Zeitschrift für Zellforschung 91, 341—365 (1968)

An Electron Microscope Study of Cells in the Matrix and Intermediate Laminae of the Cerebral Hemisphere of the 45 mm Rabbit Embryo

LARRY J. STENSAAS* and SUZANNE S. STENSAAS
Departamento de Ultraestructura, Instituto de Investigación de Ciencias Biológicas
Avenida Italia 3318, Montevideo, Uruguay

Received April 29, 1968

Summary. The morphology and intercellular relations of cells in the matrix, lower intermediate, and upper intermediate laminae of the cerebral hemisphere of rabbit embryos was studied with the electron microscope. Models of cells reconstructed from serial sections confirm previous observations made with the Golgi technique. Most cells in the matrix lamina appear to be spongioblasts; there are relatively few neuroblasts and columnar epithelial cells. Neuroblasts predominate in the intermediate lamina. Their short processes are intercalated among axons and spongioblast processes in the lower part. A large process, the preapex, distinguishes nerve cells in the upper part of the intermediate lamina, and its orientation in the direction of movement suggests that it may actively participate in the migration of neuroblasts.

Serial section analysis confirms the fact that mitotic cells in the matrix lamina are spherical and have no processes. Assuming that neuroblasts are incapable of further division, it seems probable that intermitotic germinal cells have the form of spongioblasts and columnar epithelial cells and that they give rise to neuroblasts and other spongioblasts.

The origin of nerve and glial cells from a parent or germinal element near the inner surface of the embryonic central nervous system and their migration away from this zone to form definitive structures of the brain and spinal cord was inferred by investigators soon after the inception of histologic methods for staining cell cytoplasm and nuclei. HIS (1891) distinguished two types of cells in the matrix lamina: (1) large germinal cells near the ventricle that undergo frequent division and give rise to neuroblasts, and (2) elongate spongioblasts, or primitive neuroglial elements, with somata located a short distance from the ventricle but attached to it by a process of variable length. Important new information regarding the detailed morphology of cells in the matrix lamina was provided by studies with the Golgi technique (RAMÓN Y CAJAL, 1890) which showed that two types of cells could be recognized. Epithelial cells are elongate bipolar cells with processes contacting both pial and ventricular surfaces, while neuroblasts are first unipolar and later develop a bipolar shape as they migrate from the matrix

* Supported by a postdoctoral fellowship from the United Cerebral Palsy Research and Educational Foundation and a United States National Institutes of Health fellowship No. NB 28,013—01A1.

lamina to more superficial levels. By revealing the presence of more than one type of cell in this mitotically active zone, studies with the Golgi technique seemed to support HIS's postulate that two types of cells form the matrix lamina. SCHAPER (1897), on the other hand, believed that there was a third basic cell type in the pseudostratified epithelium of the matrix lamina. He referred to it as the "indifferent" cell to distinguish it from ependymal (spongioblast) and germinal elements of this layer, and he ascribed to it a capacity for migration. Both nerve and glial elements were supposed to originate from this mobile, undifferentiated cell and to be distinguishable in higher vertebrates only after differentiation in the superficial layers outside the matrix lamina. Although SCHAPER described no morphologic features by which the indifferent cell could be recognized, it has recently been reported to be present in the cerebral hemisphere of postnatal rats (CALEY and MAXWELL, 1968) and chicken embryos (SENSENBRENNER and MANDEL, 1967).

Experiments by F. C. SAUER (1935) and WATTERSON *et al.* (1955) using colchicine to study cell division in the neural tube of chick embryos and by M. E. SAUER and WALKER (1959) with cytophotometric methods led these investigators to conclude that the matrix lamina was composed of a single cell type and that the germinal cells and spongioblasts of HIS simply represent different phases in the life cycle of a single, mitotically active cell type.

Similar conclusions were reached from recent experiments in which autoradiographic techniques were used to label cell nuclei synthesizing DNA prior to cell division (FUJITA, 1962, 1963). This method does indeed permit the dynamics of cell proliferation and migration to be studied in great detail, but it does not provide information about the external morphology of the labeled cells. Accordingly, conflicting opinions have arisen regarding the form of germinal cells and the changes they undergo in the process of giving rise to nerve and glial elements. SAUER and WALKER (1959), SIDMAN *et al.* (1959), FUJITA (1963), and LANGMAN *et al.* (1966) suggest that germinal elements have the shape of columnar epithelial cells while replication of DNA occurs in nuclei located in the external part of the pseudostratified matrix epithelium. The somata then move toward the ventricle, and the cells round up and divide, presumably to form neuroblasts, spongioblasts, or more germinal cells. On the other hand, BERRY and ROGERS (1965) suppose that germinal cells correspond to spongioblasts seen with the Golgi technique. According to their investigations, nuclei of these cells first synthesize DNA in the outer part of the matrix lamina, move toward the ventricle, and divide while the cell itself maintains contact with the pia by a long thin process. Instead of immediately forming two new cells, one nucleus remains in the matrix lamina while the other migrates toward the pia within the distal process where a new neuroblast ultimately develops by budding. This cell than develops an axon and dendrites.

Electron microscope studies of the ultrastructure of the matrix lamina have been made in a variety of embryos at different stages of development. They reveal a pseudostratified epithelium consisting of (1) round, mitotic cells and (2) elongate elements with a bipolar shape. Lack of appreciable differentiation in the nuclei

and cytoplasm of these elements seems to support the concept of a single, undifferentiated cell type (FUJITA, 1963; MELLER *et al.*, 1966a, b; and WECHSLER, 1966a, b). Therefore, dividing and columnar cells were believed to represent different forms of a single population whose morphology changes as it passes through different phases of a synthetic and mitotic cycle.

Conflicting with this interpretation is the fact that four types of cells were described in the matrix lamina of the cerebral hemisphere of rabbit embryos in a recent study of cortical development using the rapid Golgi technique (STENSAAS, 1967b). Neuroblasts undergo characteristic changes in shape as they migrate from the matrix lamina, while freely ending spongioblasts, typical spongioblasts, and astroblasts apparently represent early stages in the development of neuroglial cells. No elements were seen which could unequivocally be identified as germinal cells.

It is of considerable theoretical importance to resolve apparent discrepancies between electron microscope and Golgi results, to correlate morphologic data with autoradiographic information, and from this to ascertain the identity of cells giving rise to neuroblasts. Only a method capable of showing both the three dimensional form and the ultrastructural characteristics of cells at different levels provides the necessary information. In this study, serial section analysis and three dimensional reconstruction of cells in the matrix and intermediate laminae of rabbit embryos have been used in an effort to (1) determine the morphology of germinal cells, (2) verify recent descriptions of neuroblasts and spongioblasts made with the rapid Golgi technique, and (3) identify and describe intercellular relations of neuroblasts which might help in understanding the process of cell migration.

Material and Methods

Fourty five mm ($\pm$ 2 mm) embryos obtained from New Zealand white rabbits anesthetized with pentobarbital were fixed by perfusion for electron microscopy by slowly injecting a phosphate buffered osmic acid solution (MILLONIG, 1962) into an umbilical vein or directly into the heart. Brains of embryos in which perfusion failed, or was incomplete, were prepared with a modification of the rapid Golgi technique (STENSAAS and STENSAAS, 1968). Embryos in which darkening of the nervous tissue could be seen through the semi-transparent calvarium were carefully bisected, and the two halves of the head were immersed in the fixative for an additional two hours. Mechanical damage was minimized during dehydration by leaving the hemisphere within the embryonic calvarium and suspending it by threads in a graded series of alcohols followed by anhydrous acetone. Brains from 5 embryos were embedded in Araldite.

Precisely oriented blocks of the lateral wall of the pallium were cut from the intact hemisphere and remounted on plastic blocks with epoxy resin. The exact level and plane of orientation of serial thin sections was determined from thick (0.5 μ) sections stained with methylene blue. Alternating thin (silver to gray) and thick (blue to green) vertical, transverse serial sections were cut which extended from the ventricular surface almost to the base of the cortical plate (Fig. 1). Two strips of serial sections, each containing 30 to 40 sections, were mounted lengthwise in Sjöstrand-type one hole (1 mm $\times$ 2 mm) grids and stained with uranyl acetate (HUXLEY and ZUBAY, 1961) and lead citrate (REYNOLDS, 1963). Of several series of sections prepared with a LKB Ultrotome, only one was analyzed in detail. This consisted of 9 grids containing 450 continuous serial sections. Approximately 10 to 15 sections scattered throughout the series were lost. Their absence did not appreciably effect the continuity of the series so that a given structure could be followed without difficulty from one section to the next and from grid to grid.

Photographic montages at a total magnification of 6,000 × were prepared of vertical sections near the beginning and the end of the series and of a horizontal section of the upper part of the intermediate lamina (Fig. 13) using an RCA EMU 2C electron microscope. They were used to establish landmarks for detailed serial section analysis and to prepare the drawings shown in Figs. 3—5. For the latter certain components were mapped using colored crayons, traced in pencil onto white paper, reduced photographically and retraced on velum with india ink.

