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原　　著

Palisade Formation of Human Neurinoma Cells Cultured in Vitro

by

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One of the histological characteristics of neurinoma is palisade formation. This is more often observed in spinal tumors than in intracranial examples. In typical palisades, twenty, thirty, or more nuclei can be seen to run abreast in a single line.

Since palisade formation is doubtless closely bound up with some biological properties in the neurinoma cells, the present investigation was carried out in an effort to clarify the mechanism of this process.

Materials and Methods

Patient K. H., a 43-year-old housewife, was admitted to our Department complaining of unsteady gait,hypesthesia of right half of face, right hearing loss, dysphagia, dysarthria, and dysphonia. Neurological and neuroradiological examinations revealed a very large tumor present at the right cerebellopontine angle. The tumor, arising from the acoustic nerve, was removed subtotally by operation.

Small tissue fragments taken at operation were minced and treated with trypsin-EDTA solution for preparation of cell suspensions, as described elsewhere[1]. The harvested cells were cultured stationarily in closed Leighton's tubes at 37°C. The growth medium was composed of 85% Eagle MEM and 15% bovine serum supplemented with 1g of Glucose, 100mg of Streptomycin, 50mg of Kanamycin, 50,000 units of Penicillin G, and 2mg of Leucomycin per 1,000ml of medium. The growth medium was changed every 3 to 5 days. Cells were observed daily under an inverted microscope and fixed in methyl alcohol for staining by the May-Grünwald-Giemsa method, at the 7th, 19th, 37th, 47th, and 53th day of culture.

Results

Cells attaching well to the glass surface exhibited vivid growth as early as 24th hour of culture. They were mainly composed of B-cells of Antoni (Fig. 1.) They were discrete, stellate, or amoeboid in shape, and were distinguished by having many large granules or vacuoles in their cytoplasms (Fig. 2). They were sometimes bipolar or Y-shaped in appearance, but the shape and size of their nuclei, the size of their cell bodies and, above all, the size and distribution of their large vacuoles or granul-

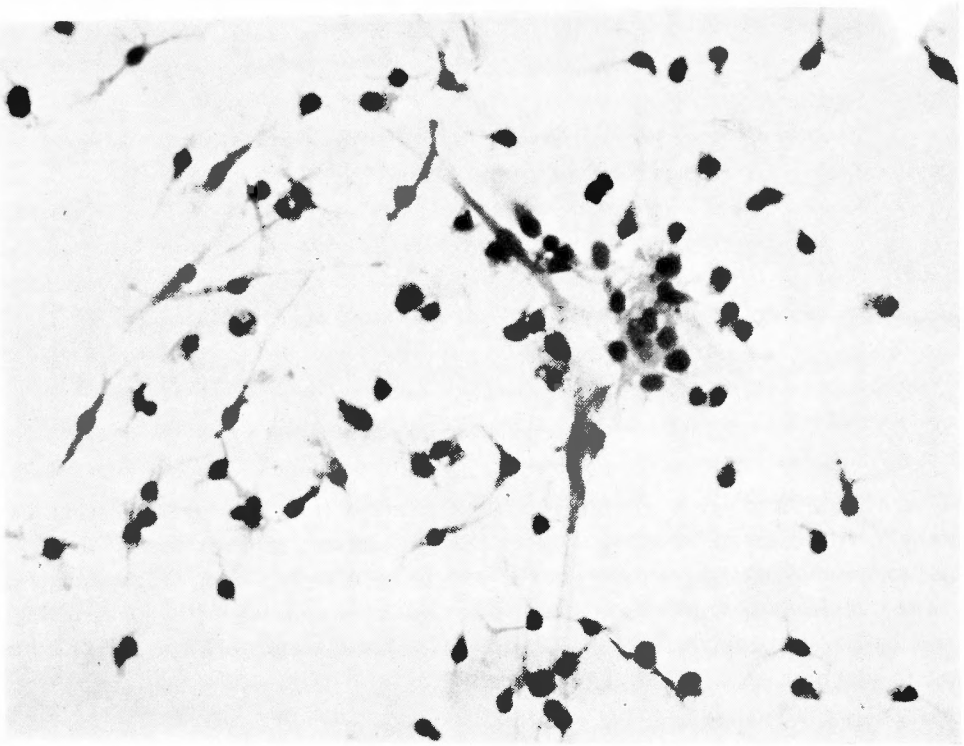


Fig. 1. Neurinoma cells, 7th day of culture. Most of them are B-cells of ANTONI. In the center there are two spindle shaped cells and one membranous and stellate cell, all of which are forming a syncitium. (x 100)

es indicated that they were B-cells of ANTONI. Their nuclei were more oval and stained a deeper purple than those of the A-cells of ANTONI. The bulging of the nuclei from their cytoplasms was usually prominent (Fig. 3).

A-cells of ANTONI were scattered among the B-cells (Figs. 4 and 5). They were filamentous, less granular or vacuolar than the B-cells, bipolar or spindle-shaped, and showed a tendency to anastomose with each other. Their nuclei were oval but more rod-like than those of the B-cells and the stained less deeply. The bulging of the nuclei from their cytoplasms was also conspicuous.

Sometimes, secreted metabolic products adhered to the cells to distutb cell visibility through the inverted microscope.

Cells of a third type, very few in number, were first observed at about one week of culture. They were identified as a specific type of B-cells of ANTONI by the following reasons: They wer membranous and stellate or sometimes bipolar in shape and multinucleated with exceedingly abundant cytoplasms. Their cytoplasms contained many granules and also very many vacuoles also (Figs. 6, 7, 8, 9, 10, 11, and 12). They were hardly to be distinguished from fibroblasts by their morphological appearance, but their cell division was clearly different from that of the fibroblasts. The nuclei of these multinucleated cells would first gather together in a cluster (Figs. 6, 7, 8, and 9). Then, each nucleus would begin to separate from the original

