Topographical and Cytological Evolution of the Glial Phase During Prenatal Development of the Human Brain: Histochemical and Electron Microscopic Study

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Abstract. An ultrastructural analysis of prenatal gliogenesis and neuronal–glial relationships in the developing fetal brain was carried out using reduced osmium and periodic acid–thiocarbohydrazide–silver proteinate to stain selectively the glycogen content of the glial population. Gliophilic neuronal migration was confirmed in the human fetus, with radial glial fibers (RGF) acting as obligatory corridors for neuronal migration in the prospective neocortex and underlying intermediate zone (IZ). With this method, the entire glial phase was differentiated from neuronal elements; this permitted a description of the evolutionary distribution pattern of RGF in the cortical plate, glial fascicles fully dissociate by 18 weeks gestation, whereas in the IZ, they remain grouped in fascicles until their transformation into astrocytes. The most conspicuous and constant developmental feature observed in the maturing glial cytoplasm between 21 and 30 weeks gestation was a radical enhancement in the abundance and activity of the lysosomal apparatus and autophagic vacuoles observed in the RGF, a cytological basis for the transformation of radial glial cells into astrocytes. These data have implications for the understanding of the ontogenesis of the neocortical vertical modules in the human brain and for the phylogenetic analysis of the vertical cortical units in terms of comparative mammalian anatomy.

Key Words: Brain, human fetal; Gliogenesis; Glycogen; Lysosomes; Neuronal–glial relationship; Neuronal migration; Osmium, reduced.

INTRODUCTION

Neuronal migration is one of the most crucial events in the development of the central nervous system (CNS) and an understanding of its underlying mechanisms is essential for the comprehension of mammalian brain development and human CNS malformations (1–3). Rakic was the first who proposed the guidance role of radial glial fibers (RGF) to migrating neurons (1, 4, 5). Despite the seminal contributions of Marin-Padilla (6), Sidman and Rakic (2), Choi and Lapham (7) and other investigators (8) who described radial glial cells (RGC) in the developing human brain, many questions about migratory mechanisms remain unanswered, particularly in the human, including the nature of glial–neuronal relationships and glial transformations (7). A major obstacle to the analysis of the glial–neuronal relationships has been the difficulty in differentiating glial fibers from dendritic and axonal processes in the developing human cortical plate (CP) at the ultrastructural level (7). The rapid Golgi–gold substitution method is difficult to use for such a study due to the capriciousness and limited number of cell impregnations obtained (9). More recently, however, immunohistochemical methods using specific antibodies to glial
fibrillary acidic protein (GFAP) and to vimentin in radial glia have been successfully used in rodents (10–13), primates (14), and human fetuses (15, 16). Nevertheless, in the human material, the immunoreactivity in the radial glia using electron microscopic immunoperoxidase methods is a function of tissue preparation; its morphological and other limitations were pointed out by Choi (16).

An essential property which differentiates the glial lineage from most neurons is the presence of particulate glycogen in glial cells (17–20). We recently applied a modified method based on Thiéry (21) and Maxwell (22), of histochemical procedures for the preservation and staining of particulate glycogen in the RGC population during mammalian CNS development, using reduced osmium and periodic acid–thiocarbohydrazide–silver proteinate (PA-TCH-SP) (17). The specificity of these methods in selectively staining the glycogen particles in nervous tissue has been recently confirmed by many investigators (20, 23). The chemical basis for glycogen contrast staining using these techniques was detailed by Cataldo (20) as well. In this study, we applied this method for the investigation of glial–neuronal relationships during migration in the human fetus and for the study of transformation of RGF into mature glial cells at the end of the neuronal migratory period.

MATERIALS AND METHODS

Light Microscopic Preparations: Forty-four normal human fetal brains from 16 to 40 weeks (wk) of gestation were examined under the light microscope in order to select the most appropriate ages and regions for the ultrastructural study of neuronal migration in its late stages and the subsequent transformations of RGC. All brain specimens were fixed in either a 10% buffered formalin or Bouin solution. After fixation, the tissues were washed with running water and dehydrated in graded alcohols. The blocks were embedded in paraffin and cut coronally in 10 to 20 μm thick sections and stained with hematoxylin and eosin (H&E) or cresyl violet.

