Virchows Arch [Pathol Anat] (1983) 400:201–211

Virchows Archiv A

Choroid plexus papilloma

Light and electron microscopic study

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Summary. Choroid plexus papilloma (CPP) was observed by light and electron microscopy, using surgically excised tissues in 7 cases and cultivated cells. CPP cells had numerous microvillous processes showing balloon-like features, lysosomes containing haemosiderin, and highly electron-dense irregular granules about 150–300 nm in diameter. Interstitial cells with highly electron-dense cytoplasm inserted their long and thin processes into the invagination of basal plasmalemma of CPP cells, occasionally breaking down the basal lamina. Many of them were located in the intercellular space among CPP cells, sometimes adhering to the ventricular surface of CPP cells. Ruthenium red stain was positive on the surface of CPP cells and was especially intense on the surface of microvilli and cilia. In culture, CPP cells and interstitial cells migrating from the CPP cell mass showed a phagocytic activity after treatment with Latex.

Key words: Choroid plexus papilloma

Tumors originating from the choroid plexus (CP) are rare. The incidence has been reported to be only about 0.4–0.8% of all verified intracranial tumors (Cushing 1932; Norlen 1949; Grant 1956; Zülch 1965; Arai et al. 1976), and 1.1–2.0% of intracranial glioma (Cushing 1932; Ringerz and Reymond 1949; Russell and Rubinstein 1977). The basic pattern of the histological features of choroid plexus papilloma (CPP) has been described in detail by many authors (Carter et al. 1972; Ghatak et al. 1976), and CP cells have been reported to have a secretory ability. Macrophage-like cells found in the interstitium and occasionally on the ventricular surface of CP have been investigated by many workers in order to determine whether these cells are derived from blood monocyte or tissue macrophage such

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Table 1. Choroid plexus papillomas examined

Case No.	Age	Sex	Site	Size of tumor mass	Symptoms and signs	Clinical course	Remarks
1.	5 years 10 months	Female	Fourth ventricle	Hen's egg	Ataxic gait, slurred speech, Vomiting, lt. trigeminus impaired, lt. Bell's sign, lt. cerebellar signs	3 years dead	Hydrocephalus with IVth vent. Occlusion
2.	5 years 11 months	Male	lt. Lateral	Walnut	Restlessness, mental retardation, Speech disturbance, hyperkinetic Tendency, rt. facial palsy, personality change	8 months alive	No hydrocephalus, avascular temporal mass
3.	26 years	Male	Fourth	Hen's egg	Cerebellar sign, tinnitus, papil-ledema	1 year 3 months alive	Hydrocephalus with IVth vent. Homogeneous enhanced mass
4.	28 years	Male	Fourth	Hen's egg	Visual disturbance, total blind, Nystagmus, cerebellar signs	7 months alive	Hydrocephalus with IVth vent. Occlusion
5.	40 years	Female	Fourth	Pigeon's egg	Tinnitus, blurred vision, headache, Nausea & vomiting, unsteady gait, Papilledema, hemianopsia	11 months alive	Hydrocephalus with IVth vent. Occlusion
6.	46 years	Male	Fourth	Pigeon's egg	Diplopia, headache, unsteady gait, Papilledema, hemianopsia	11 years 8 months alive	Hydrocephalus with partial Occlusion of IVth vent.
7.	50 years	Male	Fourth	Walnut	Weakness of lower extrem., diplopia, Headache, nausea, staggering gait, Papilledema, ataxia	11 years alive	Hydrocephalus with IVth vent. Occlusion

as histiocytic cells (Allen 1975; Schwarze 1975; Ling 1979). However, little is known about the nature of CPP cells and interstitial cells.

The present study undertakes histochemical and electron-microscopic investigations of the surgically extirpated tissues in 7 cases of CPP and the cultivated cells. Numerous interstitial cells were observed in the intercellular space among CPP cells, occasionally adhering to CPP cell surface. It was also found that the cell surface of CPP cells was stained intensely with ruthenium red, suggesting an active function of promoting fluid transport.

Materials and methods

Seven cases of histologically verified CPP were studied. As shown in Table 1, the subjects were 5 males and 2 females ranging in age from 5 years-10-months to 50 years. Six cases were IV ventricle papillomas, and the seventh was a case of left lateral ventricle tumor. In each case the symptoms and signs were due to the mass effects of the tumor. All but one case exhibited hydrocephalus.

