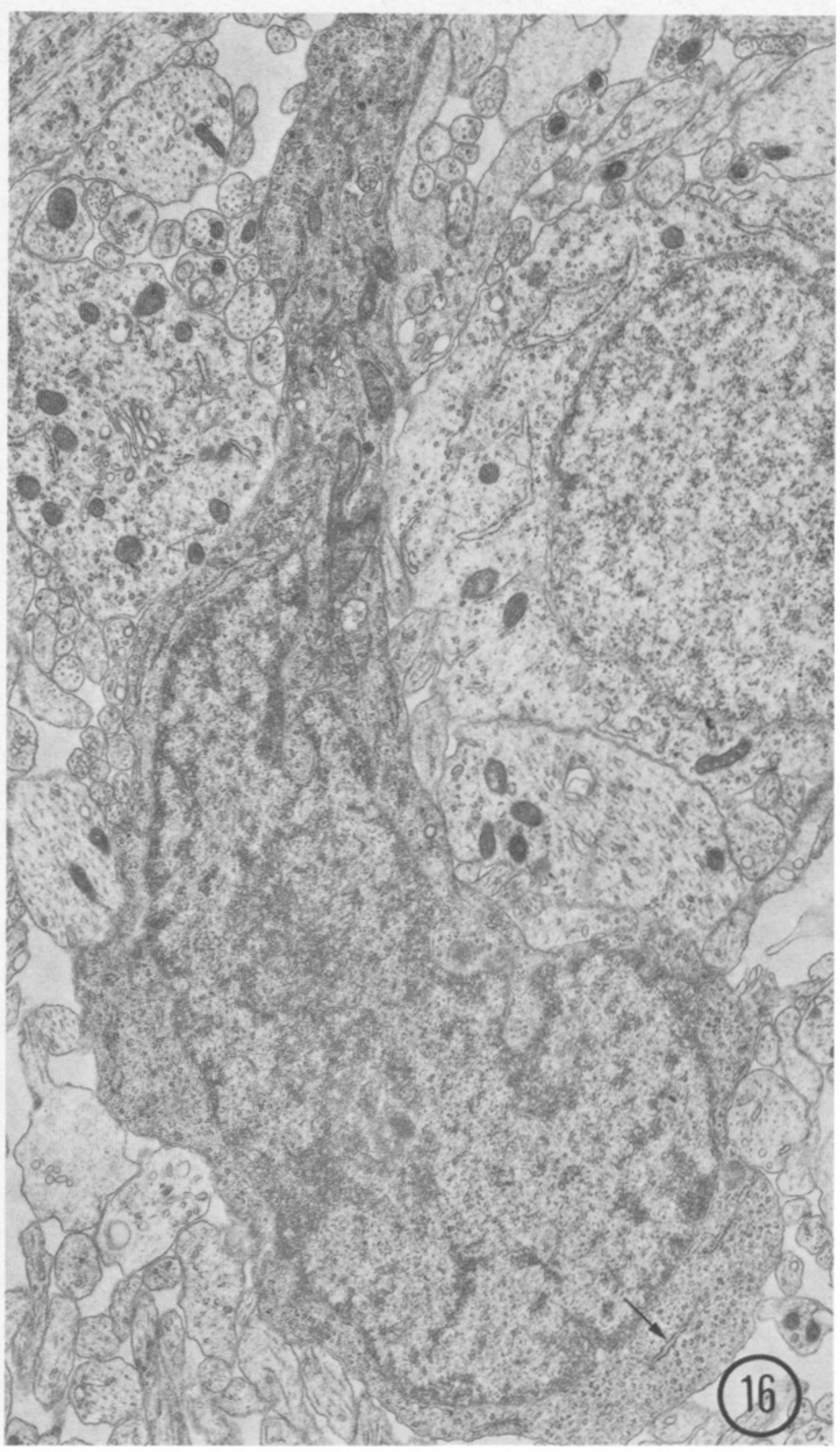


FIGURES 11–12A. For description see opposite.

