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# Gap Junctions between Human Meningioma Cells Maintained in Organ Culture

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*Summary.* Intercellular junctions were commonly observed in a human syncitial meningioma maintained in organ culture for up to 44 days in vitro (DIV) using gelatin sponge foam matrices. The junctions were identified as one of three types: desmosomes; tight junctions; or gap junctions. Of the three types, gap junctions were frequently encountered and showed preservation of their characteristic ladder-like substructure. The results suggest that organ culture provides an environment that may facilitate correlation of the structure and function of gap junctions between coupled human meningioma cells.

*Key words:* Meningioma — Organ culture — Gap junctions.

## INTRODUCTION

Meningiomas are among the most frequent and best known tumors of the central nervous system in man and animals (Rubinstein, 1971). Human meningiomas account for nearly 15 to 20% of all primary intracranial tumors and approximately 25% of intraspinal neoplasms (Russell and Rubinstein, 1971; Rubinstein, 1971). In most instances, these tumors are thought to originate from the arachnoid cells of the meninges and their derivatives in the meningeal spaces (Cervós-Navarro and Vasquez, 1969; Rubinstein, 1971; Tani at el., 1974). Because of their frequency in man, meningiomas have been the subject of considerable recent interest. The fine structure of human meningiomas is characterized by interdigitating cell processes, by fine intracytoplasmic filaments that occasionally form whorling structures within the cell, and by the presence of specialized intercellular junctions (Kepes, 1961; Napolitano et al., 1964; Cervós-Navarro and Vasquez, 1969; Poon et al., 1971). These intercellular junctions are localized modifications of the plasma membrane that occur with regularity between adjacent meningioma cells (Poon et al., 1971). A recent study (Tani et al., 1974) convincingly demonstrates the presence of at least three categories of intercellular junctions between human meningioma cells; desmosomes, tight or occludens junctions, and gap or nexus junctions.

The gap junction is a class of plasma membrane specialization that is currently the subject of considerable interest in the correlation of cell structure and function.

Although gap junctions have been encountered between a wide variety of cell types in vivo (Sheridan and Johnson, 1974; Staehlin, 1974), they are frequently observed between a number of cell types and tumor cells in tissue culture (Lowenstein, 1973; Overton, 1974). Gap junctions between tumor cells and cells in culture have served not only as a model for intercellular transfer of molecules (Johnson and Sheridan, 1971) but these transcellular pathways are also thought by some investigators to be channels for growth- and differentiation-controlling molecules (Lowenstein, 1973).

In this study, fragments of a human syncitial meningioma were grown and maintained in organ culture systems using the gelatin sponge foam matrix technique (Rubinstein et al., 1973) for up to 44 days in vitro (DIV). The presence of desmosomes, tight junctions, and gap junctions between meningioma cells grown in vitro is documented. Moreover, it is suggested that organ culture techniques may provide a favorable environment for the study of the function of gap junctions between human meningioma cells.

## MATERIALS AND METHODS

Fragments of a syncitial meningioma were obtained at craniotomy from a male age 58 years and were immersed in sterile saline. Tissue pieces were then transferred to sterile Hank's IX balanced salt solution before trimming into small fragments about 1 mm in cubic dimension. The tissue fragments were explanted to gelatin sponge foam matrices using techniques described previously (Rubinstein et al., 1973). An embryonic tissue medium (Sipe, in preparation) was used as the nutrient fluid. The conditions of incubation were standard (Rubinstein et al., 1973) and the cultures were examined twice weekly for their state of viability. Cultures selected for light microscopic study were fixed in buffered 10% formalin at 4° C for 24 h and were then processed and sectioned using standard histological techniques before staining with Hematoxylin and Eosin. Cultures for electron microscopic study were fixed in two strengths of a cacodylate-buffered glutaraldehyde-paraformaldehyde mixture at 4° C for 3 h (Sipe et al., 1973). Tissue blocks were further fixed in cacodylate-buffered osmium tetroxide and maleate-buffered uranyl acetate (Karnovsky, 1967). Standard techniques were used for embedding in Epon, thick and thin sectioning, and final staining of thin sections with uranyl acetate and lead citrate. All issue was studied in a Siemens 101 electron microscope operating at 80 KV.