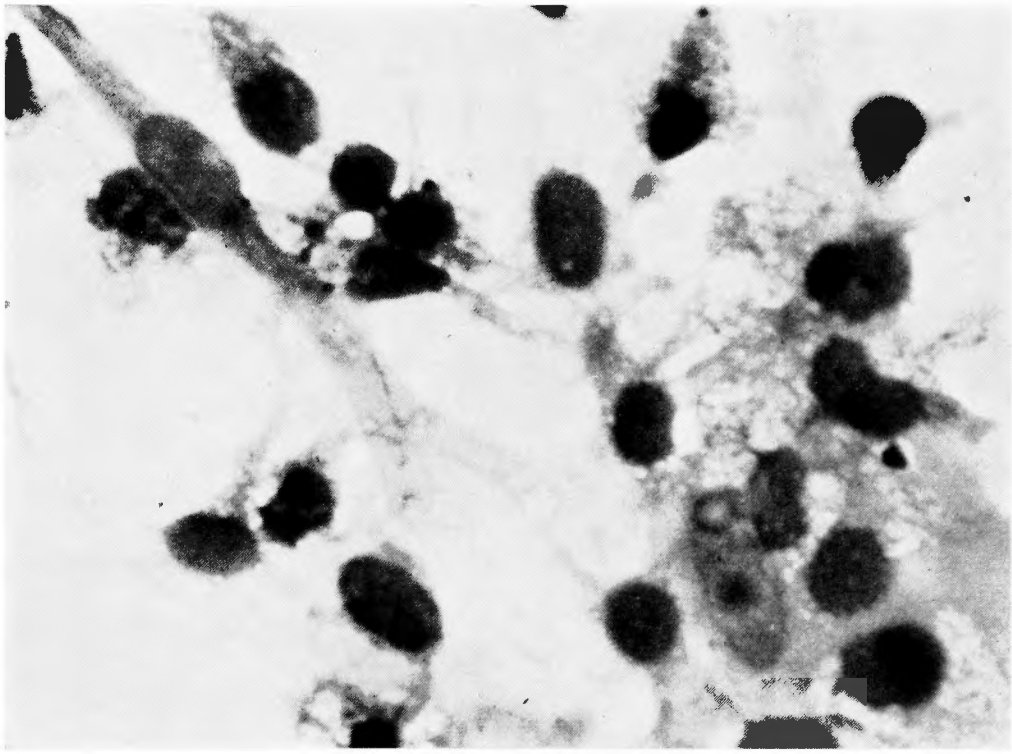


Fig. 2. Enlargement of Fig. 1. Spindle-shaped cell is continuous with membranous and stellate cell (center). Many B-cells are on top of or under them.

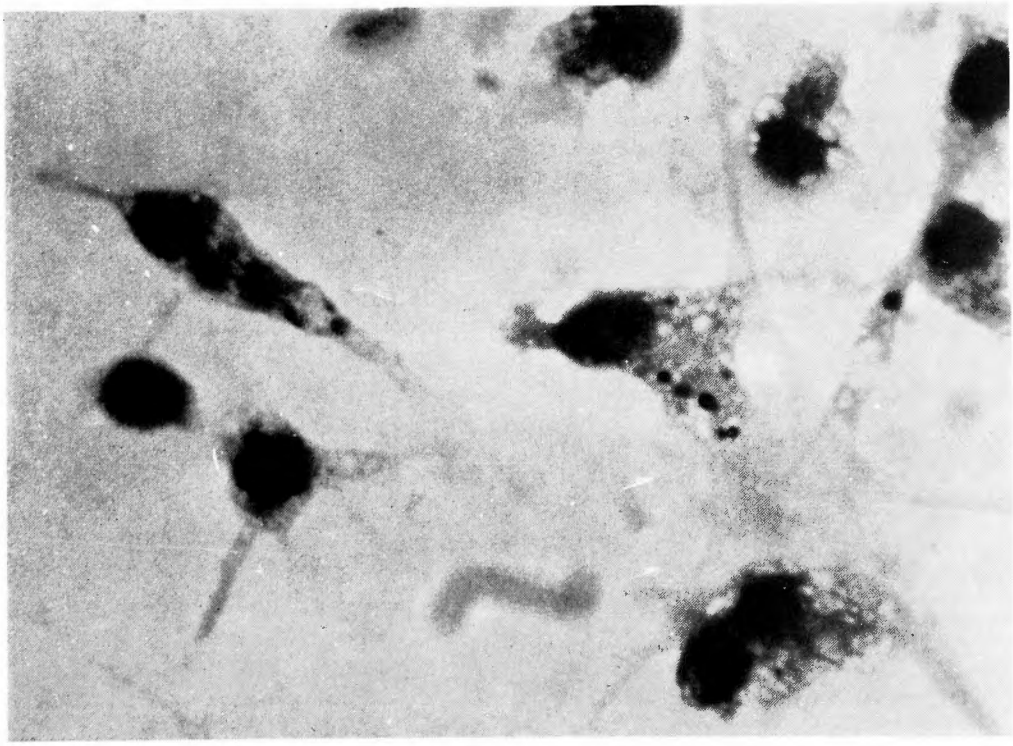


Fig. 3. All are B-cells of ANTONI ; 7th day of culture. (x 400)

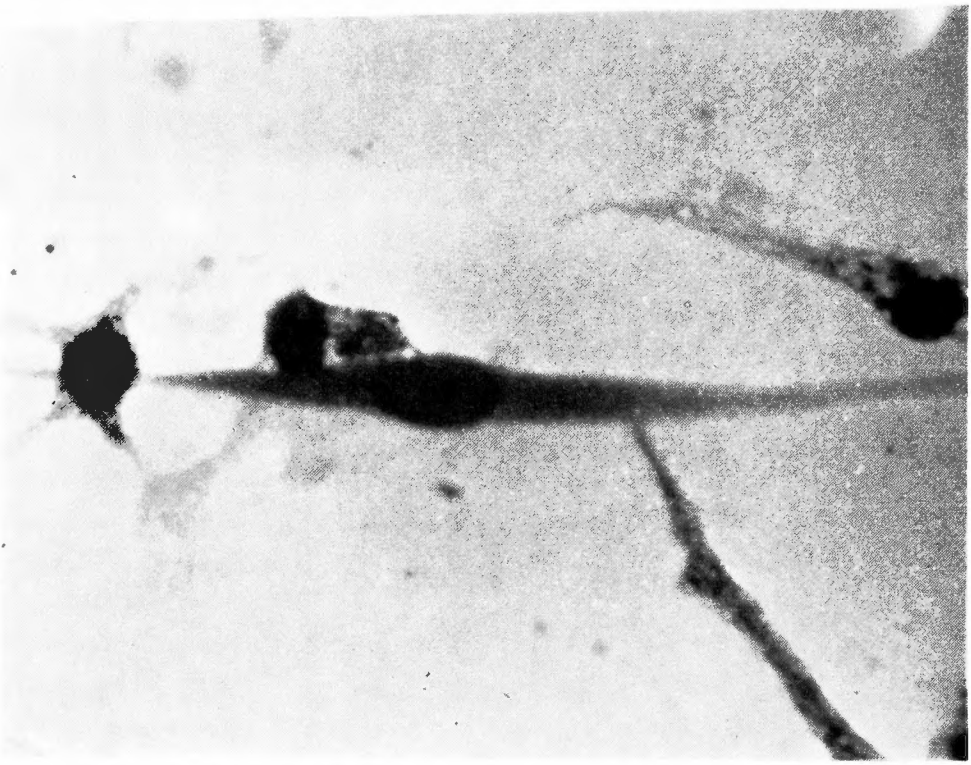


Fig. 4. Spindle-shaped A-cell of ANTONI, 7th day of culture. One B-cell is attached to it. Tendency of B-cells to adhere to spindle-shaped cells was remarkable. "Foot" of other B-cell is also adhering to the A-cell. (x 400)