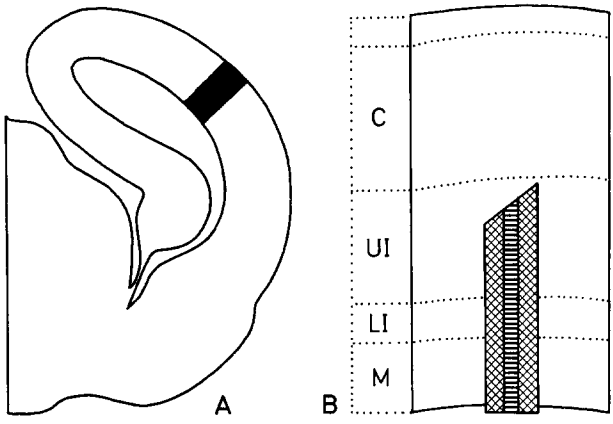


Fig. 1 A and B. The outline drawing in A represents a transverse section of the cerebral hemisphere of a 45 mm rabbit embryo and shows the area (black) from which material was selected for this study. B is a diagram showing the area in more detail. Dotted lines indicate the limits of the matrix (*M*), lower intermediate (*LI*), upper intermediate (*UI*), and cortical plate (*C*) laminae. The dark trapezoid extending from the ventricle almost to the cortical plate indicates the area from which serial sections were taken. The hatched zone shows the size of serial section 1, the crosshatched zones the size of serial section 450

Serial electron micrographs were taken at certain levels of the matrix, lower intermediate, and upper intermediate laminae; the location and approximate size of the area covered by the photographs is shown in Fig. 2. Sections having a silver or gray color were photographed at an initial magnification of 2,500, enlargements at 7,500 or 10,000 × were prepared, and individual cells, identified by number, were outlined with different colors and followed through the series. Models were constructed by tracing cell and nuclear outline onto paper, placing the patterns over warm wax sheets, and cutting them out. Nuclei whose relative proportions were known from light microscopy were assembled first in order to confirm calculations for the thickness of wax sheets. To assemble a model, individual wax profiles were heated (by dipping in a bath of very hot wax), pressed together, the surface smoothed, and the entire model dipped in white wax to provide a surface adequate for drawing.

Proper alignment of the individual wax profiles forming a cell was assured through reference to a composite tracing of that cell prepared by superimposing all outlines on a single sheet of paper. Adjacent nuclei of three or more cells were used as points of reference for orienting each section where there was doubt about horizontal or vertical displacement of the cell membrane in successive photographs.

Results

Golgi Impregnations

As described in an earlier publication (STENSAAS, 1967a), neuroblasts have a characteristic form depending on their location. Details of their morphology are

summarized in Fig. 2 in which nerve cells (black) were drawn from Golgi preparations of rabbit embryos at this stage of development. Cells in the lower part of the matrix lamina are bipolar with a short, proximal process extending toward the ventricle and a long, thin distal one, the axon, oriented vertically. The latter frequently turns near the upper limit of the matrix lamina and runs parallel to axons forming the embryonic white matter. Neuroblasts in the upper part of the matrix lamina may have a short vertical process in addition to the axon.

In the lower intermediate lamina cells generally have a horizontal, elongate shape. The presence of short, horizontal and vertical processes imparts a characteristic rectilinear profile. Most axons extend horizontally but some are vertical. Neuroblasts in the upper intermediate lamina are bipolar. A large, tapering process, the preapex, is oriented toward the pial surface while a smaller one, the axon, either descends to the lower intermediate lamina and becomes horizontal or descends a short distance before becoming vertical.

Spongioblasts (Fig. 2, hatched) in the matrix lamina are distinguished from neuroblasts by the presence of a large proximal process extending to the ventricle. In general, the distal process seems to have a somewhat larger diameter than vertical axons of neuroblasts. Two cell types have been recognized: (a) *typical spongioblasts* have a distal process that extends to the pia, and (b) *freely ending spongioblasts* have a distal process that ramifies and terminates within the intermediate lamina or cortical plate. Local enlargements along the distal process are larger and seem to be more common than similar structures situated along axons of neuroblasts.

Elements with the shape of columnar epithelial cells (Fig. 2, stippled) have somata attached to the ventricle by a process of varying length. They have either no distal process or a very short one and are therefore easily distinguished from spongioblasts. In brains in which there are few elements stained in the superficial layers it is possible to mistake incompletely impregnated spongioblasts for columnar epithelial cells. In well impregnated Golgi preparations, however, the consistency with which they are observed in the matrix lamina suggests that they should be recognized as a cell type distinct from neuroblasts and spongioblasts. These elements are referred to as columnar epithelial cells to avoid confusion in terminology since there is presently no unequivocal way to equate them with interphase germinal cells or matrix cells described by other authors.

Electron Microscopy — Matrix Lamina

Fig. 3 shows that the matrix layer primarily consists of tightly packed cells. Somata of most cells in the lower half are vertically elongate, but they are more or less equi-dimensional or cuboidal near the intermediate lamina. A narrow zone bordering the ventricle has a distinctive structure. This was recognized by His (1891) who referred to it as the "zone or arcades". In addition to numerous, large, mitotic cells, modern studies have shown it to consist of vertically oriented processes (Bellairs, 1959; Duncan, 1957; Fujita and Fujita, 1964; Lyser, 1964; Tennyson and Pappas, 1962; Wechsler, 1966a, b). Both cell somata and

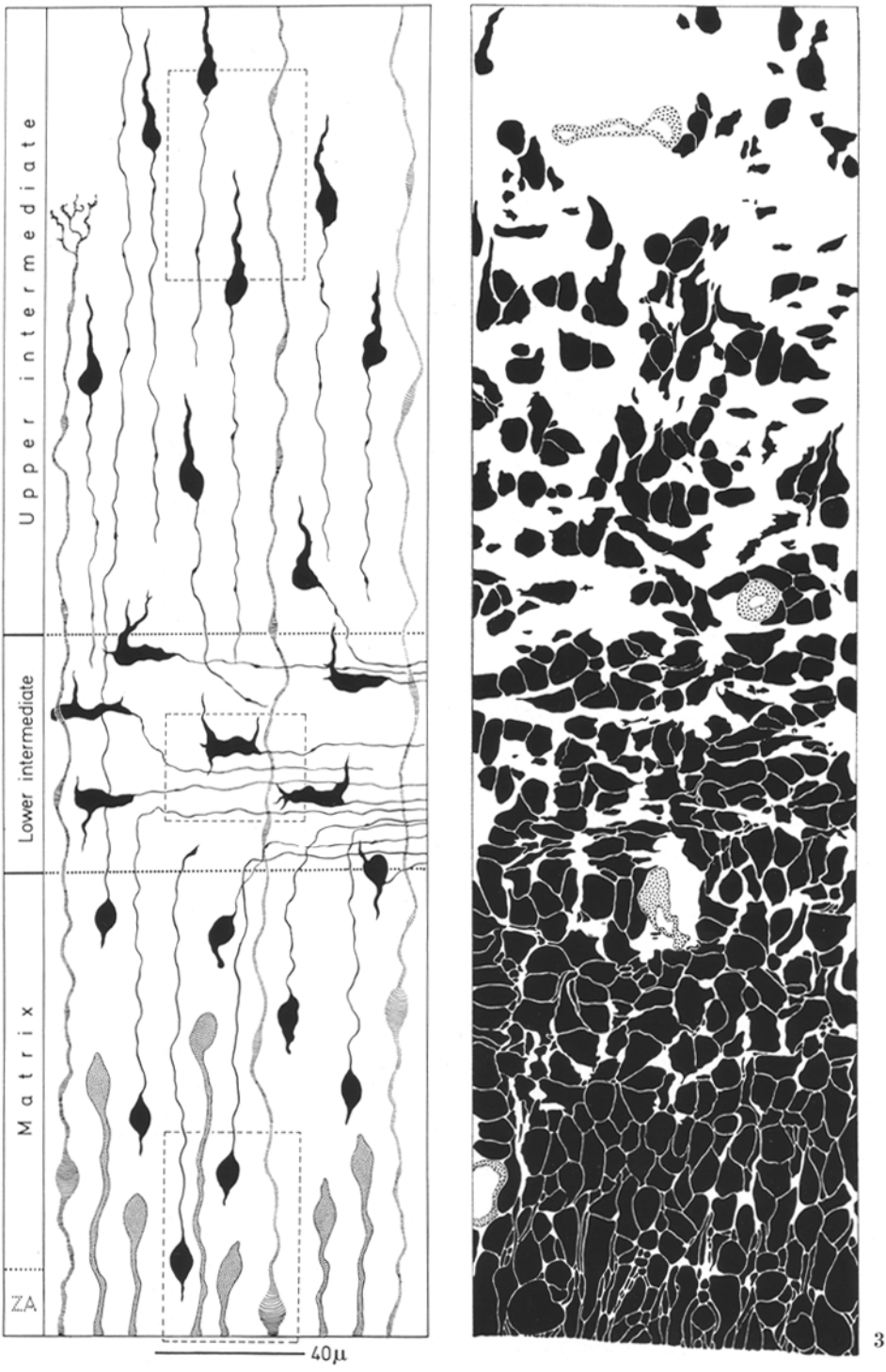
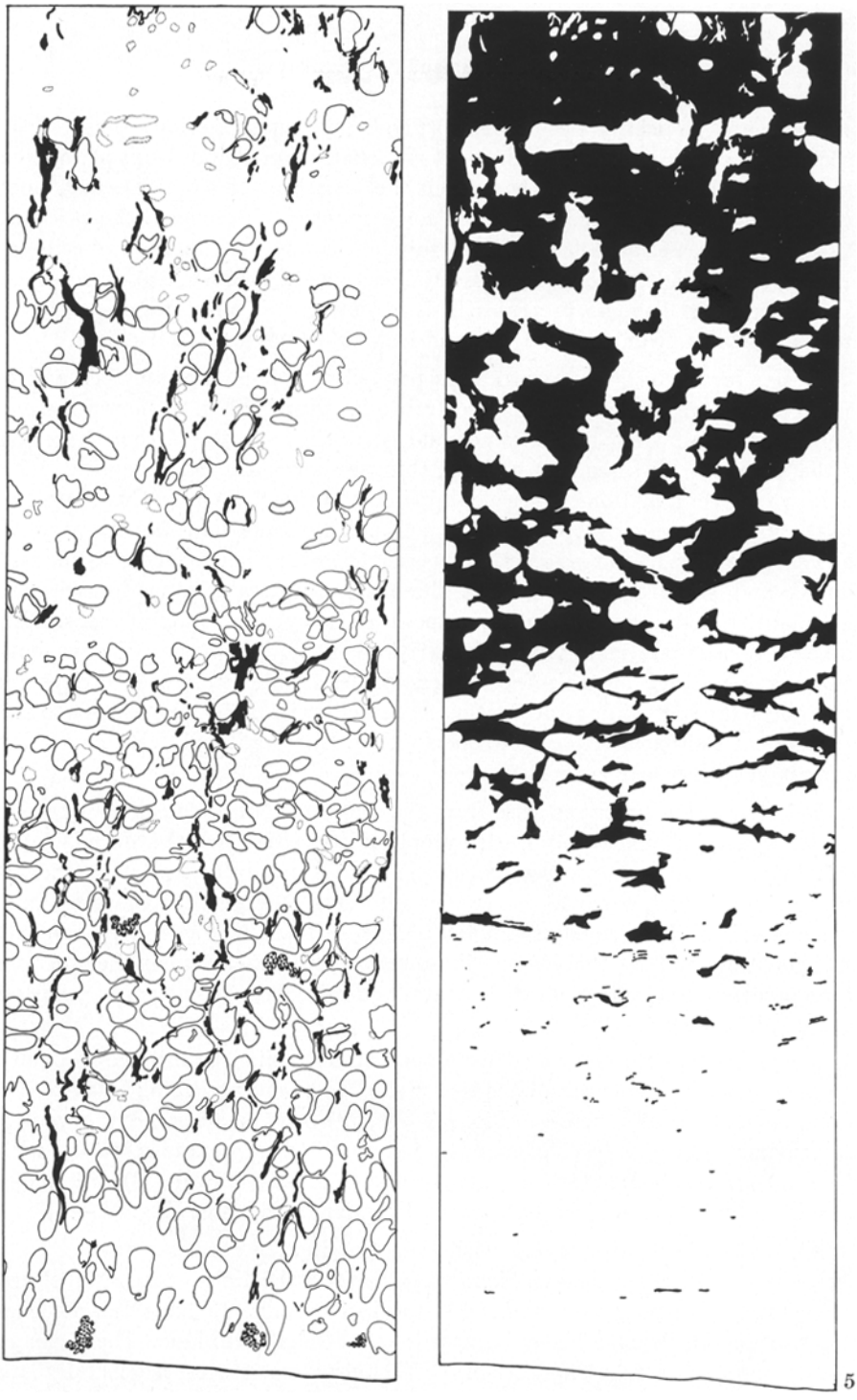


