Arch. Jap. Chir. 38(1), 12~34, Jan. 1969

Electron Microscopic Observations on Glioblastoma

by

TOKURO KUSUNOKI

Department of Surgery, Division 1, Kobe University School of Medicine
(Director: Prof. Dr. TAKAO MITSUNO)

Received for Publication Nov. 9, 1968

INTRODUCTION

A classification of the gliomas was initially described on a histogenetic basis by BAILEY and CUSHING in 1926. Their fundamental concept is still accepted, though various other viewpoints have been presented by other authors since then. The main subject of the author's observation, constitutes in frequency 20-30% of all gliomas[36] and has a high degree of malignancy. Histologically, it is a tumor composed of glioblasts, which show various grades of differentiation. It arises from the immature medulloblasts and is seen during the process of differentiation when it finally develops into mature neuroglia.

Microscopic comparison of the tumor cells of glioblastoma with normal neuroglia, the embryonal immature type of neuroglia and even mature glioma morphologically, is less analogous and cannot be distinctly affirmed as being its immature type. As the nomenclature of 'glioblastoma multiforme' signifies, its histological pattern is so complicating and various, that often gives rise to the question of its histogenesis.

It is obvious from such concepts that glioblastomas and neoplasms in general, take an anaplastic or metaplastic change during its process of development and it does not seem necessary for them to be analogous to the corresponding normal tissues. However, such concepts is barely applicable in the glioblastoma.

Main references with reports on electron microscopic studies of gliomas, was first made by FERNÁNDEZ MORÁN[9] (1947), later followed by LUSE (1958)[23] who reported on the normal astrocyte and reactive astrocyte in comparison with the neoplastic astrocyte. In 1961, LUSE[24] gave a detailed description of astrocytoma, malignant astrocytoma, ependymoma and oligodendroglioma in her report on the electromicroscopic observation of the brain tumor. NYSTRÖM (1960)[27] reported on the blood vessels of glioblastoma multifome, and RAIMONDI (1962)[30] on astrocytoma, oligodendroglioma, ependymoma, glioblastoma multiforme, etc., of human brain tumors in 98 cases.

In Japan, TUNODA (1959)[42] reported on his findings in astrocytoma, ependymoma, benign and malignant spongioblastoma, and oligodendroglioma. KOIZUMI (1962, 1963)[19][20] reported on the electron micrographs of glioblastoma and it's borderline area and further described the glioblastoma and medulloblastoma found during electromicroscopic observations of brain tumor. SAWADA (1967)[37] gave a similar report on 34 cases of the gliomas.

From these reports, it is of importance to note that the findings were not always agreeable and its explanation unsatisfactory. In spite of the amazing resolving power of

electron microscope, the visual field is extremely limited and sufficient care must be taken to identify the tumor cells. With these points taken into consideration, the morphologic charcteristics of the neoplastic cells of glioblastomas were studied electron-microscopically in comparisons with the mature type of the gliomas.

MATERIALS AND METHOD

Twenty three operated cases of gliomas experienced during the past two years with definite histological diagnosis, such as 13 cases of glioblastomas, 3 astrocytomas and 7 ependymomas, were selected as materials for this study. Two cases suspected to be ependymal origin are included in the gliolabstoma group.

Three to four specimen were taken from the tumor tissues with least invasion, and immediately divided into two blocks. One block was fixed in 10% formalin solution for light microscope use, and the other was cut into smaller pieces under 1 mm^3 immersed in 1% osmium tetraoxide of Millioig's phosphate buffer solution and fixed for 90-120 minutes for electron microscope use. After dehydrated with graded ethanol solution, the specimens were embedded in Epon. The embedded specimen were then cut into ultrathin sections, using glass knives set on Porter-blum microtome mounted on copper grids coating formbar. After double staining the specimens with uranil acetate and citratic lead, it was observed under the HU-11D type large size electron microscope made by Hitachi Co.. The same specimen was stained in toluidin-blue solution to be confirmed that it is a part of the tumor tissue under observation. A panoramic view of 3000 was prepared to compare and identify the cells.

OBSERVATION

Human normal astrocyte :

The nuclei, in general, were oval form with diffuse dispersed chromatin material and low electron density. Uniform nucleolei were occasionally seen. Cytoplasma with pale or empty appearance were poor in organellaes and tended to extend out from the narrow perinuclear zone to numerous intertwined cytoplasmic expansions.

Astrocytes are commonly classified as fibrous type and protoplasmic type under light micrographs, but no significant differencies in their type were formed in their structures; that is, the scanty cytoplasma surrounding the nucleus only contained ergastoplasmic granules, microvesicular components and rarely small mitochondria, and the cytoplasmic processes frequently contained gliofilaments of 70A in diameter (Fig. 1).

Astrocytomas :

The main difference in the fine structure of astrocytomas with that of normal astrocyte were as follow :

The chromatin material in the nuclei varied in distribution and tended to concentrate near the nuclear membrane. The cytoplasma were observed from scant to abundant and contained various size of mitochondria, rough surfaced endoplasmic reticulum (R. E. R.) consisting of paired membranes associated with ribosome, enlarged vacuoles, dense bodies and glio-filaments. The Golgi apparatus with lamellae and vesicle was seen occasionally. In most part, the cytoplasmic processes were more blunt than those of normal astrocytes and were filled with gliofilaments even in the cells of the protoplasmic type. In one case

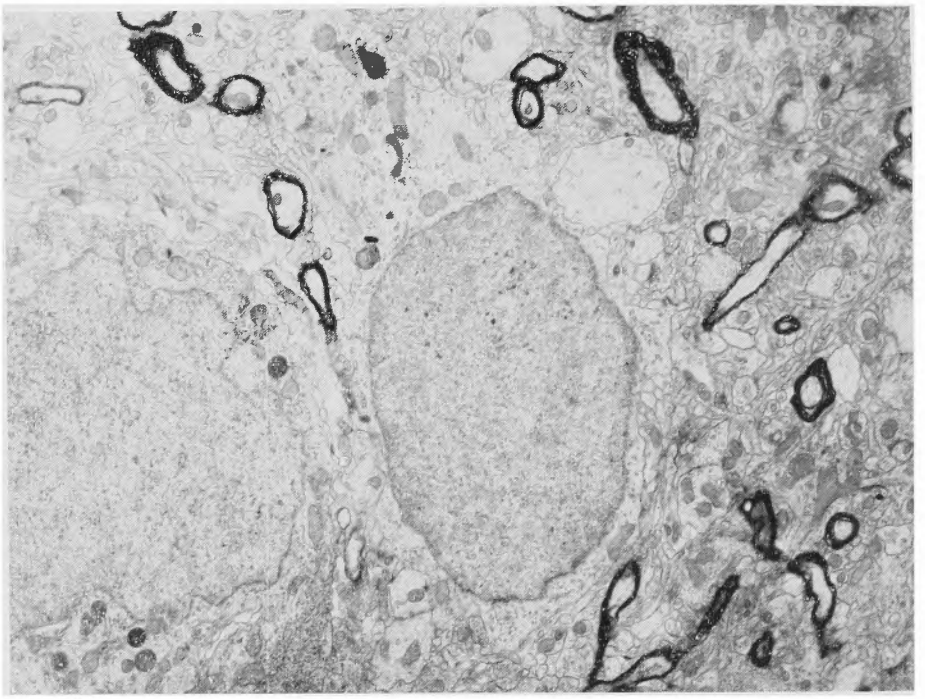


Fig. 1. Normal astrocyte in gray matter.
The nucleus is oval, and has a diffusely dispersed chromatin. The cytoplasma appearing watery have several mitochondria, free ribosomes and rough surfaced endoplasmic reticulum. Golgi apparatus is manifest just. (×7000)

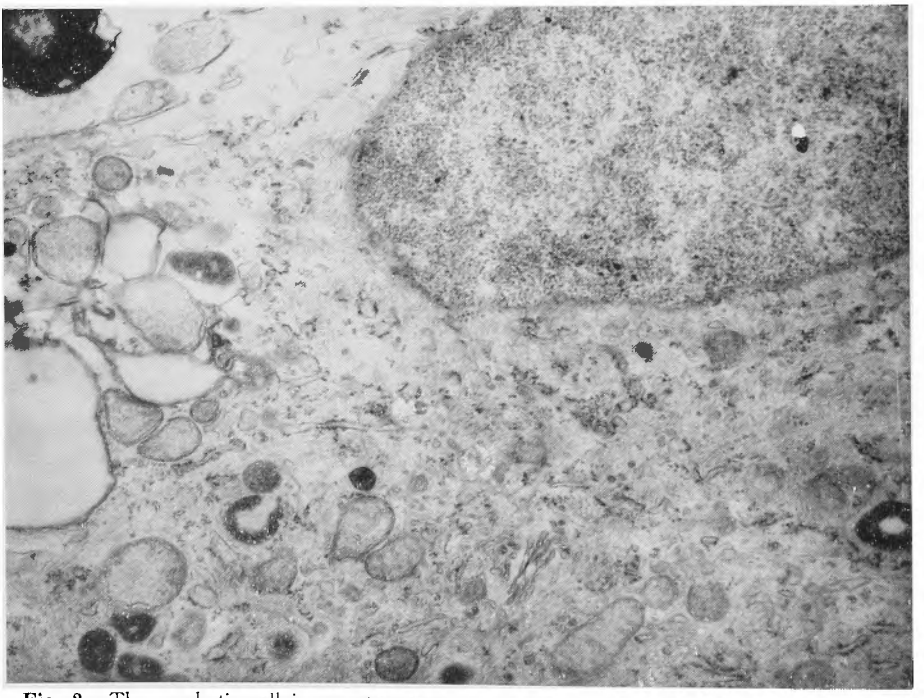


Fig. 2. The neoplastic cell in an astrocytoma.
The chromatin with high density in a nucleus is irregularly dispersed. The glio-filaments proliferate in cytoplasma. Enlarged vacuoles, a various size of mitochondria and dense bodies are observed in the abundant cytoplasma. (×20000)