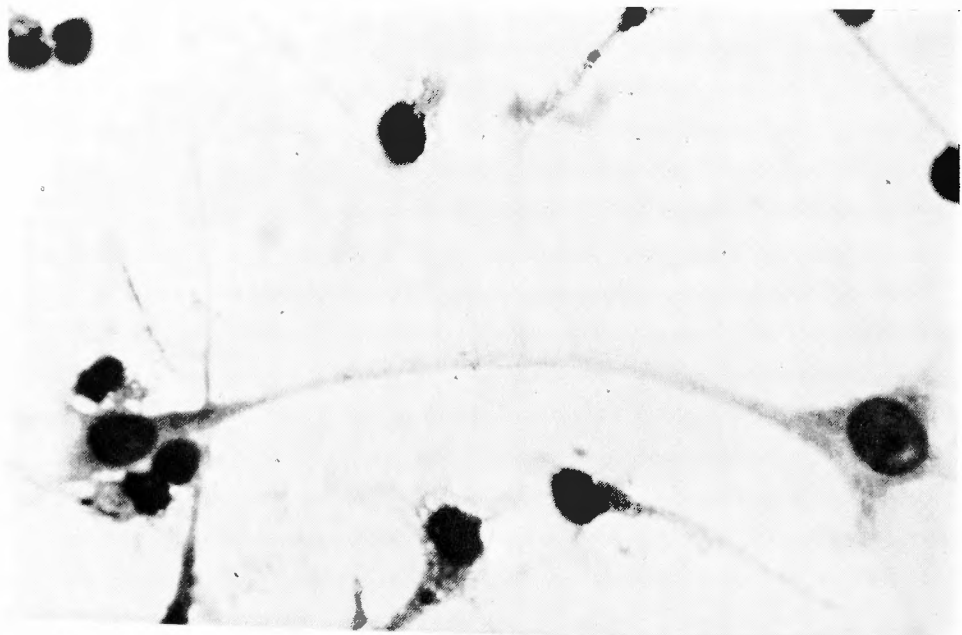


Fig. 5. Syncitium formation of A-cells, 7th day of culture. Cytoplasms show membranous and stellate configuration around their nuclei. (x 400)

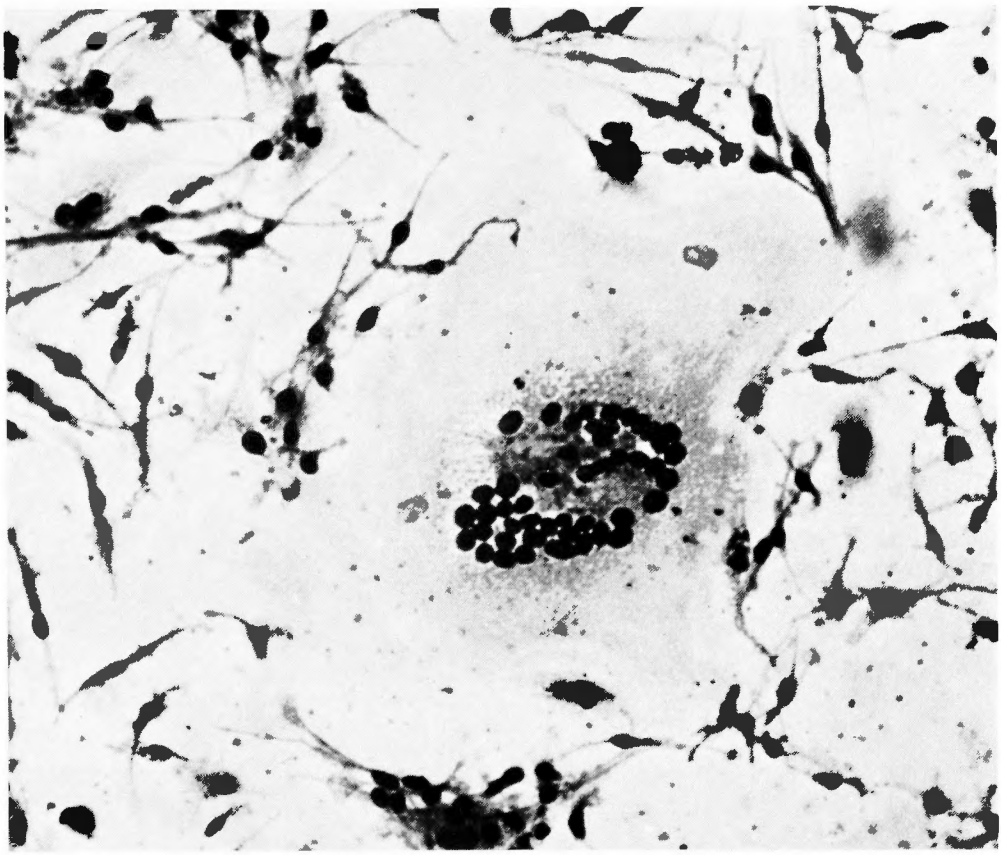
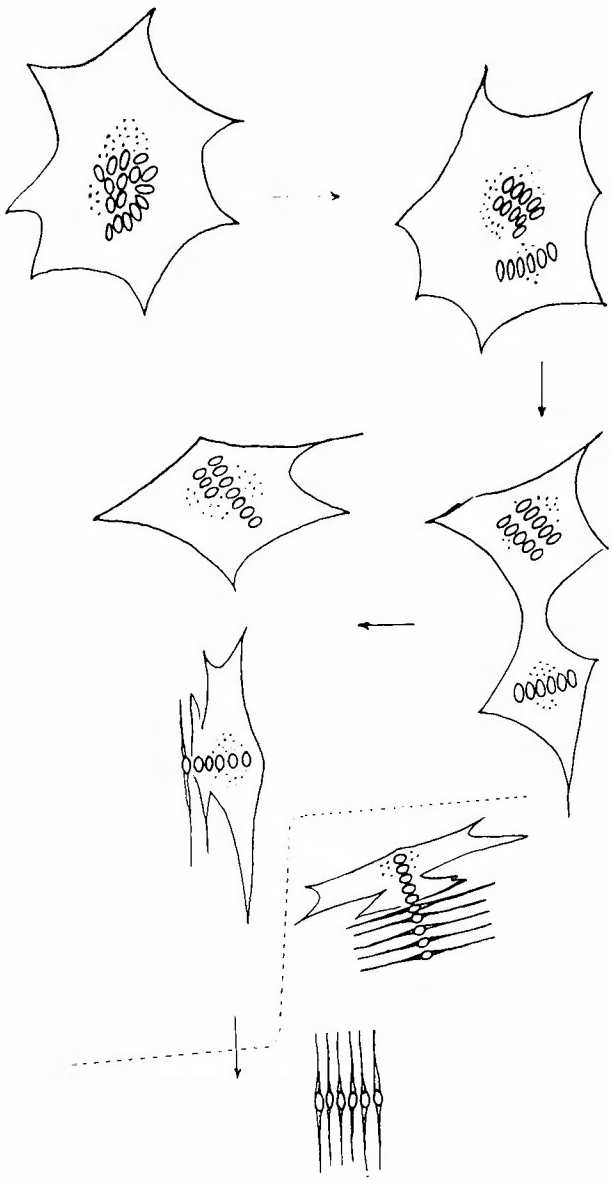