Fig. 2. Scale drawing of Golgi impregnated cells in the dorsolateral pallial region. Neuroblasts are shown in black, spongioblasts are hatched, and columnar epithelial cells are stippled. Horizontal dotted lines mark the limits of the matrix, lower intermediate, and upper intermediate laminae. Dashed lines forming rectangles indicate the level and approximate size of the area covered by serial electron micrographs used to construct cell models



Figs. 3—5 are diagrams of serial section 37. In Fig. 3 the form and distribution of cell somata and processes containing cytoplasm rich in ribosomes are shown in black. Capillary outlines are stippled. In Fig. 4 nuclear profiles are indicated by solid lines, vertical processes containing dark cytoplasm poor in ribosomes are black, and the outlines of large processes with light cytoplasmic matrix are dotted. Chromosomes of mitotic cells are outlined and stippled. Fig.5 shows the distribution of horizontal processes assumed to be embryonic axons. Individual nerve fibers in the matrix lamina can be distinguished while groups of these elements predominate in the intermediate lamina

processes are joined by desmosomes (Fig. 6); in serial sections these sites of specialized attachment are seen to form continuous bands uniting adjacent elements at the point where they reach the ventricle. In single electron micrographs irregularities in the surface of some mitotic cells suggest the possibility that small processes arise from them. However, careful examination of cell membranes of many mitotic elements in serial sections has shown that dividing cells are spherical and have no processes. This is also true of mitotic elements located at some distance from the ventricle and lacking attachment to it (Fig. 4).

Large, vertically oriented processes partially surround mitotic cells and terminate at the ventricle. In single electron micrographs (Fig. 6) they are seen to contain cytoplasm rich in ribosomes and to range from thick, short extensions of the soma (4 μ in diameter) to long, thin prolongations. Mitochondria, microtubules and accumulations of vesicles apparently belonging to the Golgi apparatus are the most common cytoplasmic organelles. A centriole can often be seen near the ventricle (Fig. 6) and is sometimes associated with a single cilium as shown by SOTELO and TRUJILLO-CENÓZ (1958). There is a considerable range in variation of the quantity and apparent density of perinuclear cytoplasm among cells. Differences in density are mainly due to variations in the cytoplasmic matrix, but in some instances they may also be related to the concentration of free ribosomes. It is not a product of uneven fixation since cells with light and dark cytoplasm are frequently situated adjacent to one another. In general, light cells have a large amount of perinuclear cytoplasm.

Relatively thin processes arise from the distal pole of some cell somata. Most are filled with a moderately dark cytoplasmic matrix, but the density of the cytoplasm varies from process to process and is generally lighter in the local enlargements which occur at infrequent intervals. The vertical processes in the upper matrix and intermediate lamina, shown in black in Fig. 4, are assumed to originate from cells at this level. Ribosomes are rare except near the site where these processes originate from the somata, and mitochondria generally occur only in the enlargements.

Models of cells reconstructed from the matrix lamina permit the recognition of three types of elements: (1) cells attached to the ventricle and having both proximal and distal processes (Fig. 7A—S), (2) cells attached to the ventricle by proximal processes having, at most, a short distal process terminating within the limits of the serial electron micrographs (Fig. 8A—E), and (3) cells lacking attachment to the ventricle, but having long distal processes (Fig. 8F—J).

Elements of the first type, with long proximal processes attached to the ventricle and distal processes which extend beyond the limits of the serial electron micrographs, are most common. Of a total of 29 cells, 19 have this form and closely resemble spongioblasts seen with the Golgi technique. Their elongate somata have an average diameter of 5 μ. The proximal process is generally larger than the distal process and ranges between 0.5 and 2 μ in diameter. It commonly terminates in a slight enlargement at the ventricle, and a single, short cilium arises from 4 of the reconstructed cells. Swellings or local enlargements up to 1.5 μ in diameter are present on both proximal (Fig. 7 M, N, Q, S) and distal (Fig. 7, B, F) processes.

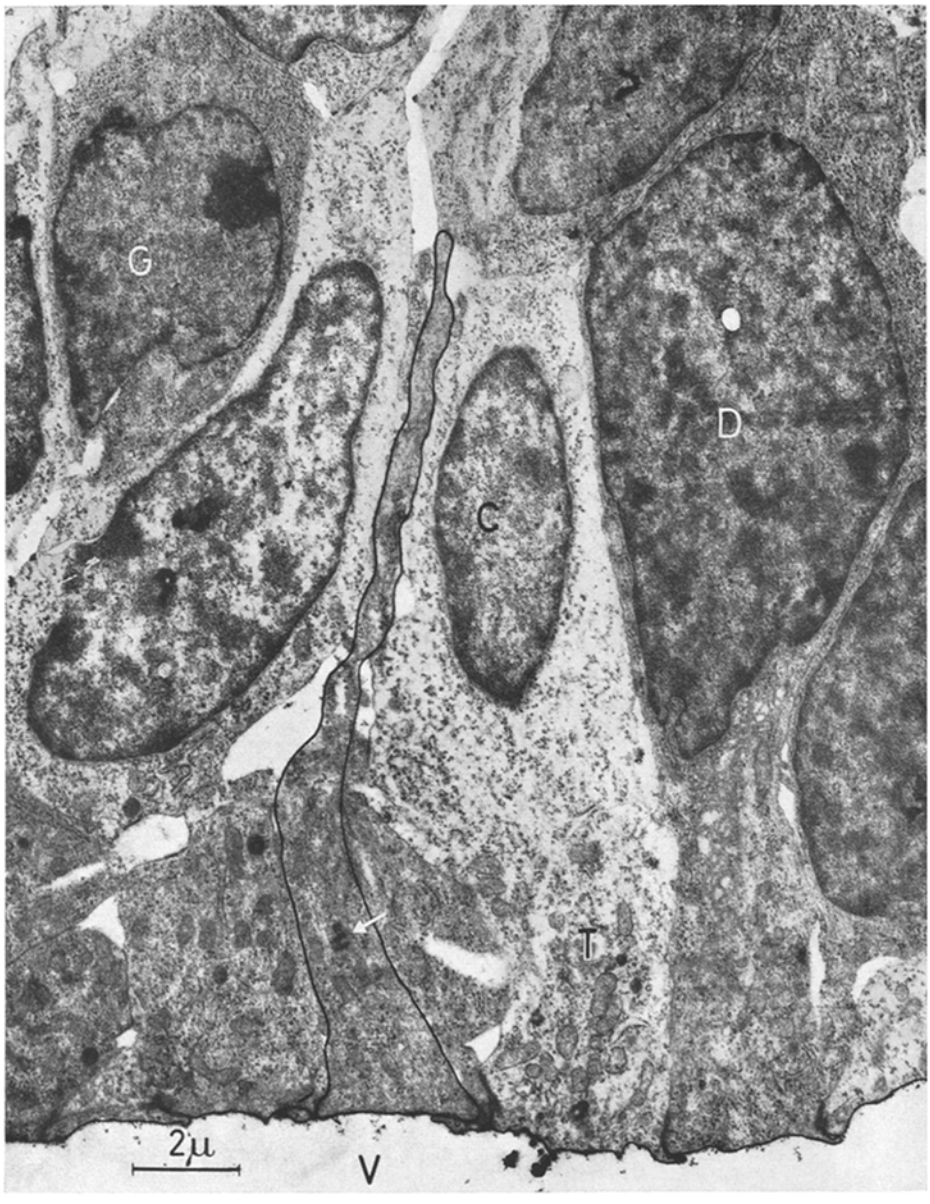


Fig. 6. Electron micrograph of cells in the matrix lamina near the ventricle (*V*) showing differences in the density of cytoplasm of adjacent cells. Cells *C*, *D*, and *G* of Fig. 7 are labeled. Cytoplasm rich in ribosomes, mitochondria and tubules (*T*) fills the processes which extend toward the ventricle. Centrioles (arrow) are also common near the ventricle and are sometimes associated with a single cilium

Five cells were reconstructed which have proximal processes extending to the ventricle and lack a well defined distal process. Their shape closely corresponds to that of columnar epithelial cells seen with the Golgi technique. They are

