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Prenatal and Early Postnatal Development of the Glial Cells in the Median Eminence of the Rat

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Summary. The development of the glial cells of the rat median eminence (ME), including the supraependymal cells, was investigated from embryonic day (ED) 14 through postnatal day (PD) 7, and pituicyte development from ED 12 through ED 17. The *anlage* of the ME and neurohypophysis shows a neuroepithelial-like structure at ED 12. From ED 13 to 15, the cells of both regions start to differentiate. At the ultrastructural level, only one cell type appears. At the beginning of ED 16, glioblasts of the oligodendrocyte and astrocyte series migrate laterally (from the region of the arcuate nucleus) into the ME. Also at this time the first distinctive structural features appear in the neurohypophysial *anlage,* the cells of which later develop into pituicytes. Starting at ED 18, tanycytes and astrocytic tanycytes arise in the ME from local glial cells, and somewhat later oligodendroblasts and astroblasts are formed from immigrant glioblasts. Due to their common features, the pituicytes, tanycytes and astrocytic tanycytes apparently represent different forms of the same parent cell type. Microglial and supraependymal cells are first seen at ED 12. Initially, they resemble the prenatal phagocytic connective tissue cells and mature in the fetus into typical electron-dense microglia and macrophage-like supraependymal cells. Both cell types are apparently of mesodermal origin. The microglial elements of the ME probably migrate from the mesenchyma through the basement into the nervous tissue. The intraventricular macrophages of the infundibular region may originate from microglia, epiplexal cells and subarachnoid macrophages.

Key words: Eminentia mediana – Neurohypophysis – Development – Pituicytes – Tanycytes – Astrocytic tanycytes – Oligodendrocytes – Astrocytes – Microglia.

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* Dedicated to Prof. I. Törö, Budapest, on the occasion of his 80th birthday

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The ultrastructure of the nerve fibers and neuroglia of the median eminence (ME) in normal adult animals and animals subjected to experimental stress has been investigated in numerous studies (Wittkowski 1968, 1972, 1974; Kobayashi et al. 1968; Daikoku et al. 1971; Monroe and Paull 1974; Léránth and Schiebler 1975; Záborszky and Schiebler 1978). By contrast, only few studies have been devoted to the development of glial cells in the ME (Monroe and Paull 1974; Bitsch and Schiebler 1979; Ugrumov et al. 1979). Little is known concerning the origin of the different types of glial cells in the ME, such as the tanycytes, astrocytic tanycytes, astrocytes, oligodendrocytes and microglia, as well as the pituicytes. The present study is focussed on the development of these cells in the median eminence of the rat. It is known that all the glial cell types in the ME and the pituicytes are already present immediately after birth (Galabov and Schiebler 1978a, b; Bitsch and Schiebler 1979). However, the differentiation of the individual glial cell types has progressed so far by that time that postnatal studies deliver very little information about their developmental history. For this reason we have investigated the development of the ME and neurohypophysis starting from embryonic day (ED) 12. Our object is to shed further light on the varying behavior of the different glial cell types of the ME and the pituicytes under conditions of experimental stress. Léránth and Schiebler (1975), for example, observed in rats subjected to adrenalectomy, castration and thirst that the tanycytes, astrocytic tanycytes and pituicytes show a similar response, while the other glial cell types show little change. In the present study attention will be focussed also on the supraependymal cells of the infundibular region, which have been previously described by Coates (1973), Bleier et al. (1975) and Mestres and Breipohl (1976).

Materials and Methods

The ME and adjacent portions of the ventricular walls were investigated in 175 Wistar rats from ED 12 to postnatal day (PD) 7; as well the development of the neurohypophysis was followed from ED 12 to ED 17. The rats used in the present study were of both sexes and were taken from colonies.

Determination of Gestational Period. The male and female animals were caged in pairs overnight (17:00 to 08:00). If spermatozoa could be detected in the vagina of the female on the following morning, this was taken to mean that conception had occurred; this day was considered as ED 1 for computational purposes. The average gestational period was 22 days.

Fixation. The animals were killed by decapitation between 09.00 and 10.00 of the specified ED or PD (between 14.30 and 16.00 on ED 12; mother under ether anesthesia). In animals between ED 12 and ED 15, the skull cap and parts of the brain were removed to expose the third ventricle, then the head was immersion-fixed in toto; in older animals the brain was dissected free, trimmed and placed into the fixative. The brains of animals from ED 12 to ED 20 were fixed in 2% glutaraldehyde plus 0.25% formaldehyde (in 0.05–0.09 M phosphate buffer, pH 7.4), and those of animals from ED 21 to ED 22 and of postnatal animals in 2% glutaraldehyde plus 0.5% formaldehyde (in 0.1 M phosphate buffer), at +4° C for about 30 min, followed by dissection of the ME and additional fixation for 2.5 to 3 h. The specimens were then rinsed in phosphate buffer (see above) for 2 h, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Durcupan®.

Semi- and Ultrathin Sections. For each ED and PD, at least one series of semithin sections (frontal sections, caudal-to-rostral series) was prepared and stained with 1% toluidine blue borax. The semithin

series was used for orientation and light-microscopic examination. Further blocks were cut for semithin sections and then ultrathin sections using a Reichert U3 microtome. The sections were stained with lead citrate and uranyl acetate and viewed on a Zeiss EM-9A electron microscope.

Results

Embryonic Day 12. On this day the walls and floor of the third ventricle consist of neuroepithelium (cf. Langman 1968). The investigated regions are free of vessels, show marked intercellular spaces and are separated from the mesenchyma surrounding the developing brain by a basement membrane.

At the beginning of ED 12, the region from which the ME and neurohypophysis arise can be discerned on the ventricular floor. It is located at the point where Rathke's pouch touches the base of the brain (Fig. 1a). The simple neuroepithelium of this region is somewhat thicker laterally than medially. Near the ventricle, mitoses are found. The neuroepithelial cells are tall. They abut broadly on the basement membrane, which is deeply folded in the region of the ME and neurohypophysial *anlage,* and extend to the ventricular lumen. The neuroepithelial cells contain numerous free ribosomes and polyribosomes, a few mitochondria, microtubules (singly or in bundles) and small, light lipid bodies (LB), as well as a few cilia at the ventricular surface. The Golgi complex is small. The few cisternae of the rough endoplasmic reticulum (RER) are irregularly distributed; some

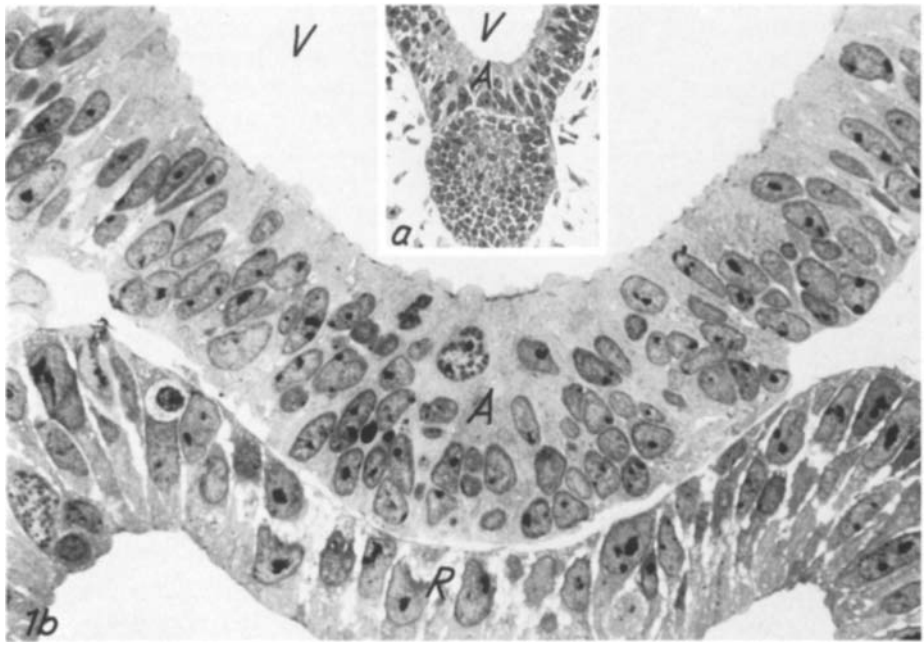

Fig. 1a and b. Survey micrograph of the anlage of the ME. Semithin sections. Toluidine blue borax. **a** Neuroepithelium in the region of the ME-anlage, ED 12. ×180. **b** Incipient differentiation of the ME-anlage, ED 13. *V* third ventricle; *A* ME-anlage; *R* Rathke's pouch. ×700

membrane portions are devoid of ribosomes. More organelles are present in the apices of the neuroepithelial cells than at the bases. In the basal regions autophagosomes are present with a light matrix and an electron-dense, flocculent or membranous internal structure, as well as ribosome-free, filament-rich areas; the latter are also found near the basement membrane in glial cells of the ME and neurohypophysis during later stages of development. The elongated nuclei of the neuroepithelial cells lie at a distance from the ventricle and contain finely flocculent, evenly distributed chromatin as well as one or two nucleoli.