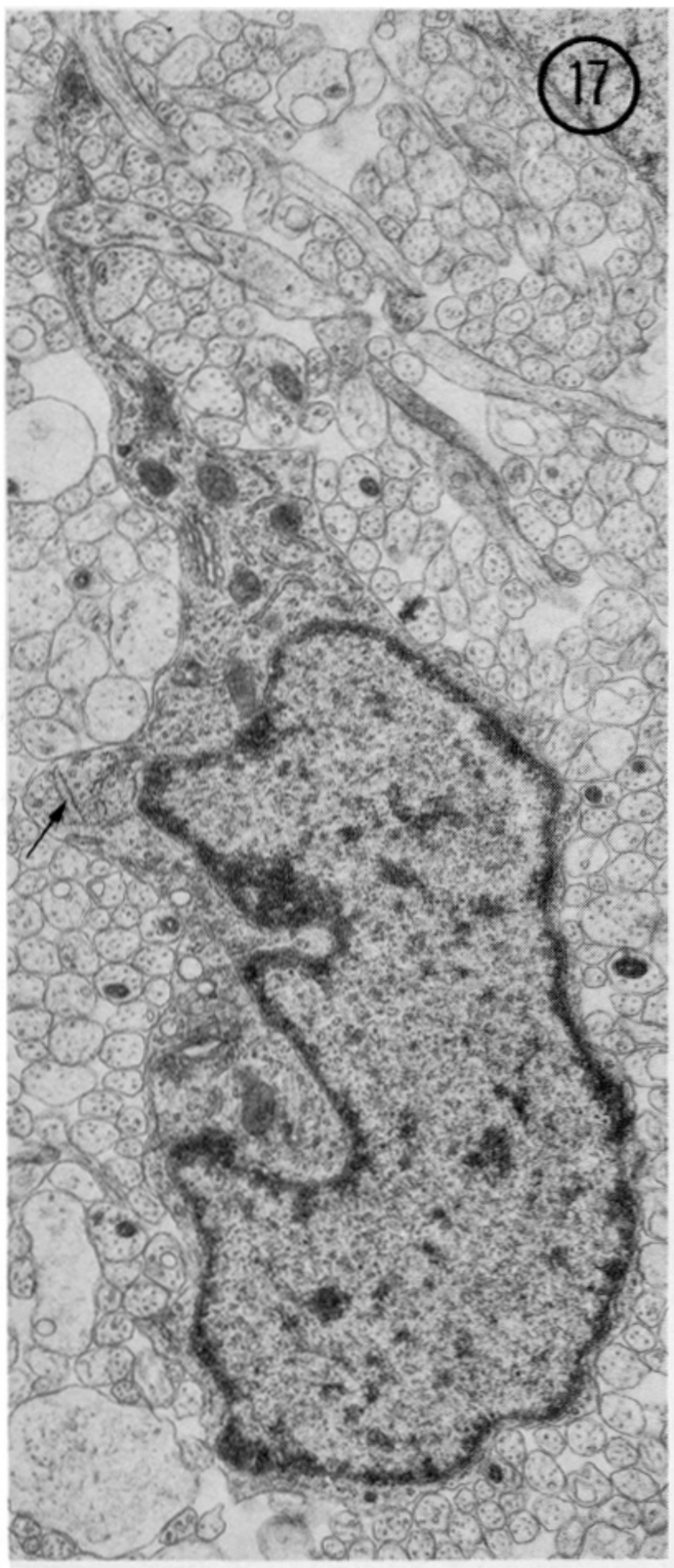


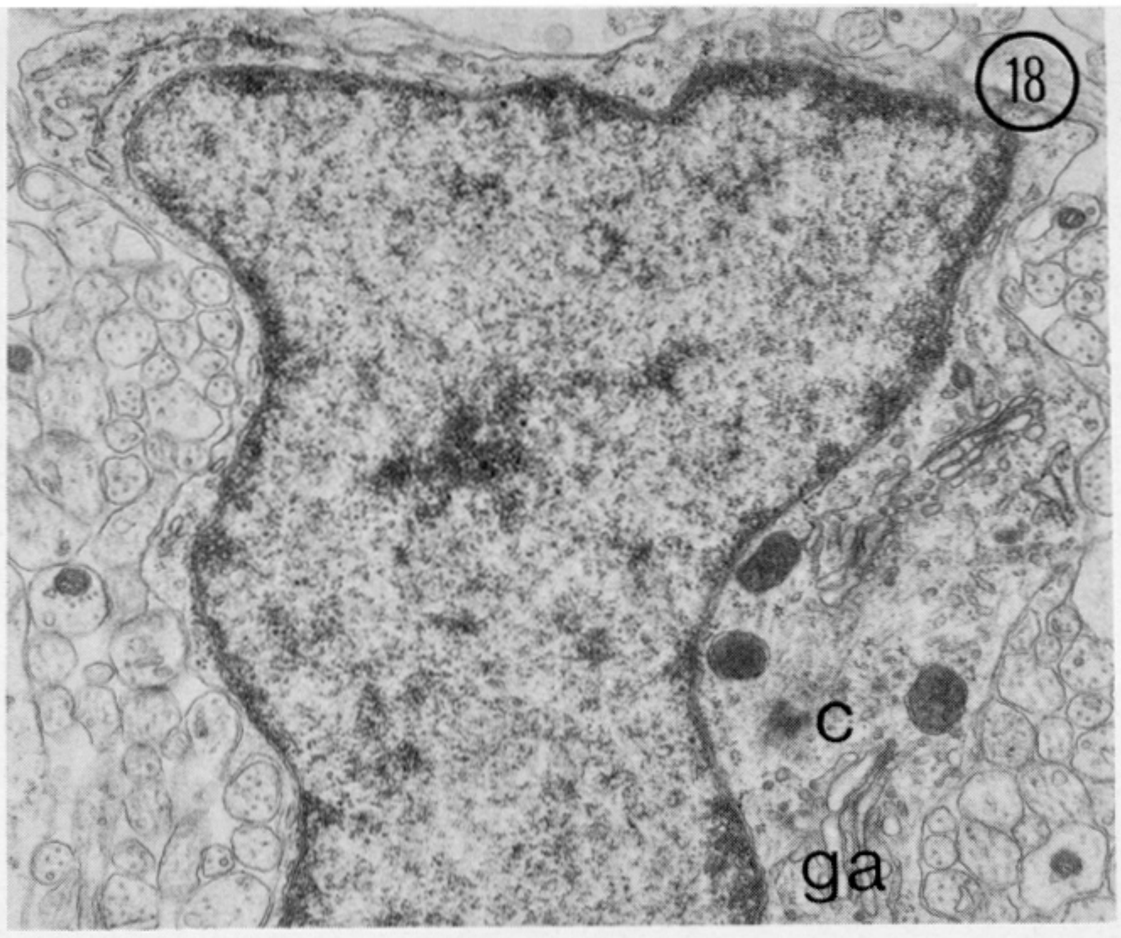


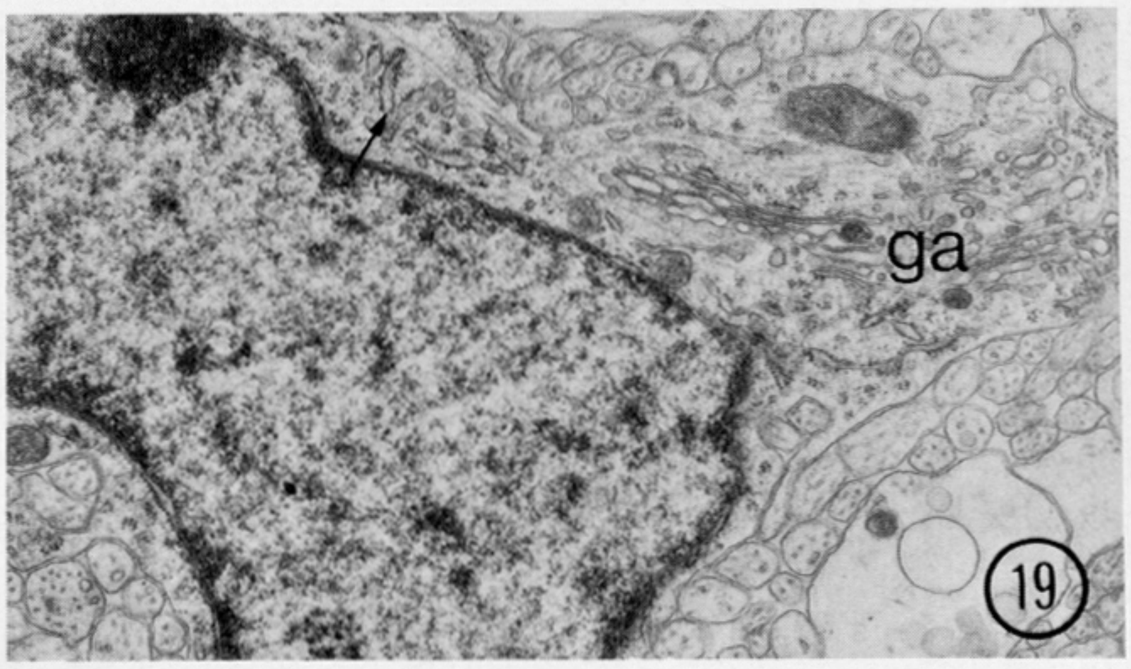


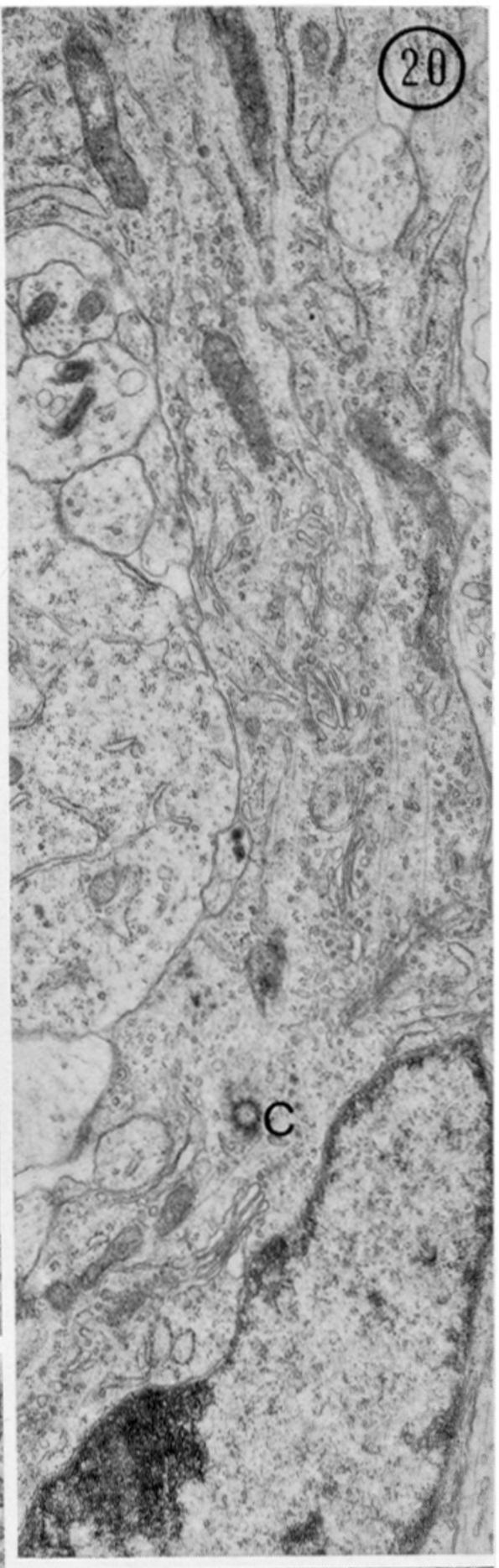


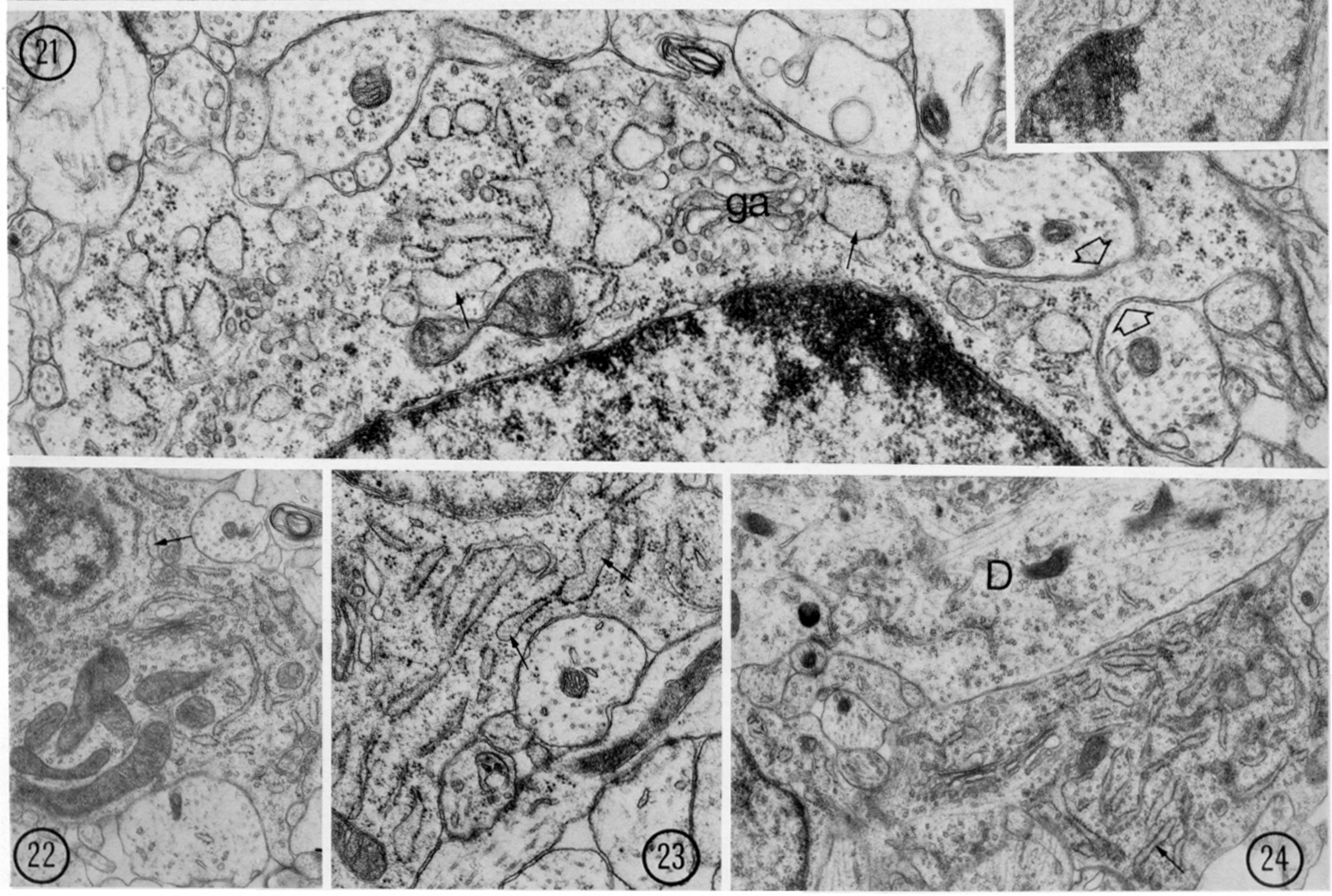
FIGURES 13–16. For description see opposite.