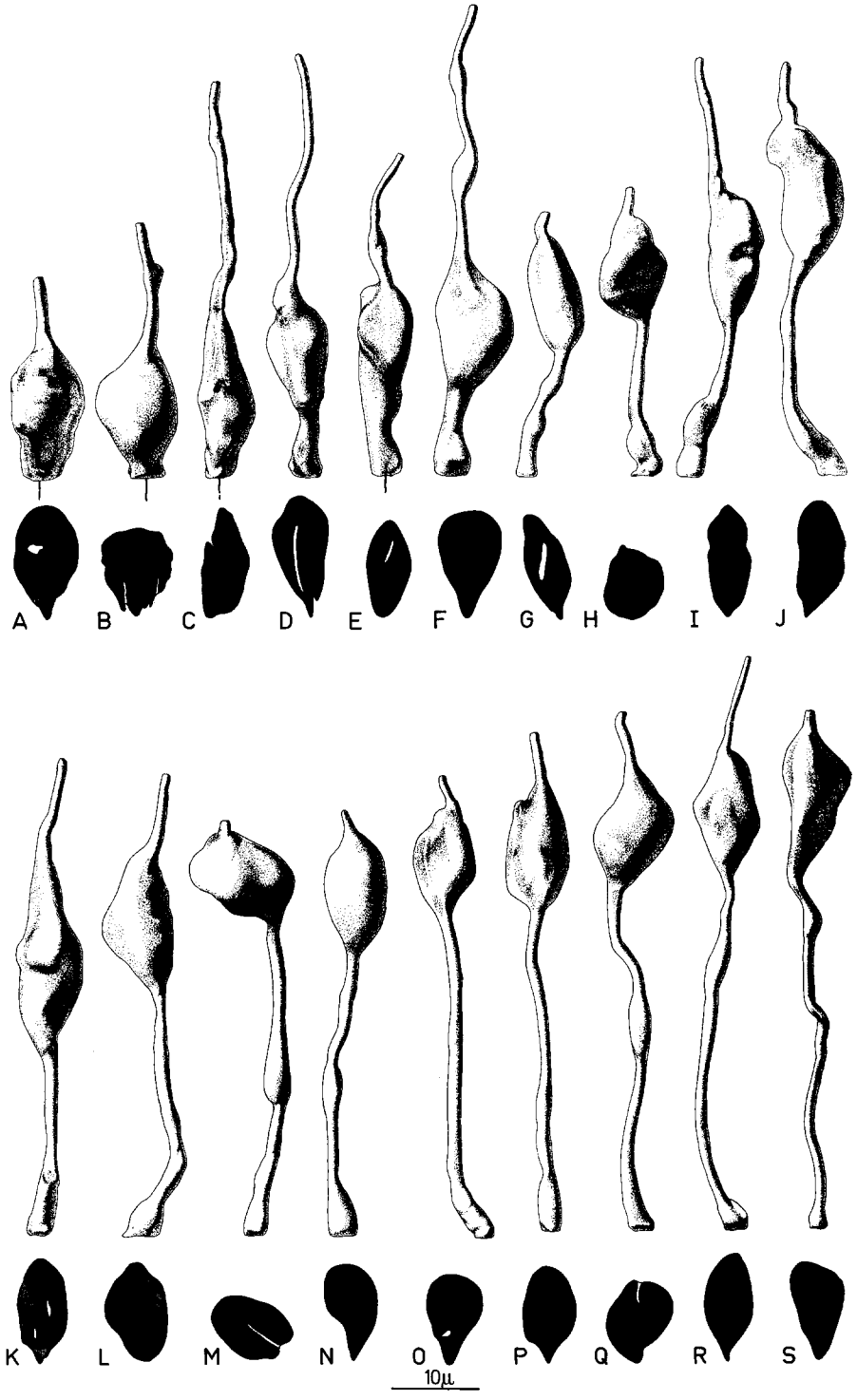


Fig. 7

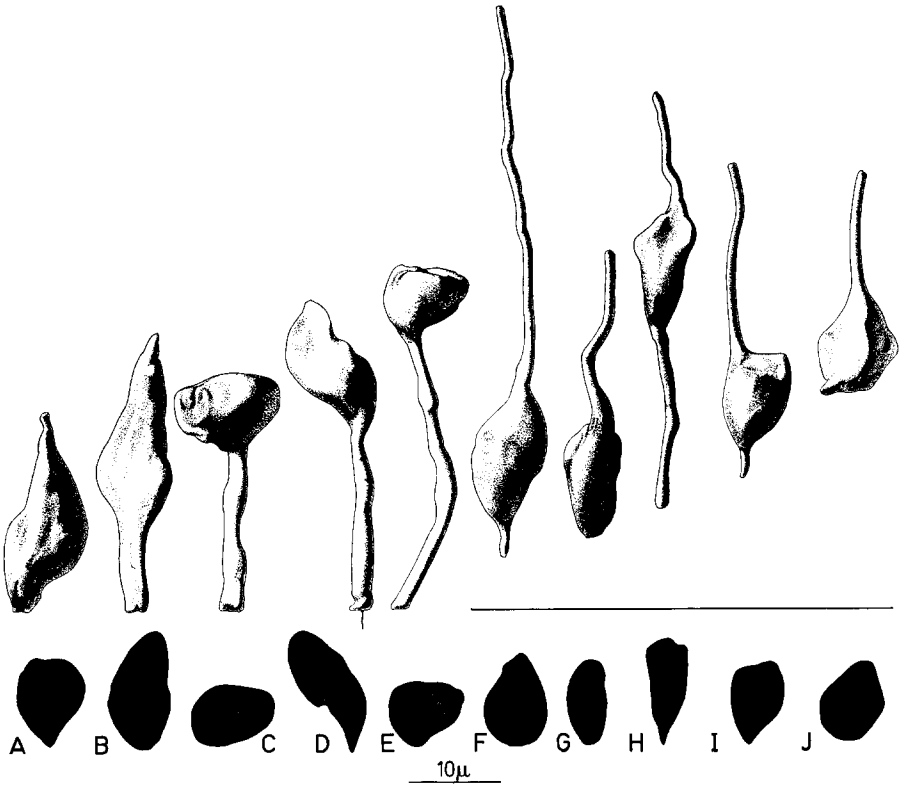


Fig. 8. Models of cells in the matrix lamina identified as columnar epithelial cells (A—E) and neuroblasts (F—J). Columnar epithelial cells are attached to the ventricle by a proximal process of variable length. They are distinguished from spongioblasts by having either no distal process (C—E) or a rudimentary one (A, B) which terminates within the limits of the serial electron micrographs. Neuroblasts have no connection to the ventricle (line) but may have a proximal (F, H, I, J) process in addition to an axon which extends beyond the limits of the serial electron micrographs. Models of cell nuclei (black) may have one or more deep invaginations (crosshatched) and are frequently pointed at one end

attached by desmosomes to adjacent elements of the ventricle, and a cilium is present on the proximal process of cell D, Fig. 8.

Cells lacking a ventricular connection are not common and appear to correspond to neuroblasts at this level in Fig. 2. Their shape and relation to the ventricle is shown in Fig. 8. Four cells (F, G, I, J) lack a well defined proximal process while that of H is 18 μ long. All have distal processes which extend beyond the limits of the photographs.

Fig. 7A—S. Models of cells in the matrix lamina identified as spongioblasts are arranged according to the length of the proximal process. The distal process extends toward the pia; all are incomplete because they continue beyond the limits of the serial electron micrographs. Localized swellings or enlargements are located at irregular intervals along the course of both proximal and distal processes. Models of cell nuclei (black) frequently have one or more deep invaginations and are pointed at one end. Invaginations on the side nearest the observer are shown in white; they are crosshatched when located on the opposite side

Models of cell nuclei in the matrix lamina generally have one or more shallow invaginations and most are pointed either at the end proximal or distal to the ventricular surface. There is no apparent correlation of nuclear orientation with a particular cell type, and there is presently no way to determine whether the asymmetry is indicative of the direction of migration. Nuclei of most cells in the matrix lamina are filled with dark, homogeneous nucleoplasm, and 3 to 5 small nucleoli may be located either centrally or near the nuclear envelope.

Small, horizontal processes identical to nerve fibers of the embryonic white matter occur at all levels in the matrix lamina, but they are not common (Fig. 5). The origin of these processes is not known with certainty, although several have been followed in serial thin sections for a considerable distance. None have been seen to arise from cells in this layer, but one descended from the intermediate lamina.

Electron Microscopy — Lower Intermediate Lamina

In single thin sections, cells at this level have a characteristic, elongate, rectangular profile which is distinct from the square or round outlines of elements in the upper part of the matrix lamina (Fig. 3). Irregular groups of 5 to 10 closely apposed cells are separated by masses of horizontal fibers forming the embryonic white matter (Fig. 5). One or more cell processes often extend parallel to these horizontal fibers in the lower part of this layer (Fig. 3) while such cell extensions are less numerous on elements in the upper part of the lamina.

Nucleoplasm is dark, finely speckled, and similar in appearance to nuclei in the matrix layer. A moderately dense, cytoplasmic matrix is typical of these cells and varies little from one element to the next (Fig. 9). Many free ribosomes occur both in perinuclear cytoplasm and within the large processes; the latter are easily distinguished from unmyelinated nerve fibers even in single thin sections. Mitochondria, cisternae of the Golgi apparatus, and endoplasmic reticulum are few in number. They are situated in the perinuclear cytoplasm near the origin of the larger processes on the side of the cell opposite the ventricle. A single, small, horizontal process filled with finely-textured cytoplasm containing no ribosomes arises from most cells. They may be quite long and are considered to be axons since they are identical to the unmyelinated axons forming the intermediate lamina.

Individual fibers forming groups of horizontal axons usually range between 0.2 and 0.4 μ. As shown in the studies of WECHSLER (1966c) they are closely apposed to one another, and no signs of incipient myelinization have been seen at this stage of development. Local enlargements are present along the course of a few fibers and are filled with a lighter, finely textured cytoplasm. Similar enlargements are found along the course of vertical processes where they are more frequent and present a greater range in the electron density of cytoplasm. Enlargements of both vertical and horizontal processes seem to be most common in the vicinity of adjacent cell somata. In the chick retina MELLER (1964) assumes that light cytoplasm is characteristic of spongioblast processes. There is no direct evidence to support such a view in rabbit embryos, but spongioblast processes have not been followed from the matrix lamina into the lower intermediate lamina. Several vertical processes run together (Fig. 9) and are usually surrounded by groups of cells and their processes.