Electron Microscopic Preparations: Five normal human fetal brains (18, 21, 24, 27, and 30 wk) and one full-term human brain (41 wk) were fixed by immersion in a fixative based on a 0.1 M cacodylate buffer with 0.01 M CaCl_2 and H_2O_2 as described by Peracchia and Mitter (24), containing glutaraldehyde 2.5% and parafomaldehyde 1%, and adjusted to pH 7.3. After a one hour immersion in the fixative, the brains were cut into full-thickness coronal sections (leptomeninges to ventricles; 3 × 1 × 1 mm) and reimmersed overnight in the same fixative at 4°C. Subsequently, the blocks were rinsed with the buffer and postfixed at room temperature in 1% OsO_4, and 0.05 M K_2Fe(CN)_6·3H_2O in a 0.1 M cacodylate buffer. This solution was readjusted to pH 7.3 as the addition of K_2Fe(CN)_6·3H_2O increased the pH to basic values. Postfixation lasted 45 to 60 minutes (min). After careful rinsing with the buffer, the blocks were gradually dehydrated in ethanol and propylene oxide. All specimens were embedded in Epon 812 (Serva). One-micrometer (μm) thick sections were cut, stained with toluidine blue, and used for topographic localization in order to select suitable areas for further ultrastructural study. Ultrathin sections were cut with a Reichert ultramicrotome.

Ultrathin sections mounted on copper grids were stained with uranyl acetate and lead citrate (ten min/one min) (U-Pb) (25). Other sections mounted on nickel grids were treated with PA-TCH-SP according to our adaptation for fetal tissues (17) of the Thiéry (21) and Maxwell (22) methods for staining polysaccharides. To compare both staining methods, successive serial sections were stained alternatively with U-Pb and with PA-TCH-SP.

The Epon blocks were first cut coronally, reoriented, and then cut tangentially when necessary.

Pellicam lozenge grids were used for the analysis of serial sections. The sections were examined with a Philips EM 300 electron microscope and montages of 2 to 20 adjacent pictures were made.

Geometrical Method: To compare semiquantitative data obtained on tangential sections at different levels of the neopallial wall, the concentric planes of section through the developing

Fig. 1. Ultrastructural identification of RGC by their glycogen content selectively preserved and stained in coronal sections of normal human fetal brain at 18 wk gestation. A. Perivascular glial sheathing (arrows), heavily populated with glycogen particles, IZ; postfixation with reduced osmium and PA-TCH-SP staining. B. A glial end-foot loaded with glycogen particles (asterisk), plexiform zone (PZ); postfixation with reduced osmium and U-Pb staining. C. A mitotic cell in the periventricular zone with glycogen particles in its cytoplasm; postfixation
cerebral hemispheres can be geometrically modelized as ellipsoids of revolution characterized by their half-great axis (B) and their half-small axis (A). The surface area of these ellipsoids of revolution is $4rAB$.

**Ethical Procedures for the Study of Human Fetal Brains:** The human brains used for this study have been collected in accordance with the ethical rules applied in our laboratory. Human fetal brains are obtained for neuropathological and neuroanatomical studies from cases involving miscarriage, abortion and deceased premature infants; when obtained following abortion, we accept specimens only if the obstetrician referring the specimen makes a formal certification that the ethical rules applicable in his institution were fully respected.

**RESULTS**

**Light Microscopic Data**

A conspicuous cohort of neurons remained constantly present in the depths of the intermediate zone (IZ) until the 21st week of gestation. From 18 to 21 wk this cohort was clearly delineated from the subventricular zone (SVZ) and from the overlying upper IZ and cortical plate (CP) (26). By 26 wk gestation, layers II and III seemed to have received their main complement of neurons. On the basis of these data, 18-, 20-, and 21-wk stages were selected for the study of neuronal migration in the IZ and CP, and the 21- and 30-wk stages were selected for the cytological study of glial transformation.

**Description of Glial Glycogen and Ultrastructural Identification of the Glial Phase (Figs. 1-5, 7-11; Tables 1-3)**

The use of postfixation with reduced osmium resulted in excellent preservation of glycogen in the glial phase (Figs. 1B, 2A, 3, 5, 9D, 10, 11) and the subsequent use of PA-TCH-SP staining permitted easy differentiation of glycogen particles from free ribosomes (Figs. 1A, 1C, 2B, 4, 7, 8, 9A-C). As summarized in Table 1, the appearance of glycogen particles remained the same at the different fetal ages, but its abundance varied.