In the lateral walls of the ventricle, the neuroepithelial cells lack the autophagosomes and ribosome-free, filament-rich areas. In other respects their fine structure is identical to that of the neuroepithelium of the ventricular floor. The basement membrane of the lateral walls follows a linear course.

Except for a few collagen fibers, no structures are found between Rathke's pouch and the floor of the third ventricle.

Embryonic Day 13–15. Cell multiplication is unsymmetrical during this phase, resulting in marked differences between the *anlage* of the ME and neurohypophysis. The cells of these two regions have a similar fine structure, but the difference between the neuroepithelium of the ventricular floor and the ventricular lateral walls becomes more pronounced.

The neuroepithelium of the ME forms multiple rows with no significant increase in thickness, as new cells form basally that have no contact with the ventricle and the long axes of which frequently are parallel to the ventricular surface (Fig. 1b). Some cells in the *anlage* of the ME still extend from the ventricular surface to the basement membrane. The basal part of these cells consists of a slender, unbranched process. Degenerated cells are also occasionally seen in the ME. The *anlage* of the neurohypophysis grows markedly in a ventral direction, and a recess appears in this structure on ED 14.

In all cells of the ME- and neurohypophysial *anlage* free ribosomes decrease and all other cell organelles increase in number. The cell region abutting on the ventricular lumen maintains its relatively higher concentration of organelles (Fig. 2). The basal region is characterized primarily by an increase in the number of mitochondria, as well as by the presence of multivesicular bodies (MVB). Near the basement membrane the number of phagosomes greatly increases on ED 13 and 14. On ED 15 the ribosome-free areas become more extensive, while the nuclei become more spherical. Distinct peripheral chromatin aggregations are present, and the nuclear envelope is frequently folded. In the median region of the ventricular floor the nuclei are generally larger, and less electron dense, and have a more even chromatin distribution as well as fewer nucleoli than neighboring cell nuclei. Large intercellular spaces are no longer evident. Desmosome-like contacts are occasionally seen (cf. Galabov and Schiebler 1978a, b). Ventricular protrusions of the neuroepithelial cells contain vesicles.

The cells of the ventricular lateral walls adjoining the ME-anlage also form multiple rows, characterized by broad intercellular spaces. The neuroepithelial cells are slender and arranged in palisades. They contain a few organelles, mostly mitochondria, but also many ribosomes. The nuclei are small and dark compared with those of the ME- and neurohypophysial *anlage;* the nuclear chromatin is coarsely flocculent.

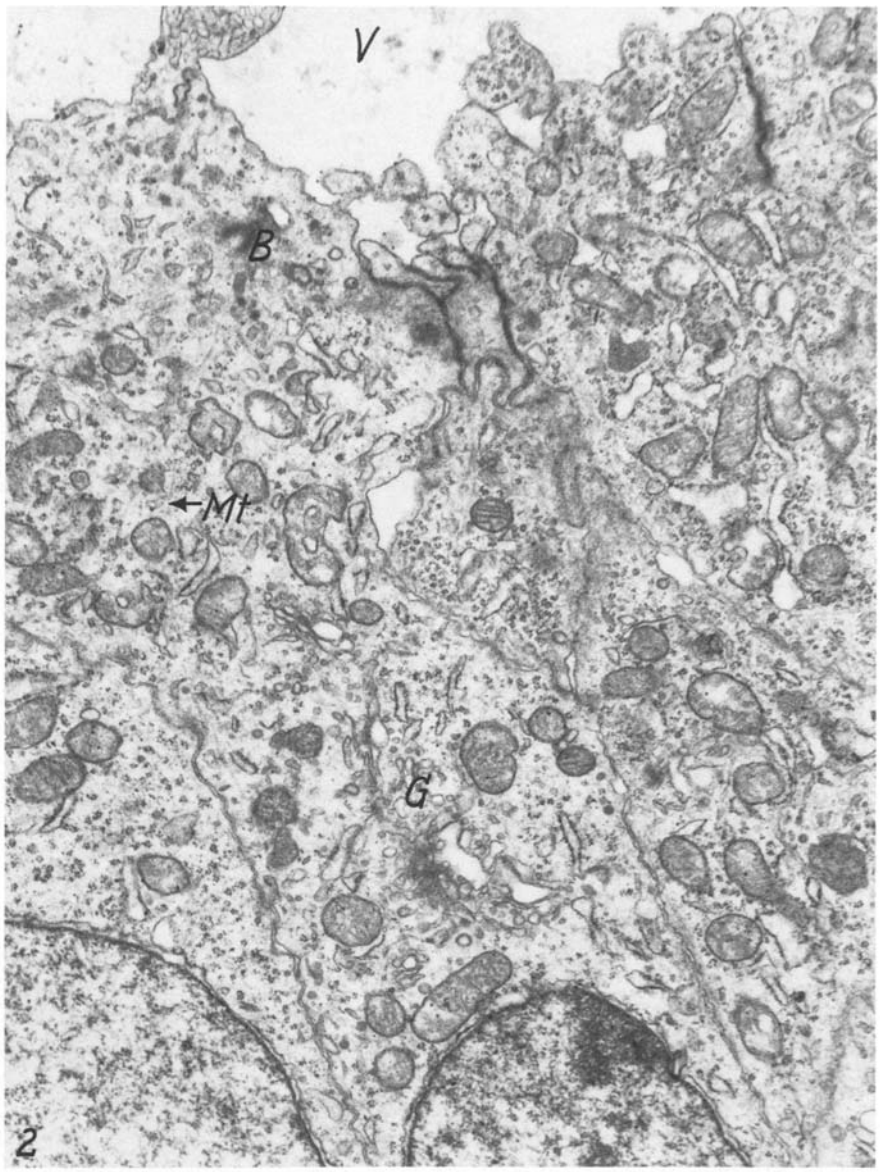

Fig. 2. Ependymal cells of the ME. ED 14. *V* ventricle; *B* basal body of a cilium; *Mt* microtubules; *G* Golgi complex. ×21,000

The first mesenchymal cells, which later develop into the mantle plexus, appear between the *anlage* of the adenohypophysis and ME on ED 14.

Embryonic Day 16 and 17. The differences between the *anlagen* of the ME and the neurohypophysis, on the one hand, and adjacent parts of the ventricular lateral walls, on the other, become increasingly pronounced and are now noticeable at the ultrastructural level as well.

During this time the ME is organized into three distinct layers: the ependyma, the subependyma and a relatively narrow acellular layer at the basement membrane. The latter consists of ependymal cell processes and individual nerve fibers of fiber bundles (cf. Monroe and Paull 1974); rostrally it occupies about one fourth of the dorso-ventral extent of the ME, while caudally it is only extremely thin. Medially, the ependyma and subependyma consist of cells similar in type, but laterally another cell type, presumably a precursor of the oligodendroblasts and astroblasts, occurs, which is also observed in adjacent portions of the lateral ventricular walls.