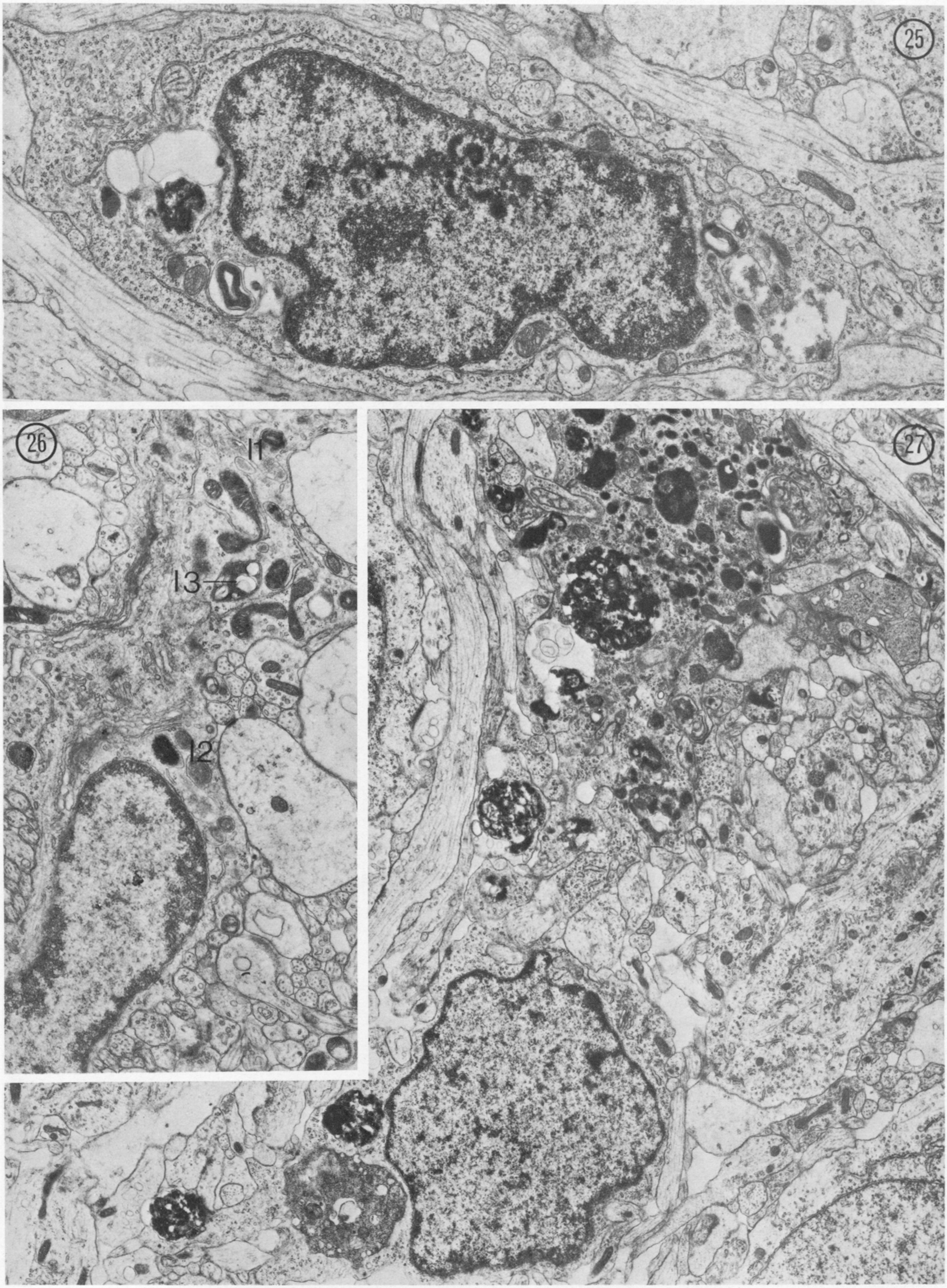




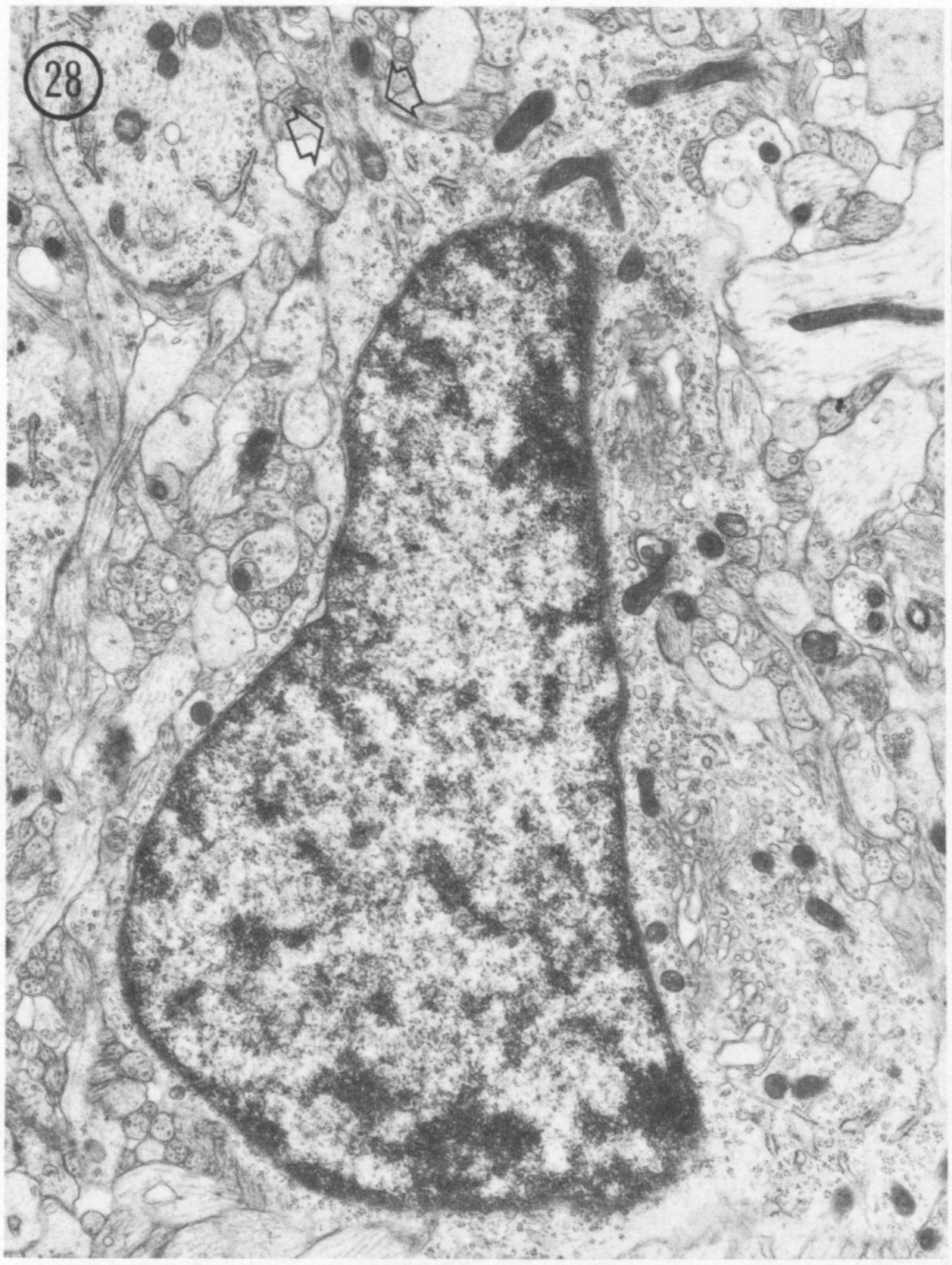


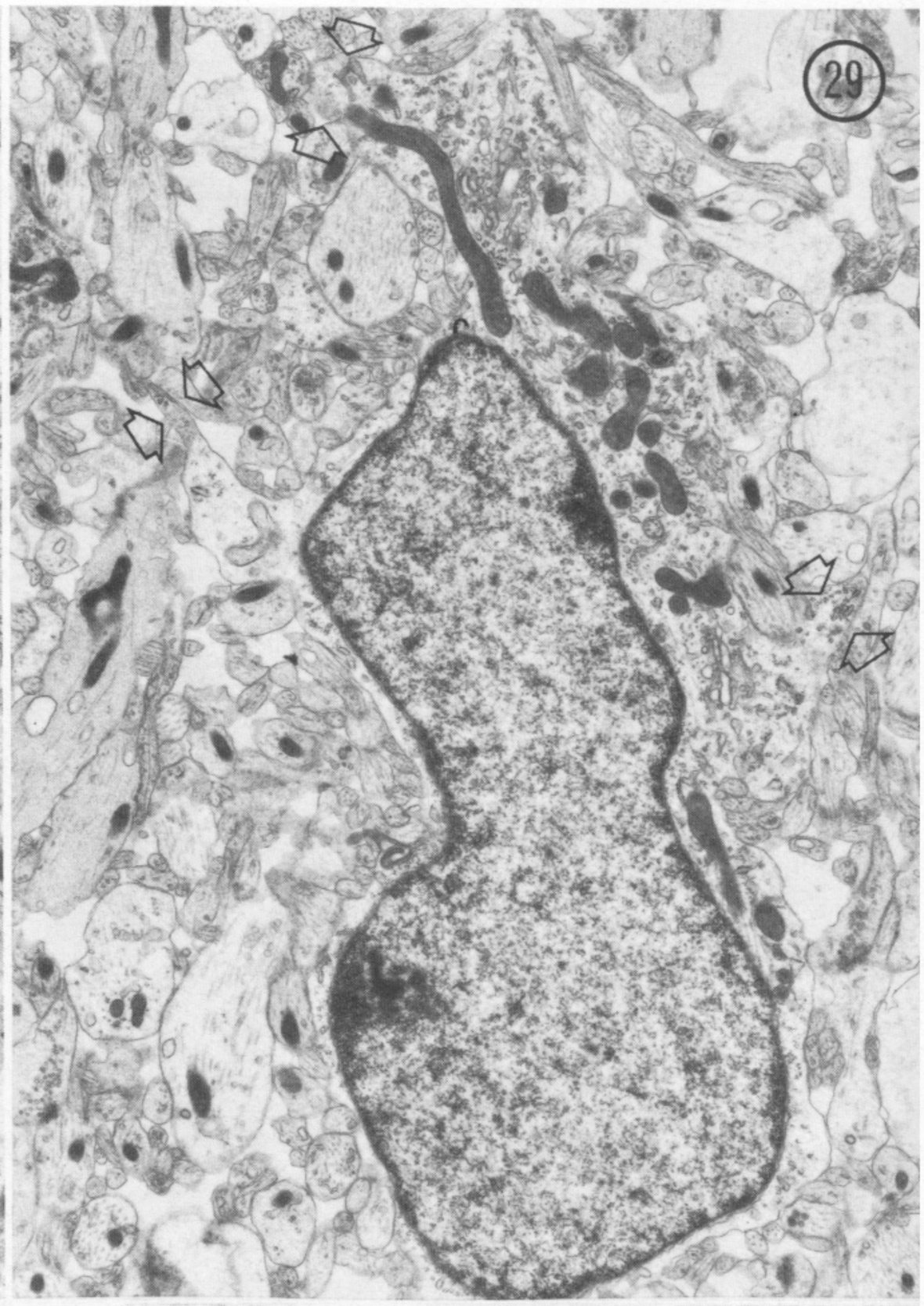


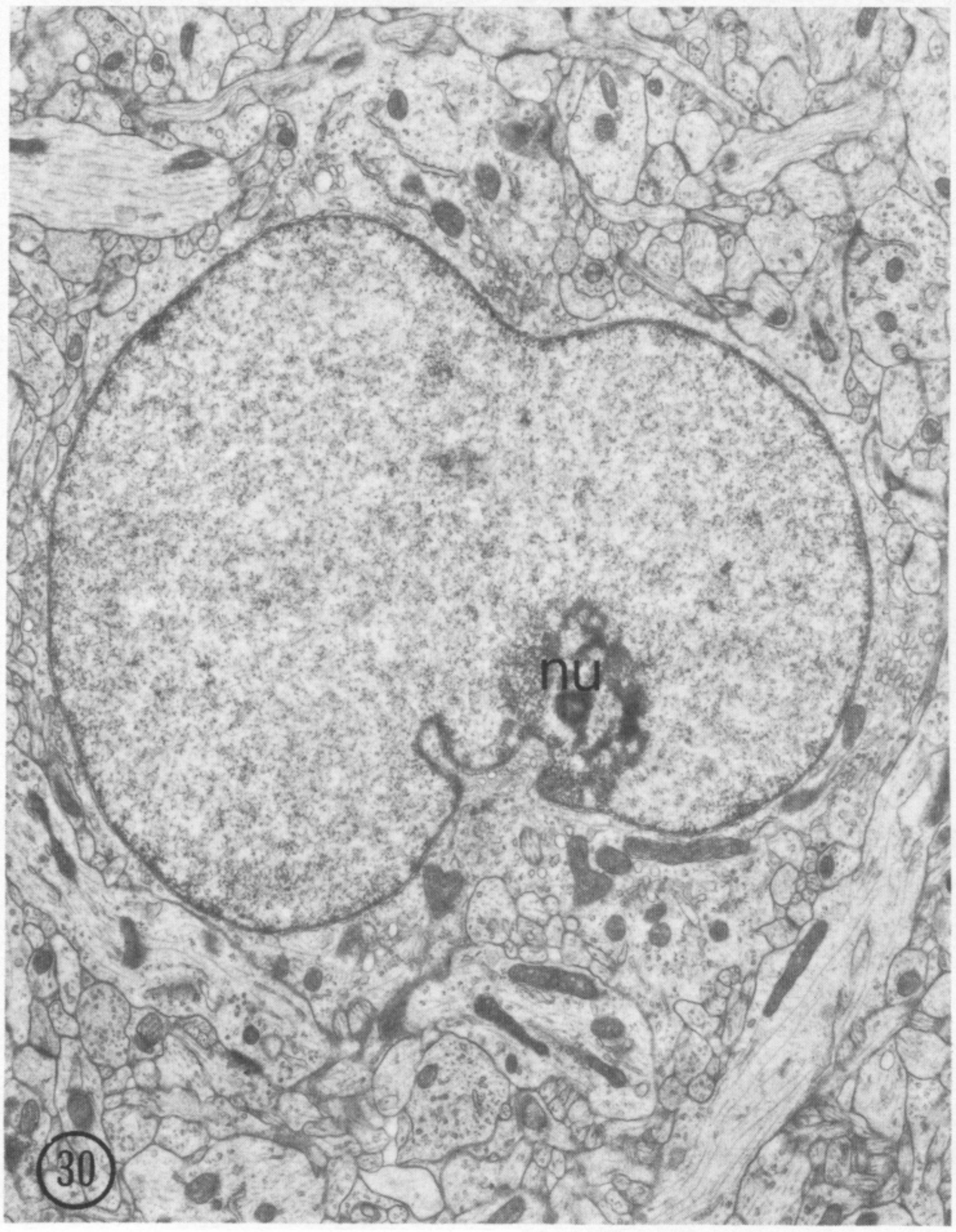
FIGURES 17–24. For description see page 66.

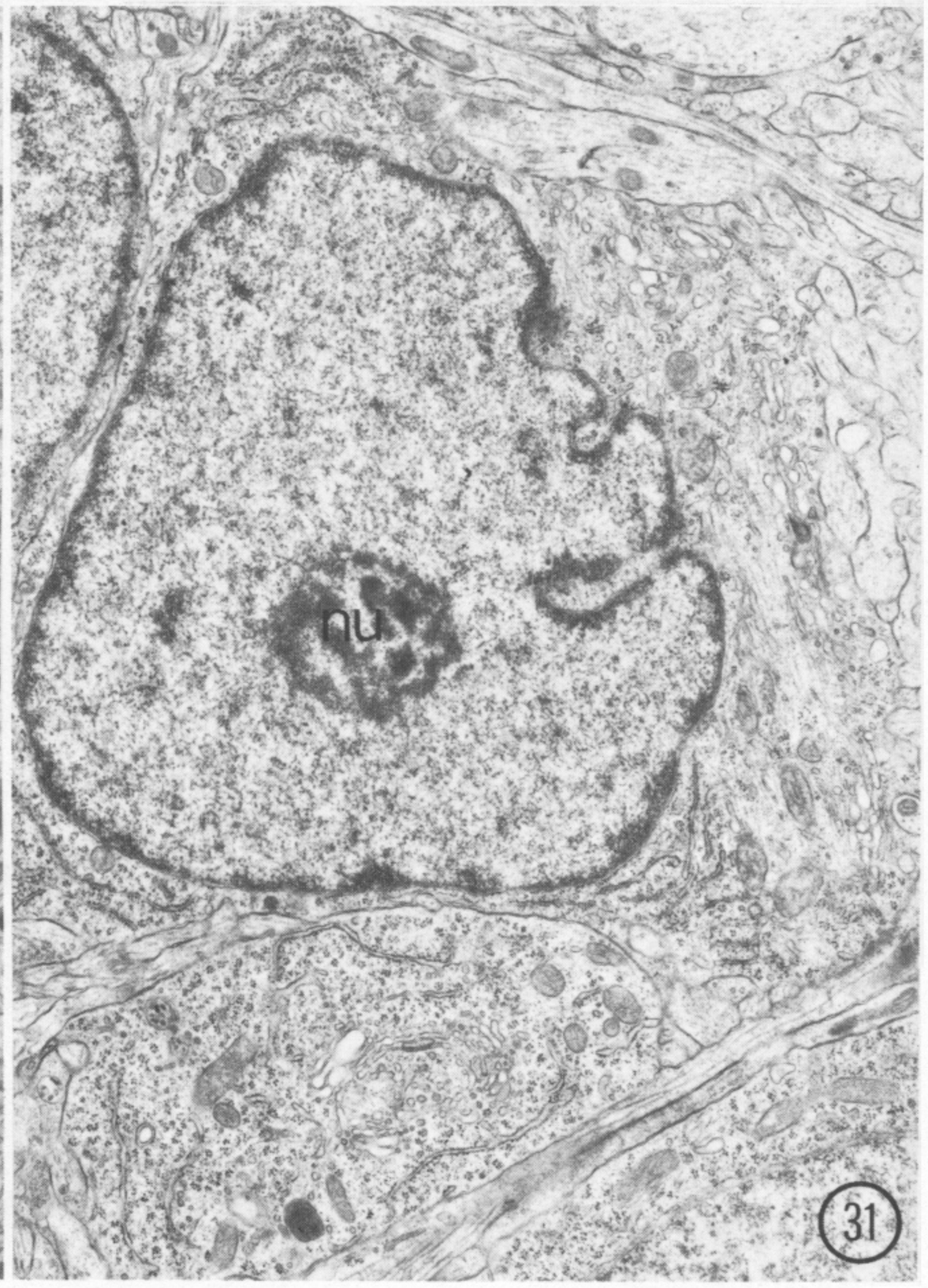


FIGURES 25–27. For description see page 66.

