Retinoblastoma
A study of two cases by electron microscopy

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The ultrastructure of two retinoblastomas includes cytologic features which are characteristic of neoplasia. Unusual degenerative changes were observed in both tumors. Rosettes were present in one case, and the cells which formed them showed some characteristics of ependyma of the primitive neural tube, and also of developing vision receptor cells. Structures resembling pericentriolar bodies were associated with basal bodies of cilia in rosettes.

Retinoblastoma, the most common intraocular tumor of childhood, has been well characterized as regards its clinical behavior and its varied structure by light microscopy. This report is to record features of two such tumors observed by electron microscopy. No previous such study appears to have been published.

Materials, methods, and observations by light microscopy

Case 1. A 3-year-old white boy was first found to have an abnormal light reflex in the left eye 1½ months before he was seen at the University of California Medical Center, Los Angeles. There was no family history of retinoblastoma or significant eye disease.

Physical examination disclosed no abnormality except the ocular lesion. X-rays of the skull were normal.

Examination of the eyes demonstrated normal vision in the right eye and no light perception in the left eye. The right eye appeared normal in all respects. The left eye contained a large creamy-white and irregularly vascularized tumor mass which filled the temporal aspect of the posterior segment, and obscured the optic nerve head. The vitreous body contained numerous, rather small, discrete white particles of neoplasm.

Enucleation of the left eye was performed under general anesthesia. Immediately after removal, the eye was opened, disclosing a soft white tumor mass nearly filling the vitreous body. Bits of tumor tissue were immediately placed in 1 per cent osmium tetroxide fixative buffered with Veronal acetate and hydrochloric acid to pH 7.36. Fixation at 0° C. was continued for 3½ hours. Dehydration in graded alcohol series was followed by propylene oxide for Epon embedding, according to the method of Luft. An estimated period of 2 minutes elapsed between cutting the optic nerve and placing the tumor fragments into fixative.

Sections about 1 μ thick were cut from the blocks on a Porter-Blum microtome and stained with toluidine blue for light microscopy. Thin sections were cut and mounted on grids coated with celloidin. Most sections were stained with uranyl acetate, though some were stained with basic lead oxide. The specimens were examined with an RCA EMU 3-B electron microscope.

Light microscopy disclosed a large tumor mass extending inward from a broad attachment to the retina (Fig. 1) and occupying most of the posterior portion of the vitreous body. The closely packed tumor cells varied only slightly in size, shape, and staining. The cytoplasm was...
sparse, faintly pink, and apparently shrunken. The nuclei were generally round or oval, though some were lobated and occasional ones appeared folded (Figs. 2 and 3). Most cells contained one or more nucleoli which were seen best in the osmium-fixed toluidine blue-stained sections (Fig. 3). Rosette formation was not observed. Occasional foci of calcium were present in necrotic areas.

Within the retina the tumor extended to the optic nerve head, but the optic nerve itself was not invaded. The tumor did not invade the choroid.

Case 2. A 2½-year-old white boy had been well throughout his life. One and one half months before admission his parents noted a "strange red reflection" from his eyes as he watched television. He was referred to the University of California Medical Center for ophthalmologic evaluation. There was no family history of eye defects or neoplasms.

Physical examination disclosed a well-developed child, apparently healthy except for the eye findings.

Examined under anesthesia, the left eye showed a large tumor mass occupying the temporal half of the vitreous body and an associated retinal detachment. Clumps of tumor could be seen floating in the vitreous body. In the right eye there was a nodular area of retinoblastoma slightly inferior to the optic nerve head.

Enucleation of the left eye was advised, and a program of treatment for the right eye was recommended. This program, subsequently carried out, included right internal carotid artery injection of triethylene melamine, x-irradiation, and photocoagulation of the tumor nodule.

The left eye was enucleated under general anesthesia and immediately after removal the eye was opened. The lower temporal sector of the retina was replaced by a white tumor mass approximately 1 cm. across. The growth lay principally on the inner surface of the retina and protruded into the vitreous body.

The technical procedures followed were similar to those described for Case 1 with the following exceptions: (1) a period of about 15 minutes elapsed between the time the optic nerve was clamped and the bits of tissue were placed in osmium fixative; (2) fixation time in chilled osmium fixative was 1 to 1½ hours.