Fig. 6. Multinucleated giant cell, 17th day of culture. It is membranous and stellate in shape and distinguished by many large vacuoles. Forty seven nuclei are included in one cell (x 100)

multinucleated giant cell from the outside either one by one or in groups (Figs. 10, 11, and 12). Cytoplasmic separation between the cells was in complete and a tendency to a preservation of the contact between them was noted. Thus, the nuclei maintained the palisade-like arrangement even after all the cells had become independent of each other, just like palisades *in vivo*. This palisade-like arrangement gradually became distorted with the migration of each cell. And the daughter cells born from multinucleated giant cells were B-cells of ANTONI just described, containing large vacuoles and granules in their cytoplasms and being bipolar or amoeboid in shape (Text-Fig. 1).

On histological examination, the tumor was composed of A-and B-cells of ANTONI. Palisades were not found in the B-areas, but frequently observed in the A-areas (Fig. 15). The nuclei of the palisaded cells were usually irregular, lightly stained, oval rod-like in shape, but not so spindle-shaped as those of the mature A-cells of ANTONI, and the nucleoplasms were not so condensed.

Not infrequently, palisades were associated with clusters of nuclei (Fig. 14). They were several to many in number and their gathering was close or intimate



Text Fig. 1. Palisade formation mechanism from multinucleated giant cell is seen *in vitro*. A membranous and multinucleated giant cell as the very beginning and the tendency of cellls to remain in contact with each other after nuclear division are the most important factors in a palisade formation.

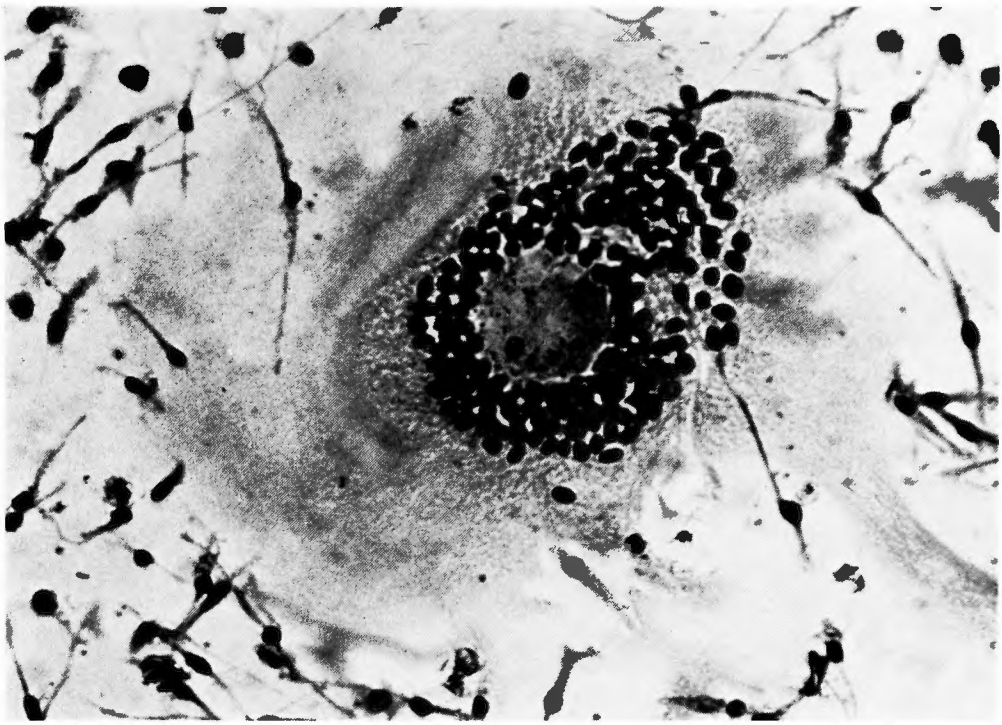


Fig. 7. Multinucleated huge cell, 19th day of culture. It is membranous and stellate in shape and distinguished by many large vacuoles. About 160 nuclei are included in one cell. (x 100)