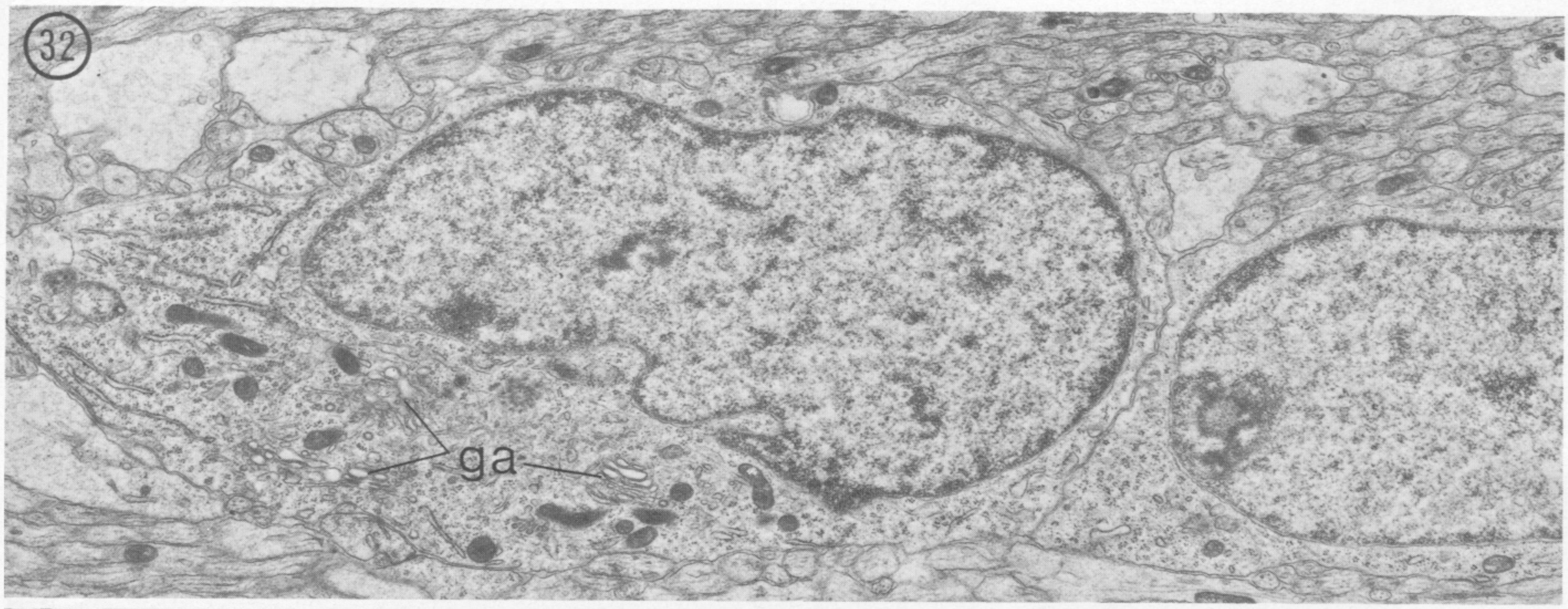


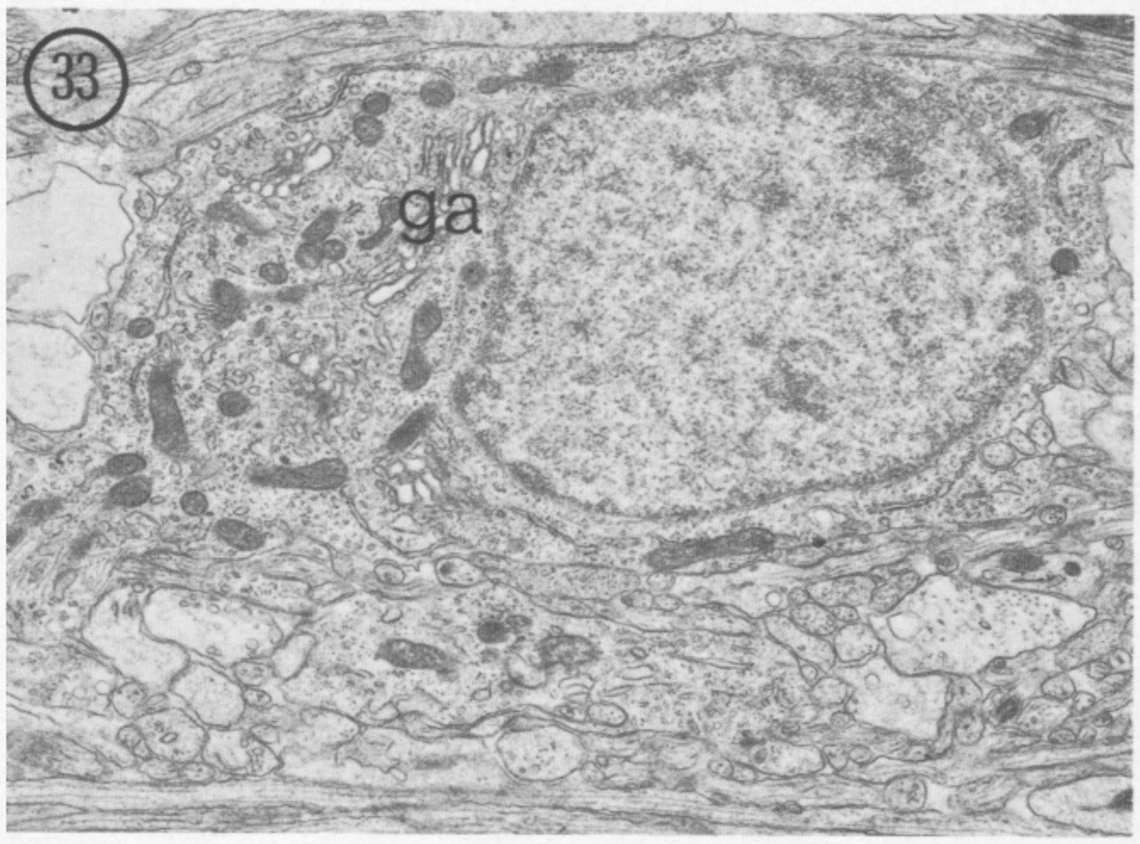


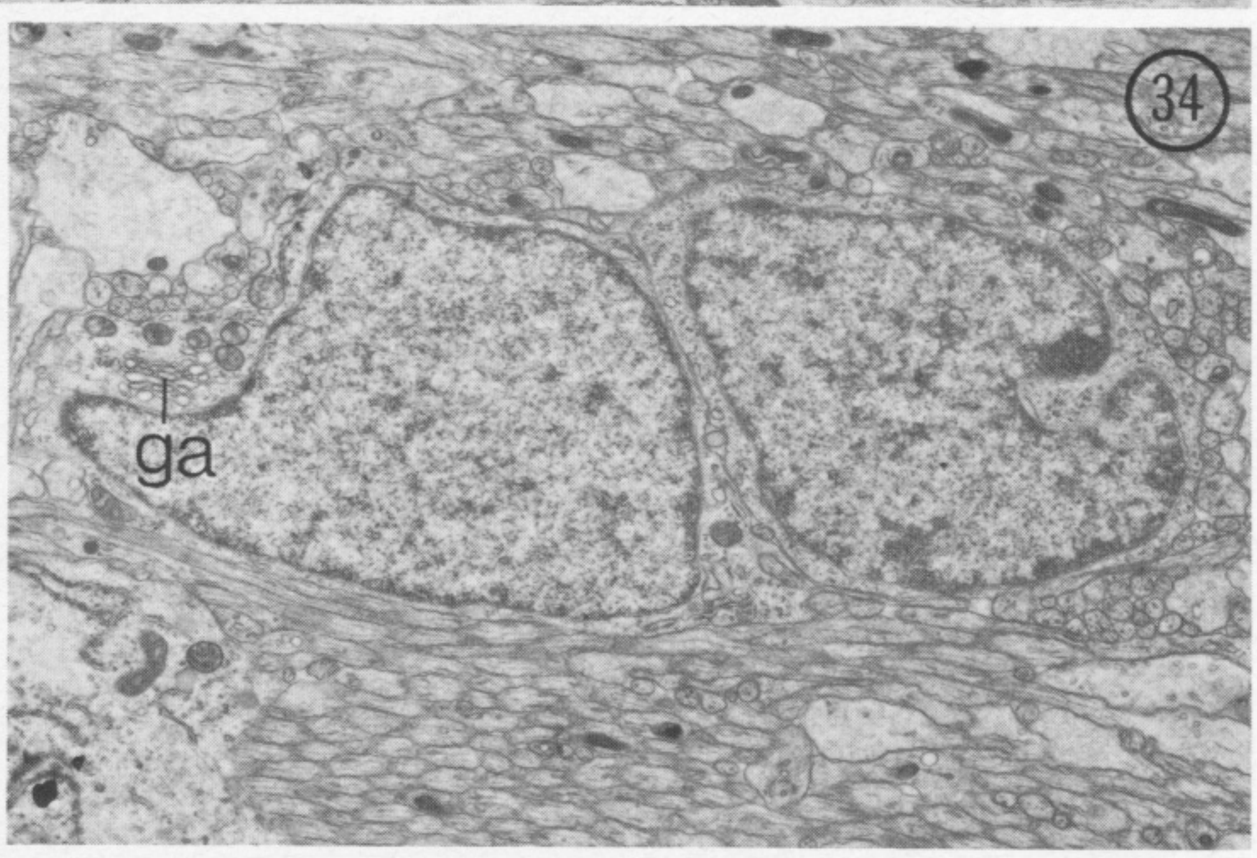


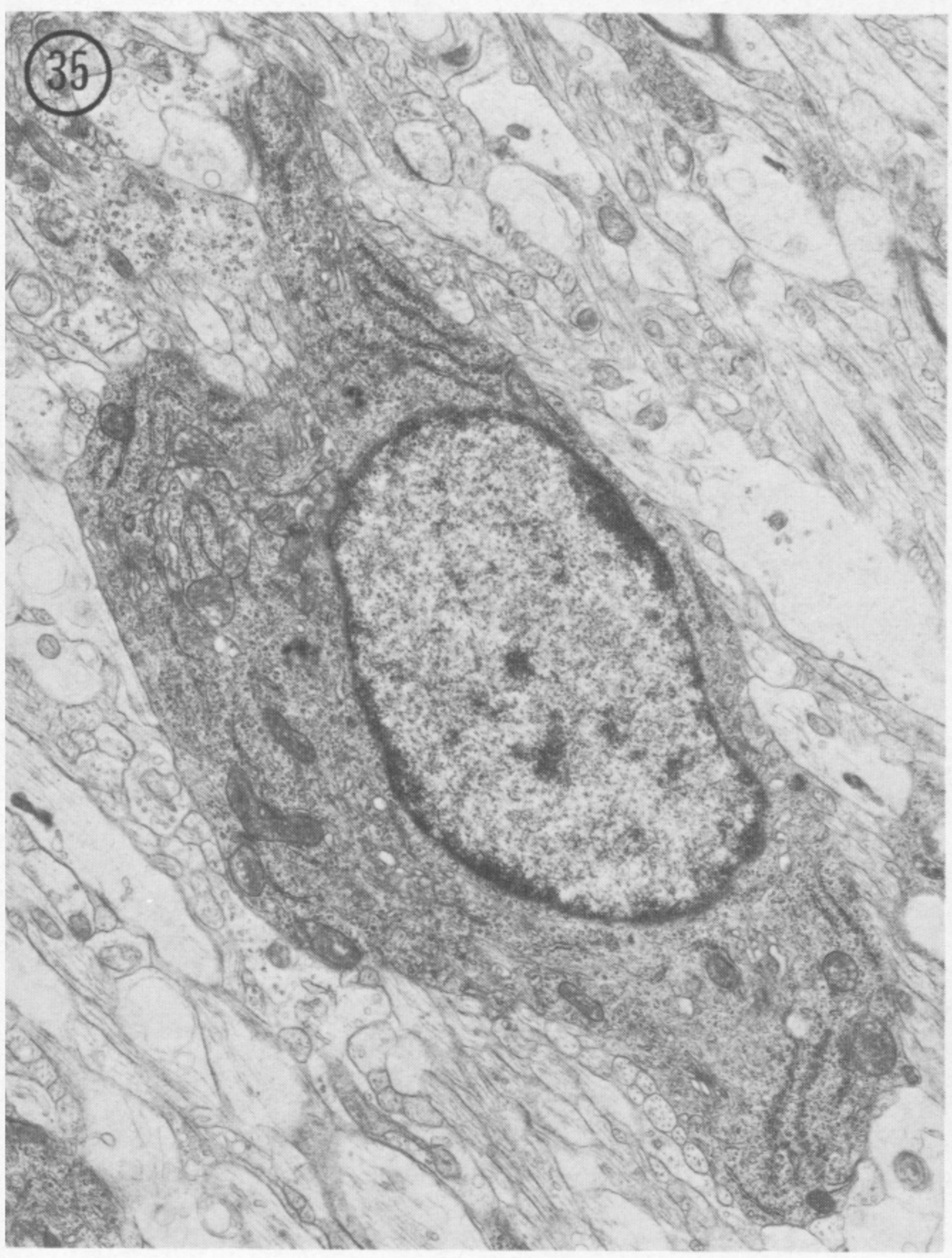


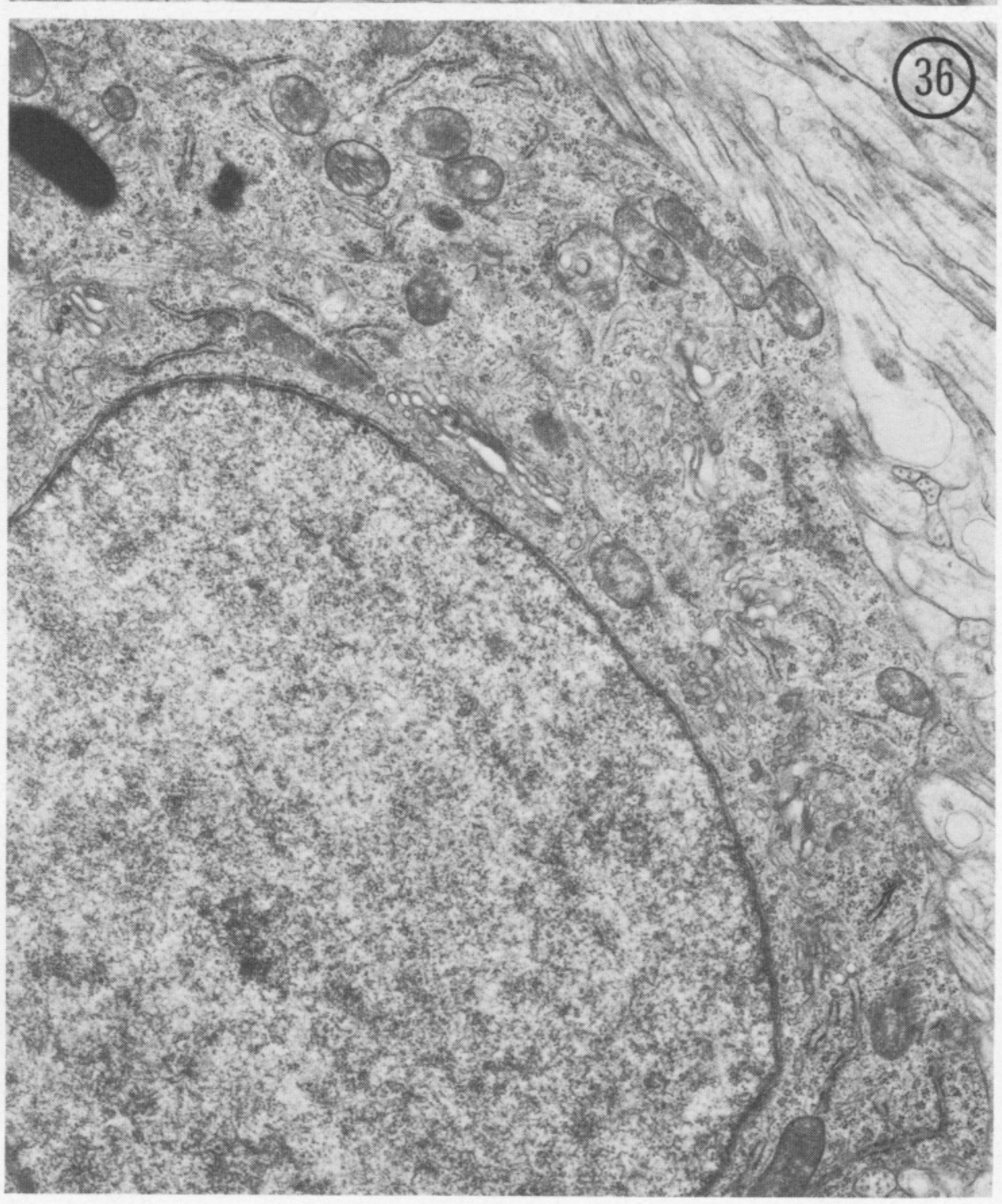
FIGURES 28–31. For description see facing plate 10.