Fig. 9. Electron micrograph of cells in the lower intermediate lamina. Cells *B*, *D*, *F*, *G*, *I* and *J* of Fig. 10 are outlined to indicate their irregular shape. A thin rim of cytoplasm containing numerous free ribosomes surrounds the nucleus except where processes arise from the side opposite the ventricle. Masses of small horizontal processes, assumed to be axons (*A*), separate groups of cells while small aggregates of vertical processes commonly parallel ribosome-rich cell extensions. Cytoplasm of the vertical processes range from light (*) to dark (arrow) with a light matrix predominating at the sites of local enlargements

In the lower intermediate lamina there is a striking correspondence between the form of the models (Fig. 10) and cells seen with the Golgi technique (Fig. 2).

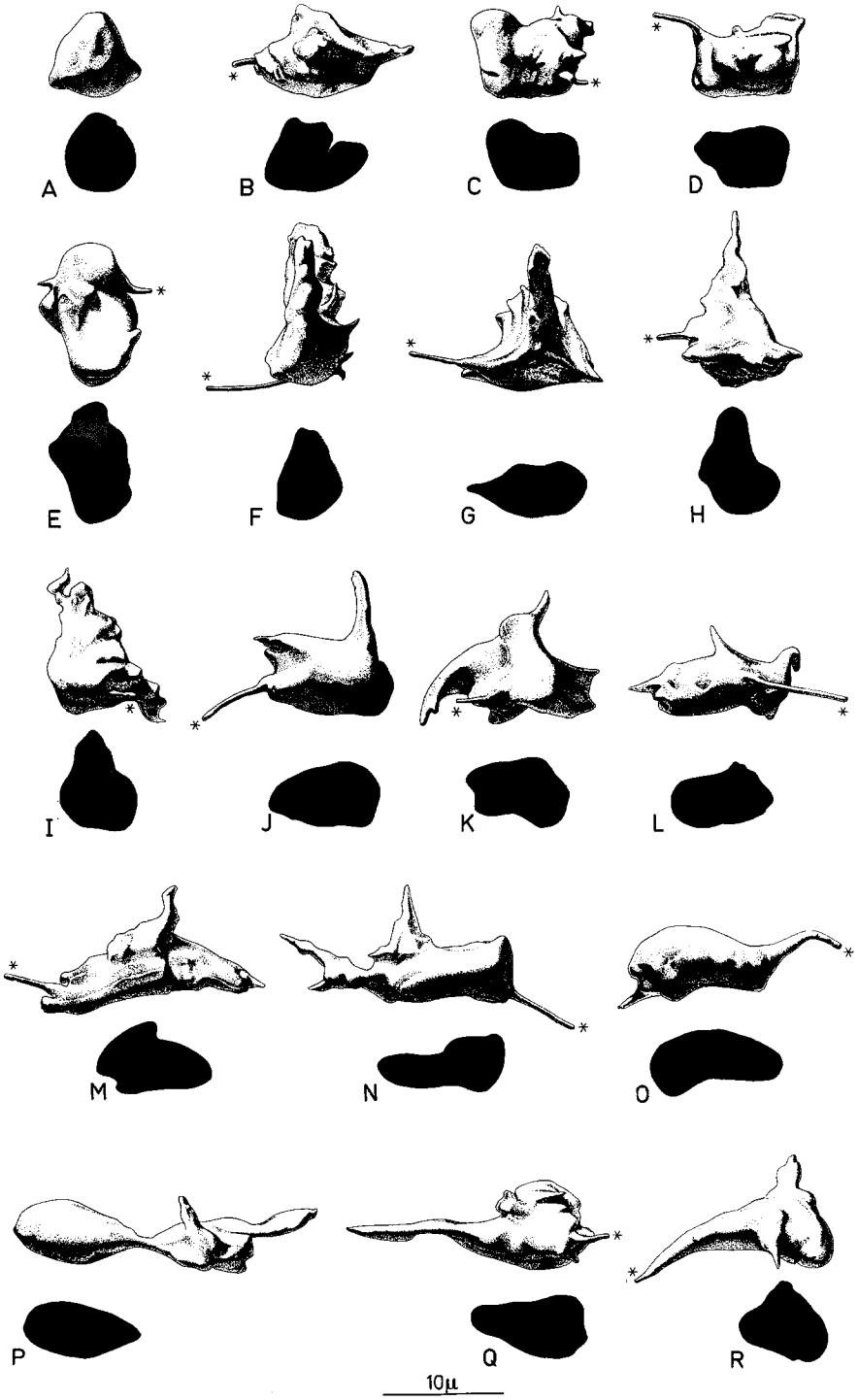


Fig. 10

Almost all have the shape of neuroblasts. Somata range from an irregular oval form to a rectangular or triangular shape. Their general configuration seems to depend on the way in which groups of cells are packed together while their processes appear to be oriented by the surrounding fascicles of vertical or horizontal axons and spongioblast processes. In contrast to cells at lower levels, many of these elements have a relatively complex profile because of one or more extensions. The latter are filled with cytoplasm rich in ribosomes, are usually oriented either horizontally or vertically, and terminate in an acute angle. With the exception of cells A and P in Fig. 10 a small, round, elongate, horizontal process (*) could be seen to arise from these elements. It was identified as an axon since its form and dark cytoplasm are identical to that of the surrounding horizontal nerve fibers.

The shape of nuclei reconstructed from serial sections also correspond to elements seen at this level with the light microscope. Most are horizontally elongate and have irregular contours closely conforming to the shape of the cell soma. Deep invaginations of the cell nuclei are present (Fig. 10 B, D, E, F), but the are not common.

Electron Microscopy — Upper Intermediate Lamina

An elongate, triangular profile is characteristic of most cells at this level in electron micrographs of single sections (Fig. 3). Nuclei are located near the base of the cell, and cytoplasm fills the apex which is pointed toward the pia (Fig. 11). Models of cells reconstructed from this level complement this finding; most have an elongate pear shape (Fig. 12) closely resembling cells seen with the light microscope at this level in Golgi preparations (Fig. 2). Of the 12 cells reconstructed at this level all but one are vertically oriented. The scant perinuclear cytoplasm contains few scattered free ribosomes within a moderately dense matrix except near the origin of the large apical processes. Ribosomes are abundant there and are associated with membranes forming a rudimentary endoplasmic reticulum. Mitochondria are most common near the tip of the apical process or preapex. In the material from which serial sections were prepared penetration of osmium tetroxide to the middle part of the pallial wall during fixation seems to have been substantially less than near the pial or ventricular surface judging by the density of staining of cell membranes. Therefore, processes corresponding to axons could not always be followed with certainty at this level because of the difficulty in clearly distinguishing the lightly stained cell membranes.

Two or three cells often occur together with their membranes apposed for an appreciable distance (Fig. 11). They are separated from other groups of cells by large masses of horizontal fibers (Fig. 5). Careful examination of the soma and preapex in serial electron micrographs shows that they are often associated with

Fig. 10 A—R. Models of cells in the lower intermediate lamina range from simple, almost round elements (A) to complex cells identified as neuroblasts (B—R). The latter have one or more extensions or irregularities intercalated among the surrounding axons and spongioblast processes and a single, round, thin process, the axon (*), which extends horizontally. Their similarity to cells impregnated with the Golgi technique at this level is apparent by comparison with cells in Fig. 2. Models of many cell nuclei (black) are horizontally oriented in contrast to the predominantly vertically elongate nuclei of cells in the matrix lamina and have fewer and less pronounced invaginations (crosshatched) of the nuclear membrane