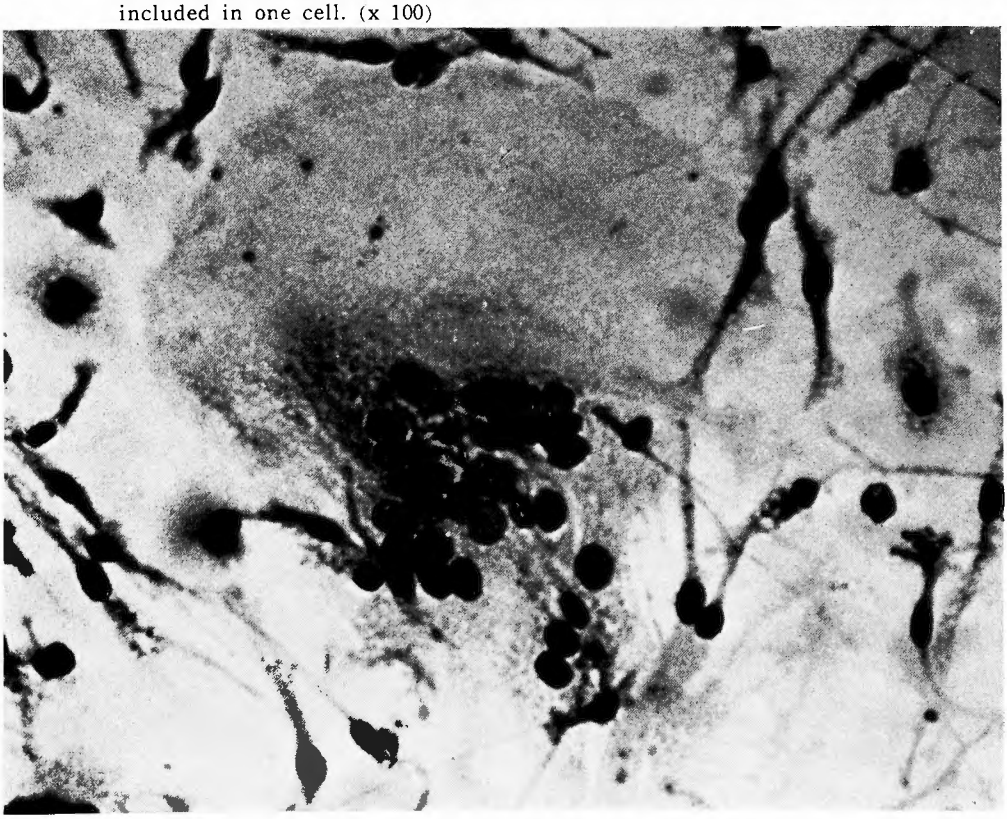


Fig. 8. Multinucleated giant cell. About 30 nuclei are included on one cell. Four of the nuclei are about to separate in a single file group. (x 100)

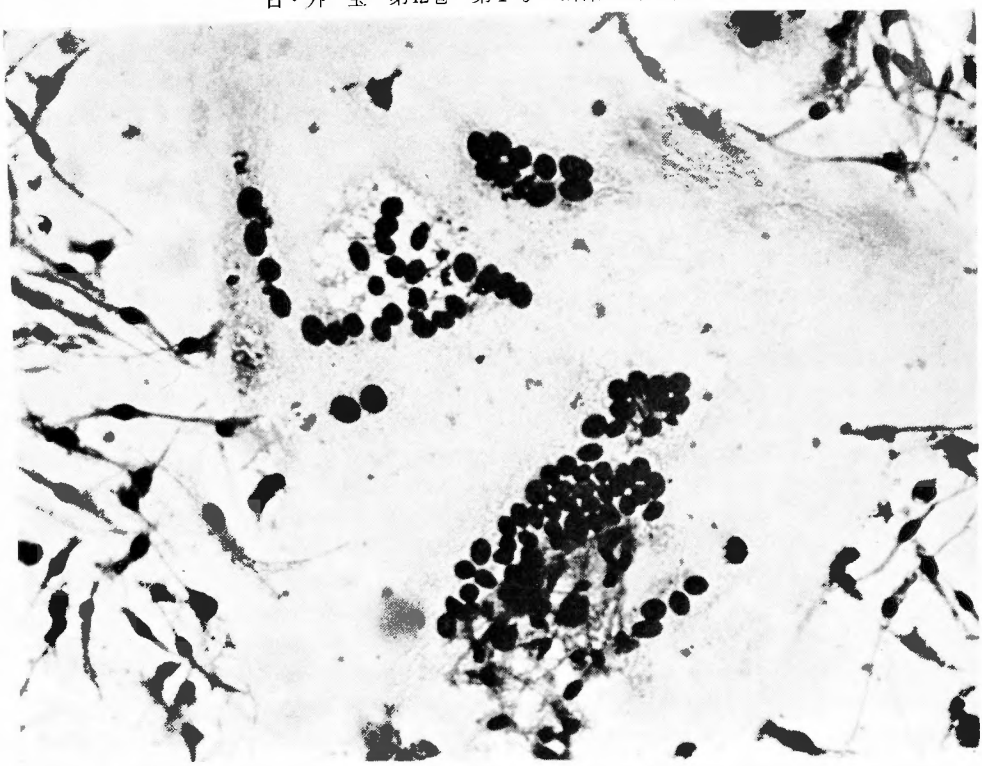


Fig. 9. Multinucleated giant cell, 19th day of culture. Their nuclei are separated into two groups. Those of the upper group are further separated into small groups, all of which show a tendency of palisade. (x 100)

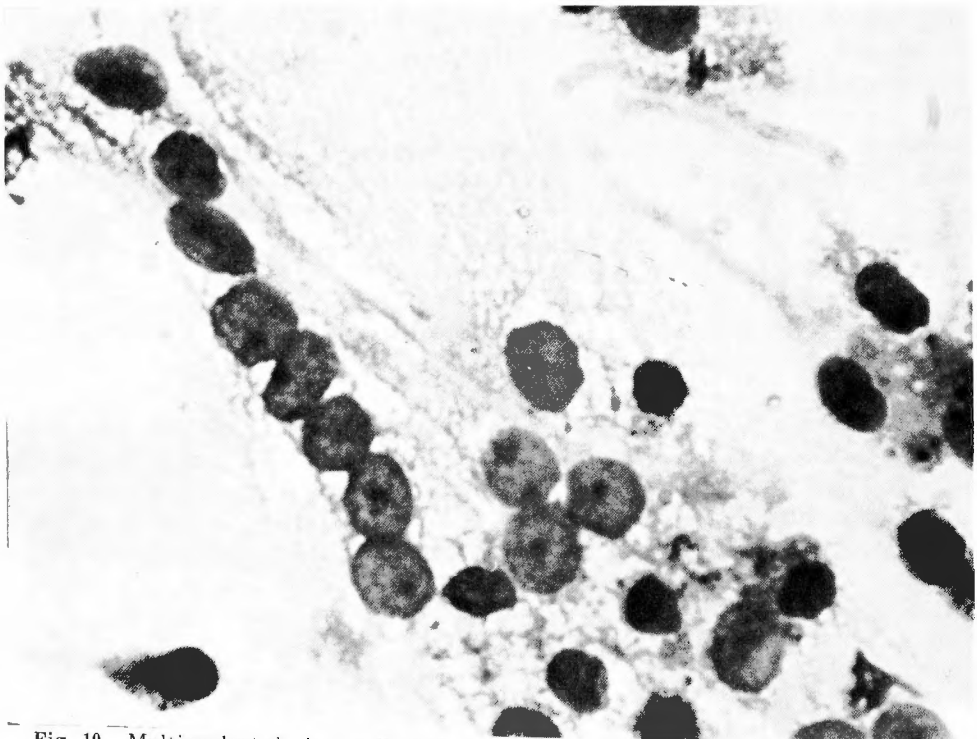


Fig. 10. Multinucleated giant cell, 19th day of culture, Eight nuclei are aligned in a palisade. (x 400)