In addition to the glycogen preservation in glial cells, the staining methods used modified and often improved the contrast of several neuronal and glial structures. Reduced osmium postfixation increased the contrast of the cell plasma membrane, endoplasmic reticulum, mitochondria, and the Golgi apparatus (27, 28) without modifying the ribosomal and chromat in staining. The distinction between young neurons with dense-staining cytoplasms and nuclei, and late postmigratory neurons with clear-staining nuclei was thereby enhanced. The combined use of reduced osmium and PA-TCH-SP resulted in dense staining of plasma membranes, mitochondria, and endoplasmic reticulum with a reduced chromatin and ribosomal staining; PA-TCH-SP also added the staining of microtubules, glial filaments, and junctions (Figs. 1A, C, 2B, 4, 7, 8, 9A-C). Consecutive serial sections stained alternatively with U-Pb and with PA-TCH-SP (Fig. 2), permitted an accurate comparison of the staining specificities of these methods. With U-Pb, glial filaments were more densely stained, and the concomitant presence of glycogen and glial filaments was systematically observed (Figs. 2A, 3, 5); with PA-TCH-SP, glycogen differentiation from other structures was particularly easy (Fig. 2B).

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with reduced osmium and PA-TCH-SP staining. Like the GFAP method, glycogen labeling permits the identification of the glial lineage from the first step of gliogenesis (subventricular mitosis). Bar: 2 μm.
Fig. 2. Comparison of the results of the U-Pb and PA-TCH-SP staining methods on successive serial sections after post-fixation with reduced osmium. Coronal sections of normal human fetal brain at 18 wk gestation, plexiform zone. BM: basement membrane. C: collagen, P: pial cell. Arrows point to glycogen particles. Inserts at higher magnification, ×2. A. U-Pb staining. B. PA-TCH-SP. Staining differences between these two methods for the study of glycogen and glial filaments are discussed in the text. Bar: 2 μm.

In sections parallel to the pial surface (tangential sections), the number of glycogen-labeled profiles per surface unit was very constant on consecutive sections at a given level of the pial wall (Table 2). In sections perpendicular to the pial surface (coronal sections) (Figs. 7, 9) when glycogen-labeled profiles are longitudinally followed across different neopallial levels, glycogen particles were systematically distributed all along that profile. Glial subpial end-feet and perivascular glial sheaths were continuously loaded with glycogen all along the pial surface and vascular walls.

Fig. 3. Glial filaments in RGC at the different neopallial levels. Human fetal brains, U-Pb staining. A. Distal segment of RGF (asterisk); PZ, coronal section, 21 wk gestation. B. Tangential section of CP, 21 wk; arrows indicate a glial profile. C. Perinuclear process of RGC (asterisk), SVZ, 18 wk, coronal section. Bar: 1 μm.

Fig. 4. Patterns of glial fascicles in upper IZ. Ultrastructural study at 18 wk fetal age. Tangential sections. Postfixation with reduced osmium and PA-TCH-SP staining method. A. Heavy arrows indicate RGF. B. Magnified insert from A; RGF are indicated by heavy arrows; light arrows indicate glycogen particles. In the IZ, glial fibers remain grouped in fascicles until the period of glial transformation; local defasciculation patterns only occur when migrating neurons transiently dissociate the glial fascicles. Bar: 1 μm.
Fig. 5. Fascicular and transient defasciculation patterns of RGF in the IZ. Transient and local dissociation around migrating neurons is shown in A. Radial glial fibers are clearly identified by their glycogen particles and intermediate filaments. Human fetal brain at 18 wk. Postfixed with reduced osmium and stained with U-Pb; coronal section. Inserts B, C, D are magnified detail from rectangles in A. Bar: 1.5 μm.

Radial Glial Fiber Distribution Pattern (Figs. 4–9, Table 2)

The unequivocal glycogen labeling of the glial phase at the ultrastructural level permitted the study of the relative glial cell distribution and the neuronal–glial relationships in the human fetal brain. A varying pattern of RGF disposition was clearly observed at different levels of the developing neopallium.
### TABLE 1

**A. Ultrastructural appearance of glycogen in the human fetal telencephalon**

<table>
<thead>
<tr>
<th>Staining</th>
<th>Type of glycogen particles at all fetal stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postfixation 1% OsO₄ + K₄FeCN₆, U-Pb</td>
<td>Glycogen beta particles, abundant, 40–80 nm, irregular contours, rosette-like appearance, composed of 10- to 15-nm subunits</td>
</tr>
<tr>
<td>Postfixation 1% OsO₄ + K₄FeCN₆, PA-TCH-SP</td>
<td>Glycogen beta particles, abundant, 40- to 80-nm diameter, rosette-like appearance, composed of 20–25 subunits of 2–4 nm</td>
</tr>
</tbody>
</table>