Light microscopy disclosed a fairly large solid tumor mass extending into the vitreous body from the posterior temporal portion of the retina.

Fig. 1. Case 1. Posterior portion of eye, showing main tumor mass. The neoplasm has a broad retinal attachment and has grown over the optic nerve head, though the optic nerve itself was not invaded. Retinal detachment was present, but shrinkage during processing accounts for most of the separation shown. (×7.5.)
Fig. 2. Case 1. Microscopic appearance of tumor, formalin-fixed paraffin embedded tissue. The cells are fairly uniform in size and staining, but cytologic detail is lacking. Scattered pyknotic nuclei are evident. (Hematoxylin and eosin. ×500; reduced %.)

(Fig. 4). The cells were similar to those of Case 1 but differed in that there was conspicuous rosette formation (Figs. 5 and 6). Areas of necrosis with calcification were present. Variable-sized clumps of tumor cells were free within the vitreous body. Large tumor cell aggregates were present in the intraretinal space, and at some sites the neoplasm had invaded the choroid. The tumor extended to the margins of the optic nerve head, but the optic nerve was not invaded.

Observations by electron microscopy

The cellular characteristics of the two tumors were essentially similar. The descriptions to follow, therefore, apply to both tumors and differences will be cited separately. The most significant difference appeared to be rosette formations which occurred in Case 2.

Where the cells were best preserved, the neoplasm consisted of sheets of polyhedral cells with interposed degenerating cells or cell processes (Figs. 7 and 9). Much of the variation in size and shape may be explained by the thin sections which included only narrow portions of some cells.

The cytoplasm. The cytoplasm generally consisted of a narrow rim around the nucleus, but some cells had angular or rounded extensions from the main cell. This latter feature was more conspicuous in Case 2, especially near rosette formations (Fig. 23).

The mitochondria were the most prominent cytoplasmic component, and showed moderate variation in number, location, and size in different cells. In some instances they appeared to occupy a sector of the cell, extending from the nuclear envelope almost to the plasma membrane, and were decreased in number or absent in other portions of the cytoplasm. Where tumor cells were in relation to small blood vessels, the mitochondria seemed more numerous on the vascular side of the cell (Fig. 22).
In section the mitochondria contained prominent cristae and showed moderate variability in size and shape. Rare bizarre forms were observed. In one mitochondrion a cluster of distinct round osmiophilic bodies could be seen (Fig. 20). In early stages of cell degeneration the mitochondria became swollen and empty (Fig. 20).

The Golgi complex could be identified in some cells, but it was generally small and inconspicuous (Fig. 20).

The endoplasmic reticulum was also a relatively inconspicuous cytoplasmic component. It consisted mainly of small vesicles and occasional cisternae with dense ribonucleoprotein (RNP) granules. Occasionally small vesicles were seen without RNP granules.

Centrioles could be identified in many cells, and were generally located near the nucleus. Cilia were present in the cells forming rosettes in Case 2. These structures will be further described in connection with the rosettes.

Several varieties of cytoplasmic inclusions were observed. The most common of these were spheroidal homogeneous osmiophilic bodies, possibly lipid. There were membranous cytoplasmic bodies containing more coarsely granular material which may represent lysosomes. One cell contained a pair of fibrillar or layered bodies which were apparently contained within a membranous sac (Fig. 8). Vacuoles of irregular size and shape were present in some cells, but were more conspicuous in degenerating cells.

Cytoplasmic fibrils were present, especially in cells forming rosettes, and were less commonly found in other areas of the tumors.

The nucleus. In contrast to the comparatively uniform appearance of the nuclei by light microscopy of the sections stained with toluidine blue (Figs. 3 and 6), the electron microscope disclosed considerable variation in shape and structure (Figs. 7 and 9). Many nuclei were round or oval, but deep cytoplasmic indentations were common. Often several indentations of varying depth were seen within a single nucleus, in some cases resulting in a U- or Y-shaped nuclear configuration. Occasional cells appeared binucleated, apparently as the result of sectioning both arms of the U (Figs. 7 and 9). Likewise, some irregular sections through cytoplasmic invaginations disclosed seemingly isolated islands of cytoplasm within the nucleus. In many cells the nuclei were bi- or tri-lobated, the lobes connected only by a thin strand of chromatin material, with its investing nuclear envelope intact. Occasionally small bosses of nuclear material were present on the surface of the nucleus. In many cells small nuclear spurs or rounded projections were connected to the main nuclear mass by a thin stalk or pedicle. More complicated nuclear forms were common with complex divisions and subdivisions of nuclear processes and cor-

Fig. 4. Case 2. Posterior portion of the eye. The retina next to the optic nerve head is almost replaced by the tumor. On the nasal side of the optic nerve the tumor is seen in the outer plexiform layer of the retina. Small masses of tumor can be seen in the subretinal space. (×20; reduced %.)
responding cytoplasmic indentations (Figs. 9 and 23).