Morphological observations. Tumors excised surgically were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin, periodic acid-Shiff (PAS), Alcian-blue, orcein, AZAN, Berlin-blue and silver impregnation.

For electron microscopic examination, small pieces of tumor tissue from Cases 2, 3 and 4 were immediately fixed in cold 2% glutaraldehyde, post fixed in 2% osmium tetroxide and embedded in Epon 812. Thin sections were observed under Hitachi electron microscope operating at 75 KV.

Ruthenium red was applied to demonstrate glycosaminoglycans according to the procedure of Luft (1971a and b). The tumor segments, cultured cells, and histologic sections were stained with ruthenium red.

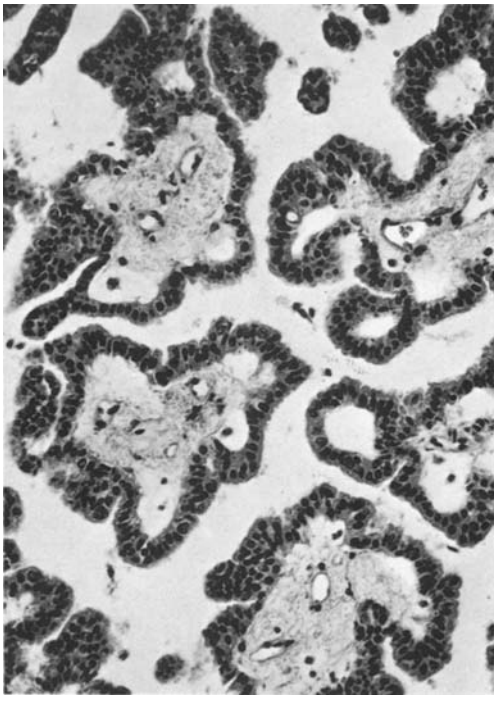

Fig. 1. Microscopic section of CPP (Case 4) showing a papillary pattern (HE, 200:1)

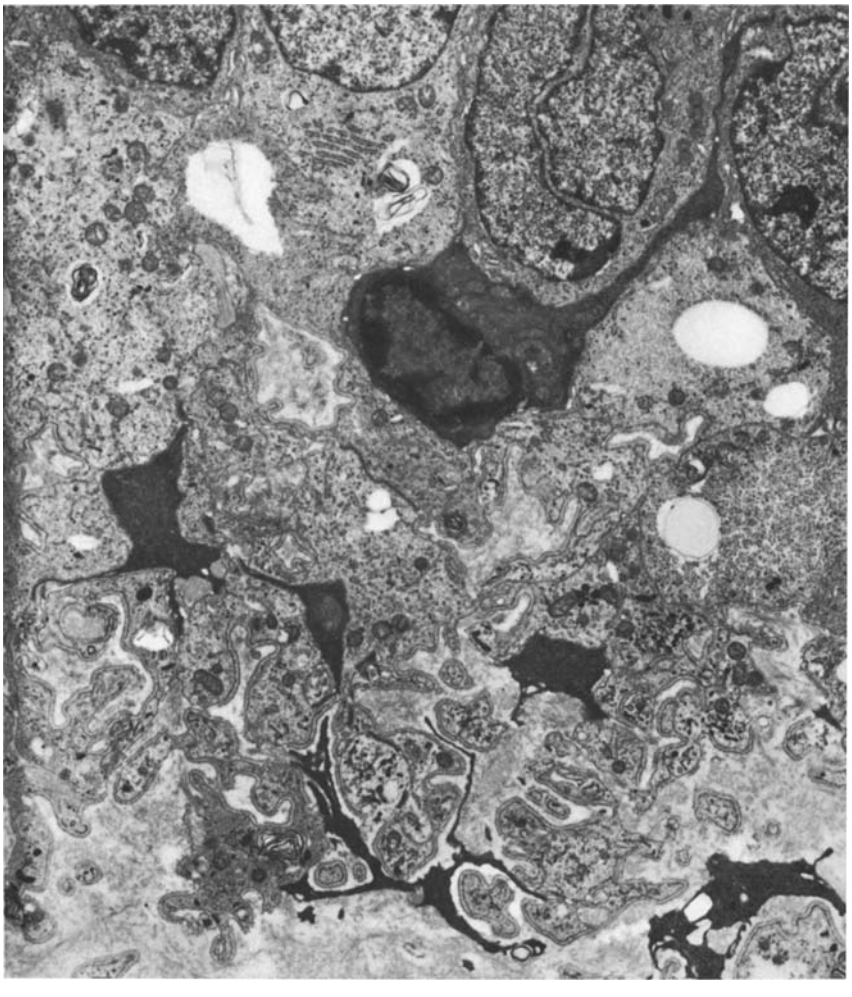

Fig. 2. Electron micrograph showing the basal surface of CPP cells (Case 2). Many invaginations of plasmalemma outlined by a basal lamina are seen. Spindle cells with highly electron dense cytoplasm are located outside the basal lamina and in between the epithelial cells of the tumor (4,250:1)