**B. Abundance of glycogen particles in glial cells at different fetal ages**

<table>
<thead>
<tr>
<th>Gestational stage (wk)</th>
<th>18</th>
<th>20</th>
<th>21</th>
<th>24</th>
<th>27</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate zone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>AV</td>
<td>++</td>
</tr>
<tr>
<td>Cortical plate and subplate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Perivascular end-feet in cortical plate, subplate, intermediate zone, and plexiform zone</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Plexiform zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subpial end-feet*</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>NP</td>
</tr>
<tr>
<td>Subpial glia limitans</td>
<td>NYF</td>
<td>NYF</td>
<td>NYF</td>
<td>NYF</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Glial profiles in lower plexiform zone</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>AV</td>
<td>++</td>
<td>AV</td>
</tr>
</tbody>
</table>

+, small aggregates of beta particles and isolated glycogen particles in all glial profiles; ++, numerous, large aggregates of beta particles in all glial profiles; ++++, heavy glycogen loading in all glial profiles, occupying up to 40% of the area of glial profiles; ++++, heavy glycogen loading in all glial profiles, occupying more than 40% of the area of glial profiles; AV, glycogen in autophagic vacuoles in some glial profiles; NYF, not yet formed; NP, no longer present.

* In our material, a part of the most superficial, subpial glial end-feet seemed to transform into a subpial glia limitans between 27 and 30 wk gestation.
In the IZ, RGF were usually disposed in fascicles or groups of two to six (mean number of RGF/fascicle = 4) (Figs. 4–6), with the diameter of individual fibers varying between 0.35 and 2.9 \( \mu \)m (mean diam. = 0.8 \( \mu \)m). Interfascicular distance ranged from 1.5 to 8 \( \mu \)m. As for their orientation and intercellular relationship, these fascicles were radially disposed, ran parallel with other fascicles, and were in contact with either cell bodies of migrating neurons or with their neuronal processes. Many horizontal axonal profiles intercepted the radial fascicles of RGF at most regions of this zone (Fig. 4). In places where neuronal cell bodies were in intimate contact with the RGF, the glial fascicles were locally dissociated or defasciculated around the neuron (Figs. 4–6).

The fasciculated pattern of RGF and the process of local defasciculation were recognized on tangential and coronal sections of the IZ before 30 wk gestation.

By contrast, in the CP, all RGF, identified by their glycogen content, were singly disposed (Fig. 7). The radial glial fascicles were already defasciculated within the CP at 18 wk gestation. The diameter of RGF in the CP varied between 0.36 and 2.9 \( \mu \)m (mean diam. = 0.94 \( \mu \)m) and were separated from one another by a distance of 0.6–11.1 \( \mu \)m (Fig. 8), a space occupied by neuronal elements and cerebral vessels. In coronal sections, RGF of the CP were radially disposed and completely parallel with other radial glial and neuronal processes and with columns of neuronal somata (Fig. 7). At upper levels of the CP, columns of neuronal cell bodies clearly alternated with dendritic bundles and axons (Fig. 9A).

Certain RGF were bordered by columns of neuronal bodies (Fig. 9A), while others were seen in the center of dendritic and axonal bundles, alternating with the columns of neuronal cell bodies. At all different developmental stages examined, contacts of
the puncta adherentia type were noted between the RGF and the adjacent neurites (Fig. 9D).

A conspicuous feature of the material before 30 wk gestation was the systematic contact between migratory neurons and RGF. In the IZ, all neurons were in contact with three to six RGF and often in the center of a dissociated glial fascicle; in the CP, almost all neurons which clearly displayed a cytological character of migrating neurons were in contact with only isolated glycogen-labeled RGF.