The nuclear envelope consisted of two distinct layers. Pores in the nuclear envelope were observed in higher magnifications. In some apparently well-preserved cells there was cisternal separation of the two layers which gave the appearance of nuclear blebs (Fig. 10). This feature did not seem accentuated in cells with changes of early degeneration.

Depending upon the stage of mitosis, the nuclear membrane was present to a varying extent or appeared absent altogether in some cells (Figs. 14 to 17). In many degenerated cells both layers of the nuclear envelope were clearly distinguishable after the nuclear chromatin had lost its organized appearance and extensive degeneration had occurred in the cytoplasm (Figs. 18 and 19).

Two fairly distinct patterns of nuclear chromatin distribution were observed: (1) a fairly even distribution of small, dense particles with one or more nucleoli (Fig. 7); (2) aggregations into irregular coarse clumps with patchy condensations along the nuclear envelope (Figs. 9 and 20).

The nucleoplasm contained fine fibrillar or granular material. Occasionally small membranous structures lay close to the inner layer of the nuclear envelope and might represent invaginations of it (Figs. 11, 12, and 16).

In cells with evenly distributed nuclear chromatin several nucleoli were often apparent. Usually this structure was situated at the periphery of the nucleus, often adjacent to the nuclear envelope. It was generally round or oval, but it occurred in a variety of less regular shapes.

Mitotic figures. These were fairly numerous in both tumors. Most of them were in metaphase or telophase, but occasionally earlier stages were noted (Figs. 14 and 23).
**Cellular relationships.** The relationships of the cells appeared comparatively simple. Surface differentiation was not observed except in the rosettes where terminal bars were present (Figs. 23 and 24). Mostly the cells were fairly close together, the plasma membranes of adjacent cells forming more or less parallel lines. Portions of other cells were sometimes interposed where the intercellular spaces widened. The tumor cells apparently did not form intercellular material.

**Degenerative changes.** Degeneration was widespread in both tumors, and in practically every field there were cells or portions of cells where such changes were present to some extent (Figs. 7, 10, and 14).

One common form of degeneration (Figs. 18 and 19) consisted of fine and coarse clumping of the nuclear chromatin with disorganization. Both layers of the nuclear envelope were usually preserved, but convoluted and partly collapsed. The cytoplasm showed loss of fine detail, though occasional vesicles and clusters of RNP remained. The plasma membrane remained, irregularly convoluted but apparently intact. At a later stage of degeneration the nucleus appeared as irregularly clumped dark granules within a membranous sac. Various-sized membranous sacs enclosed cytoplasmic constituents, some of which could be recognized as mitochondria. These latter structures appeared ballooned, and the cristae within them appeared as small cogs.

A slightly different degenerative pattern occurred when the nuclear chromatin clumped into variably sized dark homogeneous masses (Figs. 7 and 14). In these cells the membranous structures were less well preserved, and only a few small tubular structures remained in the cytoplasm, admixed with clumps of granular material. That such changes were not due to fixation was evident from the fact that such degenerating cells lay immediately adjacent to or often completely surrounded by well-preserved cells. Degenerated neoplastic cells adjacent to blood vessels were noted frequently.

Where degeneration was far more extensive and involved large areas, there was cellular fragmentation and loss of all fine cell detail. Among the cellular debris there were smudged, round, dark masses of variable size and poorly defined membranous and tubular remnants. Occasional larger vacuolated bodies and myelin figures with amorphous scattered granular material were also present (Fig. 21).

**Blood vessels.** These were fairly numerous in the main tumor mass. They were mostly capillaries, lined by a single layer of endothelial cells resting upon a well-defined basement membrane which in some instances was shared with pericytes. Both of these cell types were contiguous with the neoplastic cells.