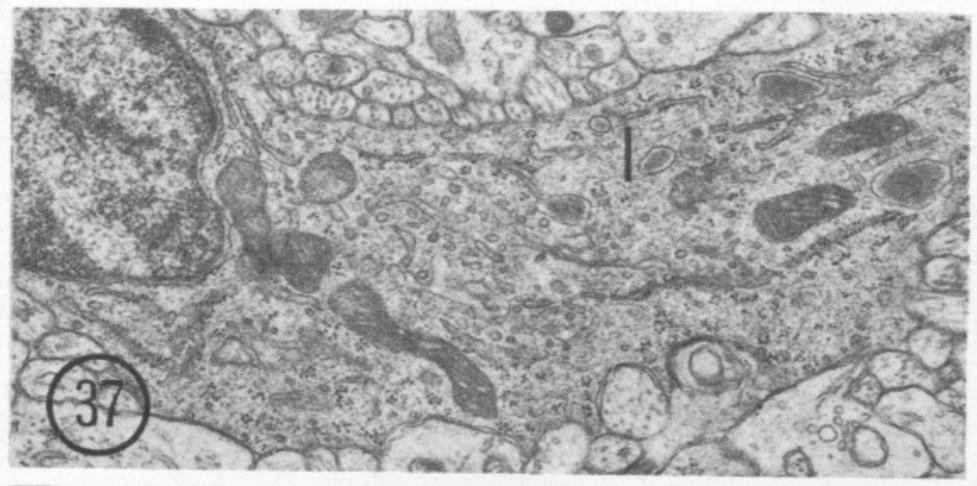


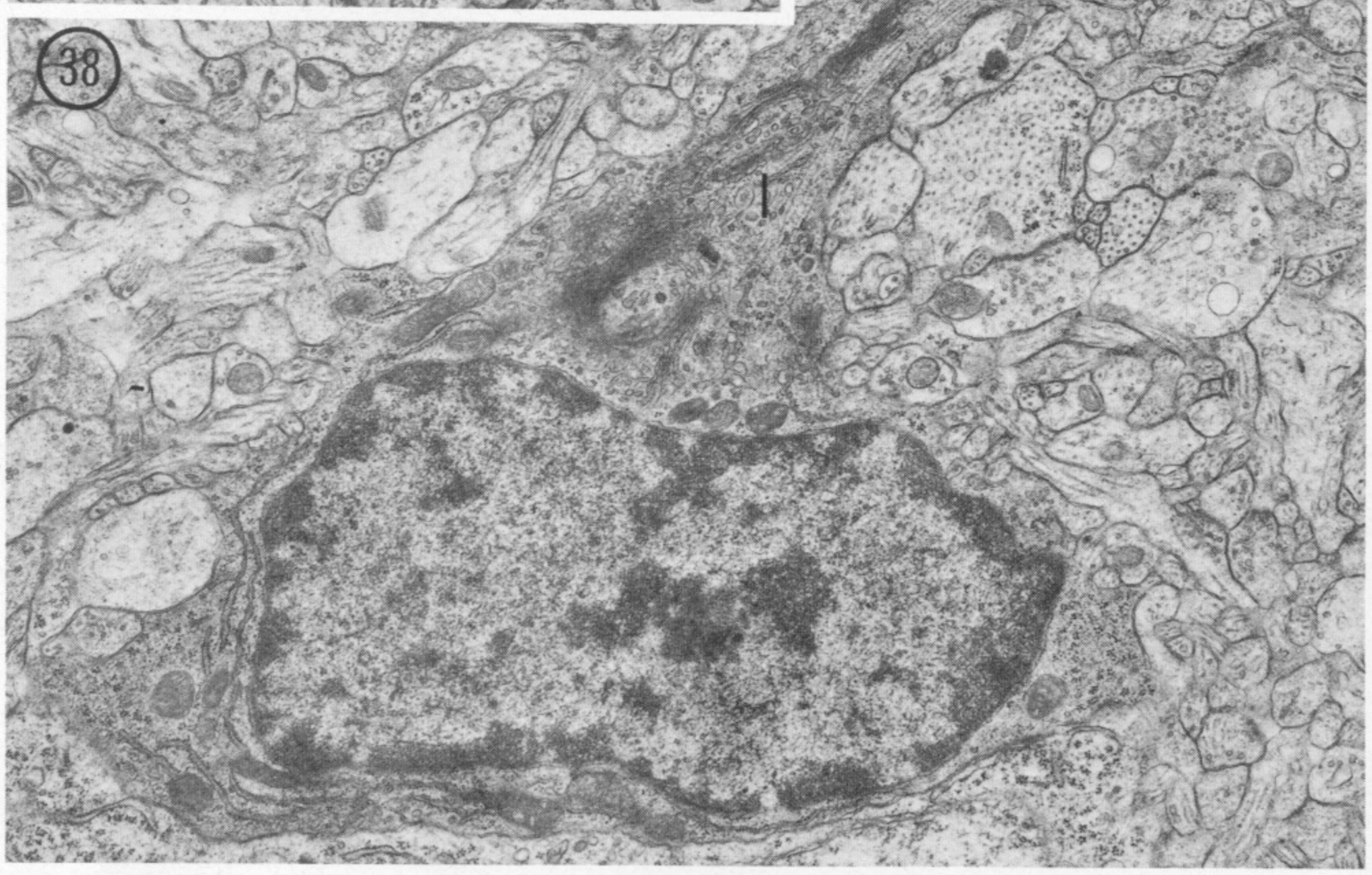


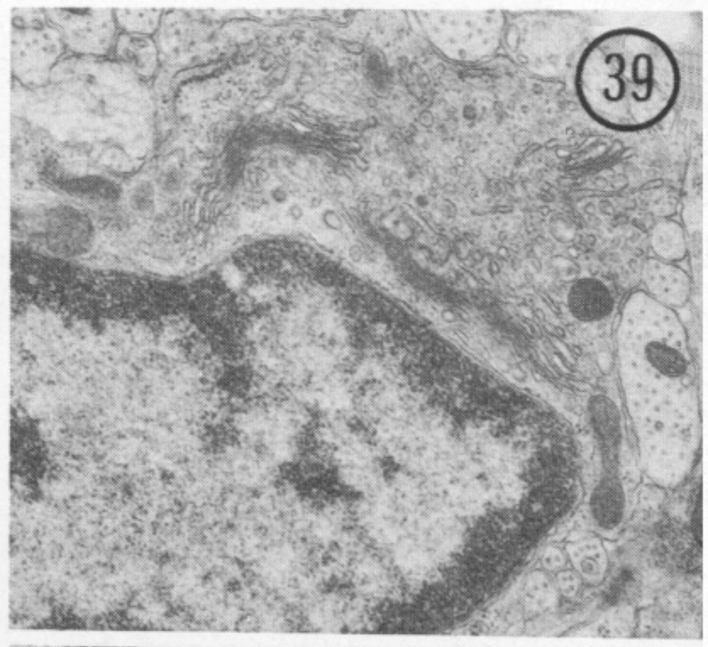


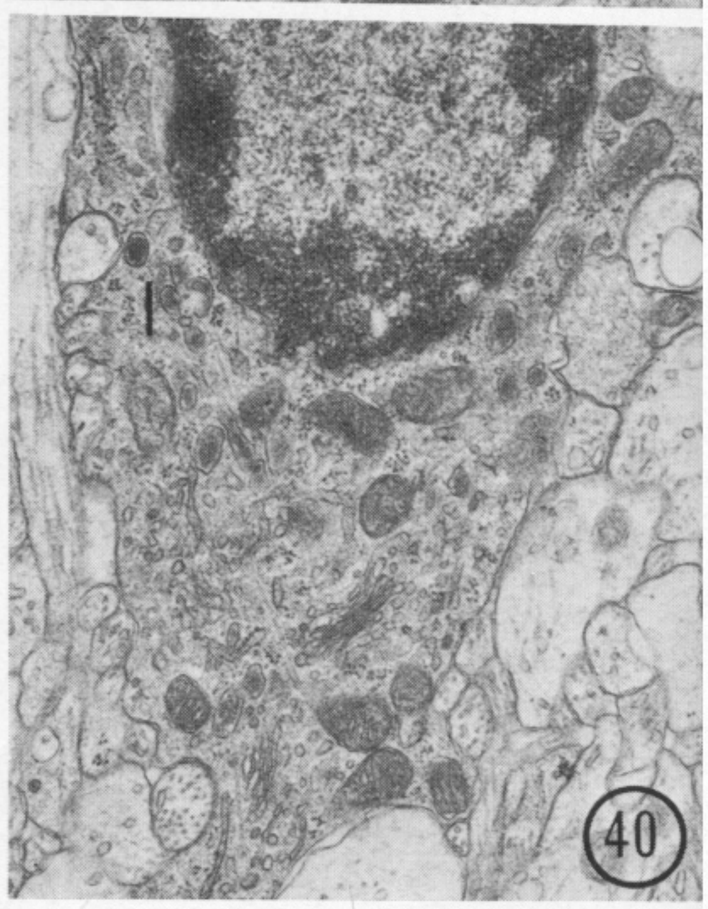


FIGURES 32–36. For description see facing plate 10.