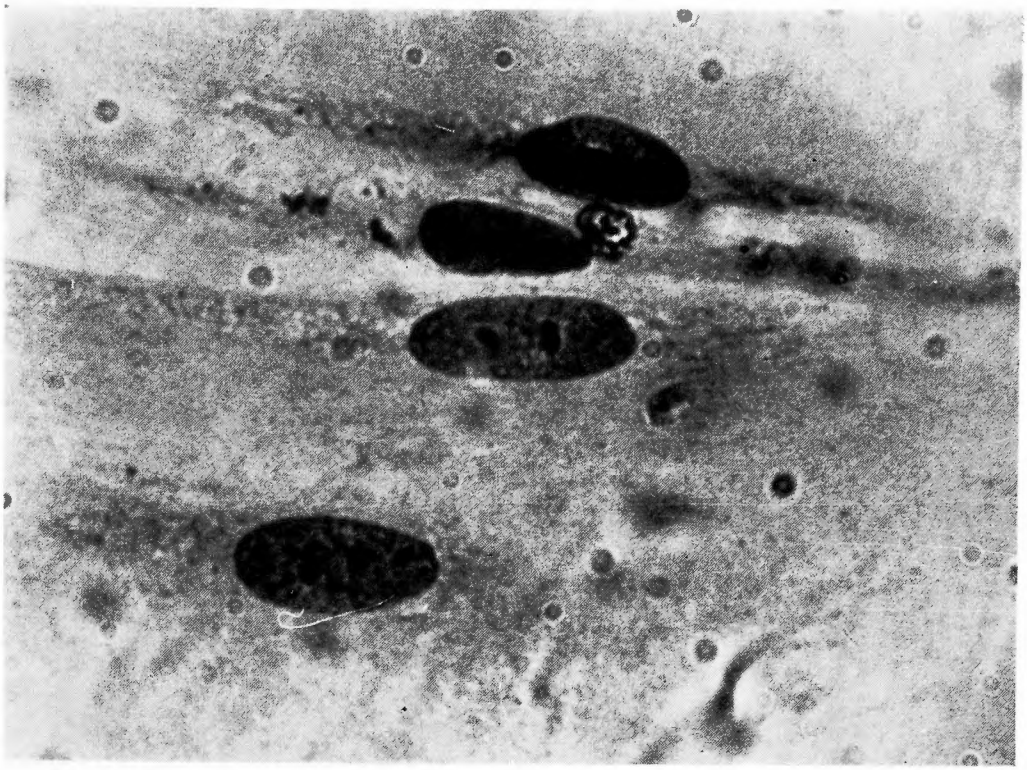


Fig. 11. One membranous and stellate cell (center) and two bipolar B-cells (below) arranged in palisade, 19th day of cuture. The two bipolar B-cells are probably separated from the membranous and stellate cell. (x 400)

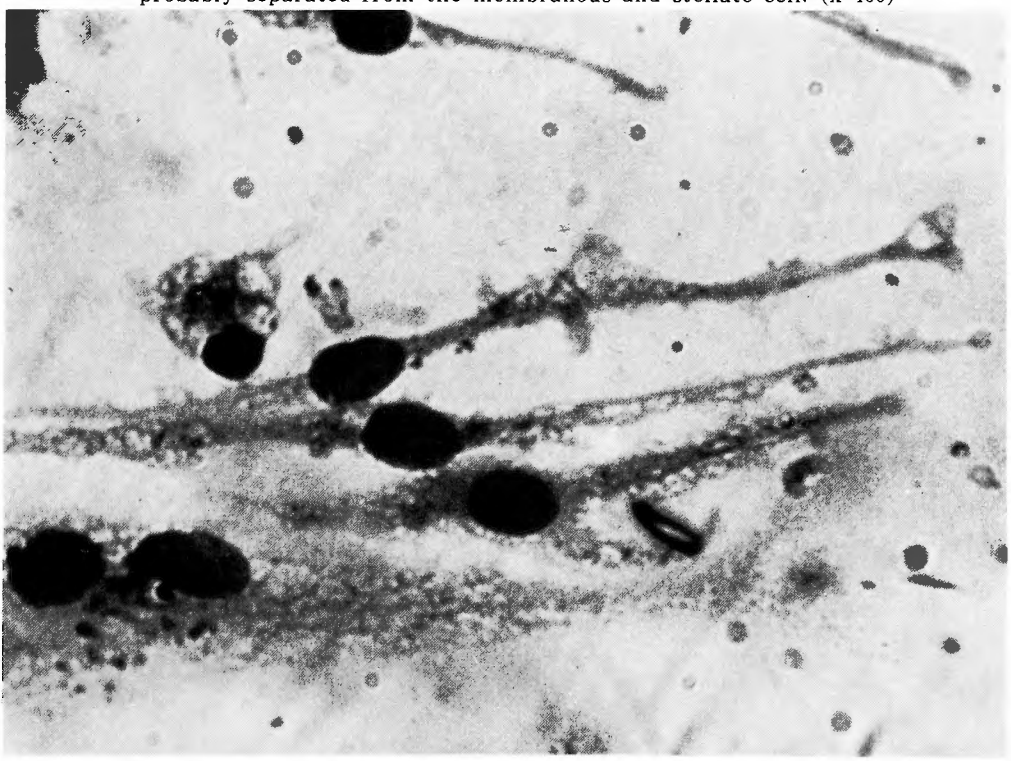


Fig. 12. Two bipolar B-cells and one Y-shaped B-cell are going to separate from the binucleated membranous B-cell. Thus, the B-cells were directly produced from mohter B-cell. (x 400)