The inner surface of the endothelium was irregular (Fig. 22) and spurs and flap-like processes projected into the lumen. In vessels which appeared collapsed, there were more complicated endothelial infoldings. Several focal densities resembling terminal bars were visible along the cleft forming the junctions of the endothelial cells. The capillary endothelial basement membranes were distinct and appeared separate from the basement membrane of adjacent tumor cells (Fig. 22).

**Rosettes.** A striking feature of Case 2, not observed in Case 1, was the rosette, which consisted of a radial arrangement of tumor cells around a central lumen. Usually the circle of cells consisted of 9 or 10 cell nuclei in a single plane, and cell processes from an additional 7 or 8 cells could be identified as apparently participating in the formation of the rosette (Fig. 23). Mitosis was often found in the cells of the rosette.

The nuclei were situated in the peripheral broader portions of the cells near the outer cell margin. Cytoplasmic indentations into the nucleus were often striking, and occurred principally on the luminal side of the nucleus, producing long fingerlike extensions pointing to the lumen. Cyto-
plasmic components also appeared oriented in the direction of the rosette lumen.

The mitochondria were mostly on the luminal side of the nucleus and had a radial arrangement. Admixed with the mitochondria and also occupying the inner portion of the cytoplasm were many tubules and vesicles, including endoplasmic reticulum. Filamentous structures frequently extended radially within the cytoplasm from the narrow luminal border of some cells (Fig. 24). Near the lumen terminal bars were prominent between the cells (Figs. 23 and 24).

The lumen of the rosette contained microvilli and broad clublike projections from some of its component cells. Only a scant amount of amorphous granular material was present within the nearly empty space.

Well-defined cilia extended into the lumen (Fig. 26). No more than one cilium was identified with any single cell. The cilia and their basal bodies had distinctive structural features (Figs. 24, 25, and 26). In one case a longitudinal section through a basal body showed structures resembling pericentriolar bodies (Fig. 25). Near the lumen surface of a nearby cell there was a structure resembling a centriole with several pericentriolar bodies cut in cross section (Fig. 24).

Discussion and comment

General features of neoplasia. As in tumor diagnosis by light microscopy, there is no known ultrastructural feature or combination of features specific for cancer cells. The various abnormalities which occur in tumor cells have been well summarized.8 These may vary greatly in different neoplasms and their presence can be considered only as general features of cancer.13

Retinoblastoma cells were characterized by the following changes in the nuclei: enlargement, lobulation, and irregularity of shape, with pseudo-inclusions of cytoplasmic organelles. Additionally, the nucleoli were enlarged and were often multiple.

Cytoplasmic changes were less striking. In general the normal cytoplasmic components (mitochondria, Golgi apparatus, endoplasmic reticulum) did not show significant alterations. One tumor cell showed stratified inclusion bodies apparently enclosed within a membranous envelope (Fig. 8) but the significance of these is not known. No inclusions suggestive of virus particles were seen. Mitotic figures were increased in number, but no abnormal forms were seen. These cytologic changes are in general agreement with abnormalities observed in other neoplasms by others.1, 8, 12, 20

Only the cells comprising the rosettes appeared to have specific orientation. The traditional view that rosette formation in retinoblastoma is a favorable prognostic feature is probably sound, since according to Oberling and Bernhard9 "polarity is gradually lost in proportion to the degree of anaplasia."

Degenerative changes. Necrosis and degeneration were conspicuous histologic features of both tumors. By light microscopy these areas were similar to such changes described by others.14

By electron microscopy degeneration and necrosis were even more widely evident, large portions of both tumors show-
Figs. 7 and 8. For legends see opposite page.
Figs. 9-13. For legends see page 741.
Figs. 14-17. For legends see page 741.
Figs. 18 and 19. For legends see page 741.
Figs. 20-22. For legends see page 741.
Figs. 23-26. For legends see page 742.
Fig. 9. Clump of tumor cells growing free in vitreous body demonstrates many nuclear irregularities which characterized both tumors studied. 1, Bilobed nucleus with thin strand-like connection. 2, Small nuclear processes connected by thin strand. 3, Apparently binucleated cells believed the result of sectioning across two lobes. Two fairly distinct patterns of nuclear chromatin are shown in adjacent cells, N and N'. (×2,600; reduced ½.)