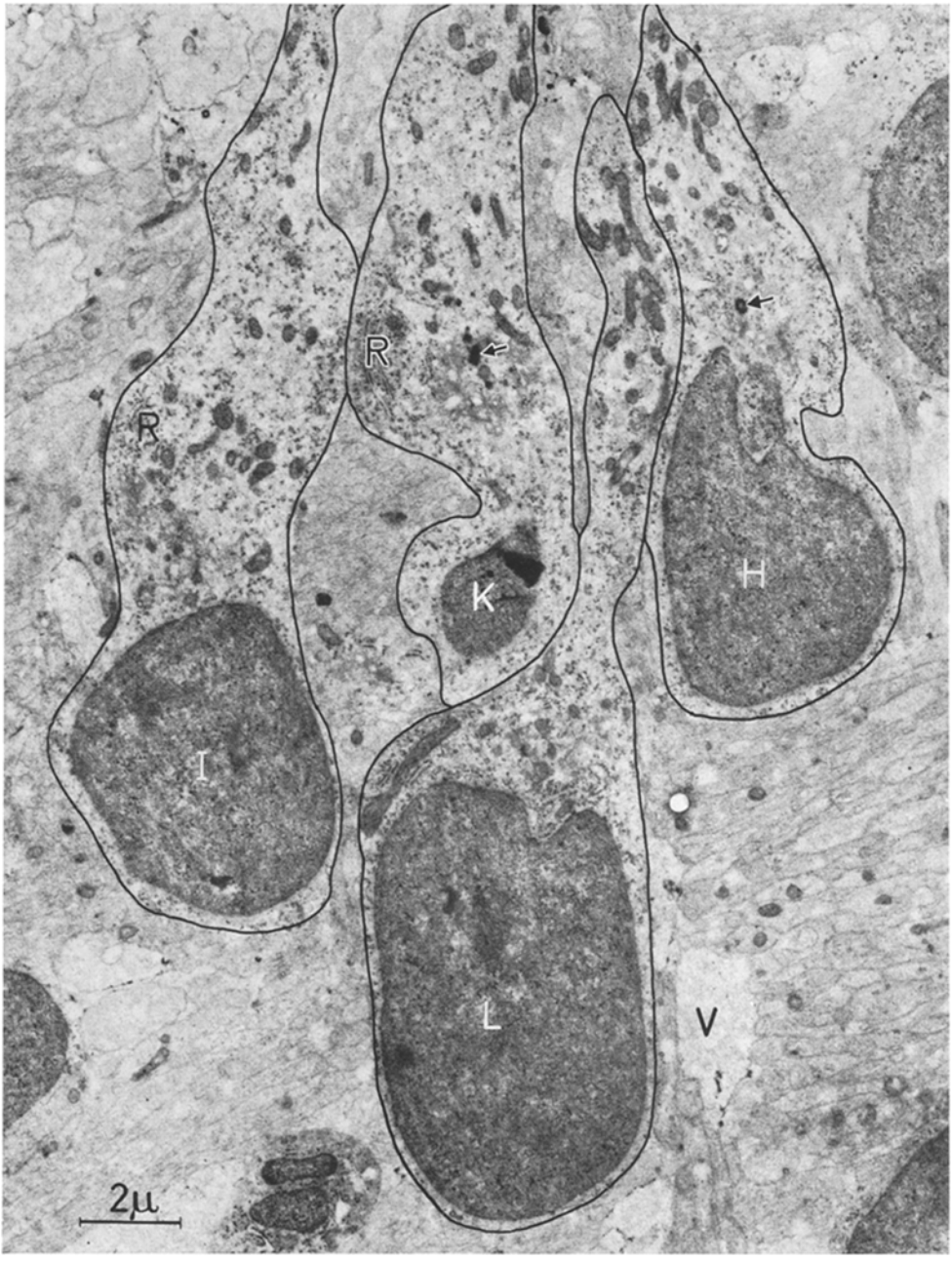


Fig. 11. Electron micrograph of cells in the upper intermediate lamina. Cells *H*, *I*, *K* and *L* of Fig. 12 have been outlined to emphasize the size and orientation of the preapex. The presence of ribosomes in the preapex allows it to be easily distinguished from surrounding vertical processes (*V*). Primitive aggregates of rough walled cisternae of the endoplasmic reticulum (*R*) are located above the nucleus together with centrioles (arrows) surrounded by an irregular cluster of tubules and small vesicles. Mitochondria are frequently concentrated near the tip of the preapex

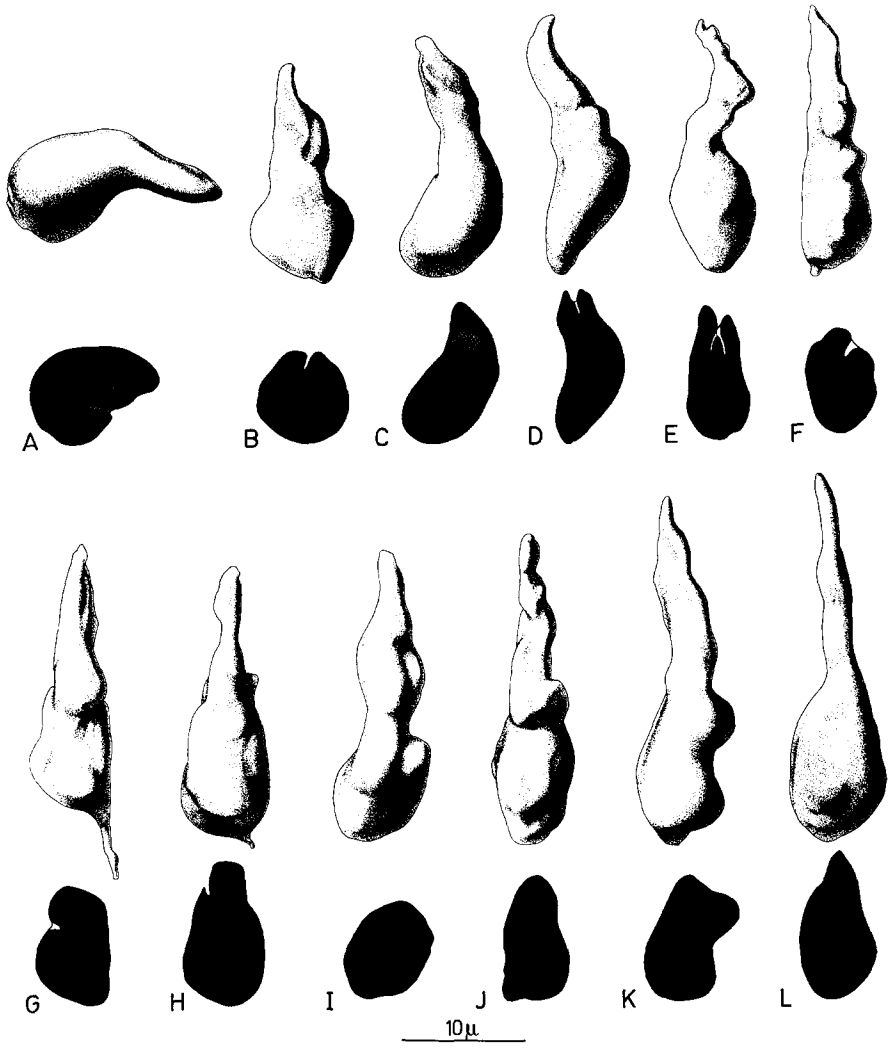


Fig. 12A—L. Models of cells in the upper intermediate lamina, with one exception (A), are vertically elongate elements whose form is similar to neuroblasts seen at this level with the Golgi technique. Their most characteristic feature is a large process, the preapex, extending toward the pia. Cell A has an elongate shape similar to other elements but lies adjacent to a capillary. Cell nuclei (black) are located in the basal portion of the cell and may have one or more invaginations (white or crosshatched) of the nuclear membrane

vertical fibers. This relationship is seen more clearly in horizontal sections. The small, vertical processes shown in black in Fig. 13 are not scattered randomly but occur in groups intercalated among the irregular rows of cells. In addition there are large processes filled with light cytoplasm (Fig. 13, dotted lines), presumably corresponding to local enlargements of axons and spongioblast processes, which are most common within the rows of neuroblasts and vertical fibers. Similar large processes with light cytoplasm contact capillary endothelial cells and resemble those described by WECHSLER (1965). They do not entirely surround capillaries and

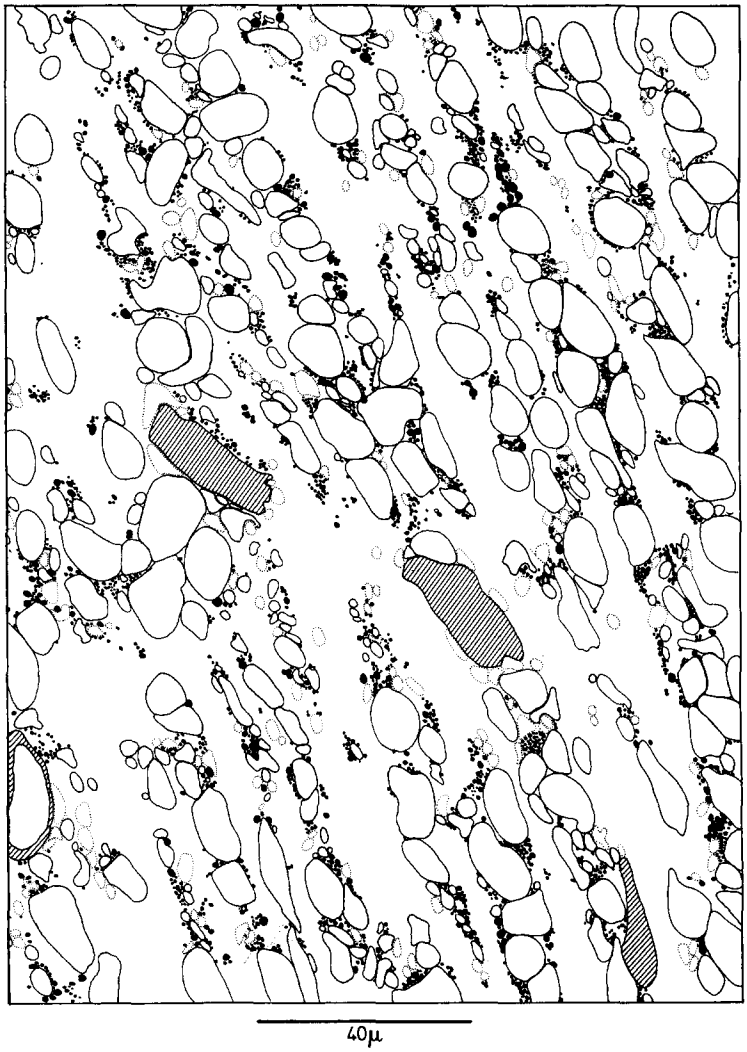


Fig. 13. Diagram of a horizontal section at the level of the upper intermediate lamina near the cortical plate showing: (1) the size and distribution of vertical processes containing cytoplasm with few or no ribosomes (black), (2) soma and preapex of neuroblasts (solid line), (3) large processes with light cytoplasm (dotted line) and (4) capillaries (hatched)

their precise form is not known since they have not been reconstructed from serial sections.

A slightly more advanced degree of differentiation for cells at this level is suggested by their appreciably larger soma, an increased number of organelles and a large preapex. The presence of the preapex gives these cells a distinctive shape and allows them to be distinguished from astroblasts since it does not continue beyond the limits of the electron micrographs. No cells have been seen with lobulated nuclei resembling glioblasts reported by MELLER *et al.* (1966a) in the migratory zone of 20 day mouse embryos.