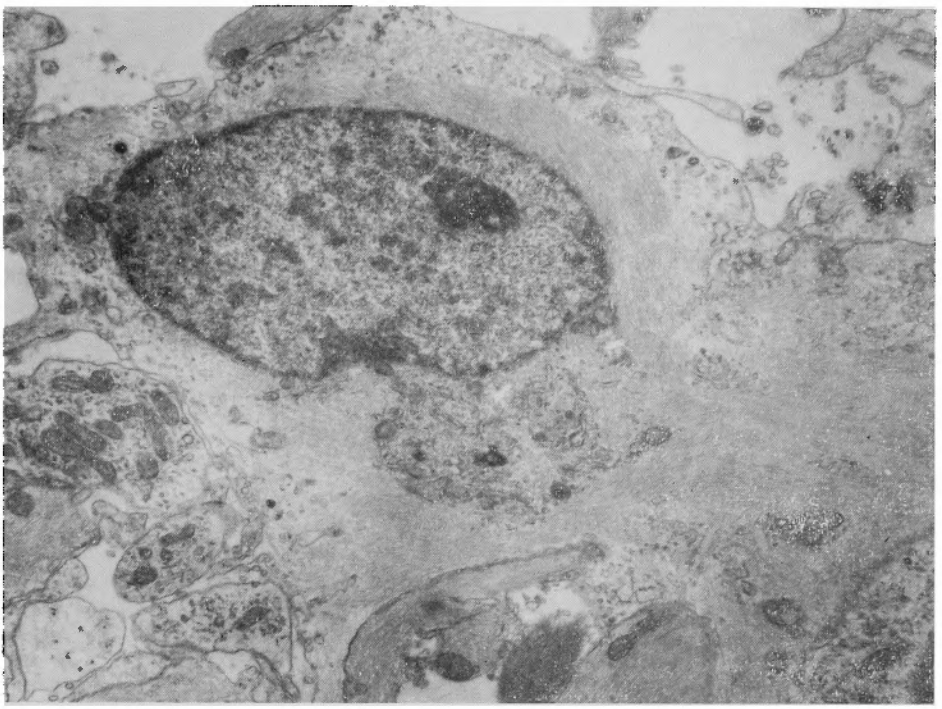


Fig. 3. The neoplastic cells in a fibrous astrocytoma.
These neoplastic cells have scant cytoplasma and one or two long cytoplasmic processes which have a concentration of glio-filaments. Many fragments of cytoplasmic processes are scattered in wide extracellular spaces. (×9000)

diagnosed as fibrous astrocytoma light microscopically, the cytoplasma was scant around the nucleus and extended to the slender processes filled with gliofilaments. In this case the cytoplasmic organellaes were scarce with the exception of mitochondria with dark matrix and free ribosomes. As extra-cellular spaces apparently existed, cytoplasmic processes were scattered, and the neoplastic cells were arranged almost untouching each other (Figs. 2, 3).

Glioblastoma :

Three groups of glioblastoma may be classified electron microscopically.

1) In light microscope this type of the neoplastic cells is recognized in most of the glioblastoma multiform consisting of multipolar cells arranging reticular nets pattern, or spindle cells with stream-like arrangement.

The nuclei were various in size, form, and distribution of chromatin material, and sometimes represented the accumulation of chromatin material near the nuclear membrane, one or more conspicuous nucleolei, and deep clefts of nuclear membrane or cross-sectionally appearing cytoplasmic inclusions. The ratio between the size of nucleus and cytoplasma was inconstant. The cytoplasmic organellaes in glioblastomas were more abundant than in astrocytomas. Some of the cells had abundant cytoplasma with few blunt processes, while other had scant cytoplasma with many slender processes. In general, well developed Golgi apparatus consisted of lamellae and vesicles, numerous R. E. R., and various size and form of mitochondria with uncertain creste were observed in the cells which had

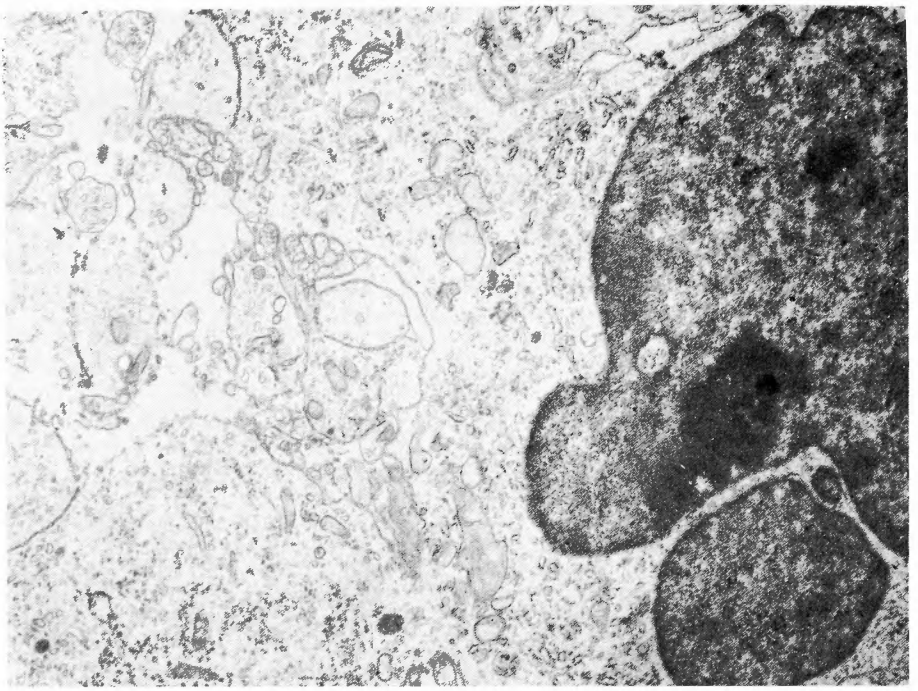


Fig. 5. Glioblastoma.
This neoplastic cell has abundant cytoplasma with blunt processes and large nucleus. Many free ribosomes, endoplasmic reticulum combined with ribosomes (R.E.R.) are scattered in the cytoplasma. Gliofilaments are less found. (×8000)

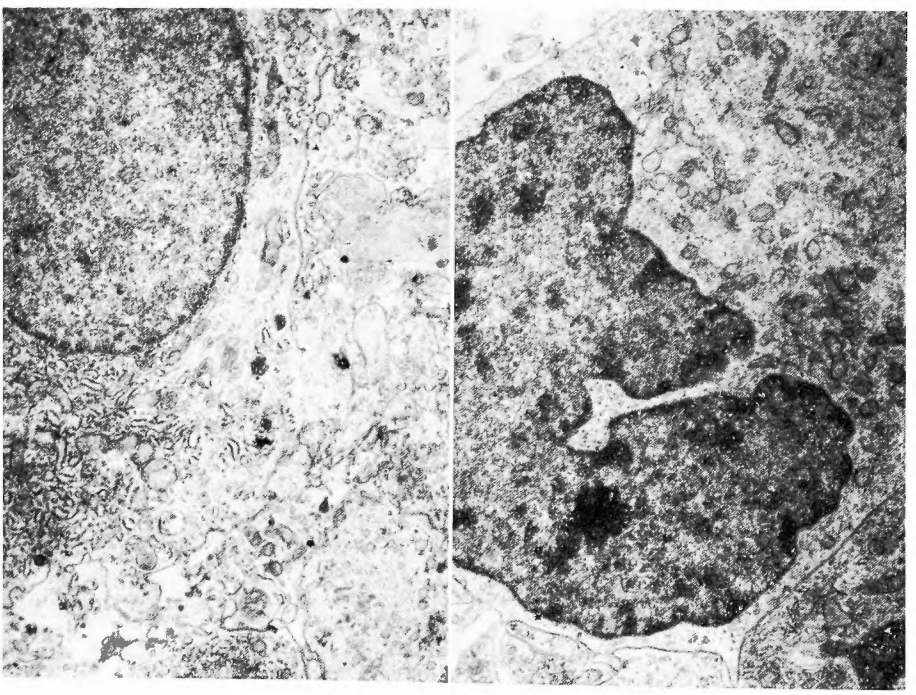


Fig. 6. Glioblastoma.
The neoplastic cell which has relatively developed rough surfaced endoplasmic reticulum is manifest.
(×10000)

Fig. 7. Glioblastoma.
The neoplastic cell which has many various sized mitochondria and microvesicles in cytoplasma is shown. The deep cleft of nuclear membrane is manifest. (×7000)

abundant cytoplasma, but were absent of glio-filament in the perinuclear area (Figs. 5, 6, 7).

But in other cells with scant cytoplasma and many slender processes, the cytoplasma was pale in appearance and had a few ribosomes, polysomes, various size of vesicles, and mitochondria which varied in form and size. In the giant cells, the large nuclei with one or more nucleolei and numerous deep clefts of the nuclear membrane, developing of R. E. R., well developed Golgi apparatus, and proliferation of the glio-filaments were characteristic in all cases. As it was in astrocytomas, the various size of processes of tumor cells were twisted confusely whether they were filled with glio-filaments or not. These processes were loosely attached to the swelling basement membrane of capillaries like astrocytic vascular feet. Extracellular spaces were seen around many degenerating cells (Fig. 4).

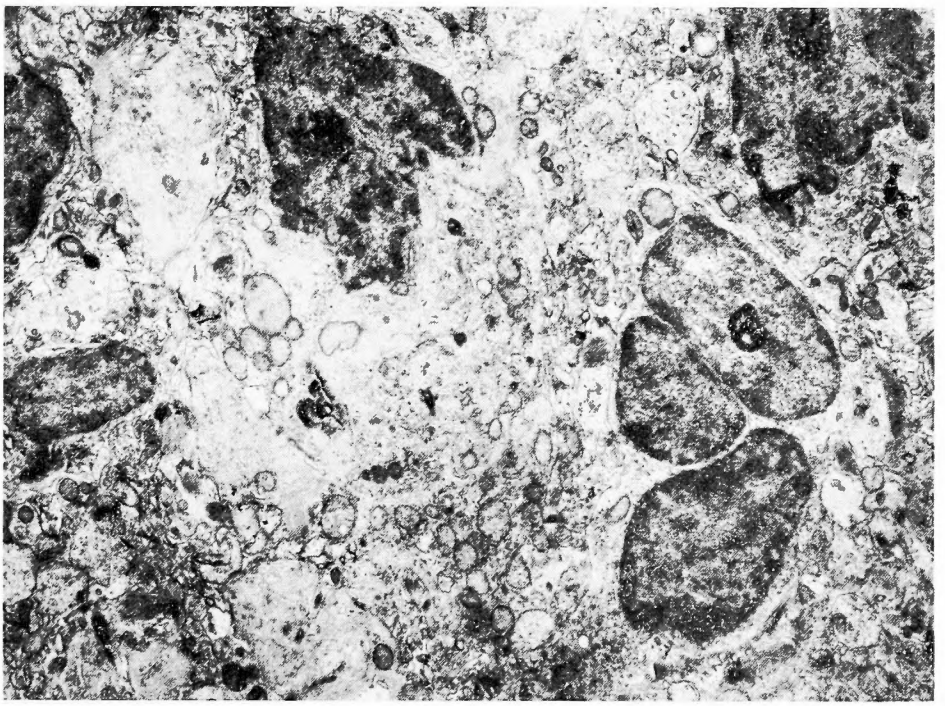


Fig. 4. Glioblastoma.
The various size and form of nuclei have rich chromatin, and conspicuous nucleolus. The cytoplasmic processes are filling with glio-filaments or without them, and intertuine each other. It tends to show numerous organellaes in more abundant cytoplasma. Tri-nuclear neoplastic cell are manifest. (×6000)