Fig. 10. Widened spaces (arrows) resembling blebs result from separation of the two layers of the nuclear envelope. Such changes seemed to occur in well-preserved cells and were not accentuated in the process of degeneration. A portion of a cell at right shows numerous vacuoles (V) and swollen mitochondria. A round dark cytoplasmic inclusion body (b) is present in cell at lower right. (×7,600; reduced ½.)

Fig. 11. Tubular structures included within nuclei are shown in this and the following figure. The space here (arrows) lies in an area of densely clumped chromatin. (×24,000; reduced ½.)

Fig. 12. Parallel tubular structures (arrows) lie wholly within the nucleus and are separated from the inner lamina of the nuclear envelope by a thin rim of nucleoplasm. Invaginations into the nucleus by the inner layer of the nuclear envelope may account for some of these structures. (×25,000; reduced ½.)

Fig. 13. This membranous nuclear inclusion is shown in lower magnification in Fig. 7. Nucleoplasm is included within the double membrane structure (arrows). (×25,000; reduced ½.)

Fig. 14. Early mitosis, late prophase (mi). The nuclear membrane can be seen on the right (arrows) but may have disappeared on the left side of the cell. The fine granules of nuclear chromatin are irregularly clumped. Degenerated cells (D) can be seen at upper right and lower left and border on cells which appear healthy. (×6,800; reduced ½.)

Fig. 15. Late mitosis (telophase). The nuclear membranes shown in higher magnifications in Figs. 16 and 17 are beginning to reform. The plasma membranes (arrows) appear wholly reconstituted. (×10,000; reduced ½.)

Figs. 16 and 17. Detail of reforming nuclear membranes of cells shown in Fig. 15. Nuclear chromatin is dense, and at several sites (arrows) the nuclear envelope appears to dip within nucleoplasm. (Fig. 16, ×27,000; Fig. 17, ×23,000; both reduced ½.)

Fig. 18. Degeneration of progressive severity can be seen in cells from left to right. Cell in lower left corner (1) is apparently normal; adjacent cell (2) shows nuclear and cytoplasmic changes of early degeneration, and the two cells (3 and 4) at right show changes comparatively far advanced. (×11,000; reduced ½.)

Fig. 19. Advanced degeneration of both nucleus and cytoplasm, with comparatively good preservation of nuclear and cytoplasmic membranes is shown. Most cells at right show both layers of nuclear envelope and well-defined plasma membranes. (×14,000; reduced ½.)

Fig. 20. The earliest changes of cellular degeneration are shown. The mitochondria (M) are swollen; a large cytoplasmic vacuole (V) is shown at left; the nuclear chromatin of all the cells is irregularly clumped. The Golgi apparatus (G) can be identified in cell at right. Small round bodies (b) are seen in mitochondrion at left. (×19,000; reduced ½.)

Fig. 21. Junction between apparently healthy cells and areas of degeneration was often sharp. Even where degenerative changes are farthest advanced (right) membranous structures can be recognized. Myelin figure (mf) is shown above. The nature of the round gray bodies (arrows) is obscure. (×7,000; reduced ½.)

Fig. 22. A capillary within the tumor is shown adjacent to a tumor cell, left. Endothelial spurs extend into the lumen (L). The layers of the basement membrane (bm) are distinct. The mitochondria (M) of the tumor cell are more numerous on the vascular side of the cell. Two centrioles (ce) can be seen. (×8,700; reduced ½.)
Fig. 23. Case 2. Typical appearance of rosette. The radially arranged cells surround a central lumen (L), into which project blunt cellular processes, microvilli and cilia. A cell in mitosis (mi) is seen at right. The mitochondria (M) are principally on the luminal side of the nucleus. Arrow 1 points to detail shown in Fig. 24, arrow 2 to detail shown in Fig. 25. (×4,200, reduced %.)

Fig. 24. Detail of lumen (L) at arrow 1 shown in Fig. 23 is seen at higher magnification. Cellular processes and microvilli (mv) can be seen within lumen. Fibrillar cytoplasm (fi) can be seen in two cells at left. Terminal bars (tb) are present between cells near lumen. In cell process at lower edge of lumen there is structure (arrow) resembling centriole with spoke-like arrangement of pericentriolar bodies (pb). Located near the surface of the cell, these structures appear associated with cilium. (×15,000, reduced %.)