The ependymal cells of the ME are oriented perpendicular to the ventricular surface. Protrusions and isolated cilia project into the ventricle. Apically, the cytoplasm is rich in RER, mitochondria, microtubules, dictyosomes and MVB. Glycogen appears for the first time. There is a further decrease in the number of ribosomes. The nuclei occupy the upper third of the cells; they are ovoid, their chromatin is homogeneous except for a narrow peripheral condensed margin. The basal processes are slender, they contain a few organelles and extend in large numbers to the basement membrane. Phagosomes are less frequent than on ED 13 and 14. In the subependyma the cells are parallel or perpendicular to the ventricular floor. Large processes are rare. The fine structure of the subependymal cells, especially that of the nuclei, resembles the ependymal cells. The cytoplasm of the subependymal cells contains somewhat fewer organelles than ependymal cells.

The cells of the neurohypophysial *anlage* have electron-lucent spherical to ovoid nuclei. The nuclear envelope is rarely folded. With the exception of fewer ribosomes, the cytoplasm of the cells of the neurohypophysial *anlage* resembles that of the cells of the ME (Fig. 3). Between the *anlage* of the ME and the neural lobe exists a transition zone (*anlage* of the hypophysial stalk), which contains precursors of glial cells of both the ME (astrocytic tanycytes) and the neurohypophysis (pituicytes).

In the lateral parts of the ME and in the ventral portions of the lateral ventricular walls (later the arcuate nucleus), a cell type appears differing from the neuroblasts of the same region (Celey and Maxwell 1968a, b) and from the previously described cells of the ME and the microglia (see below). Judging from the further development of the arcuate nucleus, these cells apparently are precursors of oligodendroblasts and astroblasts. These cells have folded nuclei with dense chromatin and a narrow chromatin margin, numerous polyribosomes, as well as processes with microtubules, mitochondria, Golgi complexes and RER (Fig. 4). These cells usually form small clusters. From ED 18 to 19, they occur in the medial part of the ME and in the hypophysial stalk, decreasing in number caudally.

Embryonic Day 18 Through Postnatal Day 1. During this period the cells of the neurohypophysis develop into a single type, the pituicytes. This process has been described in detail by Galabov and Schiebler (1978a) and will thus not be discussed again.

The organization of the ME into different layers and the differentiation of the cells into tanycytes, astrocytic tanycytes, oligodendroblasts and astroblasts becomes increasingly pronounced during this period. Precursor cells of oligodendroblasts and astroblasts are also observed. Moreover, the "neuronal layer"

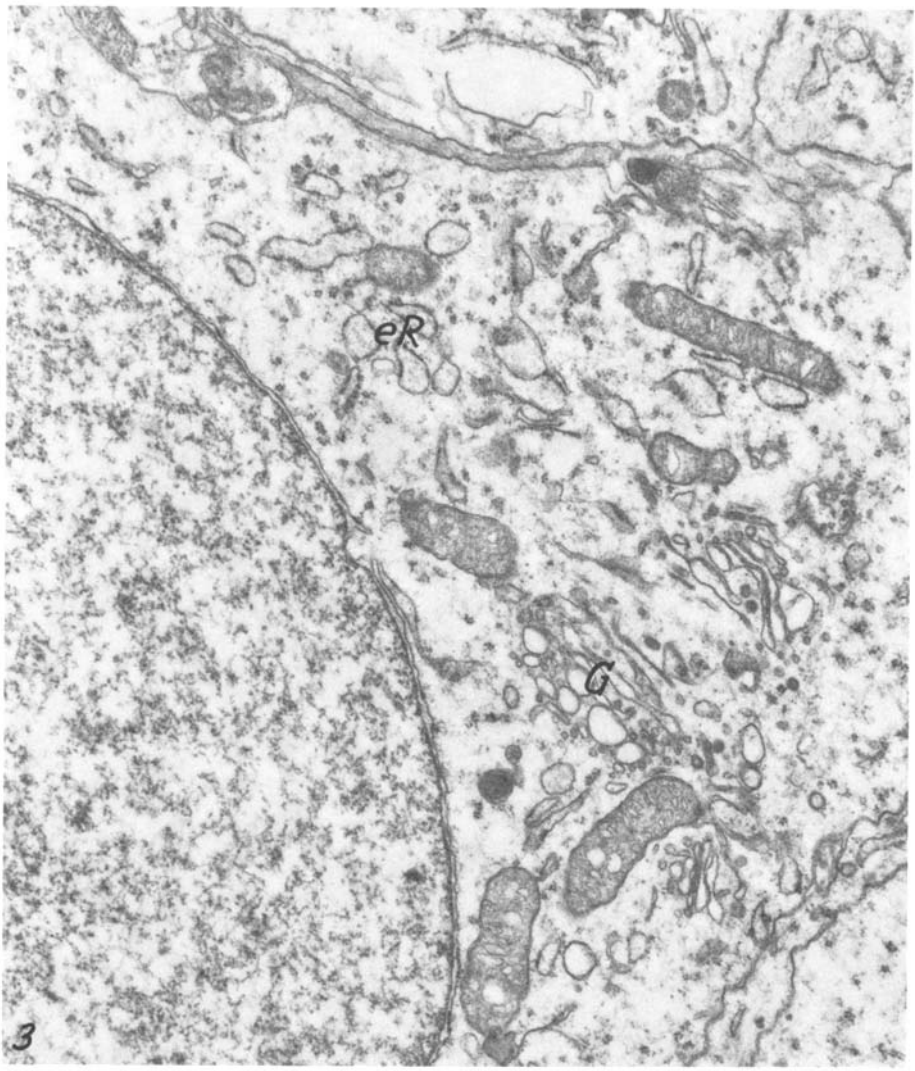

Fig. 3. Cell in the neurohypophysial anlage. ED 15. *eR* Endoplasmic reticulum; *G* Golgi complex. ×21,000

(Kobayashi et al. 1968) develops from the narrow acellular zone of ED 16–17. By ED 18 the neuronal layer makes up about half of the dorso-ventral diameter of the ME and expands to about two thirds of the distance on the first day of life (Fig. 5). The neuronal layer consists of nerve fibers, some of them extending to the basement membrane of the brain, and cells, as well as of the processes of cells of the ependymal layer. Compared with the lateral walls of the ventricle, few mitoses occur in the ME; they appear mainly in the ependyma, and less often in the neuronal layer.

The first tanycytes become visible on ED 18. They are elongated in appearance. The broad apical ventricular region contains the cell nucleus, which lies at varying