The fine structure of spindle cells in the glioblastoma multiforme were essentially the same except that they had irregular elongated nuclei and cytoplasma with abundant glio-filaments, extending out to the long cytoplasmic processes, and spacious extracellular spaces (Fig. 8). This type of the glioblastoma cells were not so different in the fine structure of the intracytoplasmic organellaes and differentiation of the processes in comparison with that of astrocytoma. It seemed to be the highly atypical type of astrocytoma by the reasons of the findings of nuclei, inconstancy of the ratio between size of nucleus and

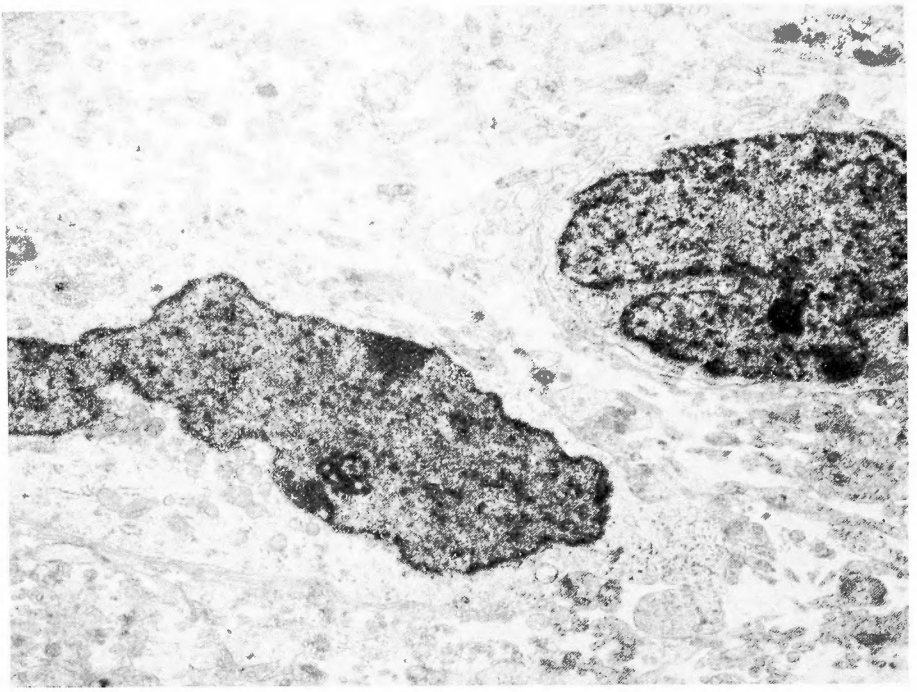


Fig. 8. Glioblastoma.
The elongeted irregular shape of nuclei and the cytoplasma which have many slender processes with gliofilaments or without them are manifest. The cytoplasma has not developed structures except mitochondria, scant rough surfaced endoplasmic reticulum and ribosomes.
(×7000)

cytoplasma and intensive cellular polymorphism and etc.

2) Light-microscopic findings of this type of neoplastic cells are observed in the glioblastoma consisting of multipolar cells seen in the first type. But the electron micrographs of these neoplastic cells were different. The nuclei were round or oval, and had fast uniformly the chromatin material with high electron density. The medium sized nucleolei and the clefts of nuclear membrane were observed. The cytoplasma were scanty around the nucleus generally containing only free ribosome and small mitochondria.

The cytoplasmic processes were short and blunt, sometimes slender, but not so developed as the first type. Neoplastic cells arranged each other tightly keeping intercellular spaces approximately 200-300A. The gliofilaments were not seen. From the findings that numerous ribosomes, absence of R. E. R., and Golgi apparatus and incomplete developing of the cytoplasmic process were presented, this type of glioblastoma might be considered as immature neoplastic cells (Figs. 9, 10).

3) In light microscope this type of the neoplastic cells is recognized in the glioblastoma consisting of polymorphic cells with hyperchromatic nucleus and scant cytoplasma arranged closely.

The nuclei were elongated oval or irregular round with a diffusely dispersed chromatin material, and a conspicuous nucleolei appearing networks. The clefts of nuclear membrane were sometimes seen. The cytoplasma were scant containing only a few ergastoplasmic

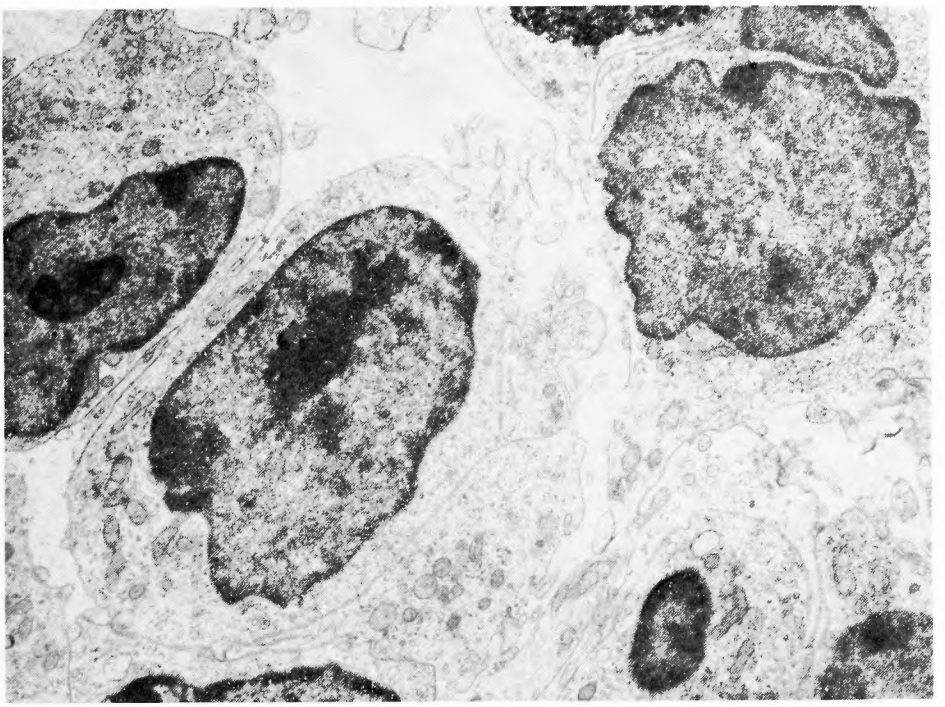


Fig. 9. Glioblastoma.
The cells are relatively round from with few processes. The cytoplasma contains ribosomes and micro-vesicles but no filaments. (×7000)

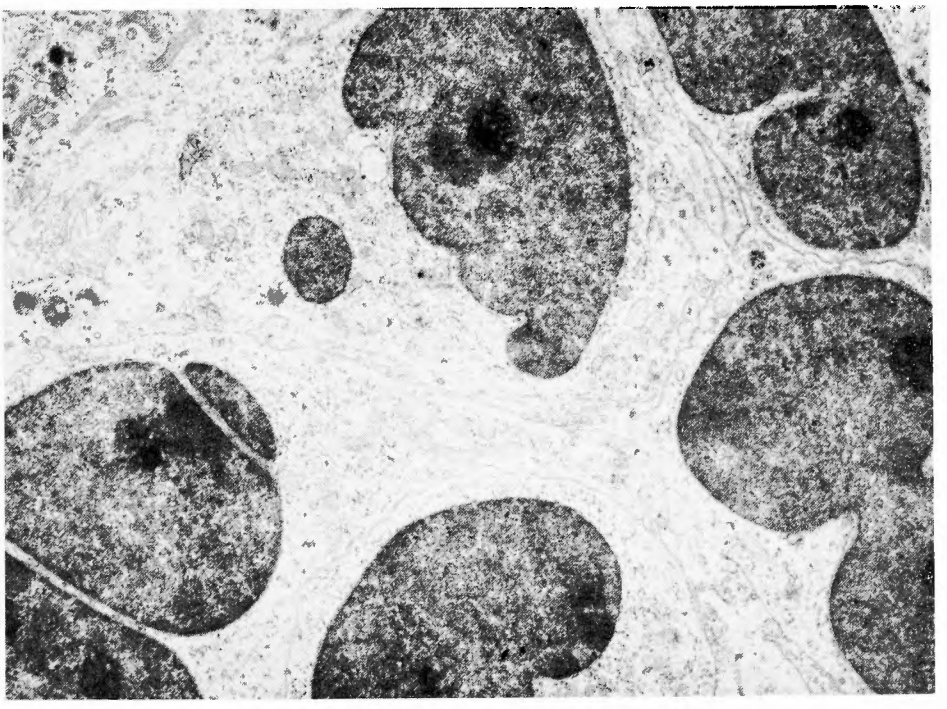


Fig. 10. Glioblastoma.
The nuclei have a homogeneously dispersed chromatin with high density. The cytoplasma have no gliofilaments and less R. E. R.. Ribosomes are scattered in the cytoplasma. (×8000)

granular components, vesicles, and various size of mitochondria. Differentiations of the cytoplasmic processes were slightly observed, but generally blunt and short. The neoplastic cells were arranged at intercellular space of 200-300A. In the perivascular area, blunt cytoplasmic processes or a part of the cell body attached to the basement membrane of capillaries. Some light micrographs of this tumor resembled medulloblastomas, but the fine structures of these neoplastic cells were not similar to those of medulloblastoma which had been observed by other investigators. R. E. R., Golgi apparatus, and ribosomes were not present in the cytoplasma. The fine structure of these neoplastic cells might result from the anaplastic differentiation as in other malignant neoplastic cells, especially in carcinoma, because of the simple findings of the cytoplasma (Figs. 11, 12).