Cytological Transformations of Radial Glia from
21 wk Gestation (Figs. 10, 11; Table 3)

The most conspicuous and constant developmental feature observed in the glial cytoplasm between 18 and 30 wk gestation was the radical transformation of the abundance and activity of the lysosomal apparatus (Figs. 10, 11). In 18- and 20-wk fetal brains, secondary lysosomes and autophagic vacuoles were absent in the entire
Fig. 8. Defasciculation pattern of RGF at CP levels in tangential sections in the normal human fetal brain at 18 wk. Postfixed with reduced osmium and stained with PA-TCH-SP. Arrows indicate RGF magnified in inserts B, C, D, E, F, G and identified by glycogen particles. Bar: 1.5 μm.
Fig. 9. Neocortical architectonics, upper levels of CP at 18 wk gestation, normal human fetal brain. A. Columns of neuronal somata alternating with bundles of neuronal and glial processes; postfixed with reduced osmium and stained with PA-TCH-SP. B. Magnified detail.
GLIAL EVOLUTION IN PRENATAL HUMAN BRAIN DEVELOPMENT

TABLE 2
Quantitative Data: Glial Fascicles and Radial Glial Fibers*

<table>
<thead>
<tr>
<th>Species</th>
<th>Prenatal development stage</th>
<th>Fascicles of radial glial fibers (N/1,000 μm²)</th>
<th>Radial glial fibers (N/1,000 μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cortical plate</td>
<td>Intermediate zone</td>
</tr>
<tr>
<td>Human†</td>
<td>18 wk</td>
<td>Absent</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>21 wk</td>
<td>Absent</td>
<td>NDA</td>
</tr>
<tr>
<td>Monkey†</td>
<td>E70</td>
<td>NDA</td>
<td>NDA</td>
</tr>
<tr>
<td>Normal mouse†</td>
<td>E14</td>
<td>19</td>
<td>NDA</td>
</tr>
<tr>
<td></td>
<td>E16</td>
<td>PCI</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>E17</td>
<td>Absent</td>
<td>NDA</td>
</tr>
<tr>
<td>Reeler mouse†</td>
<td>E17</td>
<td>13</td>
<td>NDA</td>
</tr>
</tbody>
</table>

* Counting performed on montages of tangential sections covering approximately 1,000 μm².
† All quantitative data based on our enumerations in the human material described in this paper and on our previous enumerations in normal and reeler mice (17). Data on embryonic day 70 monkey radial glial fibers derived from Levitt and Rakic (37).
‡ Sections used for these enumerations are part of a hemispheric ellipsoid of revolution whose half-axes measure an average of 11.5 and 7.5 mm.
§ Average half-axes at this level: 8.5 and 5 mm. PCI: precise counting impossible due to gradual defasciculation. NDA: no data available.

glial phase; rare primary lysosomes, however, were occasionally observed. In the 21-, 24-, 27-, and 30-wk-old fetal brains, lysosomes and autophagic vacuoles became conspicuous in a population of RGC at specific levels of the brain mantle (Table 3; Figs. 10, 11). The glial glycogen content was considerably more abundant around and within the autophagic vacuoles than in the other segments of the RGC population affected with this regional autophagic and lysosomal change (Fig. 10).

DISCUSSION

Identification of the Glial Phase

Postfixation with reduced osmium combined with the modified Thiéry histochemical staining method for fetal nervous tissue is a useful method for the ultrastructural analysis of neuronal–glial relationships during cerebral development. It is also useful for the study of radial glial transformation following the period of neuronal migration in normal and reeler mice (17) as well as in the human fetus (29). The presence of glycogen particles in glial cells is one of their essential morphological, biochemical and functional properties that generally differentiates the glial lineage from most neurons (17–20, 23, 30). For this reason, several investigators have previously attempted to use glycogen as an ultrastructural marker of glial cells in

from rectangle B in Figure 9A; RGF (asterisk) labeled by its glycogen content in the midst of a bundle of neurites. C. Magnified detail from rectangle C in A; RGF (asterisk) bordering a migrating neuron. D. Punta adherentia contact (heavy arrow) between RGF identified by its glycogen (light arrow) and dendritic profile; CP: coronal section; postfixed with reduced osmium and stained with U-Pb. Bar: 1.5 μm.

the developing cerebral cortical plate (CP), cerebellum, and spinal cord. However, these attempts (1, 7, 18, 31) to use glycogen as a glial marker have been only partially successful, due to the unpredictable glycogen preservation in glial cells following the classical steps of fixation, embedding, and staining for electron microscopy. In a previous study (17), we labeled the glial phase in normal and reeler mouse embryos with the use of reduced osmium and PA-TCH-SP methods for preservation and
Fig. 11. Sequential study of RGC transformation during the second half of gestation. Humal fetal brains, coronal sections. Postfixation with reduced osmium and U-Pb staining. A. Plexiform zone (PZ), 21 wk gestation. B. Intermediate zone (IZ), 24 wk gestation. C. PZ, 27 wk gestation. D. PZ, 30 wk gestation. Bar: 1.5 μm.