Fig. 25. Detail of edge of lumen at arrow 2 of rosette shown in Fig. 23. Portion of cilium (ci) in association with centriole (ce) can be seen. Pericentriolar bodies (pb) are seen in plane nearly perpendicular to those shown in Fig. 24. (×40,000, reduced %.)

Fig. 26. Cilium (ci) projecting into rosette lumen (L) is shown with associated centrioles (ce, and ce t). Pericentriolar bodies are not seen. (×31,000, reduced %.)

ing such changes. In considering the degenerative changes and the alterations in dead and dying cells observed in both tumors, we are unable to interpret the sequence of events. It remains uncertain at which stage a cell is "dead," if indeed cell death as such can be defined morphologically. Surprisingly little attention has been paid to the phenomenon of cellular death and the changes of necrosis which ensue.27 Studies have been made of the fine structure of postmortem alterations but the details remain to be published.10

The earliest changes which we interpreted as indicative of cellular degeneration are similar to those described by others in acute injury.2, 24 These consisted of slight clumping of the nuclear chromatin, swelling of the mitochondria, and formation of large cytoplasmic vacuoles (Fig. 20). The nuclear blebs in the tumors seemed unrelated to degenerative phenomena, but they have been observed in acute cell injury.5, 20, 21, 22

Later degenerative changes (Fig. 18, cells 3 and 4) consisted of clumping and coarsening of the nuclear chromatin, widening of the space between the 2 layers of the nuclear envelope, and marked swelling of the cytoplasm and of its components. These resemble later stages of cell injury.24, 32 Still later degenerative changes appear to be those depicted in Fig. 19. These resemble changes which have been observed in early phases of tissue necrosis.7, 9, 32 In many areas degenerative changes were those which seem to lead to pyknosis as described by light and electron microscopy.21

A different pattern of degeneration is also shown in Fig. 19. Some cells, though apparently in advanced degeneration, show remarkable preservation of nuclear and cytoplasmic membranes. The changes shown here do not seem to be related to pyknosis, but there is as yet no direct evidence to relate them to karyorrhexis or karyolysis. The remarkable persistence of nuclear and plasma membranes, and of some of the membranous cytoplasmic components, long after cellular organization is lost, is one variety of necrotic cell reaction which appears not to have been previously described.

The most advanced necrotic tumor cell degeneration is shown in Fig. 21. Bordering on cells apparently healthy or with only minimal changes of degeneration, there were wide expanses of cellular debris which included membranous fragments of cells, myelin figures, vacuolated bodies, lipoidal material, and granular material. The nature of the intermingled faintly granular gray
masses is not apparent. The appearance of these bodies by light microscopy is suggestive of the changes observed in late pyknosis.\textsuperscript{19}

**Possible cell origin**

The structural features of the cells forming rosettes provide some clues as to the possible origin of retinoblastoma. There are resemblances of these cells to the lining cells of the primitive neural tube.\textsuperscript{4, 10, 31} Some similarities, less striking, are evident when these cells are compared with ependyma.\textsuperscript{13, 25, 33}

The cell process with its centrioles and cilium shown in Fig. 26 is similar to that of developing visual cells in a 10-day-old mouse as shown by Lasansky and De Robertis\textsuperscript{18} in their Fig. 1. None of the material we viewed, however, showed the later stage of development where the cilium is expanded to contain saclike structures, eventually to differentiate into the outer segment of the visual cell (their Fig. 2).

The structures which resemble paricentriolar bodies shown in Fig. 23, and in higher magnifications in Figs. 24 and 25, are similar to those described by others.\textsuperscript{1, 5, 6} Somewhat similar, but not identical, structures have been described in association with the primitive cilia from which the rods and cones differentiate.\textsuperscript{18}

That the cells forming rosettes resemble both the ependymal cells and primitive visual receptor cells may be explained by the histogenesis of the retina. The developing pars optica retinae undergoes histologic changes similar to those which occur in other portions of the neural tube. The simple columnar neural epithelium differentiates into ependymal, mantle, and marginal layers. The ependymal zone, which is in contact with the outer pigmented layer, finally differentiates into the rod and cone cells. The visual receptor cells may therefore be regarded as highly specialized ependymal elements.\textsuperscript{13}

The controversy, therefore, as to whether rosettes represent immature visual cells\textsuperscript{35} or lining cells of the primitive neural tube\textsuperscript{20} is largely resolved to one of definitions.

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