Giant cells were recognized in almost all glioblastoma. Their characteristic fine structures were, generally, the presence of the large bizar nuclei with prominent nucleolei and deep clefts of nuclear membrane, and the cytoplasma were very abundant containing glio-filaments and numerous organellaes, such as well developed Golgi apparatus and R.E.R., various size and form of mitochondria with irregular creste, and vesicles. The fine structure of the so-called gemistcyte was that the eccentric small nuclei with ireregularly dispersed chromatin material with high electron density, had obscure nucleolei, and the abundant cytoplasma filled with numerous glio-filaments, only a few small mitochondria with dark matrix, and a few isolated R. E. R.. It was difficult to determine the nature of the cells, but they might be considered as one of the mature neoplastic cells which

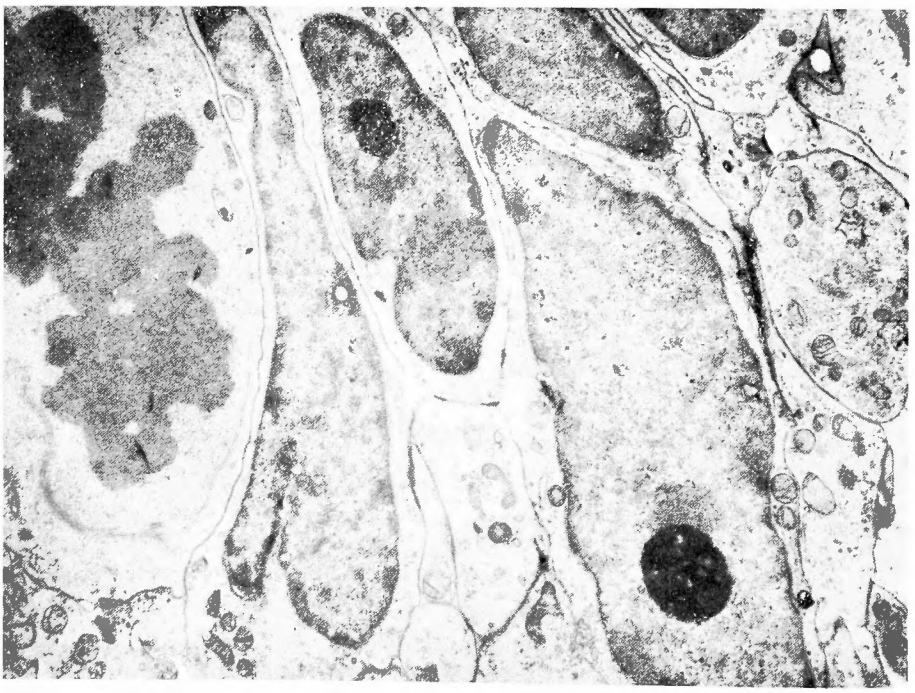


Fig. 11. Glioblastoma.
The cytoplasma are scant in comparison to the size of nuclei, and poor in organellaes. The elongated nuclei have conspicuous nucleolei appearing network. The cytoplasmic processes less extend. Mitotic figure is manifest in left-side. (×8000)

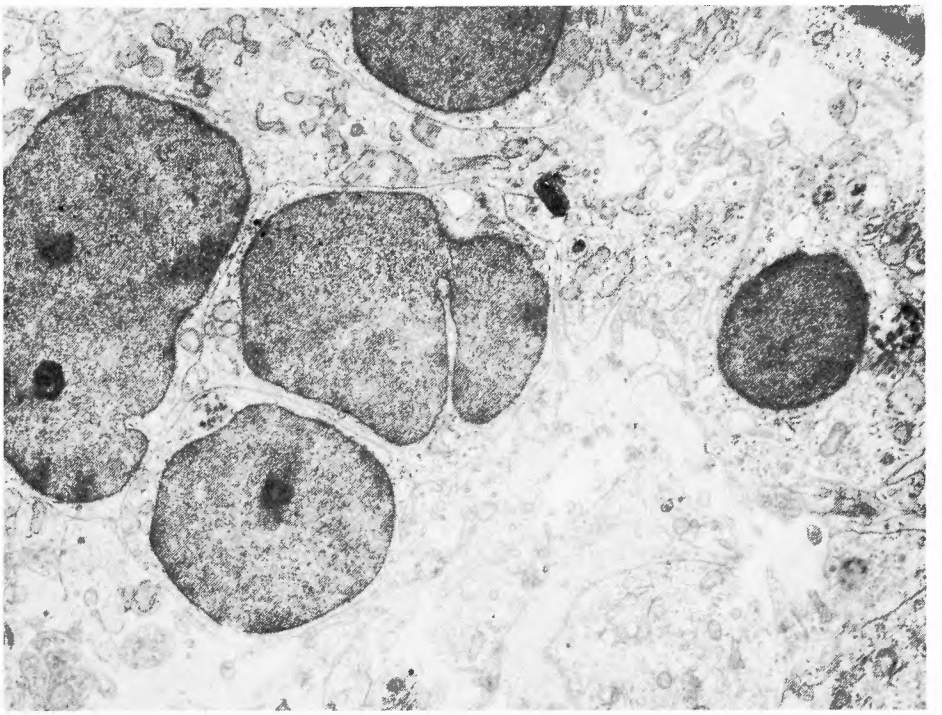


Fig. 12. Glioblastoma.
The nuclei have deep clefts of nuclear membrane appearing irregular form. The cytoplasma are scant in comparison to the size of nuclei and poor in developed organellaes. (×7000)

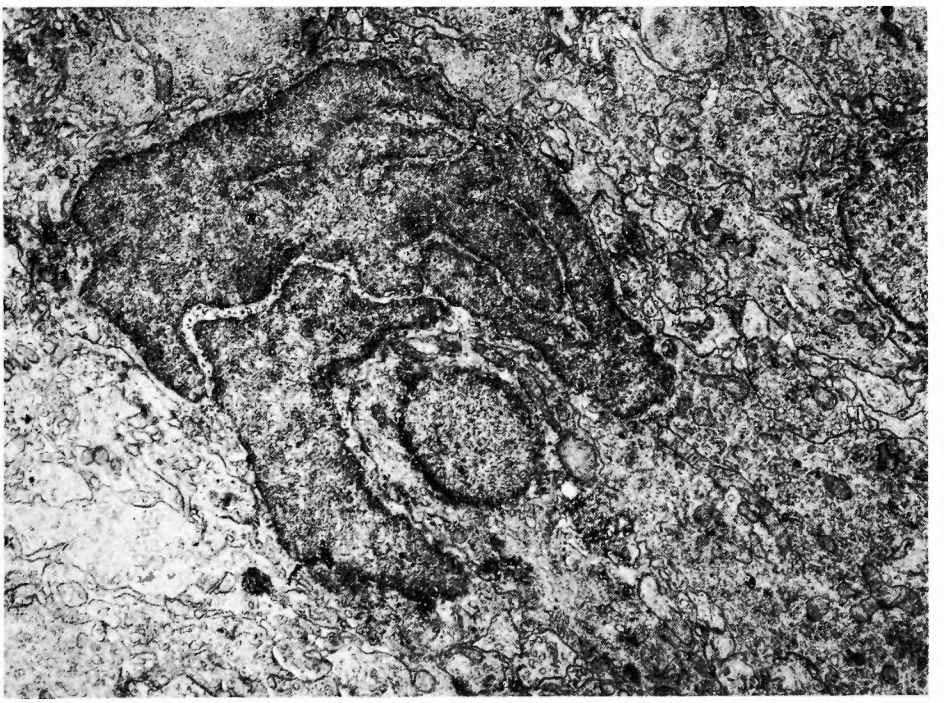


Fig. 13. Glioblastoma.
The giant cell which has bizarshaped nuceus is manifest. It tends to show elongated large mitochondria in astrocytic giant cells. (×7000)

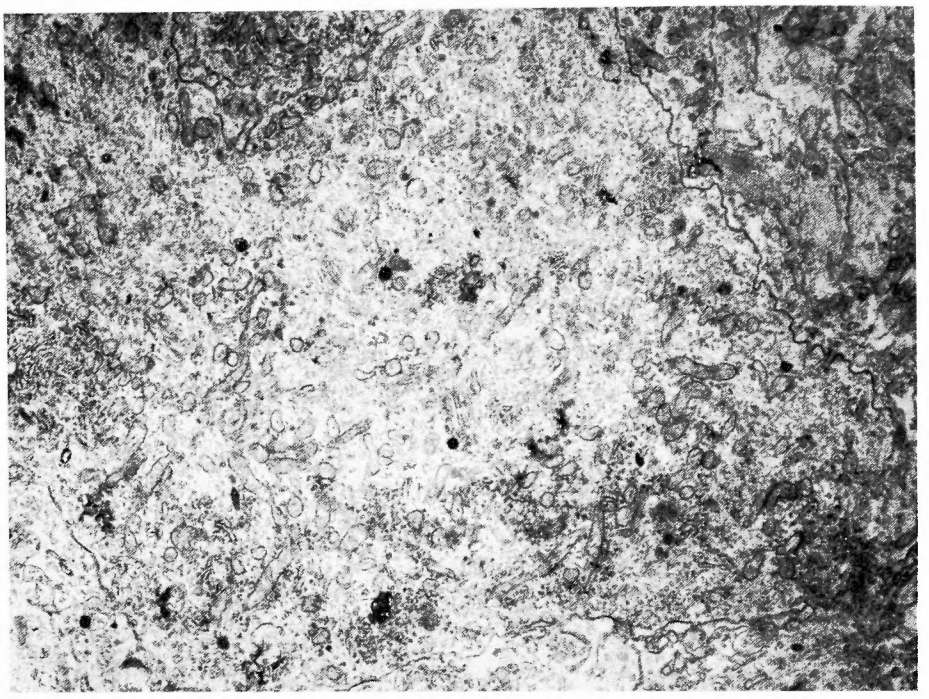


Fig. 14. The cytoplasma of giant cell.
Numerous elongated mitochondria, increasing the number of Golgi apparatus, R. E. R. and ribosomes are observed. (×7000)

accumulated glio-filaments (Fig. 15).

Except these neoplastic cells, small round cells like small lymphocyte light-microscopically were observed in the glioblastoma. On the electron microscopy, theses cells smaller than neoplastic cells presented the fine structure of resembling lymphocyte, plasmoblast, and plasa-cell (Fig. 16).

Ependymomas :

In the mature type of ependymoma, the structures of neoplastic cells were considerably similar to that of normal ependym cells. That is, the nuclei were oval with uniformly low electron density and had prominent nucleolei. The cytoplasma had abundant cytoplasmic organellaes, approximately 70A fine filaments, and sometimes multivescular body. Through the observation of cell membrane, microvilli and cilia on the free surface, terminal bar and intermediate junction on the cell membrane of contiguous cells were observed. The cytoplasma extended out some processes in which contained the filament. Generally in the ependymoma, each tumor cell tended to show similar structure in a specimen (Figs. 17, 18, 19).