staining of particulate glycogen. Based upon quantitative data concerning the periodicity of glycogen-positive glial fibers, serial sections of radial glial fibers (RGF), and the continuity of glial labeling at the border of the pial surface, our previous study suggested that the reduced osmium and PA-TCH-SP methods are able to reveal most radial glial cells (RGC) along their entire length in the murine telencephalon (17). When applying these methods to the human fetal brain, our data, which are based on morphological observations and supported by semiquantitative estimates, suggest that almost the totality of RGF is identified all along their length by their glycogen content. One supportive argument comes from the number of glycogen-labeled profiles followed in consecutive tangential sections, and from the study of glycogen distribution in longitudinal sections of RGF. The continuity of
<table>
<thead>
<tr>
<th>Gestational stage (wk)</th>
<th>Intermediate zone</th>
<th>Cortical plate and subplate</th>
<th>Perivascular glial end-feet in cortical plate and intermediate zone</th>
<th>Lower part of plexiform zone</th>
<th>Subpial glial end-feet*</th>
<th>Subpial glia limitans</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>NYF</td>
</tr>
<tr>
<td>20 NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>NYF</td>
</tr>
<tr>
<td>21 Moderate proliferation† of lysosomes and autophagic vacuoles</td>
<td>NLY</td>
<td>Massive proliferation† of autophagic vacuoles and lysosomes</td>
<td>NLY</td>
<td>NLY</td>
<td>NYF</td>
<td></td>
</tr>
<tr>
<td>24 Moderate to massive lysosomal proliferation† with autophagic vacuoles</td>
<td>NLY</td>
<td>Moderate but less proliferation† of lysosomes</td>
<td>NLY</td>
<td>NLY</td>
<td>NYF</td>
<td></td>
</tr>
<tr>
<td>30 NLY</td>
<td>NLY</td>
<td>NLY</td>
<td>Moderate proliferation† lysosomes and autophagic vacuoles</td>
<td>NLY</td>
<td>NLY</td>
<td>NLY</td>
</tr>
</tbody>
</table>

NLY: no lysosomal proliferation (no detectable autophagic vacuole and no secondary lysosomes; rare organelles resembling primary lysosomes); NYF: not yet formed; NP: no longer present.

* Glial end-feet in the subpial part of the plexiform zone.
† When there is a proliferation of the lysosomal apparatus, the radial glial profiles are divided into two groups: one glial population with active autophagy and lysosomal abundance, and another completely devoid of lysosomal activity.
glial labeling at the border of the pial surface and along the vascular walls is another argument for the reliable identification of RGF by their glycogen content. A third argument supporting this concept is based upon the fact that the number of RGF counted in the CP correspond to the number of RGF enumerated in the intermediate zone (IZ) after taking into account the divergence of RGF in more superficial levels. In Table 2, the number of RGF per 1,000 μm² on tangential sections of human cerebral hemispheres (18 wk gestation) is reported. The selected planes of section were located in the mid-IZ (at 5 mm from the center of the ventricle) and in the mid-CP (at 8 mm from the center of the ventricle) and the number of RGF at these two levels were, on the average, 21 and 12 per surface unit, respectively.

The two concentric hemispheric planes passing through the IZ and CP levels would draw two ellipsoids of revolution as described in Materials and Methods with half axes of 5 × 8.5 mm at the IZ level and 7.5 × 11.5 mm at that of the CP. Since the total number of RGF (before RGF transformation to astrocytes) around the entire cerebral hemisphere that extend from the ventricular zone into the pial surface is the same in those two concentric planes and is equal to N, the fiber densities in any of these hemispheric planes would therefore be equal to N/4πXY where X and Y are the half small and half large axes, respectively. By relating the two fiber densities of the two planes, the order of dilution of RGF can be estimated as being equal to (11.5 × 7.5) ÷ (8.5 × 5) = 2.03.

This is in agreement with the data for RGF presented in Table 2 where the density of RGF at the CP level was 12 per 1,000 μm², and 21 at the IZ level. These results are fully consistent with the fact that all or most RGF detected in the IZ remain identified within the CP with our method and indicate that the risk of error in describing the periodicity of glia and neurites is not excessive.