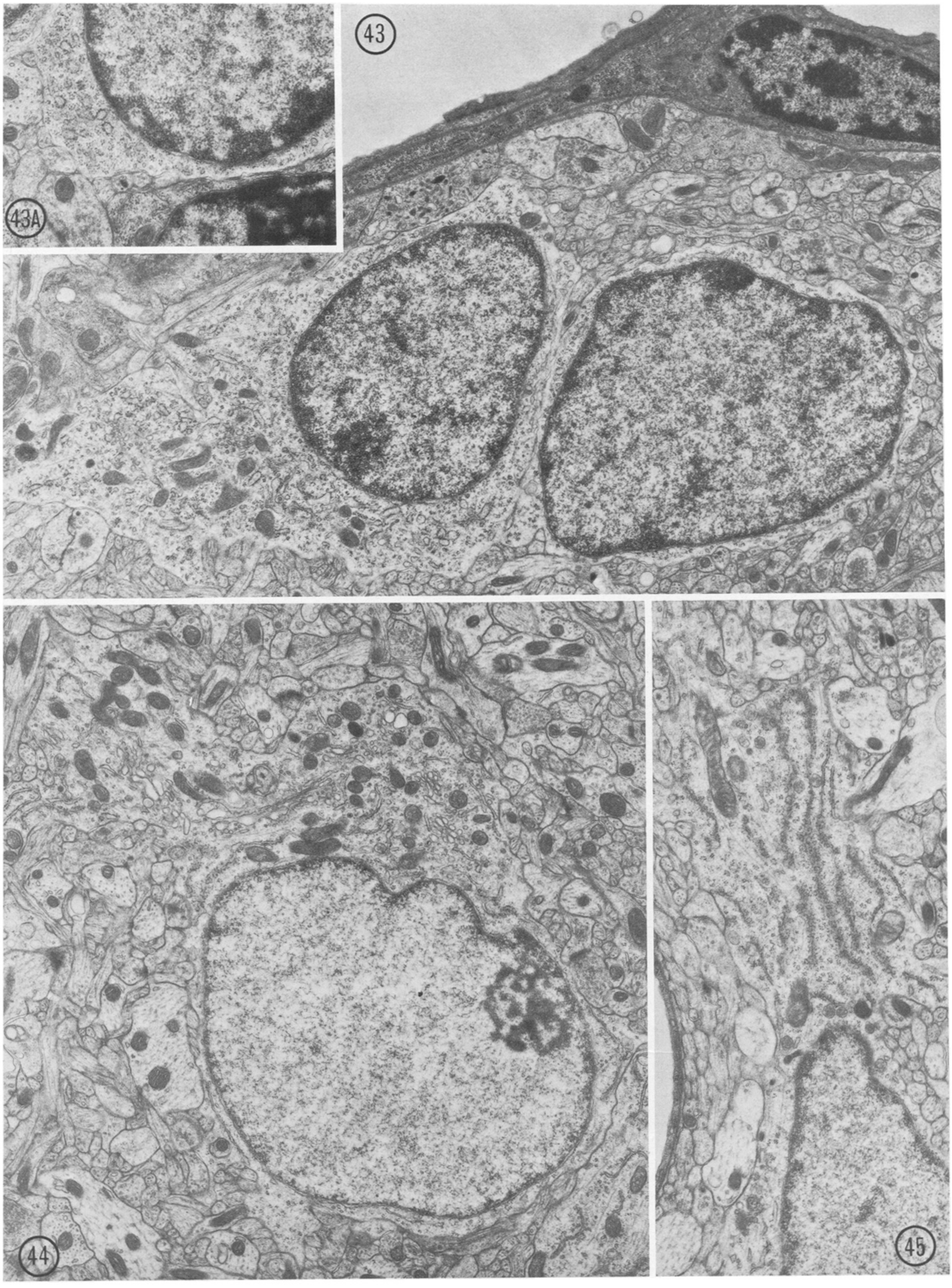




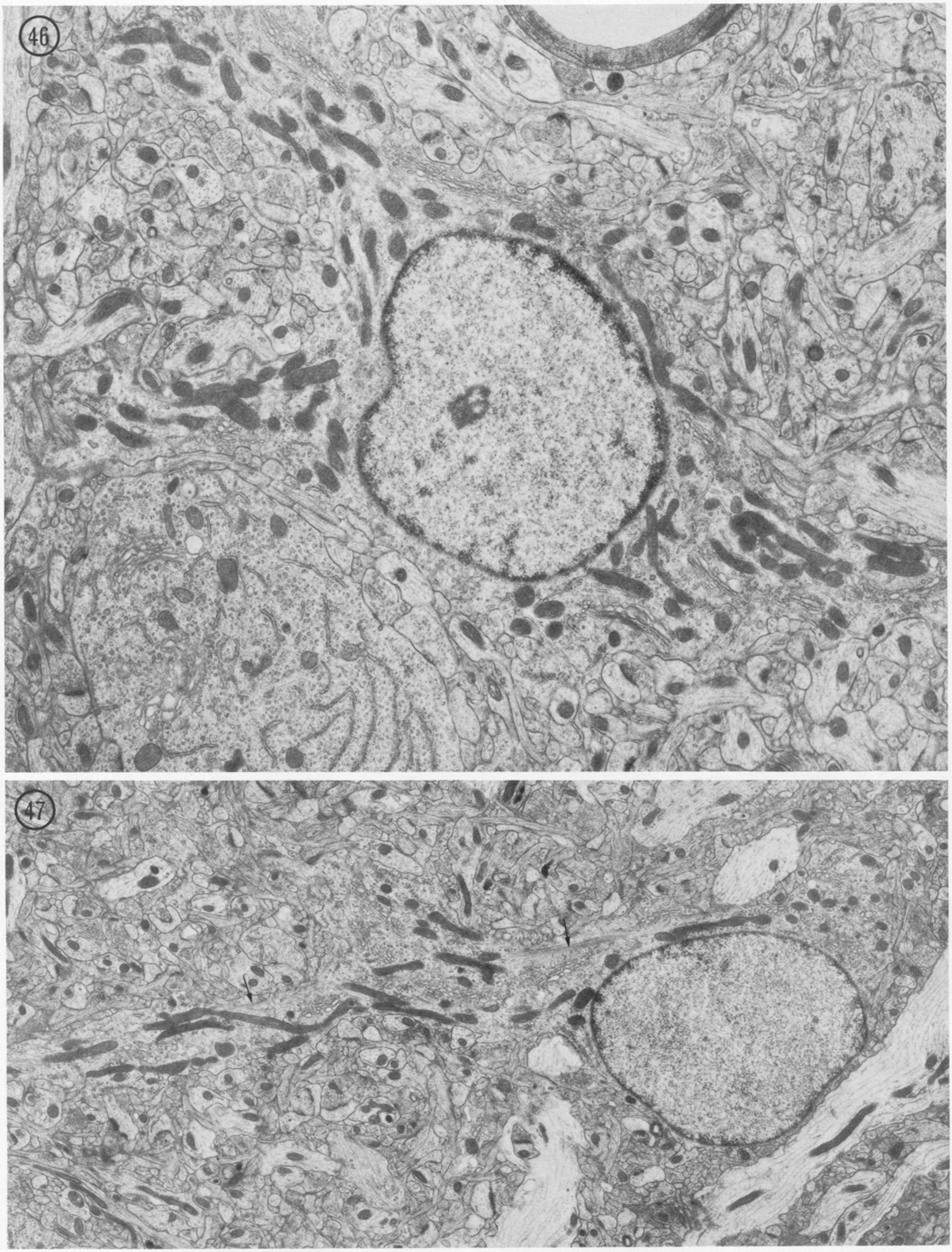




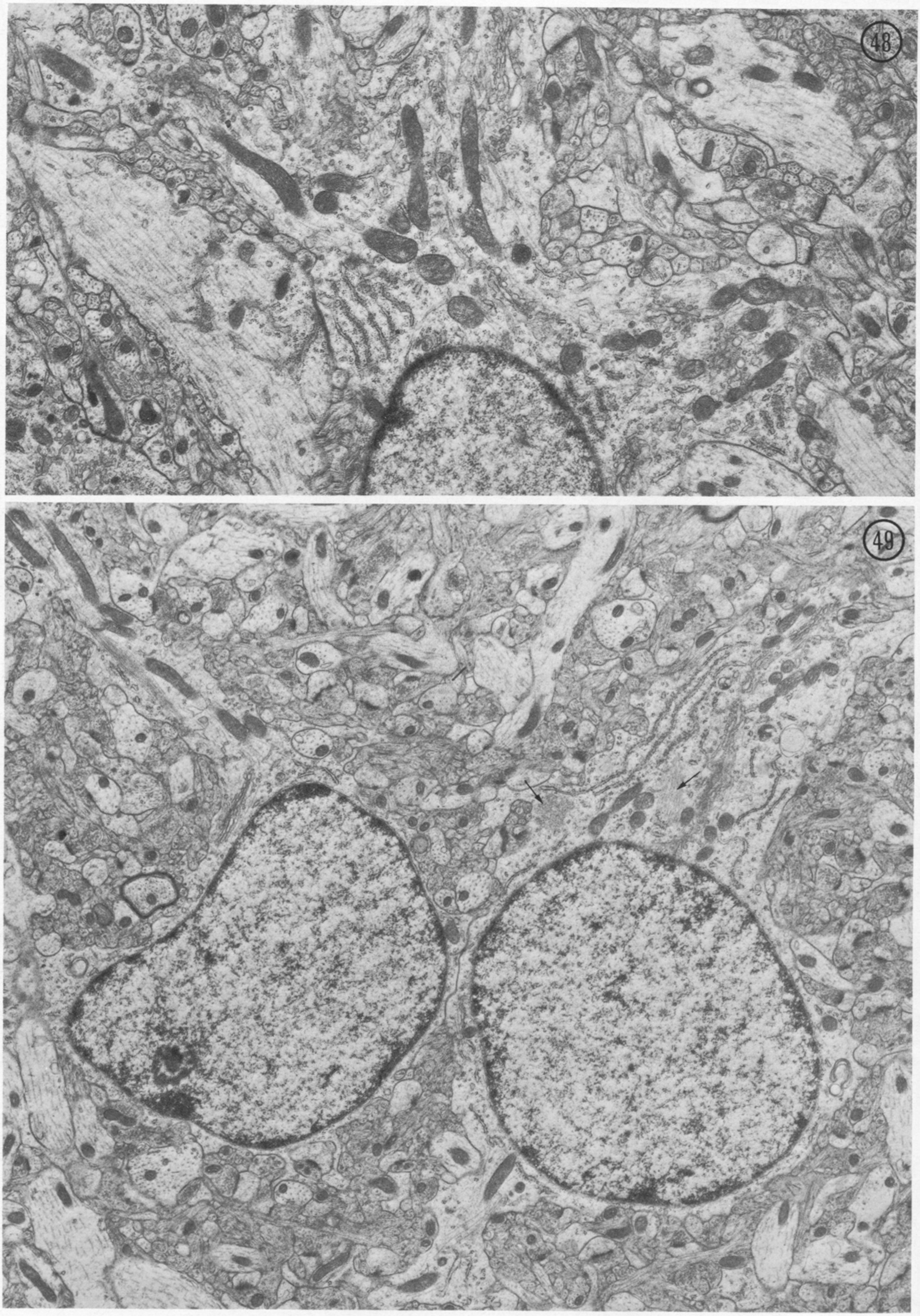
FIGURES 37–42. For description see opposite.