Ependymal glioblastoma :

In one case, the nuclei were various shaped with chromatin material variable in distribution, conspicuous nucleolei and the clefts of nuclear membrane, and the cytoplasma was relatively abundant filling with approximately 70A filaments. R. E. R., dense body, a few vacuoles, various size of mitochondria and ribosomes were observed in the cytoplasma. Microvilli, cilia, and terminal bar or intermediate junction as ependymal character, of the

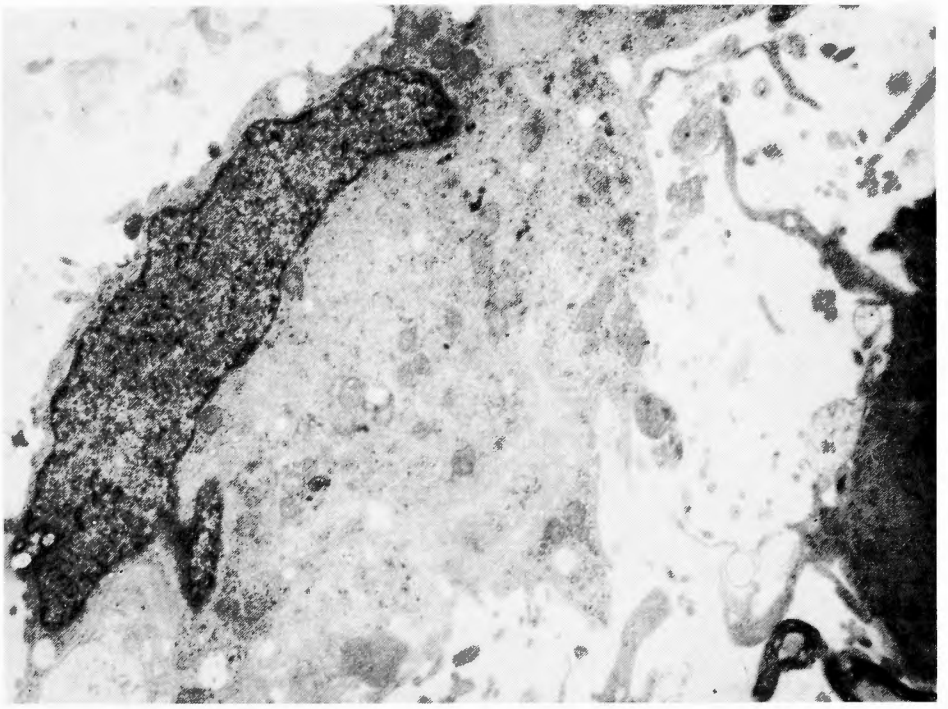
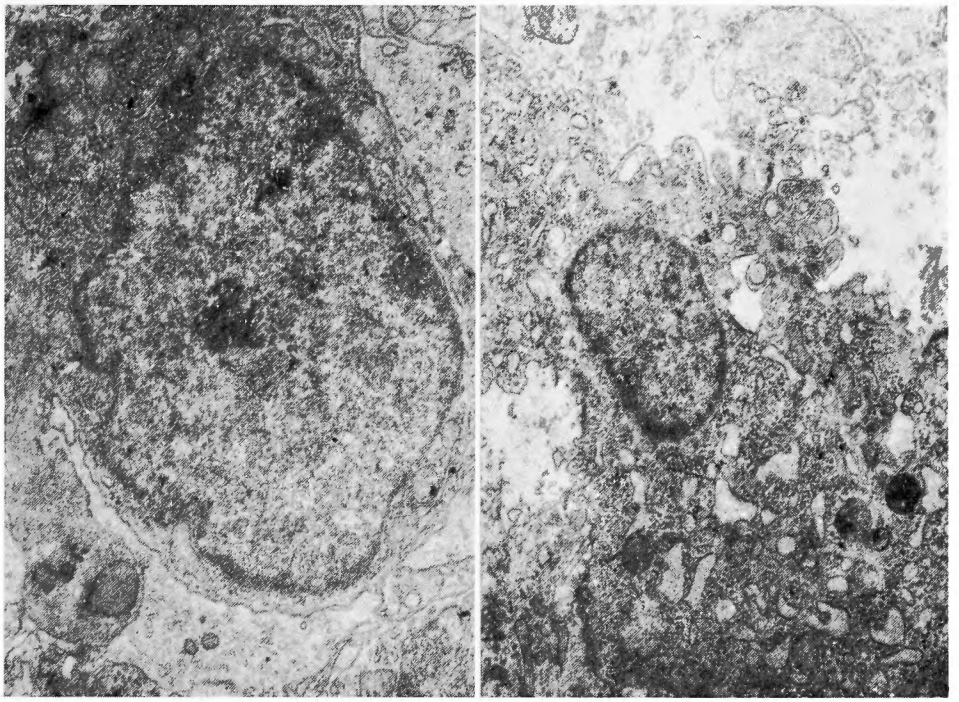


Fig. 15. So-called "Gemistocyte" The eccentric nucleus and the abundant cytoplas ma filled with glio-filaments are observed. The mitochondria with dark appearances and scant ribosomes are in the cytoplasma. (×6000)



A B

Fig. 16. The cells resembling to plasmoblast (A) and plasma cell (B) are manifest. Ribosomes are numerous in cytoplasma. The cysternae delate, containing homogeneous material with moderate density in (B). (×7000)

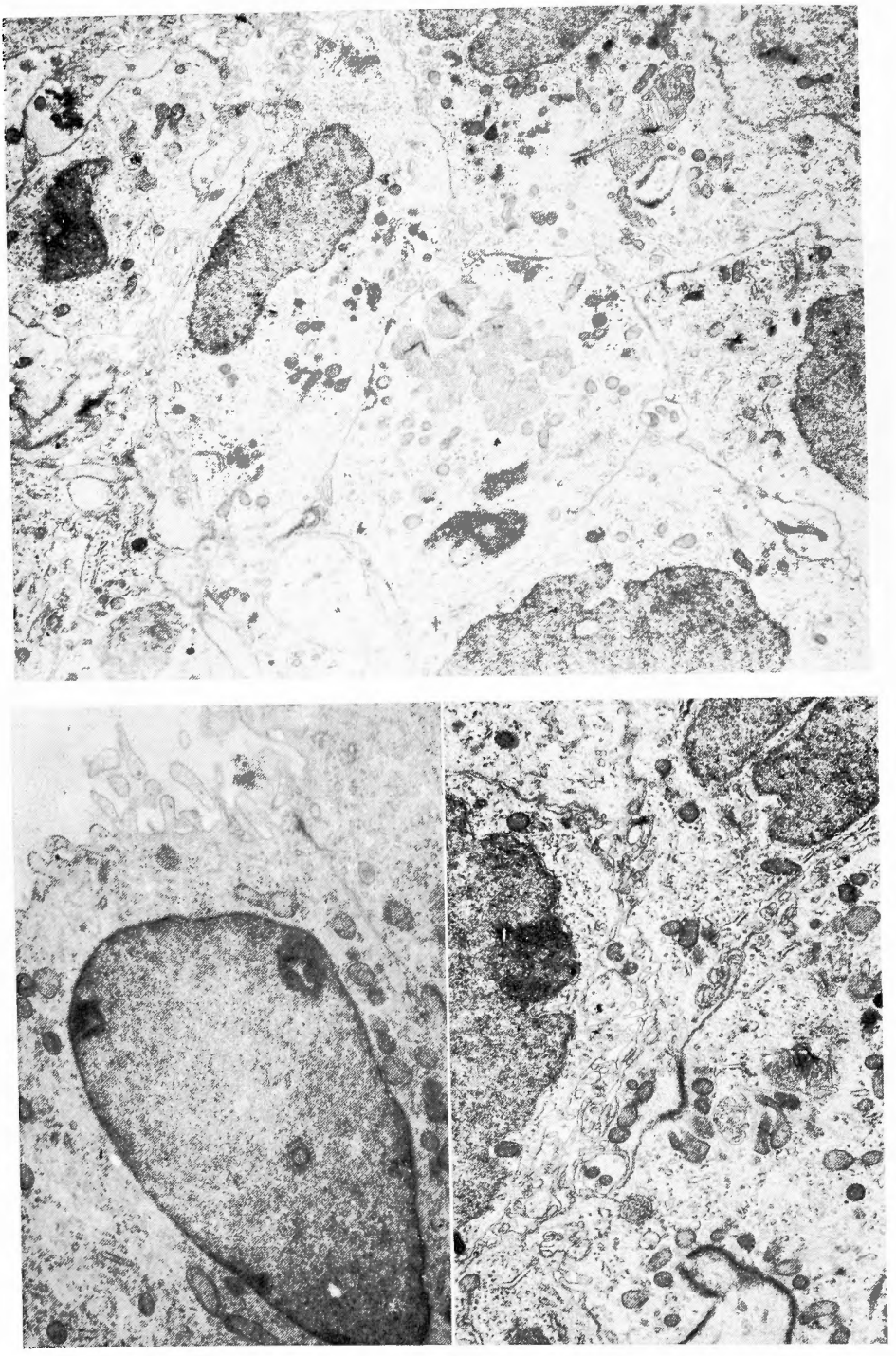


Fig. 17, 18, 19. Ependymoma.
The nuclei have a homogeneously dispersed chromatin.
Ependymal characteristics such as microvilli, cilia, terminal bar and intermediate junction, multivesicular body, and fine filaments are observed. Invagination of microvilli into cytoplasma, mitochondria with dark appearances, and Golgi apparatus are manifest.
(×7000) (×16000) (×10500)

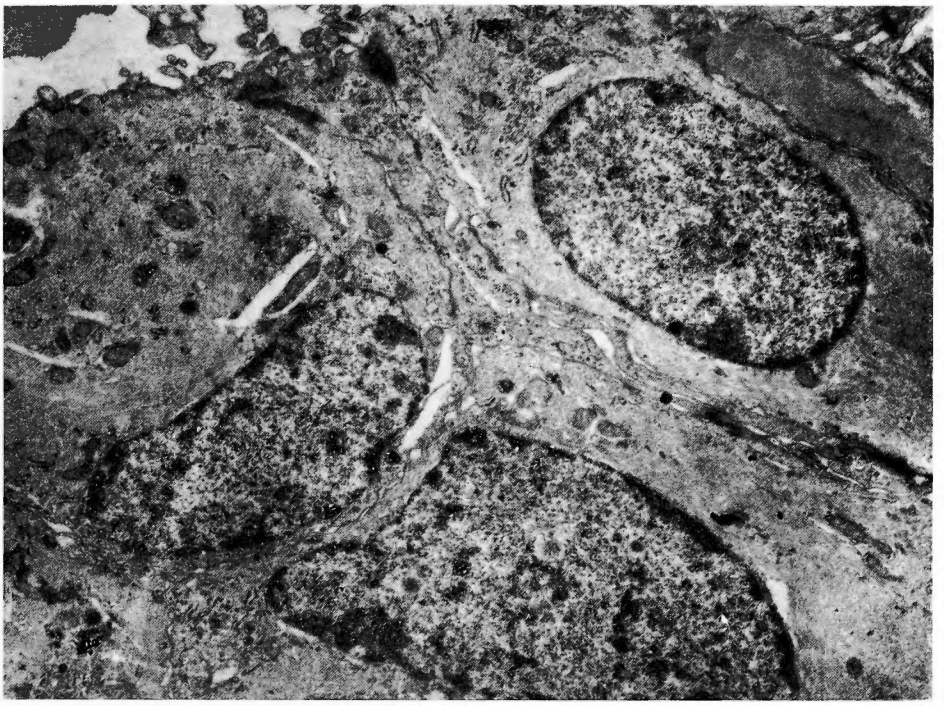


Fig. 20. Ependymoma Grade 3.
The nuclei have irregularly dispersed chromatin with high density. The cytoplasma are filled with numereus bundles of fine filaments. The microvilli on free surface, intermediate junction and terminal bar are observed. (×8000)