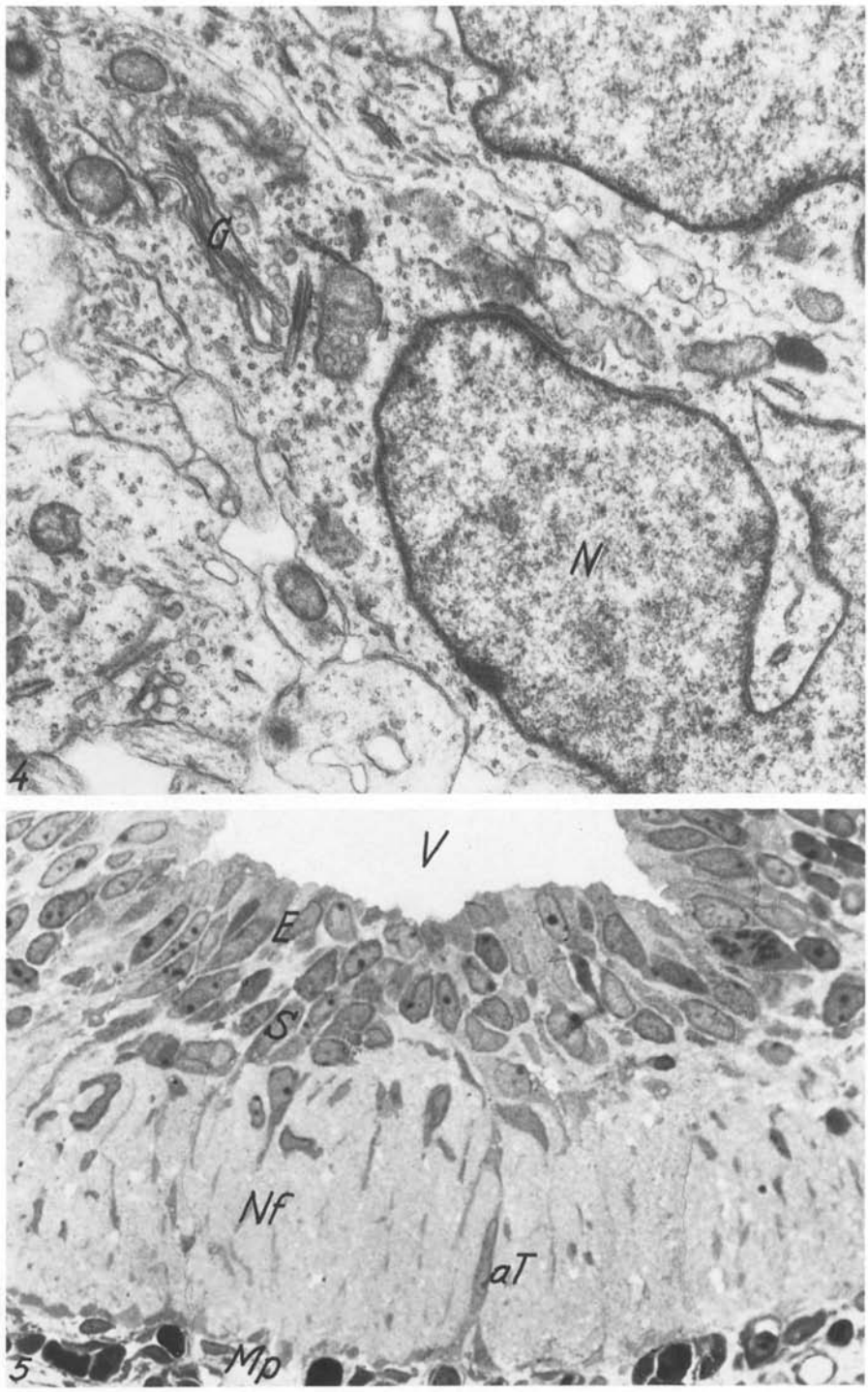

Fig. 4. Precursor cell of oligodendroblasts and astroblasts. Junction of ME and arcuate nucleus. ED 16. *N* nucleus; *G* Golgi complex. ×21,000

Fig. 5. Survey micrograph of the ME. Migration of astrocytic tanycytes into the neuronal zone. Semithin section. Toluidine blue borax. ED 20. *V* third ventricle; *E* ependyma; *S* subependyma; *Nf* neuronal zone; *aT* astrocytic tanycyte; *Mp* mantle plexus. ×700

distances from the ventricular surface. The supranuclear cytoplasm contains microtubules, stacked or irregularly distributed cisternae of RER with ribosome-free segments, dense bodies, MVB and LB (Fig. 6). Their long and slender basal processes pass through the neuronal layer without branching and divide near the basement membrane into several end-feet, which abut on this membrane. In the basal process isolated, elongated mitochondria and glycogen, as well as short and sometimes spindle-shaped profiles of smooth and rough ER are present, which are arranged in long, irregular loops or concentric circles in the end-feet. Filaments, numerous microtubules and isolated free ribosomes are also seen.

The ependyma also contains cells without processes, but the nuclear structure and organelle content of which correspond to those of the tanycytes.

The first precursor cells of astrocytic tanycytes are observed on ED 19 and 20. They are found principally in the subependyma and in the neuronal zone. All transitional stages between younger and more mature forms are observed in the subependyma. The precursor cells in the neuronal zone generally have several short, finger-like processes. Their cytoplasm contains relatively few ribosomes and few organelles (mitochondria, short cisternae of RER, Golgi complex, microtubules, LB). The polymorphic nuclei contain relatively coarse, evenly distributed chromatin aggregations and a moderately distinct chromatin margin.

By ED 21 the astrocytic tanycytes and their precursors are observed more frequently than all other cell types of the ME. The more mature astrocytic tanycytes exhibit large variations in their chromatin structure, organelle content, cytoplasmic density and structure of their processes. The long axes of these cells are usually perpendicular to the ventricular surface. One or two processes extend from the cell body to the basement membrane. Bipolar forms are also observed, especially perinatally, from which an additional process originates toward the ependyma; however, none of these processes reach the ventricular surface. The processes of the astrocytic tanycytes are broader and contain more organelles than those of the tanycytes. Numerous LB, often with accumulated glycogen granules, occur in both the tanycytes and the astrocytic tanycytes, and neuroglial contacts are seen on ED 20 (Fig. 7, inset: cf. Wittkowski 1972).

Two types of astrocytic tanycytes can be distinguished according to their electron density. Type A (Fig. 7) possesses dark, often condensed nuclear chromatin and a more electron-dense cytoplasm than the surrounding nerve fibers. The cytoplasm contains small, dark mitochondria, dense bodies, short, narrow and often spindle-shaped cisternae of SER and RER as well as numerous microtubules. The process of the cell resembles that of the tanycytes (fiber-type of extraependymal glia according to Wittkowski 1968). Type B astrocytic tanycytes (Fig. 8) have more evenly distributed and moderately to coarsely flocculent chromatin. The cytoplasm has the same density as adjacent nerve fibers. The mitochondria are larger and more electron-lucent and the microtubules less numerous. The profiles of RER are longer and of uniform width and rounded at their ends (protoplasmic type according to Wittkowski 1968). Common to both types are LB, MVB and glycogen. Occasionally, the processes of the cells are joined by desmosome-like membrane contacts. In some astrocytic tanycytes that reach the basement membrane with a long process or show synaptoid contacts with nerve fibers, mitoses could be observed.

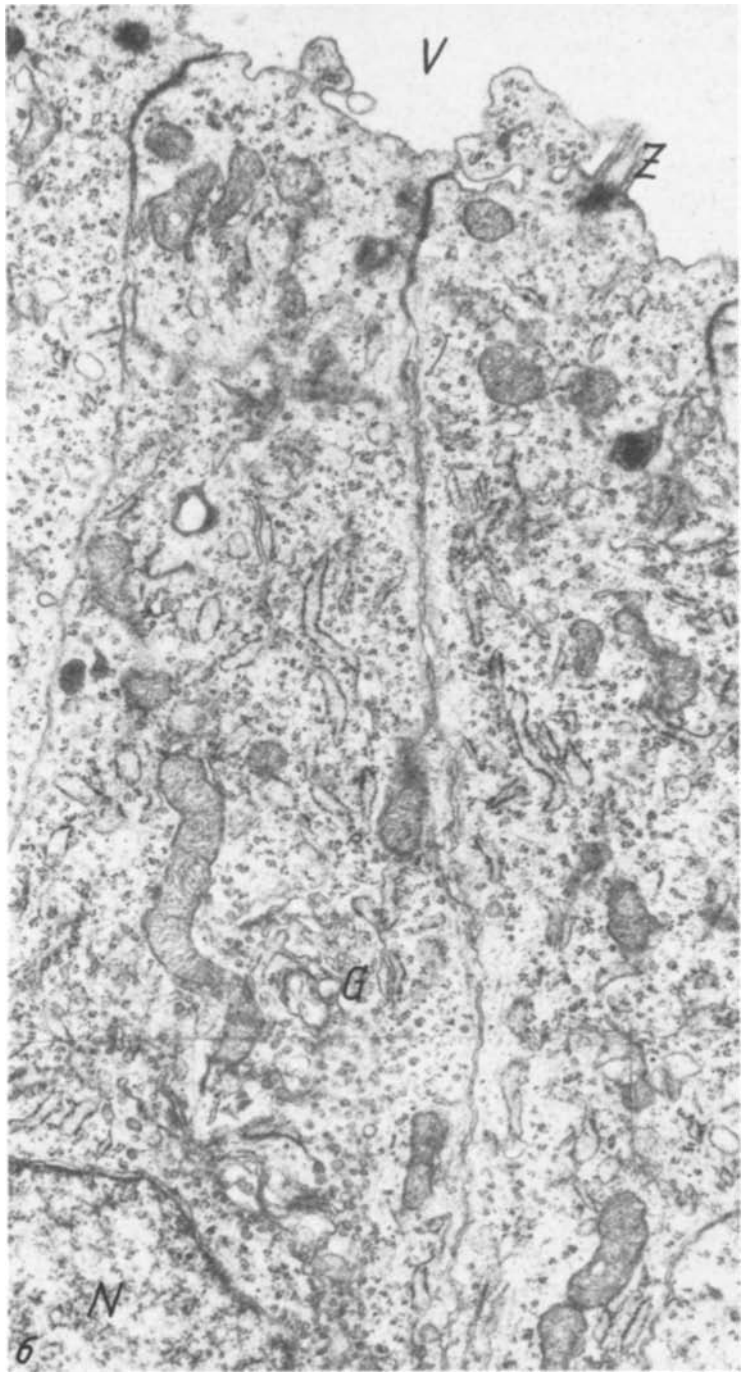

Fig. 6. Ependymal zone of the median eminence. Supranuclear cell regions of tanycytes. ED 18. *V* third ventricle; *Z* cilia; *G* Goldi complex; *N* nucleus. ×21,000