Tissue culture. Pieces of tumor tissue of Case 4 minced with blades were transfered to culture plastic bottles, and 20 ml of culture medium was added. The medium used was 20% fetal calf serum in Eagle's minimal essential medium (Gibco, Cat No. F-12) containing kanamycin (100 mg/100 ml of the medium) and penicillin (1,000 U/100 ml of the medium). The culture bottles were kept in an incubator at 37° C without gassing control, and the medium was replaced twice a week. For subculture, the cells were separated from the glass surface by EDTA-trypsin solution, collected by centrifugation, and resuspended in fresh medium for the next passage. For morphological and histochemical examination, some cells were subcultured on coverslips in Leighton tubes and stained with Sudan III, Alcian-blue and Berlin blue. Phagocytic activity was examined with Latex 0.81 μm in the same culture medium and compared with rat macrophages obtained from the peritoneal cavity after intraperitoneal injection of starch.

Results

Light microscopic observations

All 7 papillomas show a characteristic CPP pattern consisting of a papillary architecture with a single layer of columnar or cuboidal cells surrounding a slender vascular connective tissue core (see Fig. 1). In Cases 1, 2, 3, 4 and 5, many psammoma bodies are visible in the stroma. No necrosis is observed. In the younger Cases 1 and 3 the tumor cells are arranged occasionally in multilayers, which fuse with each other. However, the solid pattern observed in the malignant CPP cases described in the previous report (Nakashima et al. 1982) is not found. The cells have cytoplasm containing fine granules, and the nuclei a slightly hyperchromatic and vary in size. No mitoses are seen. On the free surface of the cells, brush borders are visible and cilia are noted in younger subjects (Cases 1, 2, 3 and 4). Hemosiderin is often observed in their cytoplasm.

The interstitium surrounded by CPP cells is edematous, closely resembling a lake of serous fluid. The spindle- or star-shaped cells, having scant cytoplasm, a small nucleus and few processes, are scattered sparsely in the edematous stroma and also in the intercellular space among CPP cells facing

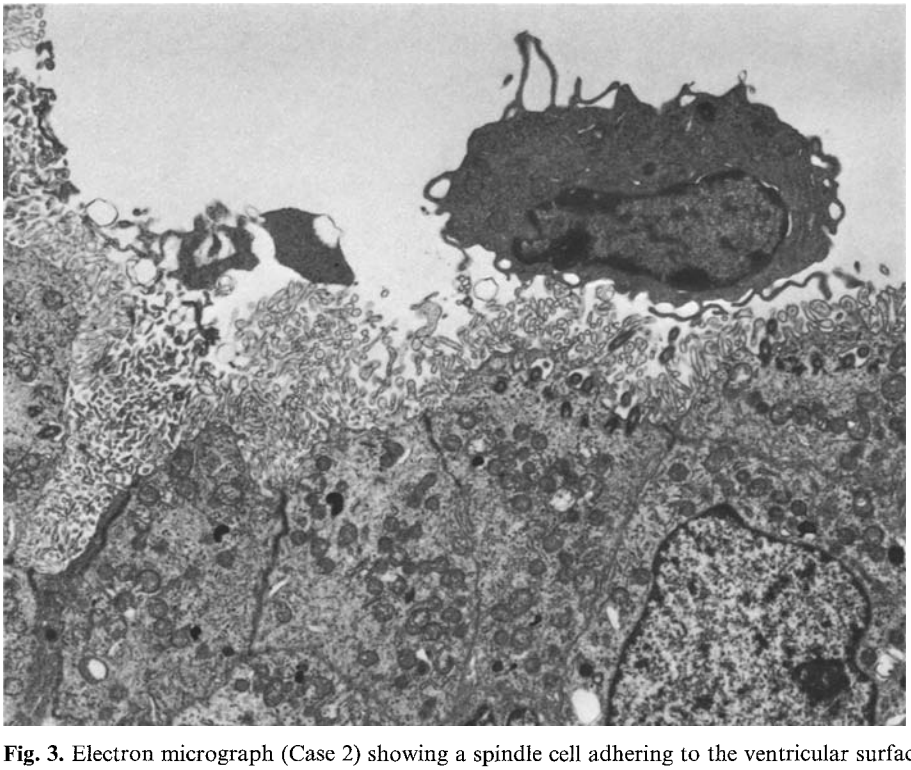

Fig. 3. Electron micrograph (Case 2) showing a spindle cell adhering to the ventricular surface of CPP cells (4,250:1)