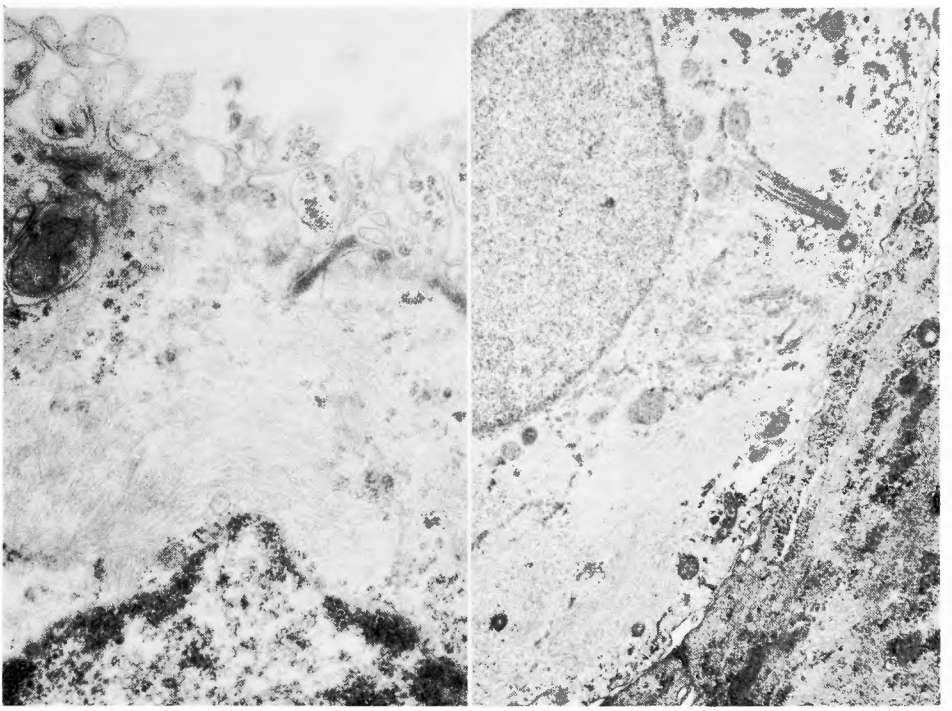


Fig. 21. Ependymoma Grade 3.
Microvilli, in which contain ribosomes but no filaments, are manifest.
(×20000)

Fig. 22. Ependymoma Grade 3.
The cross-section of cilium is seen in the cytoplasma. (×10000)

neoplastic cells tended to decrease the number and to atrophy the structure. Extracellular spaces were sometimes seen (Figs. 20, 21, 22).

The other case of ependymal glioblastoma was a so-called Giant-celled glioblastoma. Histogenetically two concepts on this tumor were found in late literatures. One was the ependymal origin, and the other a subdivision of glioblastoma multiforme. From electron-microscopic observation, this case might be considered as of ependymal origin because of the following findings. The nuclei were irregularly shaped with chromatin material distributed diffusely, and had one or two conspicuous nucleolei appearing networks. Deep clefts of nuclear membrane and cytoplasmic inclusion in the nuclei were frequently observed. The cytoplasma were abundant containing numerous organellaes and about 70A filaments in all neoplastic cells. These filaments flow from perinuclear zone to the processes and were especially numerous in the processes. The Golgi apparatus were composed of well developed lamellar structure. The mitochondria became variously large and increased in number. The R. E. R., S. E. R. (smooth-surfaced endoplasmic reticulum) and dense body like lysozome were observed here and there in whole cytoplasma. On the cytoplasmic membrane, sometimes microvilli and cilia on the free surface, invagination of microvilli in the cytoplasma and intercellular attachment as intermediate junction and terminal bar were observed (Figs. 23, 24, 25).

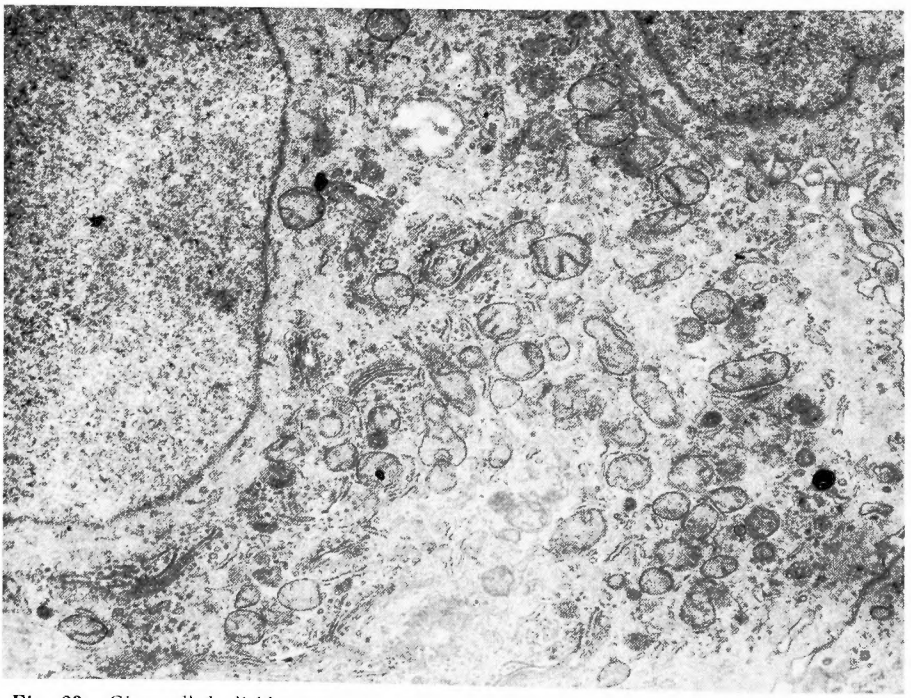


Fig. 23. Giant-celled glioblastoma.
The nucleus has diffusely dispersed chromatin and abundant cytoplasma which contain numerously well developed Golgi apparatus, large size of mitochondria and filaments are observed. Microvilli on free surface are manifest. (×7000)

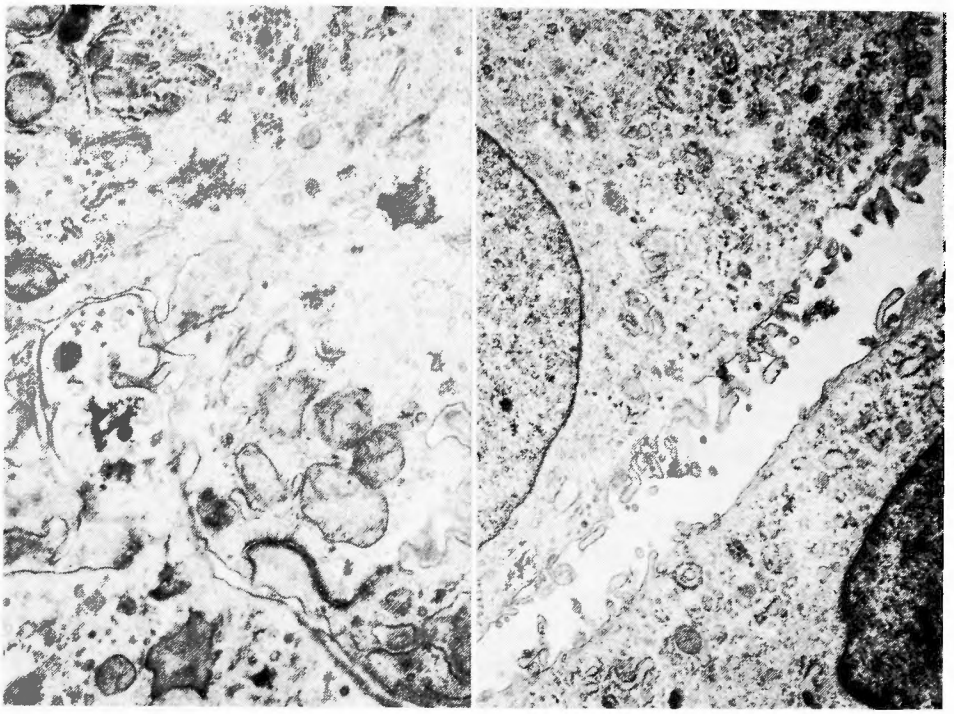


Fig. 24. Giant-celled glioblastoma.
The microvili on infolded all membrane are manifest in cross section.
Intermediate junction is observed in adjacent tumor cells. (×7500)

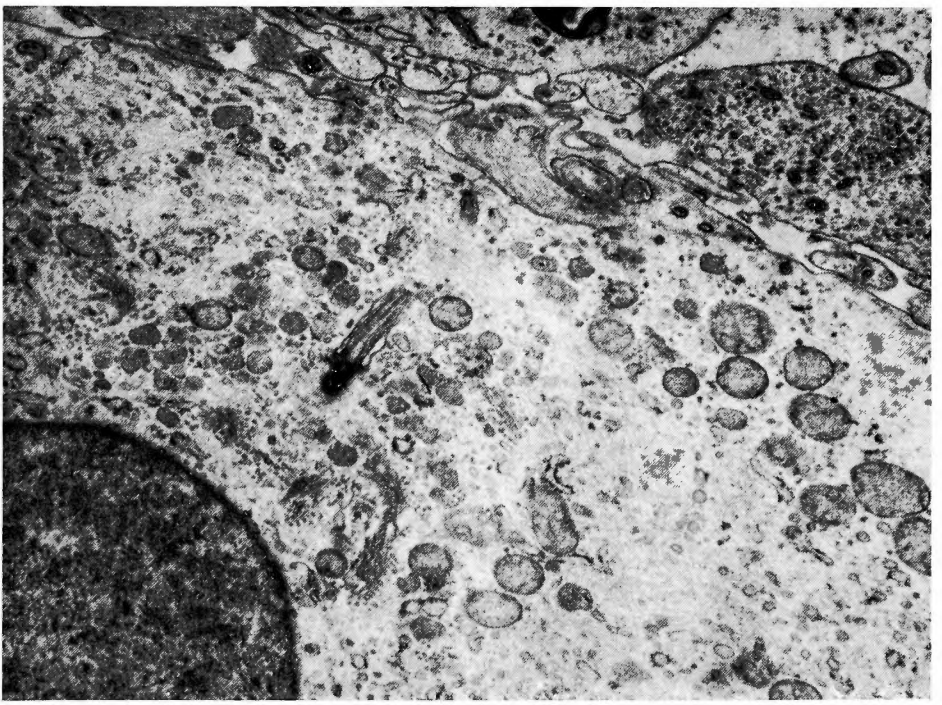


Fig. 25. Giant-celled glioblastoma.
The cilium and ciliary rootlet of giant cell are manifest. (×7500)

DISCUSSION

Up to date, a great number of investigations have been reported on the electron-microscopic observation on gliomas[6)12)16)19)20)23)24)27)30)31)33)37)41)43)] and neuroglia[7)10)13)15)38)]. The main problem in electron microscopic observation lies in the identification of cells. In relation to the identification of astrocyte and oligodendroglia, it is now understood that the astrocyte has a relatively large oval nucles with diffusely dispersed chromatin materials, while the cytoplasma is adundant and has a pale appearance with few intracytoplasmic organellaes, rarely with glio-filaments of approximately 70A in diameter[7)38)].