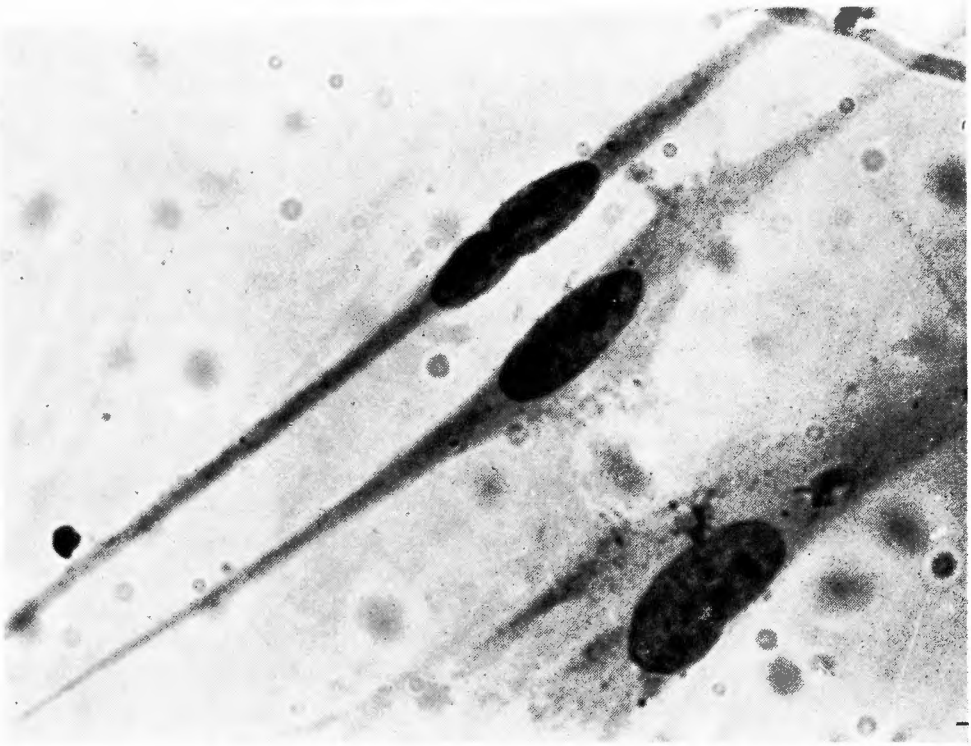


Fig. 13. Two bipolar A-cells and one membranous A-cell are arranged in a palisade, 17th day of culture. (x 400)

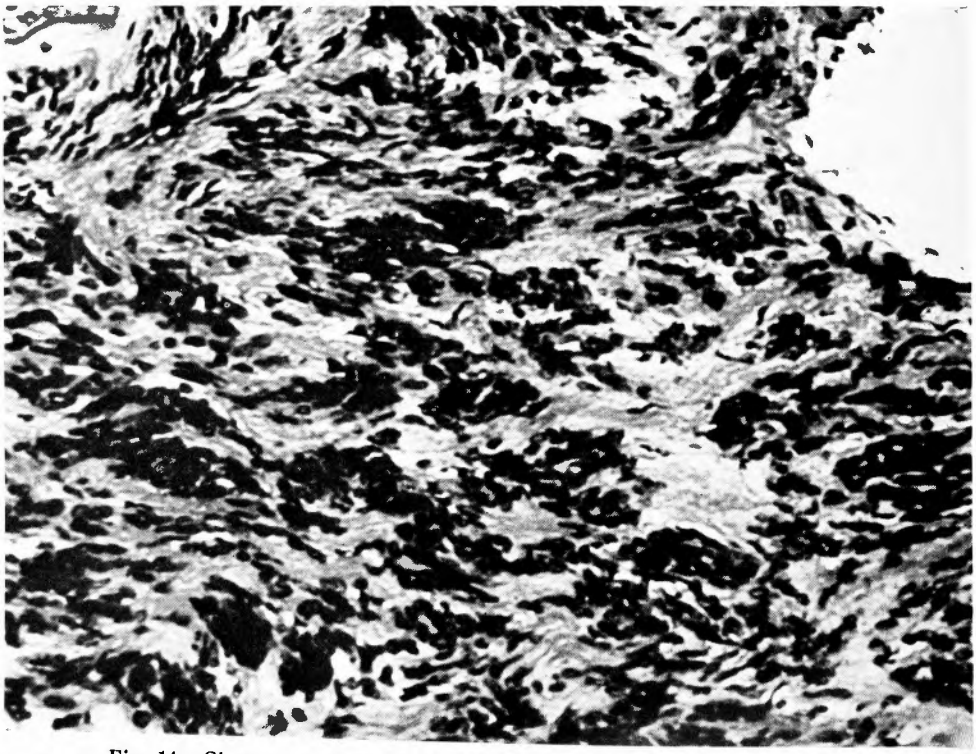


Fig. 14. Clusters of nuclei reveal the vigorous tumor growth. (x 100)

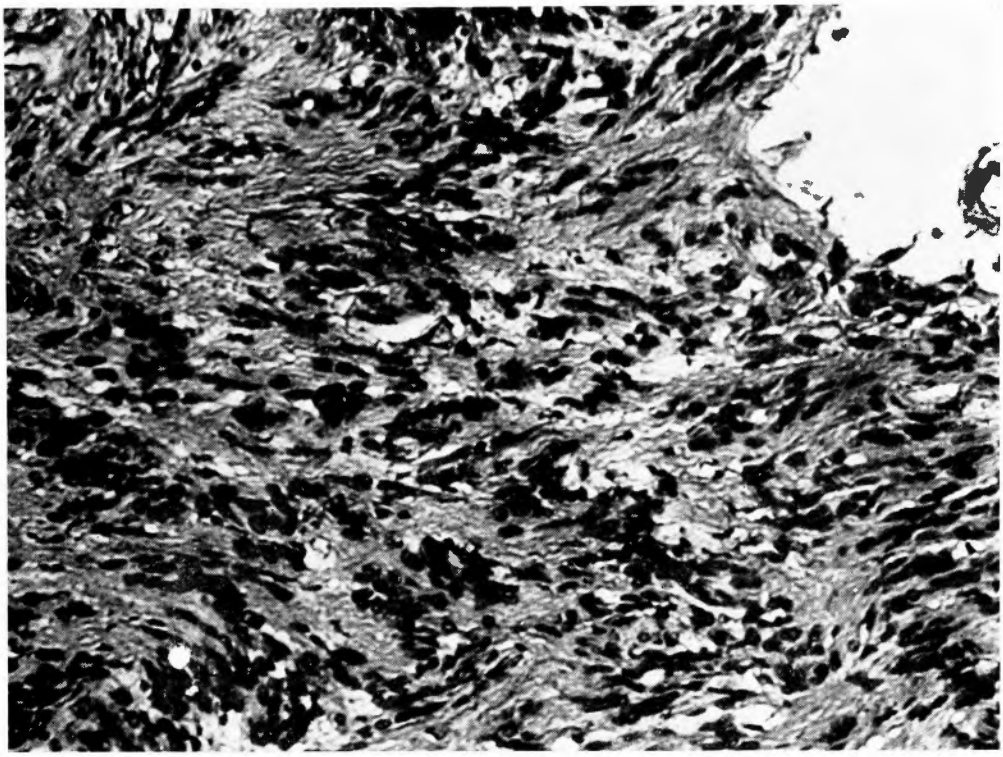


Fig. 15. Very near to the area shown in Fig. 18. Here, the nuclei show palisade arrangement. (x 100)