Discussion

I. Matrix Lamina

a) Neuroblast and Spongioblast Morphology. There is an exceedingly close correspondence between the form of models reconstructed from serial sections and cells seen with the rapid Golgi technique at each of the three levels of the cerebral hemisphere considered in this study. In the matrix lamina, spongioblasts are easily identified by characteristic prolongations. A large, proximal process is attached to adjacent elements at the ventricular surface while the thin distal one extends toward the pia. The latter continues beyond the limits of the photographs, and it is not possible to specify whether a given cell is a freely arborizing or a typical spongioblast. Cells with relatively few ribosomes were initially assumed to correspond to spongioblasts since adult astrocyte cytoplasm is generally less dense than that of neurons. However, this supposition was not confirmed by more detailed analysis which showed that there is considerable variation in ribosome content among cells having the shape of spongioblasts. Likewise, local swellings along the course of distal processes filled with very light cytoplasm at first were assumed to correspond to enlargements of spongioblast distal processes seen with the Golgi technique. However, almost identical enlargements also occur along axons of horizontal nerve fibers in the intermediate lamina. Therefore, at this stage of embryonic development it does not appear to be possible to distinguish spongioblasts from neuroblasts either on the basis of concentration of RNA in the soma or on the presence of processes containing swellings filled with light cytoplasm.

Models of cells lacking attachment to the ventricle and identified as neuroblasts are few in number. Their scarcity at this level fits well with the prediction of SIDMAN *et al.* (1959), from autoradiography, that they constitute a minority of the cell population and that, once formed, young nerve cells do not remain long at this level but migrate quickly toward the outer surface of the brain. It is conceivable that some of the cells which do not contact the ventricle are astroblasts (spongioblasts beginning to migrate toward the pia). At this and earlier stages, however, they are rarely seen in Golgi preparations and are abundant only about the time of birth. Moreover, none of the elements reconstructed from the intermediate lamina have the long distal process characteristic of this cell type.

b) Germinal Cell Morphology. The only germinal cells that can be identified with certainty in electron micrographs are mitotic cells in which chromosomes are visible. They are most abundant near the surface of the ventricle but are not restricted to this zone. Dividing cells also occur at higher levels and are commonly seen near the intermediate lamina (HAMBURGER, 1948; STENSAAS, 1967a). They generally have an irregular, round profile in single thin section, suggesting that they are devoid of processes; however, it is possible that one or more cell prolongations arise out of the plane of section. That these elements are, in fact, spherical and have no processes has been established in this study by carefully following the cell membrane of many dividing cells in serial sections. This is true both of cells near the ventricle and of elements located at more superficial levels within the matrix lamina.

The fact that mitotic cells are devoid of processes means that they cannot be confused with neuroblasts or spongioblasts. This is important since it rules

out the hypothesis of BERRY and ROGERS (1965) that mitotic division leading to the production of neuroblasts occurs in spongioblasts connected by elongate processes to both the ventricular surface and to the pia. Further indirect evidence against their concept for delayed cell division and differential nuclear migration is that no cells with two nuclei have been seen in the matrix lamina, and no vertically elongate cells with distal processes extending beyond the limits of the serial electron micrographs were seen in the intermediate lamina.

The knowledge that mitotic germinal cells have no processes makes it necessary to consider the problem of interphase germinal cell morphology. The shape of cell models reconstructed from serial electron micrographs of the matrix lamina is consistent with Golgi impregnations which show neuroblasts, spongioblasts, and columnar epithelial cells at this level. Since neuroblasts cannot divide there seem to be three possibilities. Intermitotic germinal cells may be: (1) columnar epithelial cells, (2) spongioblasts, or (3) both columnar epithelial cells and spongioblasts.

FUJITA (1963) infers from an autoradiographic study that the matrix lamina is composed of a homogeneous cell population, because the percentage of labeled cells increases linearly and eventually reaches 100%. Unfortunately the methods employed reveal nothing about the *form* of the cells which could permit the neuroblasts and glioblasts to be distinguished from one another. Moreover, the correlative experiment was not performed in which accessability to H^3-thymidine was curtailed after all cells were labeled and which allowed time for dilution of the label by continued cell division. This might have shown that heavily labeled cells remain which do not continue to participate as actively in the mitotic process. By itself the observation that all cells are eventually labeled does not rule out the presence of more than one cell type. Indeed, since neuroblasts and spongioblasts are the products of division of germinal cells near the ventricle it is obvious that both would be labeled and that both would be situated there for a finite amount of time before migrating away. This might well exceed the 25 hr experimental period reported.

Autoradiographic experiments (FUJITA, 1963; SAUER and WALKER, 1959; SIDMAN *et al.*, 1959) clearly show that nuclei of cells in the outer part of the matrix lamina undergo DNA replication and that they later become situated near the ventricle where they divide. Two patterns of development could lead to the production of neuroblasts. Both are consistent with these observations and with the morphology of cells reported in this study. For each, a cycle in the production of a neuroblast would begin with division of a germinal cell which maintained its desmosomal attachment to the ventricular surface. If *columnar epithelial cells* are interphase germinal cells the soma would be connected to the ventricle by a process of varying length as it moved to the outer part of the matrix lamina. After replication of DNA the nucleus could move back to the ventricle along this process to be followed by division. On the other hand, if *spongioblasts* are interphase germinal cells the nucleus could migrate to the outer part of the matrix lamina as the distal process grew toward the pia and return to the ventricle during or after the retraction of this distal extension. Division would occur only after the cell had achieved a spherical shape.

That interphase germinal cells are columnar epithelial cells seems more likely than that they are spongioblasts which grow and retract long distal processes between divisions. However, there is little experimental evidence which is relevant to this particular question, and it is conceivable that columnar epithelial cells are spongioblasts with have retracted their distal processes and are about to divide.

The time necessary for nuclear DNA replication between mitoses depends on the age of the embryo and the location of germinal cells within the central nervous system. In the alar plate of a 6 day chick embryo it ranges from 13 to 16 hours (FUJITA, 1962). If a comparable period exists in the rabbit at this stage of development the distal process of a spongioblast would have to travel approximately 500 μ to achieve contact with the pia and then retract an equal distance. On the other hand, freely ending spongioblasts have been seen whose distal processes extend only 150 to 200 μ into the intermediate lamina and cortical plate, and appear to be likely candidates for interphase germinal cells.

Autoradiographs show that more than 80% of the cells in the outer two thirds of the matrix lamina are labeled 1 hr after administering H^3-thymidine to 11 day mouse embryos and that few are labeled in the inner half (SIDMAN *et al.*, 1959). However, labeled nuclei were found predominantly in the inner half of the ependymal zone 6 hr after injection of thymidine-H^3. If a similar pattern exists in the 45 mm rabbit embryo, it can be assumed that most cells in the outer part of the matrix lamina are replicating DNA and will undergo division only after they migrate to the ventricular surface. Since the majority of cells reconstructed from the matrix lamina have the shape of spongioblasts and columnar epithelial cells, it would seem that they are the interphase germinal cells. However, it is impossible to determine from this study which elements have recently divided and are moving to the outer part of the matrix lamina to begin nuclear DNA synthesis, which will continue to migrate away from this lamina and form the cortical plate, and which elements have completed the DNA synthetic cycle and are about to divide. The most direct method to achieve a conclusive answer to this problem seems to be one in which autoradiography and electron microscopy are combined so that the identity of labeled cells, according to their external morphology, can be established by the use of serial sections.

c) Intercellular Relations. Only in the matrix lamina are specialized membrane contacts observed between ectodermal cells. They are the well known desmosomes, or terminal bars. An analysis of serial sections shows these specialized connections to exist as a continuous band between all processes extending to the ventricle. Of particular interest is the fact, already reported by WECHSLER (1966b) and FUJITA and FUJITA (1963), that large, round mitotic cells at this level are also attached to surrounding elements by these membrane thickenings. This arrangement suggests that desmosomes may have some function other than that of simply maintaining physical continuity of the ventricular surface. One possibility is that they serve to anchor germinal cells and thereby facilitate migration of somata toward the ventricle from the outer zone during the "elevator" movement of nuclei within the matrix lamina. One could assume that only somata of cells anchored to the ventricle by the proximal process are capable of active migration toward the ventricle by virtue of special properties of this process. Since fibrils

and tubules are present within its cytoplasm they could provide the physical substratum for movement. Migration of the remaining cells away from the ventricle might then result from simple displacement. Since cell size is fairly uniform, the number of somata displaced from the ventricle for any given period of time would equal the number actively moving toward it assuming that the volume of the matrix lamina remains relatively constant and that there is a uniform rate of mitosis. Such a scheme is purely hypothetical, but it is consistent with what is presently known of the dynamics of this population and would seem to be a simple way to account for the anatomical peculiarities of cells in this lamina.

II. Intermediate Lamina

a) Lower Intermediate Lamina — Neuroblast Morphology and Intercellular Relations. A number of investigators (PATON, 1900; HATAI, 1902; BONNÉ, 1907) have pointed out the striking change in cell orientation that occurs at the upper limit of the matrix lamina. Studies with the Golgi technique also indicate that somata of young nerve cells at this level rotate around their axons before continuing their migration toward the cortex (STENSAAS, 1967b). Electron micrograph montages of single sections of the matrix and lower intermediate laminae show that, while still tightly packed, the elements abruptly change from vertically elongate to horizontally elongate. Models of cells reconstructed from this zone also reflect this change. Most have forms coinciding with elements identified as neuroblasts with the Golgi technique thereby confirming the impression that a qualitative change in cell composition also occurs at this level. Since none of the models have the form of spongioblasts or astroblasts they provide additional, indirect evidence that cells lacking connection with the ventricle are neuroblasts.

The appearance of Golgi impregnated elements and cell models suggest that the short processes and irregularities arising from the somata of neuroblasts represent an intermediate stage of development before the appearance of the preapex. Since they parallel surrounding vertical and horizontal fibers, the change from a simple, oval shape in the matrix lamina to a complex, horizontal form in this zone may be related to the presence and relative number of the surrounding axons.

b) Upper Intermediate Lamina — Neuroblast Morphology and Intercellular Relations. A careful review of the literature led BONNÉ (1907) to surmise that there is a characteristic cell process arising from neuroblasts in the upper intermediate lamina and oriented in the direction of movement. He gave the name preapex to this cell prolongation and supposed that it played an important role in migration. Golgi preparations confirm the presence of this process and elucidate certain details of its morphology, but they reveal nothing of its relations to surrounding elements that would help to understand its orientation or the way it could promote cell movement. Therefore, during the course of this investigation special attention was devoted to the intercellular relations of the preapex for clues about the way neuroblasts migrate.