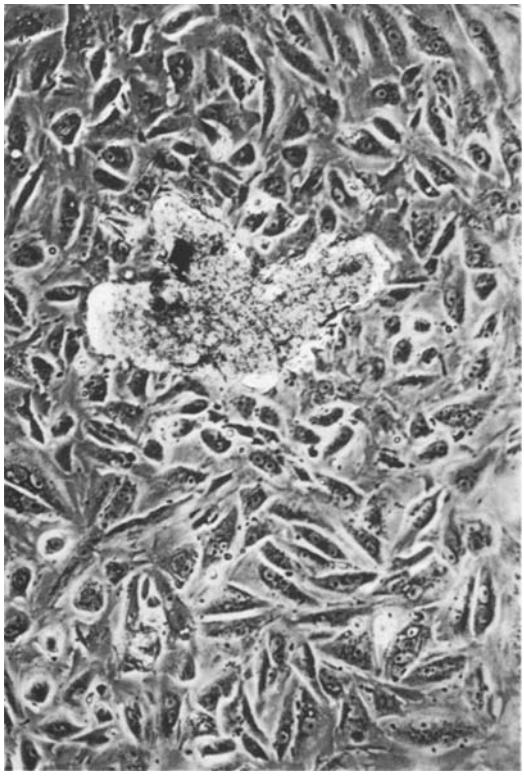

Fig. 4. Cultured cells from Case 4. The CPP cells are migrating from a tissue segment (Phase contrast microscopy, 200:1)

a ventricular cavity; their cytoplasm often contains brown pigments, which are stained with Berlin blue.

Electron microscopic observations

Cases 2, 3 and 4 were examined by electron microscopy. CPP cells maintain an apical-basal polarity. The ventricular surfaces of the cells are regular, but the microvilli are irregular in length, variously oriented and tortuous, sometimes with secondary branching. The tips of some microvilli are dilated and have balloon-like features. Cilia (9+2 microtubes) can be easily observed, and at the base of each cilium a basal body is seen. On the lateral surfaces of the tumor cells, tight junctions are noted and highly developed interdigitations serve to conjoin the tumor cells. Highly electron-dense irregular granules about 150–300 nm in diameter occasionally exist in the cytoplasm. The basal surface of CPP cells contains many invaginations of plasmalemma outlined by a basal lamina (Fig. 2). The interstitial spindle cells have highly electron-dense cytoplasm, with well-developed rough endoplasmic reticulum, electron-dense lipid granules, myelin bodies and many free ribosomes. Their cytoplasm also features a strikingly large number of small granules, and actin-like filaments. The granules are much smaller than ribo-