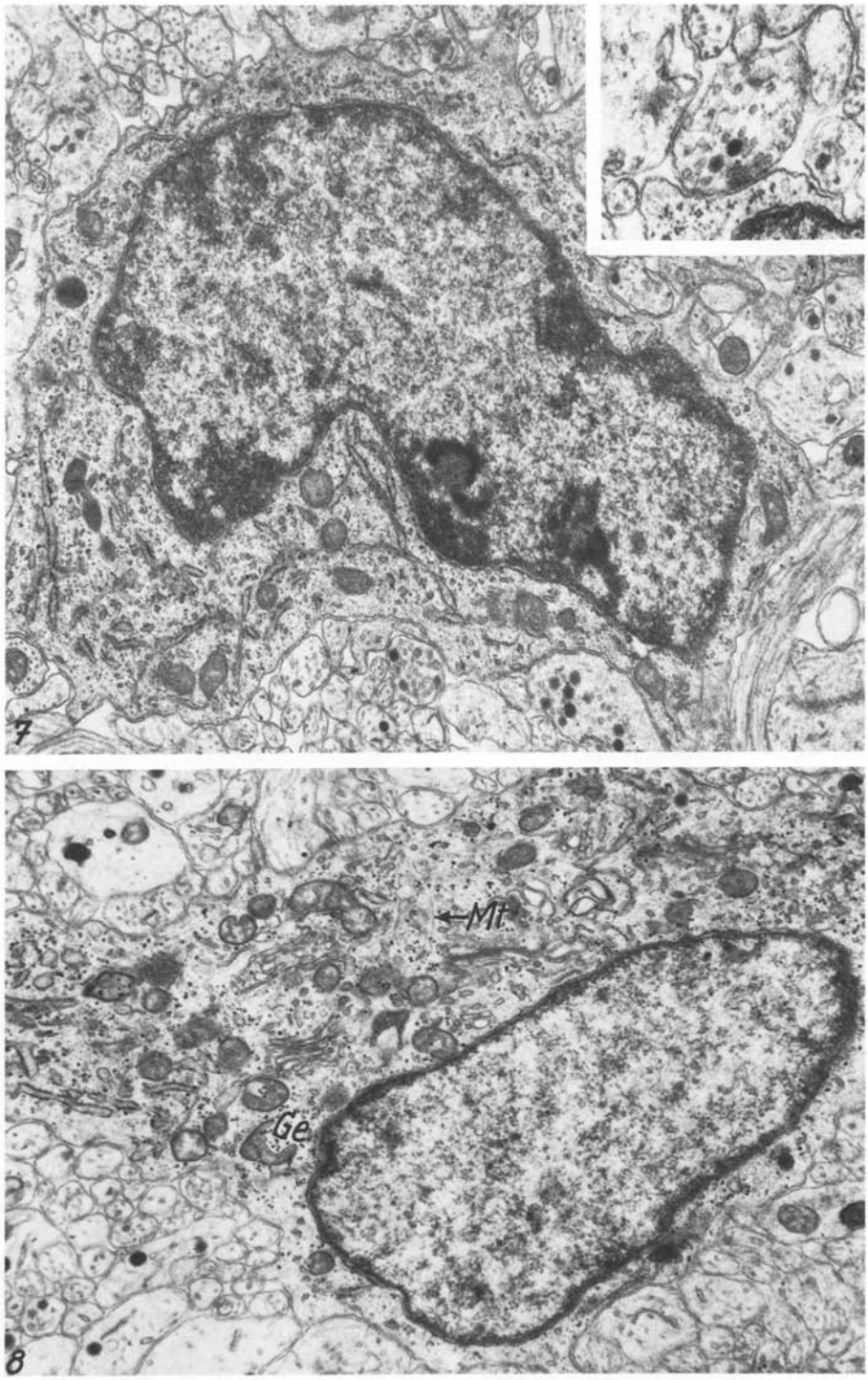

Fig. 7. Astrocytic tanycyte (Type A). Median eminence. ED 22. *Inset*: Neuroglial contact. ED 20. × 21,000

Fig. 8. Astrocytic tanycyte (Type B). Median eminence. ED 21. *Ge* glycogen; *Mt* microtubules. × 17,500

Oligodendroblasts are first seen in the ME on ED 19 (Fig. 9). They were identified according to the descriptions of Mori and Leblond (1970) and of Vaughn and Peters (1971). The cells often occur in pairs (immediately following mitosis) in the neuronal layer of the ME. The oligodendroblasts generally have a coarsely flocculent chromatin, which is condensed into electron-dense aggregates and an electron-lucent cytoplasm with numerous ribosomes and few organelles. By ED 22 the cell body and processes contain numerous organelles: ribosomes, mitochondria, a Golgi complex with clear vesicles, RER, as well as isolated lipid granules.

Between ED 20 and PD 1 astroblasts are observed only sporadically in the lateral portions of the ME, but are more frequent in the arcuate nucleus. They have small cell bodies and a few processes. A striking feature are their dense nuclei with homogeneous chromatin (cf. Kozik 1976); they are not found in any other cell type of the ME at this time. The cytoplasm of these cells is electron-lucent and contains mitochondria and microtubules. The Golgi complex has expanded sacculi and vesicles.

Oligodendroblasts and astroblasts appear later in the caudal portions of the ME (hypophysial stalk) than in the rostral portions.

Postnatal Day 2–7. The ependyma and subependyma are reduced to two cell layers by the end of this period, with a particularly pronounced decrease in the number of subependymal cells. Meanwhile, the number of cells in the neuronal zone continuously increases. The cell bodies of the tanycytes and of ependymal cells without processes become increasingly cuboidal and contain many LB, an extensive Golgi complex and a large number of the previously mentioned organelles (see ED 18 – PD 1). The cells of the subependyma have the typical structure of astrocytic tanycytes or their precursors, despite their frequent lack of processes. Mitoses are rare in the ependyma (although they are still observed on PD 7).

The first capillaries appear in the subependyma between PD 4 and PD 6. On PD 7 the vessels of the mantle plexus start to bulge into the external zone of the ME.

Astrocytic tanycytes are far more numerous than other cell types in the subependyma and neuronal zone. During this period they also occur in the two variations described above. If the nucleus of type A cells lies in the dorsal part of the neuronal zone or in the subependyma, they cannot always be clearly distinguished from tanycytes. The more mature forms are distinguished from the few precursor cells and the young astrocytic tanycytes by their numerous LB and organelles. Details on the postnatal development of the astrocytic tanycytes were given by Bitsch and Schiebler (1979).

The astrocytes rapidly increase in number during this period. They usually have a large cell body from which many small processes project into the surrounding intercellular spaces with no apparent directional preference (Fig. 10). Compared with the previous period, the Golgi complex in particular has become more extensive, and numerous phagosomes are seen, some of which contain prominent cellular debris. Typical protoplasmic astrocytes appear on PD 6, but mature fibrous astrocytes are not yet seen (Bitsch and Schiebler 1979).

Oligodendrocytes with long, thin processes, electron-dense cytoplasm and abundant organelles (mitochondria, Golgi complex, ribosomes, RER, microtubules, lipid granules) first appear on PD 3. Myelin sheath formation is not observed in the ME until PD 7.