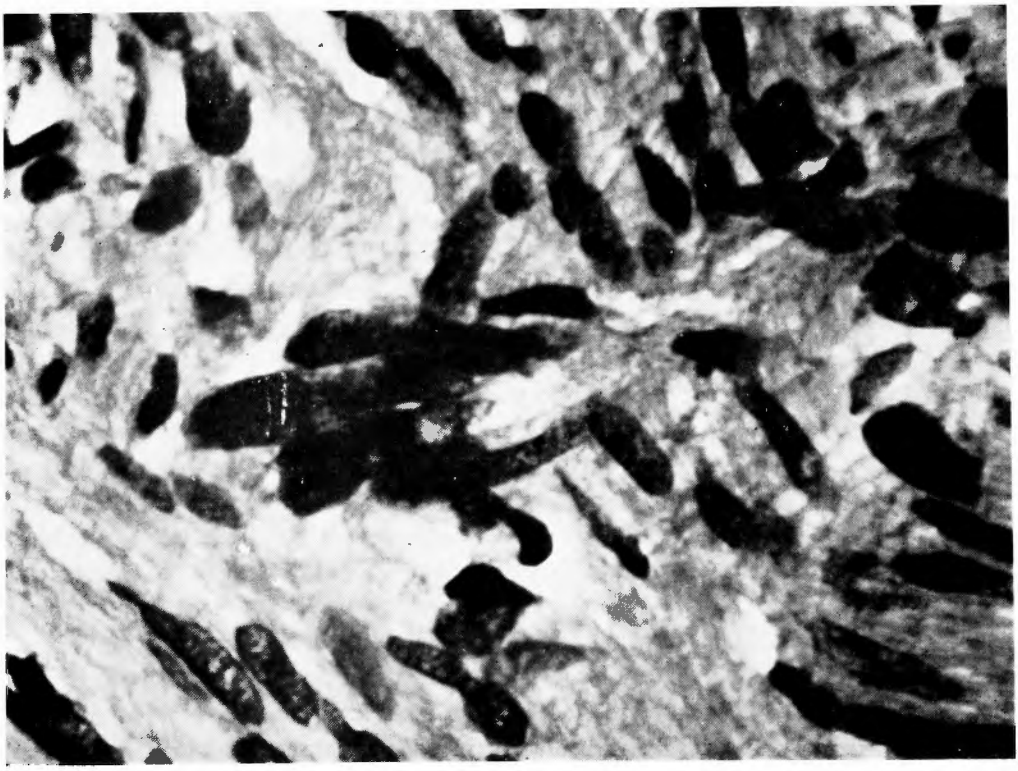


Fig. 16. A cluster of nuclei. They have already shown their tendency to form palisades. (x 400)

(Fig. 16). They were larger, more irregular, and more. oval than those of e palisades, and with more abundant nucleoplasms, signs of vigorous multiplication.

On the other hand, the nuclei of the mature A-cells of ANTONI were regular and spindle rod-like in shape, smooth in texture, and their nucleo-plasms were well condensed.

Discussion

Histological examination *in vivo* of neurinoma seemed to support the same mechanism of palisade formation as seen *in vitro*. Palisades *in vivo* were frequently seen in areas where the cells were vivid and active and cell multiplication had been vigorous, showing themselves to be a result of cell division in very near past.

Moreover, the palisades *in vivo* were often histoarchitectologically associated with the nucleic clusters to reveal the same relation to the multinucleated giant cells that the palisades do *in vitro*.

The nuclei of these palisaded cells would be an intermediate form between that of clustered cells in vivid multiplication and that of mature A-type cells. The clustered nuclei were probably multinucleated giant cells. And these multinucleated giant cells were probably the very beginning of palisades.

Palisades of B-cells cultured *in vitro* were found as shown in the Figures. The same finding was also obtained in another culture of human neurinoma cells. They were mainly composed of A-cells of ANTONI. They were fixed in methyl alcohol and stained by the May-Grünwald-Giemsa method on the 17th day of culture. And we found the palisades which indicated the same formation mechanism in both the A- and B-cells (Fig. 13). Thus, the A-and B-cells revealed similar cell division, syncitium formation, and palisade formation. But transformation from A-cells to B-cells or vise versa was not obsered.

Thus, tissue culture was seen to be most the appropriate method to clarify the mechanism of palisade formation of neurinoma, but few reports have referred to it. MURRAY and STOUT[2] observed on their neurinoma cultures that "it is more common for proliferating cells to remain in contact with one another after nuclear division has taken place". And MURRAY[3] frequently found multinucleated cells *in vitro*. But they did not correlate this to palisade formation. Their views on the mechanism of palisade formation seems to have been that solitary cells gradually lined up or fell into palisade-like arrangements because of their great tendency towards syncitial formation. WEISS and WANG[4] observed Schwann cells of rats cultured *in vitro* emerging from the neurilemmal tubes in tandem file, but further observations on the palisades were not made. CRAVIOTO and LOCKWOOD[5] investigated human acoustic neurinomas *in vitro* with time-lapse cinematography. They observed that bipolar spindle-shaped cells of the A-type of ANTONI could align themselves in a palisade fashion. CRAVIOTO and LOCKWOOD[6] found membranous and stellate multinucleated giant cells in their cultures of normal mouse sciatic nerve. They referred them as a variety of Kite-shaped cells (=fibroblasts) and made no further comment.

In the present investigation, it was found that neurinoma cells cultured *in vitro* were sometimes morphologically fibroblastic in cell division, but that the daughter

cells produced by this cell division were typical A- or B-cells. And the demonstration of S-100 protein, one of the brain specific acidic proteins seen in the membranous and stellate mononucleated cells as well as in the spindle-shaped cells, by the immunofluorescent method[1] further served to support the conclusion that human neurinoma cells are not fibroblasts.

Acknowledgement

we are indebted to Miss TOMIE ISHII for technical assistance in our tissue culture preparation.

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和文抄録

神経鞘腫の組織培養

京都大学医学部脳神経外科学教室（主任：半田　肇教授）

武内重二，内田泰史，半田　肇

人神経鞘腫の培養により次の三種の細胞が得られた．

1)．アメーバ状 microglia 様細胞
2)．紡形細胞
3)．星状単核ないし多核細胞

1) は B type cell of Antoni に 2) は A type cell of Antoni に相当するものと思われ，3) はそれらの細胞の細胞分裂時の形態に相当するものと思われた．細胞分裂時の多核巨細胞はときには数十個の核を細胞内に有し，分裂後それらがばらばらに遊走するのではなく，数個ないし十数個の細胞が一列横隊となって即ち palisade をなして遊走した．即ち神経鞘腫細胞は合胞細胞を作り易いこと，多核巨細胞を作ること．細胞分裂後数個ないし十数個ときには数十個の細胞が一塊となって行動すること，遊走速度が同じであるらしいことなどが palisade をなす要因と思われる．