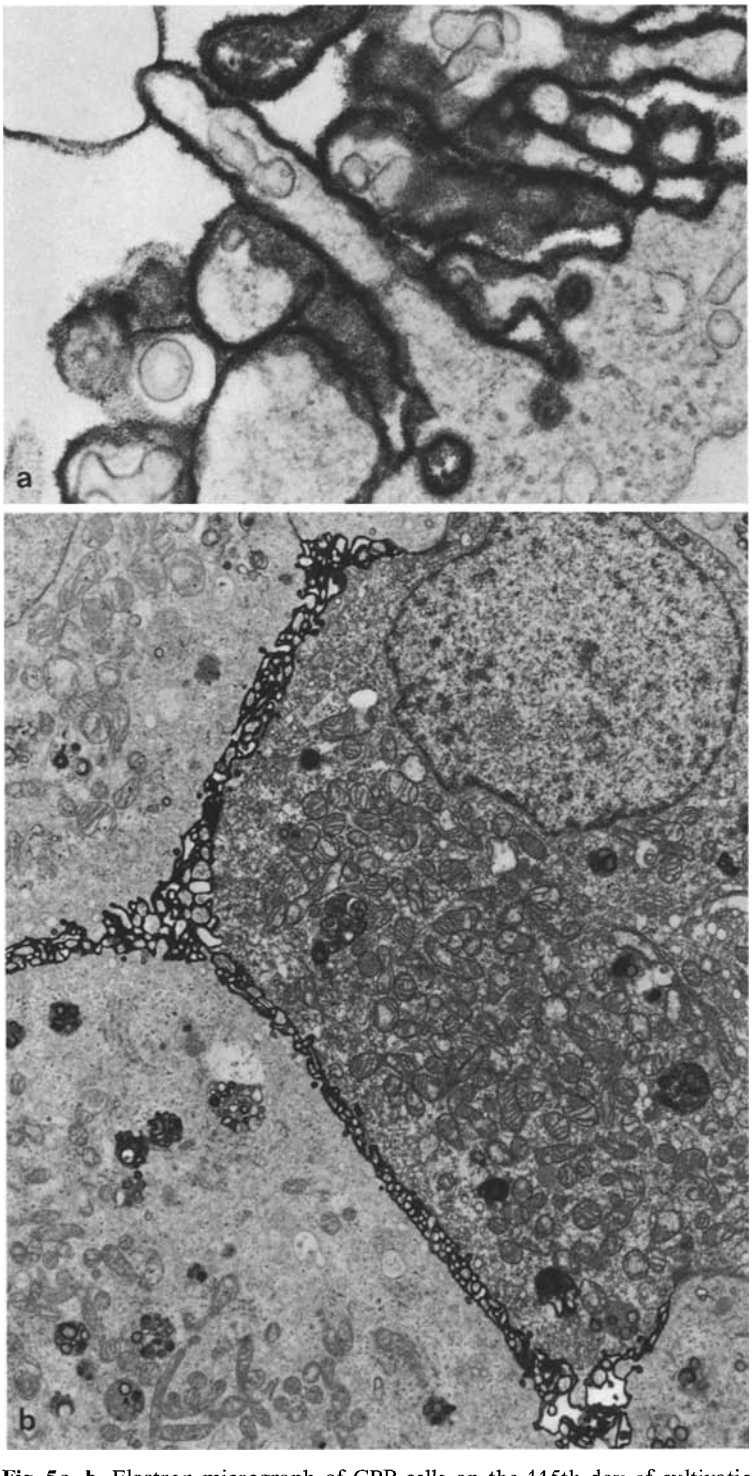

Fig. 5a, b. Electron micrograph of CPP cells on the 115th day of cultivation. **a** The surfaces of microvilli are stained intensely with ruthenium red (48,000:1). **b** Well developed interdigitations are also stained with ruthenium red (6,000:1)

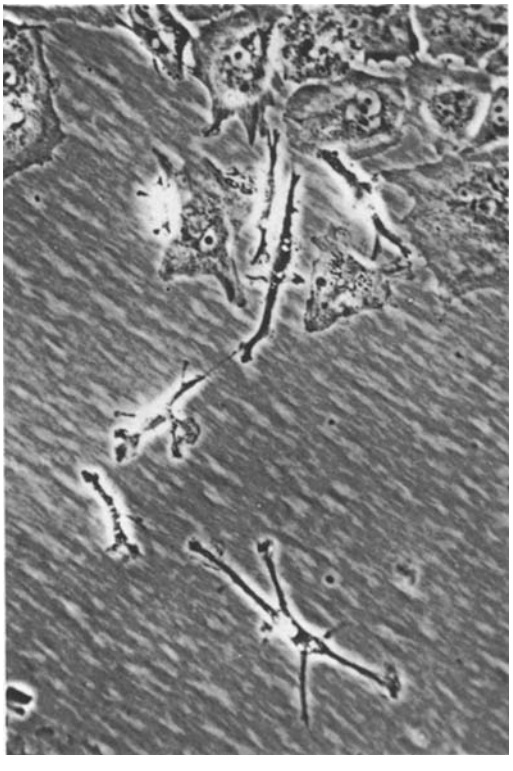

Fig. 6. Phase contrast microscopic feature of cultured CPP cells after 10 days. Spider-like cells are migrating beyond the foci of epithelial cell sheets (200:1)

somes, and their presence presumably explains the high electron density of the cytoplasm. Spindle cells with highly electron-dense cytoplasm are usually located outside of the basal lamina of tumor cells, and their long, thin processes seem to cover the outer surface of the basal lamina (Fig. 2). In some places, the long processes with highly dense cytoplasm are inserted into the invaginations of the basal plasmalemma of tumor cells (Fig. 2), and other processes extend to contact the wall of a blood vessel. It is of interest that these spindle cells with highly dense cytoplasm break through the basal lamina by their long processes with microvillous projections. Occassionally, the spindle cells are found in the intercellular space between CPP cells, and adhere to the ventricular surface of CPP cells (Fig. 3).