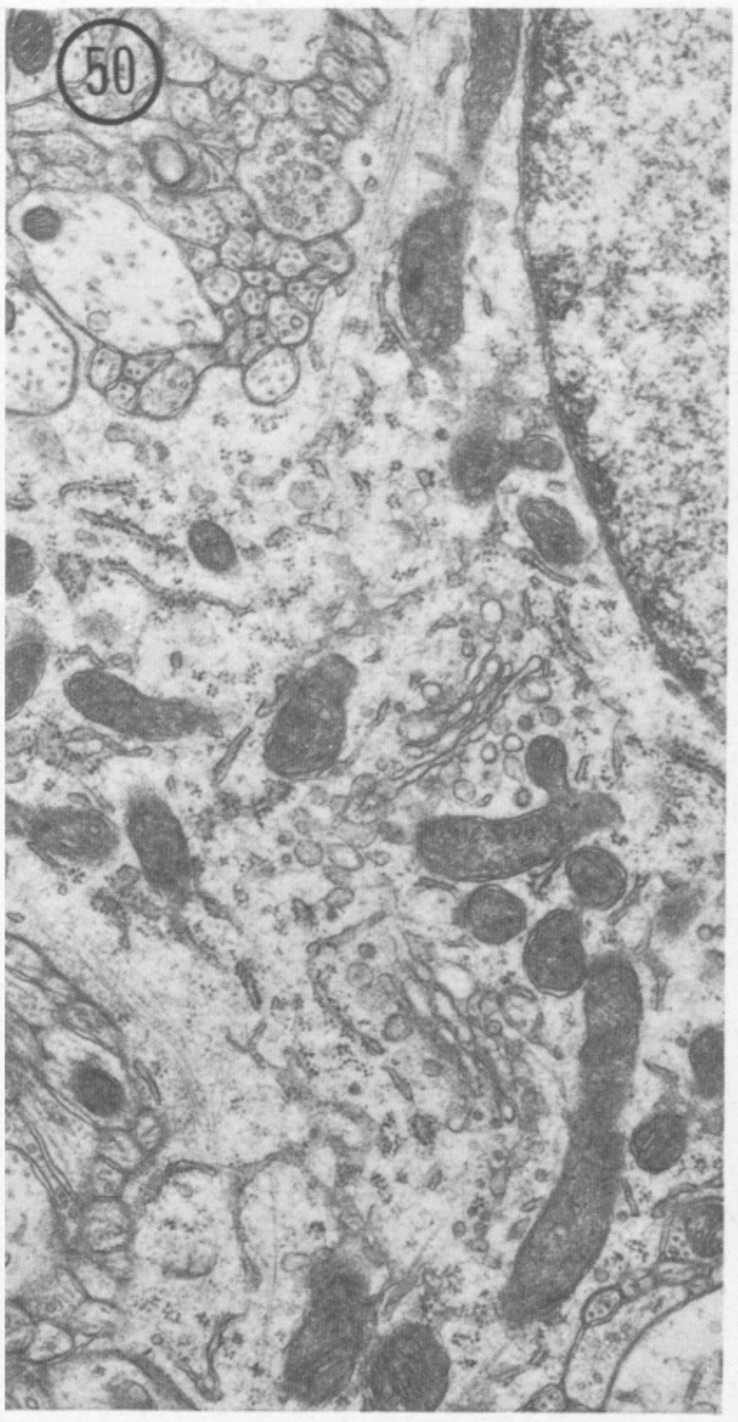
FIGURES 43–45. For description see facing plate 10.

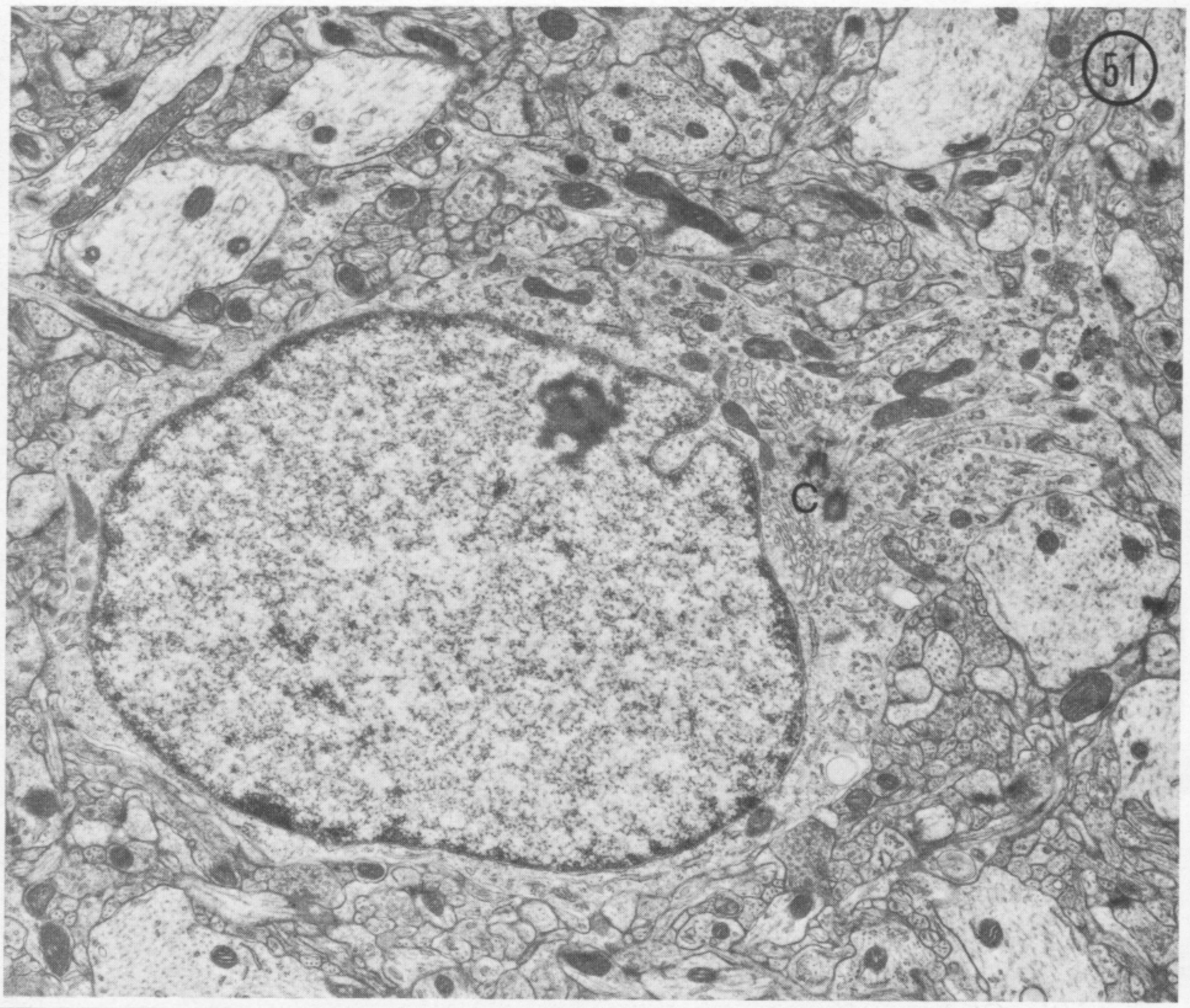


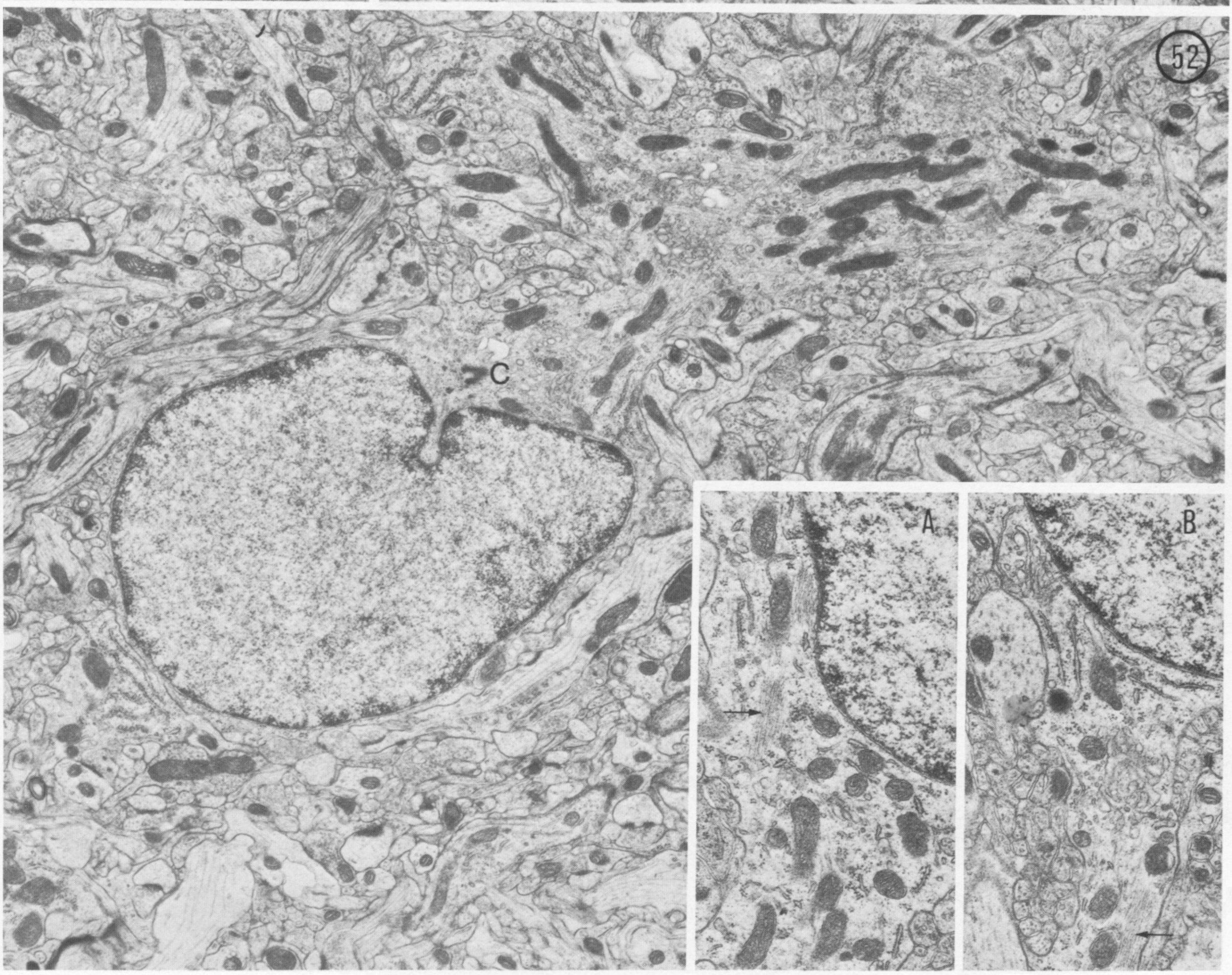
FIGURES 46 AND 47. For description see facing plate 10.



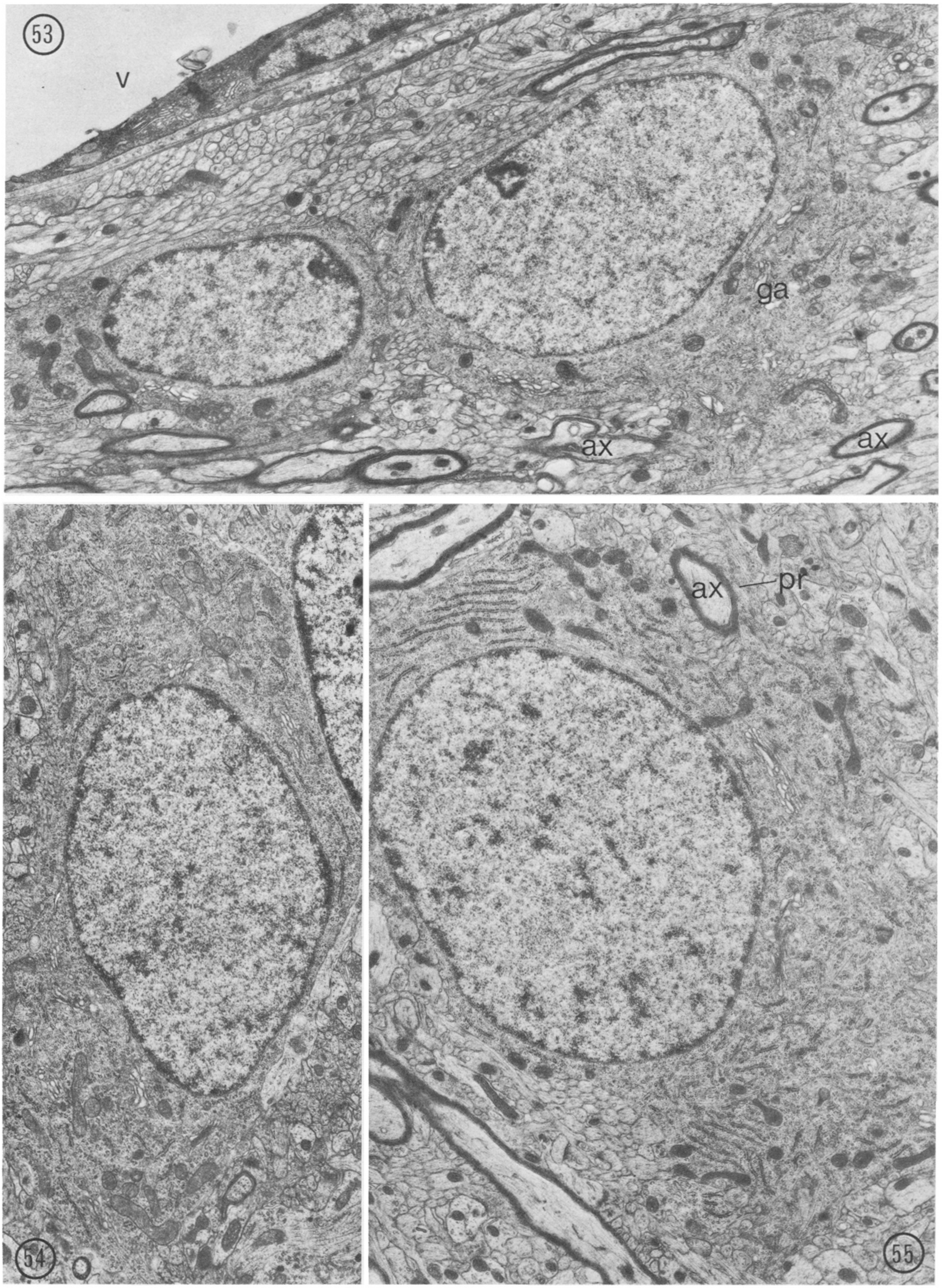
FIGURES 48 AND 49. For description see page 67.