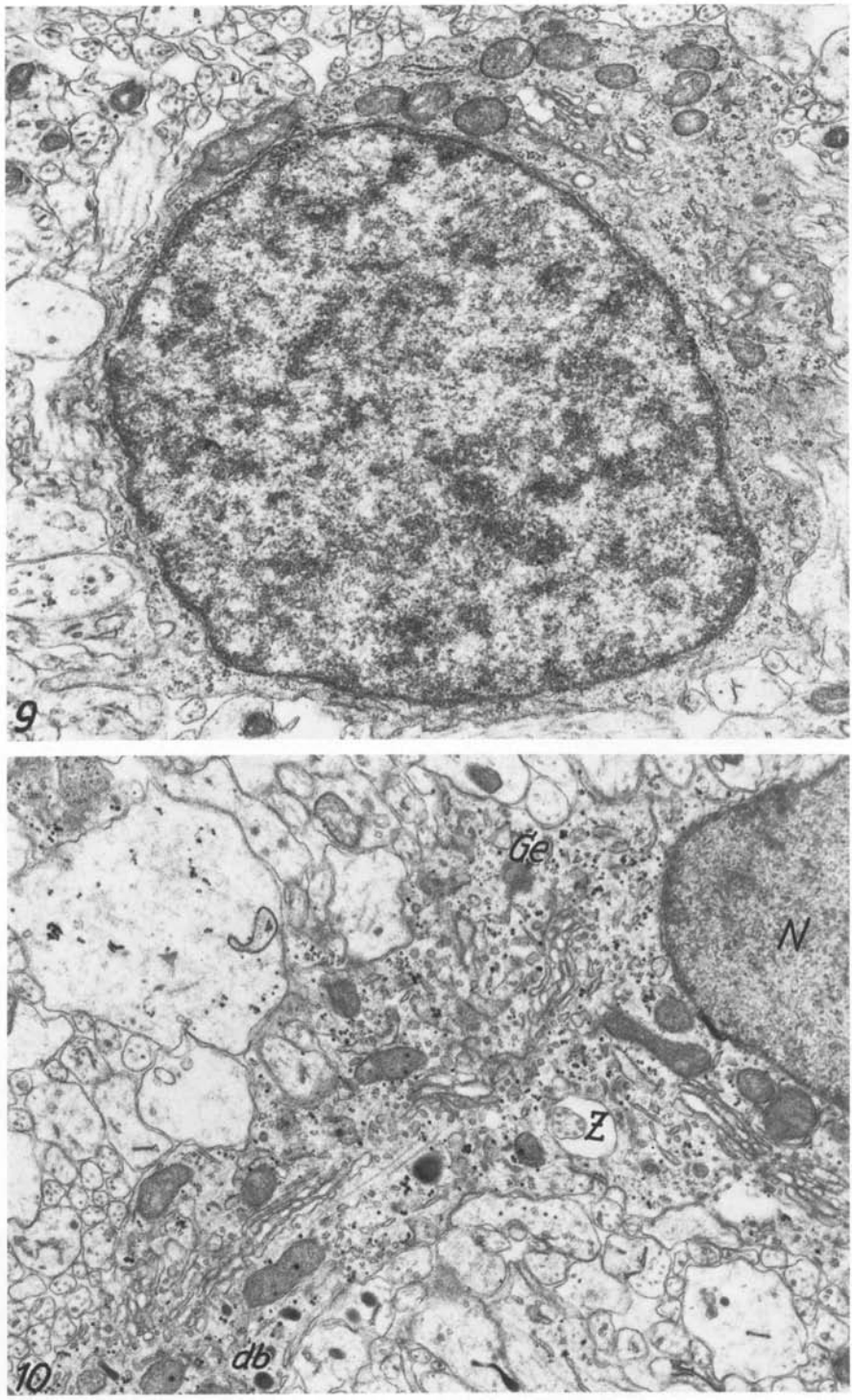

Fig. 9. Oligodendroblast. Median eminence. ED 22. ×21,000

Fig. 10. Astroblast. Median eminence. PD 6. *Z* cilia; *Ge* glycogen; *dh* dense body; *N* nucleus. ×17,500

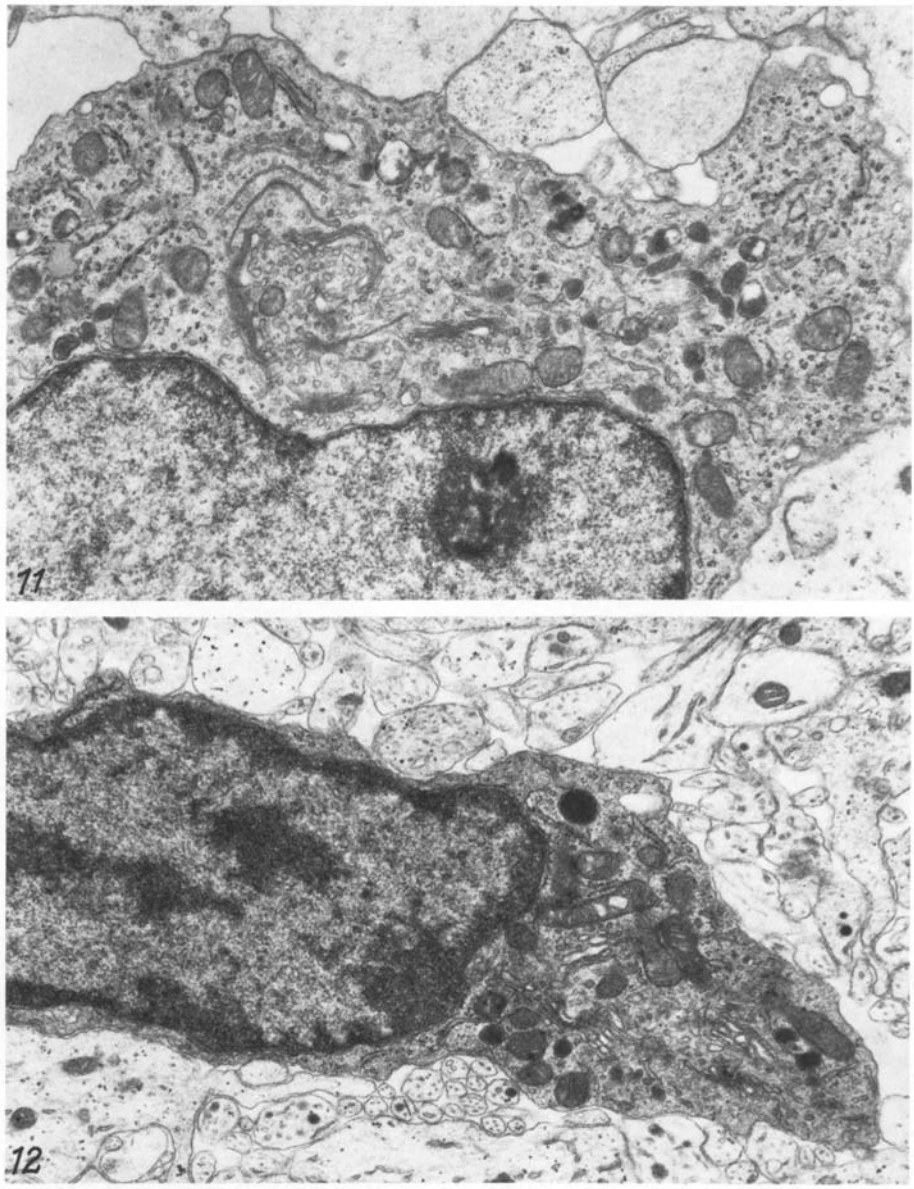

Fig. 11. Young microglia. Junction of ME and arcuate nucleus. ED 16. ×17,500

Fig. 12. Mature microglia. Median eminence. PD 4. ×17,500

Microglial Cells. As early as ED 13, microglial cells can be seen in the ME and ajdacent regions of the lateral ventricular walls. They usually lie close to the basement membrane that separates the nervous tissue from the surrounding mesenchyma. During the following days, as the blood vessels first appear in the ME, the microglial cells are mainly found in a pericapillary location. Morphologi-