The identification of the protoplasmic astrocyte and fibrous astrocyte through light-microscopic classification has been reported by PENFIELD (1932)[28)] and GLESS (1955)[15)]. The fibrous astrocyte is abundant in white matters, while the protoplasmic astrocyte mostly in gray matters. In the author's series of electron microscopic investigations in normal human brain, the neuroglia in white matter generally had scarce cytoplasma and slim cytoplasmic processes. On the other hand, the neuroglia in gray matters had abundant cytoplasma with blunt processes, and no essential differences were found between the organellaes of neuroglia in both matters as reported by HAMA et al.[7)15)38)] The light-microscopical struture corresponding to the gliar-fibrils constitutes one of the criteria for their classification and may be appear more as extended cytoplasmic processes rather than the glio-filaments as commonly referred to on electron-micrographs. FARQUHAR and HARTMANN (1957)[7)] assumed that glio-filaments might possibly be an artificial product aggregated by fixing certain element to the cytoplasms. FUJITA (1962)[11)] observed the area near the superior nucleus of the optic tract in rabbits and found no neuroglia with filaments in the perinuclear cytoplasma, but sometimes 60-100A filaments in the processes. It, however, was difficult for him to determine whether the processes belonged to the nerve cell or to the neuroglia. In the author's observations glio-filaments were not clearly noted in the cytoplasma of normal astrocyte. Thus, it would be unnecessary to do a electron microscopic identification for these two types.

The difference between neoplastic cells of astrocytoma and normal astrocyte lies in the fact that in neoplastic cells the nuclei have numerous chromatin materials with high electron density, while the cytoplasma contains abundant organellaes such as various size of mitochondria and developed R. E. R., and glio-filaments in the perinuclear area. According to BERNHARD (1958)[1)], mitochondria in the tumor cells are struck by the extraordinary variation in their number, size, shape and matrix density, and by frequent lesion they present. This is agreeable as one of the features of tumor cells.

In one case microscopically diagnosed as fibrous astrocytoma, the cytoplasma was filled with gliofilaments. Many authors[6)23)24)30)37)43)] reported that glio-filaments were found in fibrous astrocytoma, but, so far, the significance of proliferation of glio-filaments have not been discussed. Glio-filaments are also observed in the cytoplasmic processes of neoplastic cells in protoplasmic astrocytomas. Thereas, there is no significance in distinguishing these two. As such proliferation of glio-filaments was also noted in one case of ependymal glioblastoma in the studies, it may be considered as one of the patterns of neoplastic differentiation.

Basically electron micrographs of glioblastomas can be classified into 3 groups. In

the first group, though the irregularity of nuclear structure, variety in ratio between the size of nucleus and cytoplasma, cellular polymorphism, etc. were observed in the neoplastic cells, the fine structure of intracytoplasmic organellaes was not different from that of astrocytoma. These findings agree with the astrocytoma which was called "malignant astrocytoma" by RUSSELL and RUBINSTEIN[35]. The neoplastic cells in this group were generally atypical, and then the cells with clear or dark cytoplasma and with or without glio-filaments were observed in the same specimen, but their stage of differentiation were identical.

LUSE (1961)[24] stated that the tumor cells lacking glio-filaments were abundant in mitochondria. But in the author's observations, mitochondria had a tendency to increase the number in such cells with abundant cytoplasma as giant cell. Some workers[24][37] had distinguished the tumor cells of glioblastomas, to those with or without glio-filaments. But though glio-filaments are absent in perinuclear cytoplasma, they are often observed in the processes, and it is unnecessary to distinguish these two.

The second group of glioblastoma cells is the immature type. There has been no special record on the immature type in late reports. But RAIMONDI (1962)[30], in his reports on glioblastoma multiforme, stated that no glio-filament was found in the immature-appearing cells while mitochondria was abundant. In the author's observations, the neoplastic cells of this type contained numerous ribosome though glio-filaments in cytoplasma was deficient. In cells with abundant cytoplasma there were numerous mitochondria with dark matrix.

In 1958, BERNHARD[1] stated that "the malignant tumor cells, in general, have a tendency to lose their organized lamellar ergastoplasma, and basophilia is more often linked to RNA granules only which are, as in normal embryonic cells, diffusely scattered in the cytoplasma or grouped in small clusters." Such proliferation of free ribosomes in cytoplasma may be said to be one of the characteristic features in neoplastic cells. Golgi apparatus is usually absent, but at times the Golgi apparatus may consist of microvesicular structure.

The third group of glioblastoma cells is a type with anaplastic differentiation. There was no difference in their nuclear structure when compared with the former two types, but the organellaes were scarce in the cytoplasmic structure. The Golgi apparatus and R. E. R. were not seen, except for a few mitochondria, ribosome and dense body. Changes in the cell membrane and formation of cytoplasmic processes were minimal. Such findings that tumor cells lose their organized lamellar ergastoplasma may be called an anaplastic de-differentiation, as seen in the fine structure of other cancer cells with poorly developed E. R. (endoplasmic reticulum) and only a few ribosomes and mitochondria in the cytoplasma[1]. This explanation coincides with the concepts of ALBERTINI[2] in the de-differentiation of cancer cells.

In some parts, this type of glioblastoma resembled the medulloblastoma light-microscopically, but through electron micrographs, these cells differed from the description obtained by some workers[30][37]. These tumor cells looked like the fine structure of medulloblastoma reported by KOIZUMI (1963)[20] as far as the findings of the cytoplasmic organellaes were concerned. Morphologically, these findings seemed to explain the reason why the anaplastic de-differentiation and the embryonal de-differentiation of adult cells[26] should not be distinguished.

Glioblastomas have been classified into the previously stated three groups. It was interesting to note that these tumor cells were never found together in the same specimen.

The giant cells were observed in all cases. Their general findings were that the large nuclei had deep clefts of nuclear membrane and cytoplasmic inclusions, and the cytoplasma had numerous organellaes and glio-filaments. RAIMONDI (1962)[30] reported that the cells with numerous degeneration, nucleolei, ribosomes, E. R., mitochondria, etc. were observed in the rapidly growing tumors. It is true that such cells with abundant cytoplasmic organellaes as giant cell and necrotic area were observed in malignant gliomas. But, tumors having few organellaes as shown in the third group, had much more mitotic figures, which indicated a very quick growth. The cells with numerous organellaes seemed to be rather active and brisk in metabolism. These have no relation with the speed of growth. Nuclear inclusions often seen in glioblastoma multiforme were resulted from the increased invagination of nuclear membrane, as reported by ROBERTSON (1965)[33].

It is not known whether or not the small round cells appearing like lymphoid cell in light micrographs are equivalent to the lymphocyte, plasmablast or plasmacyte observed electron microscopically. However, it is certain that they are encountered very frequently in glioblastoma multiforme.

The tumor cells of ependymoma are easily identified in tumor tissue. In the fine structures of normal ependym cells, the delicate network of branching filaments, multivesicular bodies near the ventricular surface, cilia and ciliary rootlet, microvilli on free surface, prominent Golgi apparatus, zonula adherens and zonula occludens are characteristically observed[18)39].

In ependymomas, the structure of each tumor cell is relatively uniform and preserves the charcteristic of ependymal cell which appears in microvilli and cilia on the free surface, prominent terminal bar, intermediate junction and multivesicular bodies. The author has observed one case of ependymoma, in which numerous proliferation of microvilli and the invagination of microvilli to the neighbouring tumor cells was seen. These findings may also be considered as a pattern of neoplastic differentiation.

The ependymal glioblastoma, first named by RACK and YATES (1959)[29], is one type of the glioblastoma with ependymomatous nature. This is equivalent to Grade IV ependymoma of the KERNOHAN's classification. In one case of ependymal glioblastoma, numerous filaments filled the entire cytoplasma were observed in almost all tumor cells. RAIMONDI has reported on the type of ependymoma with high proliferation of filaments in the cytoplasma (1962)[30]. Such proliferation of filaments seems to be a manifestation of the metaplastic differentiation, that is, the ependymal cells lose their ependymal characters and become more characteristic as a supporting cell. This is because the ependymal cell originating on the ventricular wall develops independent with the ventricle, and as the result, microvilli and terminal bar atrophied, while the supporting mechanism of the filament increases.

The giant celled glioblastoma, named by RUSSELL and RUBINSTEIN[35], is one type of glioma, and was called in several names in the past. But, even now, there are three views as to the nature of this tumor; one is that it is a subdivision of the glioblastoma[35], the second is of ependymal origin[29], and the last is sarcoma[17)45]. From light microscopic findings this giant-celled glioblastoma is characterized as a plump or pyramidal shaped

giant cell having vesicular nuclei with eosinophilic nucleolei, existing together with fusiform cells, and presents such findings as cellular polymorphism, mitosis, palisading around necrosis, proliferation of capillaries etc.[35]

The fine structure of this giant cell presents a large nuclei with diffusely dispersed chromatin materials and prominent large nucleolei appearing clear network, while the abundant cytoplasma was filled numerous organellaes, especially well developed Golgi apparatus and various size and form of mitochondria.

The giant cells are different from the astrocytic giant cells because the nuclei have homogeneous dispersed chromatin materials and the mitochondria generally maintains a round form. Ependymal nature such as, microvilli on the free surface, invagination of microvilli into the cytoplasma, terminal bar and intermediate junction, and sometimes cilia were observed in these tumor cells. In 1964 FUKUMITSU[12] reported on two cases of giant-celled glioblastoma. He concluded that one was a sarcoma and the other a glioma, describing the latter case that such structure as desmosome and cilia were not observed. The nucleolei in this giant cell are large and have a clear network structure appearance and at times with nuclear membrane attachment. But these findings are not pathognomonic for malignancy.[3]

SUMMARY

With the exception of Gemistocyte, the tumor cells of glioblastomas contrary to the micrograph shows a same structure and a uniform stage in their differentiation in electron-microscopic findings of the same tissue.

On electron-microscopic observations, the glioblastomas were classified into three groups ; an highly atypical type of astrocytoma, an immature type, and a type which seems to have undergone an anaplastic de-differentiation or metaplastic differentiation. The astrocytoma may be classified to a fibrous type and a protoplasmic type through light microscope, but no difference was observed, electron-microscopically.