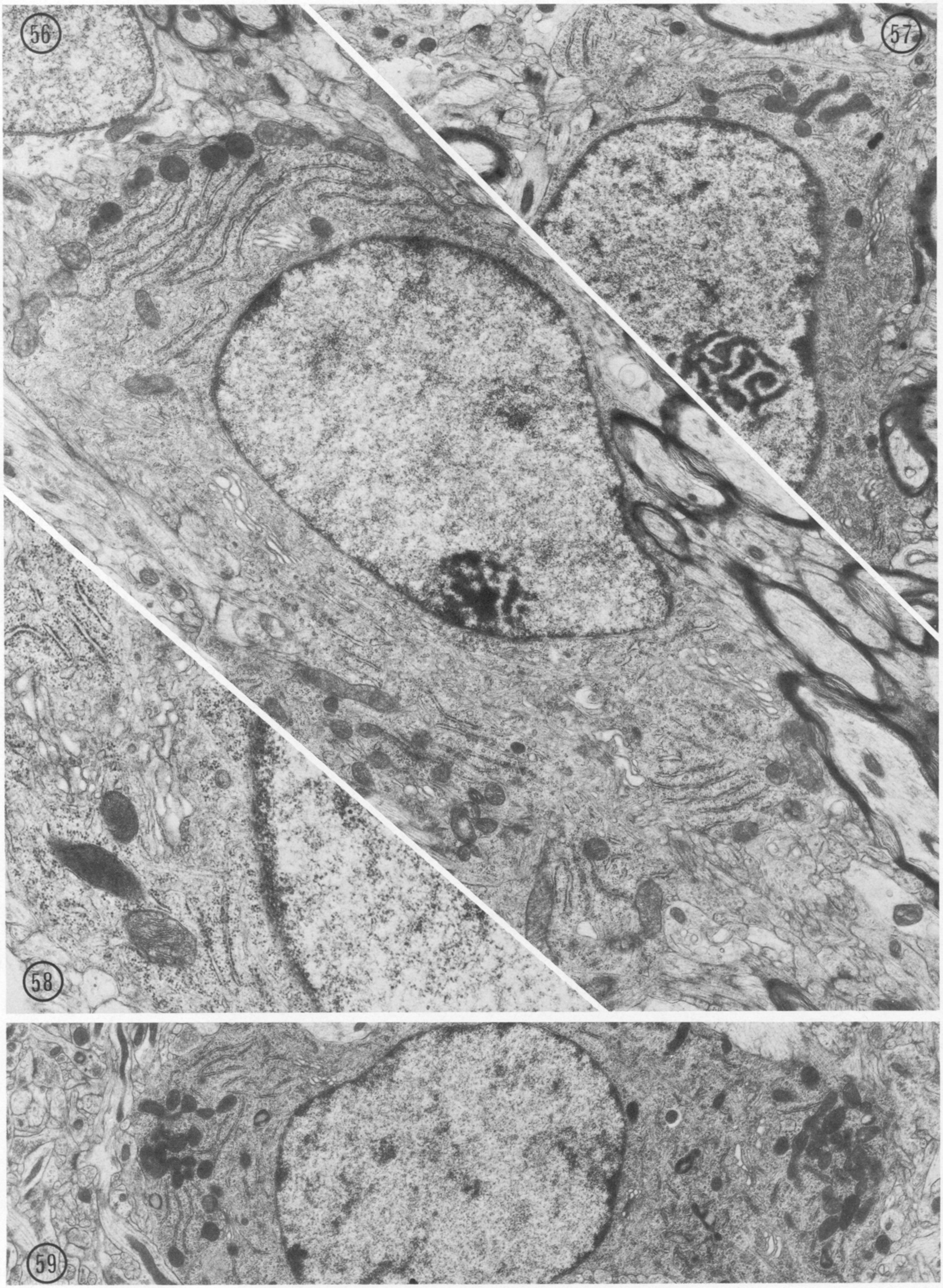




FIGURES 50–52. For description see page 67.



FIGURES 53–55. For description see opposite.



FIGURES 56–59. For description see opposite.

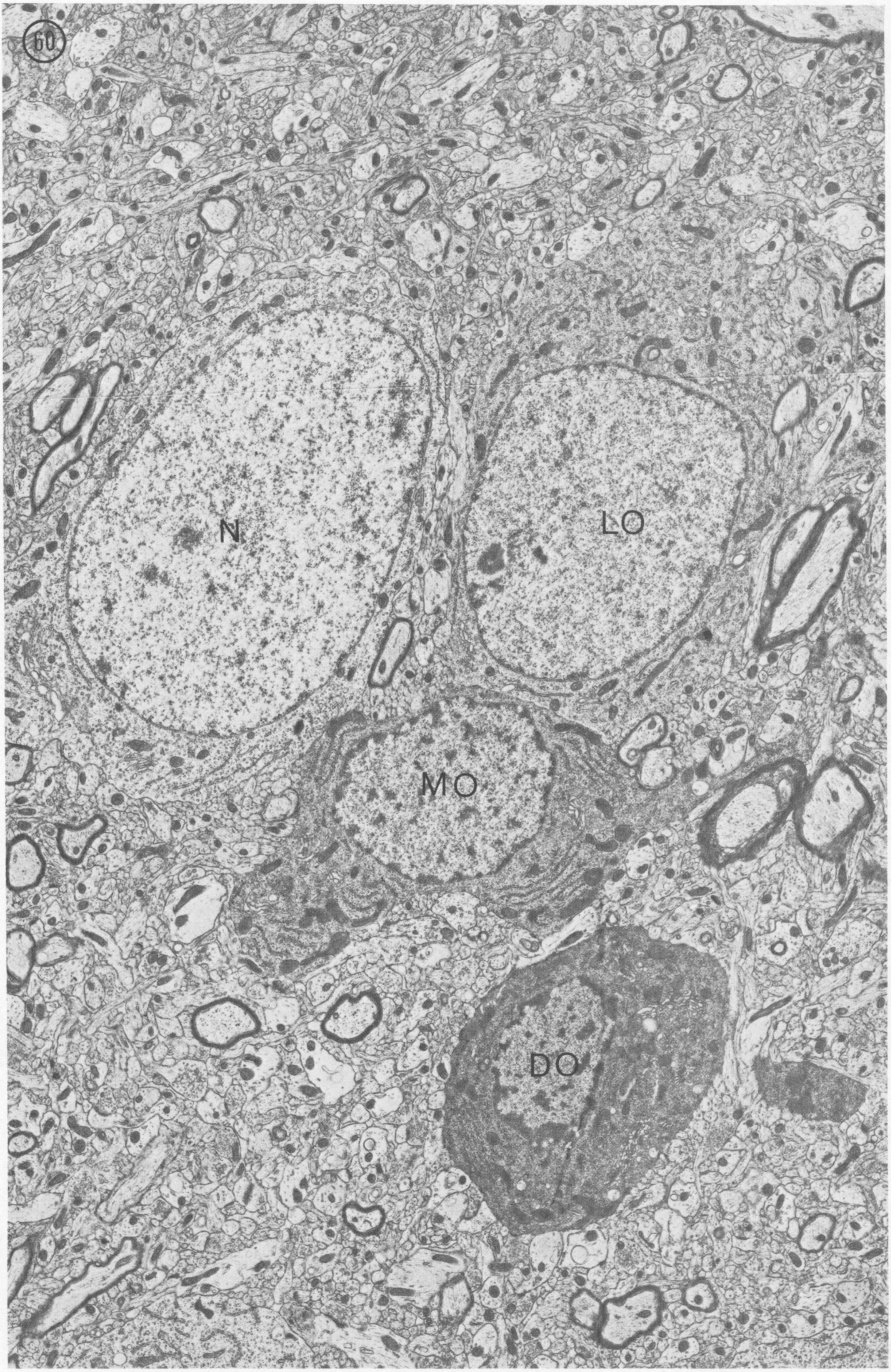
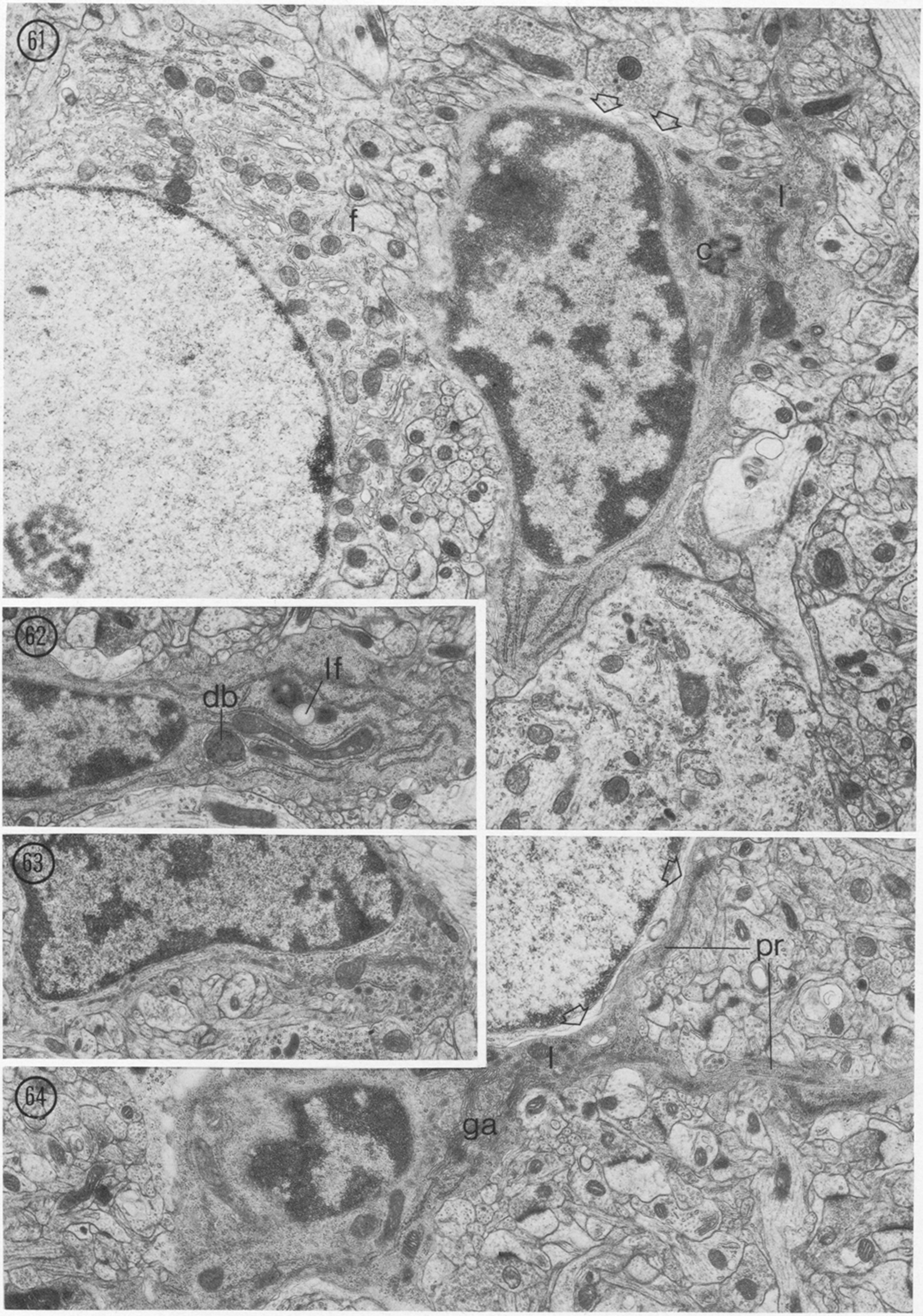


FIGURE 60. For description see opposite.



FIGURES 61–64. For description see opposite.