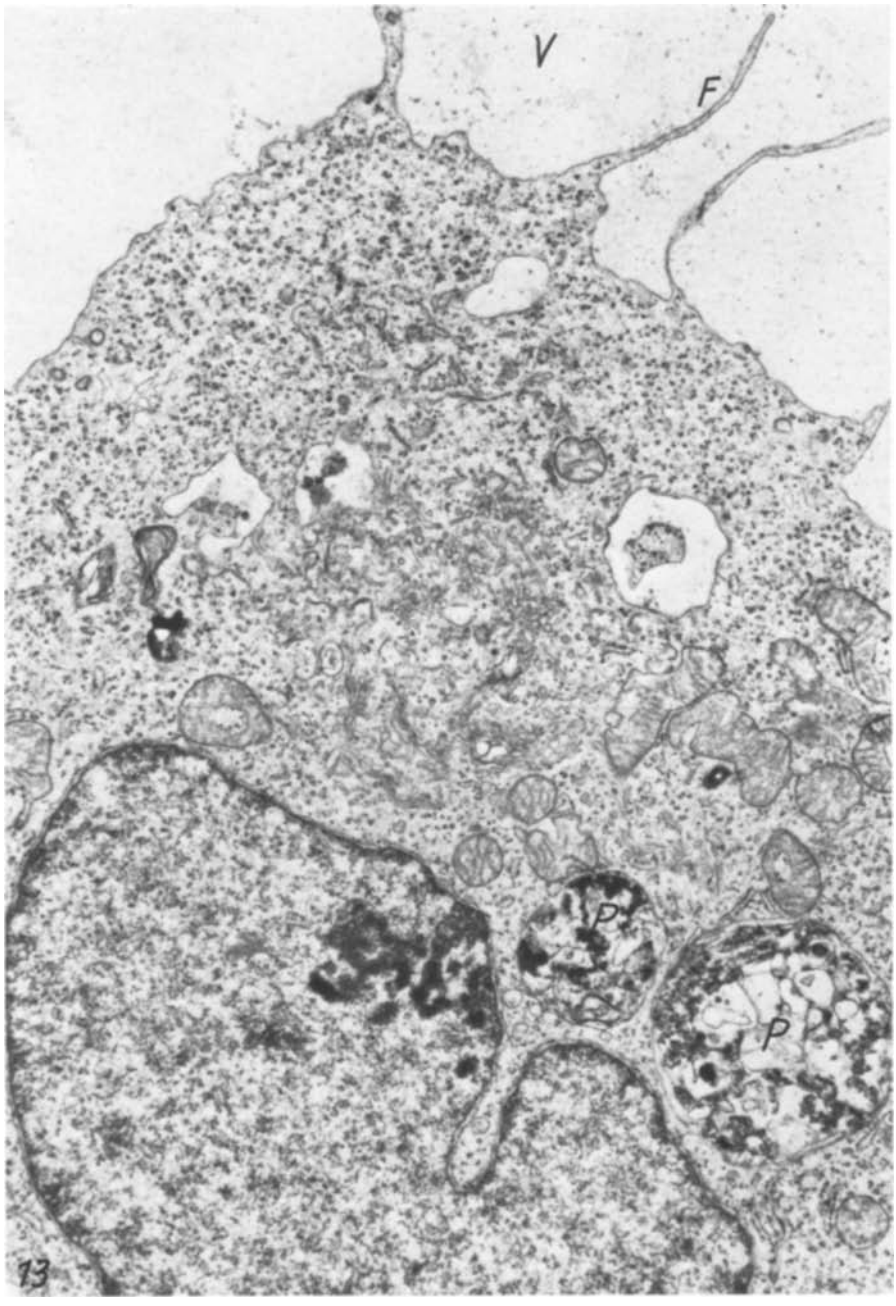

Fig. 13. Young supraependymal cell. Infundibular region. ED 14. *V* third ventricle; *F* filopodia; *P* phagosomes. ×21,000

cally, these cells resemble the free macrophages of the surrounding connective tissue and the supraependymal cells (see below). The cells are large, irregular in shape, and have an electron-lucent nucleus; their cytoplasm contains evenly distributed free ribosomes, a prominent Golgi complex, numerous large mitochondria with an electron-lucent matrix, as well as isolated, partly long cisternae of RER (Fig. 11). Sometimes, large numbers of electron-lucent LB are seen. Phagosomes with a granular or membranous internal structure occur in all sizes, and vesicles and small vacuoles are found beneath the plasmalemma, suggesting a strong pinocytotic activity. In the pericapillary space of the mantle plexus only one single incident cell was observed on ED 15 to penetrate the basement membrane and to pass between the cell processes of the ME into the brain *anlage*.

By the end of the embryonic period, the appearance of the microglial cells has changed. The nucleus is electron-dense and exhibits distinct marginal chromatin condensation. The cytoplasm is denser, the ribosomes are decreased in number, while Golgi complexes, RER, smaller phagosomes and dense bodies are more frequent. The mitochondria are smaller and more electron-dense, and the RER often consists of very long profiles that follow an irregular course. Small empty vacuoles occur more frequently beneath the cell membrane. A large process is usually present. In peri- and postnatal life small microglial cells are seen characterized by an electron-dense nucleus and dense cytoplasm with a markedly diminished number of organelles (Fig. 12). Mitoses of microglial cells are occasionally found.

Numerous vacuolized amoeboid cells occur perinatally in the subependyma of the walls of the third ventricle (Booz and Felsing 1973; Schmitt 1973).

Supraependymal Cells. The supraependymal (intraventricular) cells first appear on the floor of the third ventricle on ED 13. Initially, they resemble intramesenchymal macrophages and young microglia. Unlike the latter, however, they possess filopodia (Fig. 13). Later, chromatin and RER develop as in the microglia, except that the nucleus becomes larger and more electron-lucent. Increased numbers of phagosomes, dense bodies, filopodia and vacuoles of various sizes appear. The number of ribosomes varies considerably. These intraventricular cells may closely resemble the macrophages of the subarachnoid space (Morse 1972) or peritoneum (Carr 1973), as well as activated epiplexus cells (Carpenter et al. 1970) during the prenatal period. On the other hand, we also find cells lacking filopodia and possessing all the characteristics of microglial cells.

Discussion

The present investigation shows that the median eminence of the rat differs markedly from adjacent brain regions during prenatal development. One difference is that the matrix phases described by Keyser (1972) occur in a different manner in the ME. The marginal, mantle and matrix zones do not form in the typical way; instead, clear signs of a specific differentiation appear in all cells of the ME as early as ED 13 and 14. Cell proliferation is also lower in the ME than in adjacent regions. Moreover, throughout its development the ME contains cells (tanycytes and their precursors) extending from the ventricle to the basement membrane. This

observation led Fink and Smith (1971) to postulate a neuroepithelial structure for the pre- and postnatal ME.

The ME is also unique in terms of the conclusion of its development. Bitsch and Schiebler (1979) consider the ME of the rat to be fully mature only by the end of the third week of life. Mitro and Schiebler (1972) point out that the ventricular floor in the area of the ME is included among the regions that do not reach their final form until the animal has practically attained adulthood, while the ependyma of other regions of the hypothalamus has largely completed its differentiation by birth.

Although we are unable to offer conclusive proof, the induction for the development of the ME seems to arise from Rathke's pouch. In any case, our findings show that the differentiation specific for the ME takes place only in regions of the brain *anlage* that lie in the vicinity of Rathke's pouch.

As for the schedule of the development of the ME and neurohypophysis, it is clear that the first processes of cellular differentiation occur as early as ED 12 in the neuroepithelium adjacent to Rathke's pouch. By ED 15, an essentially uniform cell type is present in this region. The cells here are distinguished by a relatively electron-lucent nucleus, a high organelle content, and a progressive differentiation from adjacent cells. The region of the future neurohypophysis is distinguished on ED 13 by increased cell proliferation, but not by cells of other types. On ED 16, cells are first observed in the ME-*anlage* that also occur in adjacent brain areas. These cells have folded nuclei with dense chromatin, numerous polyribosomes, as well as processes with various cell organelles. As their further development indicates, these cells are precursors of oligodendroblasts and astroblasts. It is noteworthy that these cells are first seen only in the boundary regions of the ME, but in the days that follow are also observed in the center of the ME and finally in the hypophysial stalk. All evidence points to the fact that these *astroblast and oligodendroblast precursors enter the ME from surrounding regions,* particularly since initially the number of these cells decreases from a lateral to a medial and caudal direction, and toward the *anlage* of the hypophysial stalk. These cells are never found in the *anlage* of the neurohypophysis. We have no evidence that precursors of astrocytes and oligodendrocytes arise from the ependyma of the ME.