Therefore, we assume that although an occasional glial profile might possibly be devoid of detectable glycogen particles, the RGF periodicity described in this study is a good approximation of the distribution of the entire glial phase in the developing central nervous system (CNS). The use of this method of glycogen preservation and staining permits a more accurate ultrastructural description of the process of glial defasciculation during corticogenesis. During the developmental epoch when the nuclei of the RGF are situated in the periventricular zone, the glycogen staining method permits the unequivocal differentiation of neuroblasts from cell bodies of such RGF, even during the mitoses (Fig. 1C). At the time when radial glial transformation to astrocytes (7) begins, this staining method also permits the differentiation of astrocyte cell bodies from neuronal ones (Fig. 3C).

Glial Phase Distribution Patterns and Glial–Neuronal Relationships (Fig. 12)

During the period of neuronal migration in the human neopallium, the dependence of migrating neurons on glial elements in zones of gliophilic migration (3) could be confirmed (32). Those neurons with cytological characteristics of migrating neurons were in direct contact with one or more glial fibers identified by their glycogen content at the ultrastructural level. In the light of such preferential association, it can be inferred that RGF act as “obligatory corridors” for neuronal migration in the neopallium. While RGF were grouped in fascicles at IZ levels from 18 to 30 wk gestation, they were totally isolated and dissociated in the CP by 18 wk. In the IZ, however, RGF within fascicles seem to undergo transient dissociation by neurons migrating along these fascicles. Our data demonstrated that the process of full defasciculation in the human CP occurred before 18 wk gestation, whereas it takes place between 15 and 17 days gestation in the mouse (17). It also seems that those temporarily
Fig. 12. Schematic hypothetical representation of RGC distribution pattern during the different developmental stages in the mammalian neocortex. A. Early embryonic stages; RGC regularly aligned. B. Migration stage of neurons destined for layers VI to IV; RGC grouped in fascicles throughout the entire thickness of the neural tube, ventricular zone to pial surface. 
C. Mammalian RGC distribution during migration period of neurons destined for layers III and II; migrating neurons defasciculate RGF in the CP by gradual neuronal saturation and glial dilution. In the human, this stage starts after 15 wk gestation; in the mouse, the transition occurs at the embryonic days 16 (stage B) and 17 (stage C). D. RGC distribution in the reeler mutant mouse when last waves of migrating neurons reach the CP (embryonic day 17); No intracortical defasciculation of RGC in this mutant. E and F. Gradual transformation of RGC into astrocytes after the end of neuronal migration along RGF. In the IZ of the normal human fetus (E), normal mouse (E) and reeler mouse (F), and in the CP of the reeler mouse (F), glial profiles seem to maintain their fasciculated pattern. In the CP of the human fetus (E) and normal mouse (E), this cytological transformation or “involution” of glial elongated fibers occurs on previously defasciculated RGF. In the human brain, this glial transformation occurs gradually between 21 and 40 wk of gestation, while in the mouse, this takes place between one and three wk postnatally. G. Stage of full transformation into mature glia. V: lumen of neural tube; ♂: neurons; ♀: glial cell body; dotted line: transition between CP and IZ.

dissociated fascicles at IZ levels never totally defasciculate, as RGF seem to transform into astrocytes without preceding defasciculation. Dissociated RGF in the human CP are more separated and diluted than in the monkey CP at embryonic day 70 (E70), which, in turn, are more diluted than in E17 fetal mice (Table 2). The phenomenon of gradually increasing RGF dilution in the CP seen in different animal species according to their phylogenetic ascension may be due to the increase in the total number of neurons throughout evolution, to the evolutionary changes in the size and differentiation of the neuronal elements, or to evolutionary changes in the quantitative ratio between neurons and RGC within each glial–neuronal unit. The collection of additional quantitative data from other animal species will help to define and clarify the role of these three factors in the phylogenetic progression of the neuronal vertical columns and modules. Furthermore, in the human brain, the early formation of layers VI to IV in comparison with the lengthy gestational period as well as the comparatively early occurrence in the transformation of RGF into astrocytes may also contribute to that glial dilution.