The author has the opinion that abnormal proliferation of glio-filaments is a result of neoplastic differentiation as has been found in one case of fibrous astrocytoma, one case of ependymal glioblastoma or giant cell.

The giant cells observed in all glioblastomas seemed to be active cells ; because the abundant cytoplasma contained numerous well developed organellaes and the large nuclei have prominent nucleolei and deep clefts of nuclear membrane.

Those of ependymal origin are included in so-called the giant-celled glioblastoma, from the basis of electron-microscopic observations. Astrocytoma or glioblastoma classified as a highly atypical type indicates infiltrative growth in the boundary areas, while glioblastomas classified as an immature type or an anaplastic type had comparatively distinct boundaries.

The mitotic figures were numerous in glioblastoms classified as an anaplastic type.

ACKNOWLEDGEMENT

The author wishes to thank Professor TAKAO MITSUNO, Department of Surgery, Kobe University School of Medicine, for his constant guidance and advice during this investigation, and also wishes to thank TAKEO YAMORI, Department of Pathology, Division 1, Kobe University School of Medicine, for his valuable guidance and advice

in this investigation. The author wishes to express my thanks to Professor NAOKI KAGEYAMA, Department of Neurosurgery, Kansai Medical College, for his providing material and relevant information.

REFERENCES

1) Bernhard, W. : Electron microscopy of tumor cells and tumor viruses. A review. Cancer Research, **18** : 491, 1958.
2) Brachet, J. and Mirsky, A. J. : The cell. Vol. 5. Academic Press, New York and London, : 442, 1961.
3) Busch, H., Byvoet, P,, and Smetana, K. : The nucleolus of the cancer cell : A review. Cancer Research, **23** : 313, 1963.
4) Dahl, E., Flora, C. and Nelson, E. : Electron microscopic observatians on normal human intracranial arteries. Neurology, **15** : 132, 1965.
5) Donahue, S., Zeman, W. and Watanabe, I. : Alterations of basement membranes of cerebral capillaries. J. Neuropath. Exp. Neurol., **26** : 397, 1967.
6) Duffel, D., Farber, L., Ohou, S., Hartmann, J. F. and Nelson, E. : Electron-microscopic observations on astrocytoma. Am. J. Path., **43** : 539, 1963.
7) Farquhar, M. G., and Hartmann, J. F. : Neuroglial structure and relationships as revealed by electron microscopy. J. Neuropath. Exp. Neurol., **16** : 18, 1957.
8) Farquhar, M. G. and Palacle, G. E. : Junctional complexes in various epithelia. J. Cell Biol., **17** : 375, 1963.
9) Fernandez-Moran : Examination of brain tumor tissue with the electron-microscope. Arkiv for Zoologi., 40A : **6**, 1, 1947.
10) Fujita, H. : The fine structure of neuroglia. Saishin Igaku, **16** : 831, 1960 (in Japanese).
11) Fujita, S.: The histogenesis of central nervous system and the classification of brain tumors. Shinkei Kenkyu., **7** : 117, 1962 (in Japanese).
12) Fukumitsu, T. : Nature of so-called giant-celled glioblastomas. An electron microscopic study. Arch. Jap. Chir., **33** : 350, 1964.
13) Gonatas, N. K., Martin, J. and Evangelista, I. B. A. : The osmiophilic particles of astrocytes. J. Neuropath. Exp. Neurol., **26** : 369, 1967.
14) Gonzalez-Angulo, A., Reyes, H. A. and Reyna, A. N. : The ultrastructure of ganglioneuroblastoma. Observations on neoplastic ganglion cells. Neurology, **15** : 242, 1966.
15) Hama, S. : The fine structure of central nervous system. Kagaku, **33** : 522, 1963 (in Japanese).
16) Herman, M. M., Adams, W. R. and Manuelidis, E. E. : The ultrastructure of human glioblastoma multiforme-derived tumor heterologously transplanted to ginea pig eye and brain. Acta Neuropath., **8** : 321, 1967.
17) Kernohan, J. W. and Uihlien, A. : Sarcomas of the brain. C. C. Thomas, Springfield, 1962.
18) Klinkerfuss, G. H. : An electron microscopic study of the ependyma and subependymal glia of the lateral ventricle of the cat. Am. J. Anat., **115** : 71, 1964.
19) Koizumi, J.: Electron microscopic studies on human glioblastoma and its border area. Brain & Nerve, **14**: 31, 1962 (in Japanese).
20) Koizumi, J. : Electron microscopic studies of brain tumor. Shinkei Byori, **7** : 61, 1963 (in Japanese).
21) Lapham, L. W.: Subdivision of glioblastoma multiforme on a cytologic and cyrochemical basis. J. Neuropath. Exp. Neurol., **18** : 244, 1959.
22) Leighton, J., Kalla, R. L., Turner, J. M., Jr. and Fennel, R. H., Jr. : Pathogenesis of tumor invasion, 2. Aggregate replication. Cancer Research, **20** : 575, 1960.
23) Luse, S. A. : Ultrastructure of reactive and neoplastic astrocyte. Laborat. Investigation, **7** : 401, 1958.
24) Luse, S. A. : Electron microscopic studies of brain tumors. Neurology, **10** : 881, 1961.
25) Meller, K. und Haupt, R. : Die Feinstruktur der Neuro-. Glio-, und Epedymoblasten von Huhmanembryonen in der Gewebekultur. Z. Zellforsch., **76** : 260, 1967.
26) Morehead, R. P. : Human pathology : McGrow-Hill Book Co., New York, 53, 1965.
27) Nyström, S. H. M.: Pathological change in blood vessels of human glioblastoma multiforme. Acta Pathol. Microbiol. Scand., Suppl. **137**, 43, 1960.
28) Penfield, W. : Neuroglia : Normal and pathological. In cytology and cellular pathology of the central nervous system. New York. Paul B. Hoeber, **2** : 422, 1932.a.
29) Rack, J. H. and Yates, P. O.: Ependymal glioblastomas of giant cell type. J. Path. Bact., **78** : 151, 1959.

30) Raimondi, A. J., Mullan, S. and Evans, J. P. : Human brain tumors. : An electron-microscopic study. J. Neurosurg., **19** : 731, 1962.
31) Raimondi, A. J. : Correlation of structure and function in selected tumors of the human nervous system. Acta Neuropath., **8** : 149, 1967.
32) Ramsey, H. J. : Fibrous long-spacing collagen in tumors of the nervous system. Acta Neuropath. Exp. Neurol., **24** : 40, 1965.
33) Robertson, D. M. and MacLean, J. D. B. A. : Nuclear inclusions in malignant gliomas. Arch. Neurol., **13** : 287, 1965.
34) Rubinstein, L. J. : The development of contiguous sarcomatous and gliomatous tissue in intracranial tumors. J. Path. Bact., **71** : 441, 1956.
35) Russell, D. S. and Rubinstein, L. J. : Pathology of tumors of the nervous system. Arnold E. Ltd., London, : 93, 1963.
36) Sano, K. : A statistical observation of intracranial tumors. Brain & Nerve, **14** : 751, 1962 (in Japanese).
37) Sawada, T. : The fine structure of gliomas. Igaku Kenkyu, **37** : 47, 1967 (in Japanese).
38) Schultz, R. L., Maynard, E. A. and Pease, D. C. : Electron microscopy of neurons and neuroglia of cerebral cortex and corpus callosum. Am. J. Anat. **100** : 369, 1957.
39) Tonnyson, V. M. and Pappas, G. D. : An electron microscopic study of ependymal cells of the fetal, early postnatal and adult rabbit. Z. Zellforsch., **56** : 595, 1962.
40) Tokoso, Y. : Brain tumors, Igakushoin, Tokyo, 1961 (in Japanese).
41) Torack, R. M. : Ultrastructure of capillary reaction to brain tumors.
42) Tunoda, H. : Electron microscopic studies on brain tissue and intracranial tumor. Niigata Daigaku Bryorigaku Kenkyu Hokoku, **70** : 1, 1959.
43) Woolf, A. L. : Remarks on the electron microscopical appearances of brain tumors. Acta Neurochir., **10** : 75, 1964.
44) Yamamoto, T. : The structure of the plasma membrane. Igaku no Ayumi, **63** : 693, 1967 (in Japanese).
45) Zülch, K. J. : Biologie unt Pathologie der Hirngeschwülate. in Handbuch der Neurochirurgie. Vol. 3. Springer, Berlin, 1956.

和文抄録

Glioblastoma の電子顕微鏡的観察

神戸大学医学部第1外科(指導：光野孝雄教授)

楠 徳 郎

光学顕微鏡的に glioblastoma と診断されるものにも，その発育態度は様々である．又，光顕上その組織像は多形性が強く，複雑な変化を伴つている．そこで著者は一般的な腫瘍の概念にもとずいて，glioblastoma の腫瘍細胞の変化を電子顕微鏡的に観察し，その分類及び特性を調べた．研究材料は光顕的に正確な診断を得た glioblastoma 13例，astrocytoma 3例，Ependymoma 7例を選らび出した．

glioblastoma はその微細構造より，次の3群に分類できた．即ち第1群は成熟形 astrocytoma が高度の異型性を示すと思われる型．第2群は blast cell と思われる微細構造を示す未分化な型．第3群は anaplasiaの強い脱分化の所見を示す型．この内 mitosis は第3群に多く認めた．又，発育態度は一般に第一群は浸潤性のものが多く，第2群，第3群は比較的その腫瘍境界は分明であつた．又，上記3群の他に脳室上衣性を思わせる glioblastoma もあり，著者の経験したいわゆる "Giant-Celled Glioblastoma" は脳室上衣性であつた．

腫瘍細胞中，巨細胞は殆んど全て，胞体内の小器官の発育が良く，又，顕著な大きい核小体を持つ大きい核を持ち，生活力の強い細胞と考えられる．

Glio-filament の増生した像は fibrous astrocytoma の1例，脳室上衣性と思われる glioblastoma の腫瘍細胞及び腫瘍全ての gemistocyte において認めた．この glio-filament の集積像の意義は判明しがたいが，gemistocyte の場合は，核，胞体内，小器官の所見より，変性或は老熟に伴う所見の一つでないかと考えられ，又，他の腫瘍細胞では，一種の腫瘍的分化の像でないかと考える．

腫瘍中，反応性細胞と思われる lymphocyte, plasmocyte, plasmoblast 等が混在するのを認め，glioblastoma に特に多くあつた．

従来 glioblastoma multiforme と呼ばれる如く，複雑で多様な組織像を示すものも，同一腫瘍では個々の腫瘍細胞の微細構造には有意差を認めない．しかしglioblastoma の中にはその微細構造よりいくつかの性格の違つた型が含まれており，又，分化度の違つた型を区別出来た．