The preapex of cells in the intermediate lamina always contacts small groups of vertically oriented processes and is aligned parallel to them. This was first observed in serial electron micrographs of transverse sections where the preapex

is intercalated among groups of vertical fibers. Horizontal sections substantiate this finding and show that neuroblast somata are generally surrounded by the vertical processes as well. MUGNAINI and FORSTRØNEN (1967) noted a similar relation between migrating neuroblasts and vertical processes in the chick cerebellum. The latter contain lightly staining cytoplasm rich in glycogen particles and were identified as astrocytic fibers of Bergmann. A similar correlation is not possible in 45 mm rabbit embryos inasmuch as vertical processes containing both light and dark cytoplasm are present in electron micrographs and since the Golgi technique shows that both axons of cortical nerve cells and distal processes of spongioblasts occur in the intermediate lamina. However, it is logical to assume that many of the vertical processes belong to spongioblasts and that migrating neuroblasts frequently contact them.

MUGNAINI and FORSTRØNEN (1967) speculate that movements of glial membranes, as revealed in tissue culture, may be of significance for neuroblast migratory movements. Glial cells might facilitate migration in several ways. One possibility is that the proximity of glial cell processes to migrating neuroblasts provides the opportunity for the exchange of metabolites vital to the young nerve cells. This is a simple extrapolation to embryos of the concept that astrocytes participate in the exchange of substances between nerve cells and capillaries. However, relations of spongioblasts to capillaries are only rudimentary at this stage, and it seems likely that if transfer of metabolites occurs, it would take place mainly from the cerebrospinal fluid through processes which are apposed both to pial and ventricular surfaces.

The actual mobility of neuroblasts would seem to depend primarily on the presence of a cell process, such as the preapex, oriented in the direction of movement and on the characteristics of its cytoplasm. In this regard it is interesting to point out that centrioles associated with numerous tubules are conspicuous features of this element. Little is known about the way in which physical changes in relatively unstructured cytoplasm can provide the basis for movement, although recent experiments appear to provide certain clues (SIMARD-DUQUESNE and COUILLARD, 1962). Chemical (RAMÓN Y CAJAL, 1893), electrical (ARIËNS KAPPERS, 1921), and mechanical (WEISS, 1934) factors have been postulated to account for the migration of young nerve cells in a particular direction, but they are substantiated by no current experiments. Therefore, it is fair to state that nothing definite is known about the qualitative nature of agents which orient, control, or regulate movement of neuroblasts.

Bibliography

ARIËNS KAPPERS, C. U.: On structural laws in the nervous system: the principles of neurobiotaxis. Brain **44**, 125—149 (1921).

BELLAIRS, R.: The development of the nervous system in chick embryos, studied by electron microscopy. J. Embryol. exp. Morph. **7**, 94—115 (1959).

BERRY, M., and A. W. ROGERS: The migration of neuroblasts in the developing cerebral cortex. J. Anat. (Lond.) **99**, 691—709 (1965).

BONNÉ, C.: L'Écorce cérébrale. I. Developpement, morphologie et connexions des cellules nerveuses. Rev. gén. Histol. **2**, 291—581 (1907).

CALEY, D. W., and D. S. MAXWELL: An electron microscopic study of neurons during postnatal development of the rat cerebral cortex. J. comp. Neurol. **133**, 17—44 (1968).

DUNCAN, D.: Electron microscope study of the embryonic neural tube and notochord. Tex. Rep. Biol. Med. **15**, 367—377 (1957).

FUJITA, H., and S. FUJITA: Electron microscopic studies on neuroblast differentiation in the central nervous system of domestic fowl. Z. Zellforsch. **60**, 463—478 (1963).

— Electron microscopic studies on the differentiation of the ependymal cells and the glioblast in the spinal cord of domestic fowl. Z. Zellforsch. **64**, 262—272 (1964).

FUJITA, S.: Kinetics of cellular proliferation. Exp. Cell Res. **28**, 52—60 (1962).

— The matrix cell and cytogenesis in the developing central nervous system. J. comp. Neurol. **120**, 37—42 (1963).

HAMBURGER, V.: The mitotic patterns in the spinal cord of the chick embryo and their relation to histogenetic processes. J. comp. Neurol. **88**, 221—283 (1948).

HATAÏ, S.: Observations on the developing neurones of the cerebral cortex of foetal cats. J. comp. Neurol. **12**, 199—204 (1902).

HIS, W.: Histogenese und Zusammenhang der Nervenelemente. Verh. X. Int. med. Congr. Berlin 1891, S. 93—114.

HUXLEY, H. E., and G. ZUBAY: Preferential staining of nucleic acid-containing structures for electron microscopy. J. biophy. biochem. Cytol. **11**, 273—296 (1961).

LANGMAN, J., R. L. GUERRANT, and B. G. FREEMAN: Behavior of neuro-epithelial cells during closure of the neural tube. J. comp. Neurol. **127**, 399—412 (1966).

LYSER, K. M.: Early differentiation of motor neuroblasts in the chick embryo as studied by electron microscopy. I. General aspects. Develop. Biol. **10**, 433—466 (1964).

MELLER, K.: Elektronenmikroskopische Befunde zur Differenzierung der Rezeptorzellen und Bipolarzellen der Retina und ihrer synaptischen Verbindungen. Z. Zellforsch. **64**, 733—750 (1964).

— W. BREIPOHL, and P. GLEES: Early cytological differentiation in cerebral hemisphere of mice. An electronmicroscopical study. Z. Zellforsch. **72**, 525—533 (1966a).

— J. ESCHNER, and P. GLEES: The differentiation of endoplasmic reticulum in developing neurons of the chick spinal cord. Z. Zellforsch. **69**, 189—197 (1966b).

MILLONIG, G.: Further observations on a phosphate buffer for osmium solutions in fixation. Int. Congr. Elec. Micr., vol. 5, P—8. New York: Academic Press 1962.

MUGNAINI, E., and P. F. FORSTRØNEN: Ultrastructural studies on the cerebellar histogenesis. I. Differentiation of granule cells and development of *glomeruli* in the chick embryo. Z. Zellforsch. **77**, 115—143 (1967).

PATON, S.: The histogenesis of the cellular elements of the cerebral cortex. Johns Hopk. Hosp. Rep. **9**, 709—741 (1900).

RAMÓN Y CAJAL, S.: A quelle époque apparaissent les expansions des cellules nerveuses de la moelle épinière du poulet? Anat. Anz. **5**, 609—639 (1890).

— La rétine des vertébrés. Cellule **9**, 119—258 (1893).

REYNOLDS, E. S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208—212 (1963).

SAUER, F. C.: The cellular structure of the neural tube. J. comp. Neurol. **63**, 13—23 (1935).

SAUER, M. E., and B. E. WALKER: Autoradiographic study of interkinetic nuclear migration in the neural tube. Proc. Soc. exp. Biol. (N.Y.) **101**, 557—560 (1959).

SCHAPER, A.: Die frühesten Differenzierungsvorgänge im Centralnervensystem. Arch. Entwickl.-Mech. Org. **5**, 81—132 (1897).

SENSENBRENNER, M., and P. MANDEL: RNA biothesynthesis during differentiation of various cell types of chicken embryo in cerebral hemispheres. Histoautoradiographic study. Z. Zellforsch. **82**, 65—81 (1967).

SIDMAN, R. L., I. L. MIALE, and N. FEDER: Cell proliferation and migration in the primitive ependymal zone; an autoradiographic study of histogenesis in the nervous system. Exp. Neurol. **1**, 322—333 (1959).

SIMARD-DUQUESNE, N., and P. COUILLARD: Amoeboid movement. II. Research of contractile proteins in *Amoeba proteus*. Exp. Cell Res. **28**, 92—98 (1962).

SOTELO, J. R., and O. TRUJILLO-CENÓZ: Electron microscope study of the development of ciliary components of the neural epithelium of the chick embryo. Z. Zellforsch. **49**, 1—12 (1958).

STENSAAS, L. J.: The development of hippocampal and dorsolateral pallial regions of the cerebral hemisphere in fetal rabbits. II. Twenty millimeter stage, neuroblast morphology. J. comp. Neurol. **129**, 71—84 (1967a).
— The development of hippocampal and dorsolateral pallial regions of the cerebral hemisphere in fetal rabbits. III. Twenty-nine millimeter stage, marginal lamina. J. comp. Neurol. **130**, 149—162 (1967b).
—, and S. S. STENSAAS: Astrocytic neuroglial cells, oligodendrocytes and microgliacytes in the spinal cord of the toad. I. Light microscopy. Z. Zellforsch. **84**, 473—489 (1968).
TENNYSON, V. M., and G. D. PAPPAS: A electron microscope study of ependymal cells of the fetal, early postnatal and adult rabbit. Z. Zellforsch. **56**, 595—618 (1962).
WATTERSON, R. L., P. VENEZIANO, and A. BARTHA: Absence of a true germinal zone in neural tubes of young chick embryos as demonstrated by the colchicine technique. Anat. Rec. **124**, 379 (1955).
WECHSLER, W.: Die Entwicklung der Gefäße und pervivasculären Gewebsräume im Zentralnervensystem von Hühnern. Z. Anat. Entwickl.-Gesch. **124**, 367—395 (1965).
— Die Feinstruktur des Neuralrohres und der neuroektodermalen Matrixzellen am Zentralnervensystem von Hühnerembryonen. Z. Zellforsch. **70**, 240—268 (1966a).
— Elektronenmikroskopischer Beitrag zur Differenzierung des Ependyms am Rückenmark von Hühnerembryonen. Z. Zellforsch. **74**, 423—442 (1966b).
— Elektronenmikroskopischer Beitrag zur Histogenese der weißen Substanz des Rückenmarks von Hühnerembryonen. Z. Zellforsch. **74**, 232—251 (1966c).
WEISS, P.: In vitro experiments on the factors determining the course of the outgrowing nerve fiber. J. exp. Zool. **68**, 393—448 (1934).

Dr. L. J. STENSAAS
Dept. of Physiology, University of Utah Medical Center
Salt Lake City, Utah 84112, USA