The surface of the tumor cells is stained with ruthenium red, indicating the presence of glycosaminoglycan. The ruthenium red stain is intense on the surface of microvilli and cilia. The lateral surface of the cells is also stained weakly with ruthenium red.

Cultured cells from Case 4

During the first 20 days, the tumor cells migrated from the tissue segments, showing a monolayer epithelial arrangement (Fig. 4). Many cells retained

many microvilli, a few cilia and well developed interdigitation, as shown in Fig. 5. The tips of some microvilli were expanded, showing balloon-like features. In the cytoplasm, electron-dense globules were conspicuous, and sudanophil fine granules were observed to be stippled diffusely. Ruthenium red stain was found on the whole surface of the cells and was especially intense on the surface of microvilli, as shown in Fig. 5a, b. By treatment with Latex 0.81 μm; phagocytic activity of the cells, albeit weak, could be detected.

During the first 5 days after inoculation, the outgrowth of spider-like cells from the tumor segments was observed in many places. Most of these cells migrated beyond the foci of the epithelial cell sheet. Their processes displayed spider-like features (Fig. 6), in some places attaching to the epithelial cells. By phase contrast microscopy, their cytoplasm was so dark that the nucleus was often difficult to recognize. They had many coarse granules stained with both Sudan-III and Alcian blue in their cytoplasm. The sudanophil materials were occasionally packed together in the cytoplasm. Almost all were intensely stained with Berlin blue, reflecting the presence of hemosiderin. By Latex treatment the cells showed active phagocytic ability.

Discussion

In the present study, CPP cells contained numerous lysosomes with hemosiderin, and showed phagocytic activity by Latex treatment, albeit weak, in vitro. This result suggests that CPP cells have a phagocytizing ability in vivo. Ruthenium red stain was positive on the whole cell surface, being especially intense on the surface of microvillous projections. Our previous study (Takeuchi et al. 1977) demonstrated that MDCK cells, derived from epithelial cells of dog kidney, synthesized GAG, which was mainly located on the cell surface or in the intercellular matrix; The cell surface, especially the surface of microvillous projections, was stained intensely with ruthenium red. It has been reported that microvilli are well developed on the free surface of intestinal epithelium and on the proximal convoluted tubule of the kidney, the function of which is absorption (Fawcett 1965; Ito 1965). The developed mucopolysaccharide surface coat was found to be present on the villous processes. It was conceivable that ruthenium red positive material on the cell surface plays an important role in promoting fluid transport. CPP cells are considered to have an active function of secreting the cerebrospinal fluid.

The interstitial cells showed a unique biological ability in vitro and in vivo with highly electron-dense cytoplasm forming long and thin processes. They were generally located outside of the basal lamina of CPP cells, but many of them sometimes broke through the basal lamina. They were also found in the intercellular spaces of CPP cells. In their cytoplasm, both Berlin-blue and Sudan-III-positive granules were easily observed, and phagocytic ability was intense in cultured cells which showed a spider-like feature. These spindle cells were considered to be macrophage with a vigor-

ous phagocytizing activity. They moved about frequently and were located not only in the interstitium between CPP cells and vascular tissues, but also among CPP cells. They sometimes migrated into a ventricular cavity surrounded by the tumor cells. Ling (1979), observing the epiplexus cells after intravenous injection of carbon particles, showed that carbon-labelled epiplexus cells were derived from circulating monocytes. Liu (1981) showed two separate lines of phagocytes in the brain: The phagocytes which respond to acute tissue damage are mostly blood monocytes, whereas under conditions of slow and long-standing tissue break down, the tissue macrophages originating from a pool of perivascular stem cells plays a dominant role. They migrate away from the vessels and become mobile phagocytic cells known as microglia, which are derived embryologically from mesenchymal cells accompanying choroid plexus and meningeal elements. In the proliferating CPP tissue in the present study, the interstitium consisted of edematous loose connective tissue which contained a large amount of orcein positive material, both granular and fibrillar as described in our previous paper (Nakashima et al. 1982). In this interstitium, fibroblastic cells were scanty, and many of the interstitial cells mentioned above were found. These interstitial cells are considered to have an active function of phagocytizing, scavenging, controlling fluid transport and serving as mediators between the CPP cells and the capillaries.

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Accepted February 23, 1983