According to our observations the parent cells from which the various cell types of the ME arise are present in the ME by ED 16. On ED 16 and 17, differences first appear between the cells of the ME and the neurohypophysial *anlage*. Also at this time the ME is organized into an ependymal, a subependymal and a narrow, acellular layer at the basement membrane. In the days that follow, the various cell types differentiate, the organization of the ME progresses, and differentiation of the neurohypophysis occurs.

Subsequently, the development of the different cell types of the ME and neurohypophysis was examined in detail. *Tanycytes* can be recognized for the first time on ED 18 on the basis of their typical slender, basally directed processes (Ugrumov et al. 1979), which contain numerous microtubules but few organelles. The tanycytes are also characterized by LB, neuroglial contacts (after ED 20), and by the high organelle content of their cell bodies. Their precursor cells, like the tanycytes themselves extend from the ventricular surface of the ME to the basement membrane; the basal process is broad, unbranched and contains few organelles before ED 18. Because the tanycyte precursors resemble the other cells of the ME-*anlage* in their nuclear structure and organelle content, it cannot be stated with

certainty which of the cells that extend from the ME ventricular surface to the basement membrane on ED 13 will eventually differentiate into tanycytes.

The *astrocytic tanycytes* start to form from the subependymal cells on ED 18. During their differentiation they migrate into the neuronal zone, where they sometimes divide. It is possible that young tanycytes also lose contact with the ventricle and appear in the internal zone as bipolar astrocytic tanycytes (Bitsch and Schiebler 1979).

Up to now the origin of the astrocytic tanycytes could not be conclusively determined (Záborszki and Schiebler 1978; Bitsch and Schiebler 1979); the present findings indicate that this cell type develops locally in the ME and thus belongs to the tanycyte series. Marked differences exist between the early forms of the astrocytic tanycytes and the astroblasts: The astroblasts show a homogeneous chromatin structure (Kozik 1976) and electron-lucent cytoplasm with evenly distributed glycogen. In the astrocytic tanycytes the glycogen is often clumped together or concentrated around LB in the cytoplasm; these cells also receive synaptic contacts, contain LB, and have a polarity, lacking in the astroblasts. The protoplasmic type of young astrocytic tanycytes resembles astroblasts. Young astrocytic tanycytes, however, cannot be confused with oligodendroblasts because even early forms of oligodendroblasts are distinguished from the astrocytic tanycytes by their striking chromatin structure and their high ribosome content.

Although it is now clear that the astrocytic tanycytes do not represent a transitional form between tanycytes and astrocytes, as was assumed by Záborszki and Schiebler (1978), but instead belong to the tanycyte series, we shall retain the name "astrocytic tanycytes". At least in morphological terms, the astrocytic tanycytes form a distinctive cell type with an astrocyte-like structure and never come into contact with the ventricular surface. The question of whether they differ functionally from the tanycytes is still open to discussion.

The subependyma is the site of origin of astrocytic tanycytes. However, this layer is probably not a true matrix zone, for it lacks the cell proliferation characteristic of a true matrix. Mitotic figures are very rarely seen in this region. Instead, it appears that the cells of the subependyma arise from the ependyma through mitosis. The undifferentiated cells of the subependyma mentioned by Bitsch and Schiebler (1979) are probably precursors of astrocytic tanycytes. Whether they also appear in this form in the adult animal is unknown.

In the present work we traced the development of the *pituicytes* only up to ED 17. Until ED 15 the cells in the neurohypophysial *anlage* are morphologically identical to the cells of the ME-*anlage*. Afterwards, however, they follow a separate line of development, which was described in detail by Galabov and Schiebler (1978a, b). An important conclusion of the present work is that the *pituicytes arise from the same precursors as the tanycytes and astrocytic tanycytes.* During development, a boundary zone forms in the *anlage* of the hypophysial stalk in which tanycytes and astrocytic tanycytes (as characteristic cells of the ME) as well as pituicytes (cells of the neurohypophysis) coexist.

Our investigation indicates that the *astrocytes* and *oligodendrocytes* do not arise from the ependyma of the ME. Instead, the present findings suggest that they originate from neighboring regions, perhaps the lateral portions of the ME. Oligodendroblasts and astroblasts are first found in the ME at the end of the embryonic period; they do not occur in large numbers until after birth.

With regard to the functional development of the ME, the following observations were made: The first capillaries of the mantle plexus form between the adenohypophysis and ventricular floor on ED 14 and 15 (cf. Fink and Smith 1971). On ED 16 the first nerve fibers start to grow into the ME (cf. Monroe and Paull 1974). In subsequent days they reach the basement membrane of the mantle plexus (in small numbers at first), and by ED 20 they form the first neuroglial contacts with tanycytes and astrocytic tanycytes.

The microglial and supraependymal cells represent a special case. *Microglial cells* are usually considered to be of mesodermal origin (Rio-Hortega 1932; Mori and Leblond 1969; Barón and Gallego 1972; Carr 1973; Ling 1979). However, a neuroectodermal origin has also been proposed for these cells (e.g., Vaughn and Peters 1971). Our observations support the classic theory of a mesodermal origin, at least in the regions of the rat brain investigated in the present study. Specifically, we found that the young forms of the microglial cell bear a close morphologic resemblance to the free macrophages in the developing pia mater. These macrophages might be extracerebral microglial precursors demonstrable by the silver carbonate method (Rio-Hortega 1932). Moreover, clearly differentiated microglial cells appear prenatally among completely immature neuroepithelial cells, and no transitional forms between these cells are visible. Finally, we interpret their proximity to the basement membrane within the nervous system as evidence of migration from the pia mater and vascular connective tissue (cf. also Boya et al. 1979). In one case, we observed a cell penetrating the basement membrane and glia limitans in the region of the ME. A similar observation was reported by Barón and Gallego (1972) in the cat cortex. This conflicts with the findings of Vaughn and Peters (1971), who favor a neuroectodermal origin for the microglia partly because this cell form occurs prenatally in brain regions still devoid of vessels.

The mature form of the microglial cell that occur perinatally correspond to the descriptions of Mori and Leblond (1969) and of Záborszky and Schiebler (1978).

The existence of *supraependymal cells* was first mentioned by Kolmer (1921; epiplexus cells). Although they have since been studied by many investigators (Noack et al. 1972; Leonhardt and Lindemann 1973; Coates 1973; Scott et al. 19745), the debate concerning the nature and origin of these cells is not yet resolved. Based on evidence to date, we are apparently dealing with a heterogeneous cell population of nerve cells and macrophages occurring throughout the ventricular system, but exhibiting distinct regional differences.

The infundibular region contains almost exclusively macrophage-like cells (Bleier et al. 1975; Scott et al. 1975; Mestres and Breipohl 1976). Since these cells are regularly found in the normal animal and are present prenatally, we are justified in speaking of a local phagocytic system. The abundance of these cells in the infundibular region may well be due to the cellular debris that commonly occurs in the infundibular recess.

The many features common to supraependymal cells and microglial cells suggest that both forms may originate from a common mesodermal precursor. It still remains to be determined how these cells enter the ventricular system. Certain transitional forms would indicate a passage of the microglia into the ventricle, and this assumption is supported by our observations (cf. also Bleier 1971). It has been suggested that these cells arise from the epiplexus cells (Kolmer 1921;

Carpenter et al. 1970; Merker 1972) and from the macrophages of the subarachnoid space (Morse 1972).

In *conclusion,* the present study indicates that the glial cells of the median eminence are of varying origin. The tanycytes and astrocytic tanycytes develop locally. Astroblasts and oligodendroblasts migrate into the ME from surrounding tissues. The microglial and supraependymal cells may be of mesenchymal origin. Finally, our study shows that the pituicytes derive from local glial cells in the ME. This means that pituicytes, tanycytes and astrocytic tanycytes have the same origin in terms of their developmental history. Even in the adult animal, these three cell types show striking similarities. They possess numerous LB and neuroglial synapses (Wittkowski 1967, 1968, 1974), and perform comparable functions in the hypothalamo-hypophysial system. Léránth and Schiebler (1975) also point out similar reactions of tanycytes and pituicytes under various experimental conditions.

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