Transformation of the Radial Glial Cells During the Second Half of Gestation

Significant changes in the lysosomal apparatus within the RGF, often including massive proliferation of secondary lysosomes, autophagic vacuoles, and primary lysosomes were noted in the various developmental stages from 21 wk forward (Figs. 10, 11). This characteristic and systematic topography could be contrasted with most peripheral late subpial glial end-feet and the perivascular end-feet which were virtually free of such lysosomal changes. Lysosomal proliferation started at 21 wk in the distal connections of some RGF which were situated in the depths of the molecular layer or plexiform zone (PZ), a portion which seemed to be undergoing a process of autolysis. The IZ also seemed to be a site of such lysosomal proliferation in some RGF, with autophagic vacuoles beginning to appear at 21 wk gestation, while a certain number of RGF had no such lysosomal activity. In our 30-wk material, lysosomal hyperactivity was still present in the depths of the PZ, but seems more attenuated and on a smaller scale. The well-known process of RGF transformation to mature glia, which has been extensively studied using many methods and techniques (1, 7, 12, 33–37) undoubtedly implies the resorption of long RGF segments. The intense lysosomal hyperactivity and proliferation coupled with the presence of abundant autophagic vacuoles appearing in RGF during certain well-defined periods suggests that the lysosomal apparatus is responsible for glial transformation and resorption of parts of those structures. Moreover, early lysosomal activation seems to be a common feature of developmental cell death as well (38). Similar phenomena have been previously described in other species during embryogenesis or metamorphosis, including the resorption of certain organs and cell-groups (39–41). During the process of glial transformation towards the end of neuronal migration, the upward movement of RGF nuclei has been well described (7, 14, 36, 37). In our material, the process of lysosomal proliferation and the autophagic process in glial fibers seems to be initially prevalent in the upper parts of the RGF, possibly reflecting resorption of the distal portions of the fibers which are transforming and, probably evolving concomitantly with the upward migration of the glial cell body. These results are in line with the findings of Marin-Padilla (6) suggesting that distal glial processes disappear between 20 and 28 wk.

Based on the chronological sequence of cytological transformations, two distinct populations emerged in the glial phase. In the first group, early lysosomal proliferation and autophagy became conspicuous in the distal RGF segments at 21 wk. The second RGF population remained devoid of detectable cytological transformations at that stage. This suggests an all-or-none phenomenon which appears plausible since there remained a significant number of neurons that had not yet migrated upwards. The intact RGF could continue to guide late-arriving neurons.

The existence of a late transforming population of RGF, presumably related to late-migrating neurons, seems to be in harmony with the concept of a gradual process of RGF transformation previously described in rodents and primates. Schmechel and Rakic (33, 36), studying that process of transformation in the developing monkey brain, found that although glial transformation to astrocytes and ependyma begins during the first half of gestation, it may be completed as late as the second postnatal month, long after the end of neuronal migration. The late subpial as well as perivascular end-feet were seen in our material to be almost totally free of autophagic and lysosomal hyperactivity. It might, therefore, be inferred that the subsequently developing subpial and perivascular glia limitans are formed from that RGF population and later-to-be transformed glial cells that do not undergo lysosomal autolysis. This may partially explain the presence, at earlier stages, of two types of distal

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RGF terminations in the PZ: one with autophagy and lysosomal proliferations, and a second one without, implying that these latter intact RGF participate in forming the subpial glia limitans membrane. Only the end-feet of the intact RGF population may be needed to form that membrane. At 30 wk gestation, the distal connections of RGF seem to be less numerous with less intense lysosomal and autophagic hyperactivity. Palay, in describing the glia limitans in the postnatal rat cerebellum (42), depicted two forms of glial end-feet that formed the subpial and the perivascular glia limiting membranes: one with a leaf-like appearance, termed "foot-process" and a second with a "bulbous terminal." Pointing out the abundance of membranous whorls and lysosomes, particularly in the second type of glial termination, and based upon evidence from tissue culture, he speculated that the potential pleomorphic capacity of such end-feet and the ability to change in form and intercellular relations may well be an essential feature of these foot-processes.

Although our present analysis of human material did not include an electron microscopic study of lysosomal acid phosphatase, we believe that our observations should remove most doubts regarding the significance of autophagic and lysosomal proliferation. However, it remains a moot point whether certain lysosomes that show fine granular content might, in fact, be primary lysosomes in proliferation or, alternatively, whether they represent lysosomes containing some undigestable material, for example, the intermediate filaments which accumulate within the digestive apparatus (Fig. 10A). Animal studies using histoenzymologic methods are presently underway in our laboratory to study the functional role of lysosomal acid-phosphatase in RGF at the end of neuronal migration.

If one assumes that glycogen in detectable amounts is shown in most glial elements with optimal fixation methods, then the ultrastructural identification of the entire glial phase and the neuronal–glial interactions herein described may lead to new insights into human cytoarchitectural development. This method may now be applied to investigate pathological human fetuses, particularly in cases with malformations related to disturbances in neuronal migration.

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