## RESULTS

Organ cultures derived from the original tumor were maintained for up to 44 DIV using the gelatin sponge foam matrix technique. The meningioma cells exhibited the most vigorous growth up to 17 DIV, thereafter showing progressive degeneration and sclerosis up to 44 DIV. Light microscopic study of early cultures showed excellent preservation of the three dimensional pattern of tissue organization of the original tumor (Fig. 1). A syncitium of meningioma cells rested upon the supporting matrix and in cultures on gelatin sponge foam, tissue growth down into the lacunae of the matrix was observed. The three dimensional mass of meningioma cells was formed by a sheet-like arrangement of polygonal cells (Fig. 1). Individual cells were characterized by ill-defined granular eosinophilic cytoplasm containing

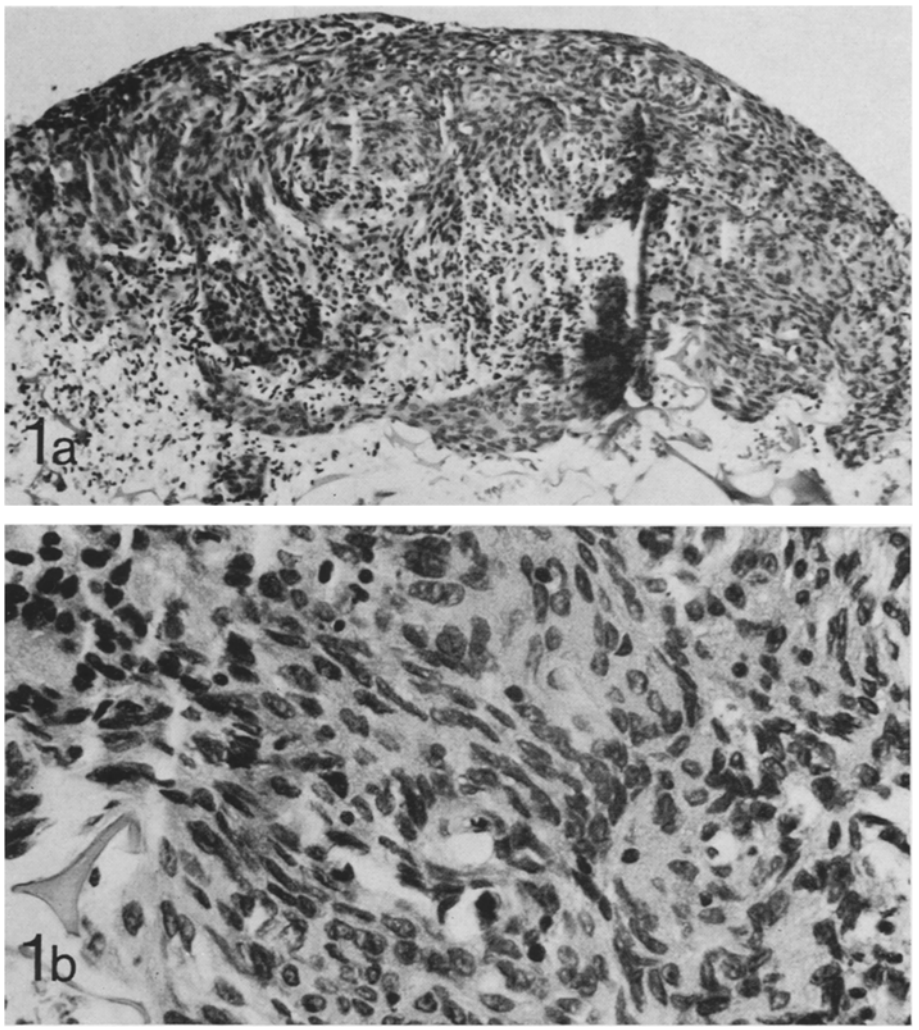


Fig. 1. (a) Human meningioma cultured on gelatin sponge foam, 3 days in vitro, showing preservation of the three dimensional pattern of histological organization characteristic of this tumor in vivo. × 180. (b) Same culture as Fig. 1a demonstrating the sheet-like arrangement of meningioma cells at higher magnification. × 585

round or oval nuclei with pale nucleoplasm and one or two small, dense nucleoli. The cytologic characteristics were benign and included the absence of mitotic figures. Early formations suggestive of whorling were occasionally seen but psammoma bodies and collagen were not evident in the cultures.

The fine structure of the meningioma cells in organ culture is identical to that reported by several observers (Kepes, 1961; Napolitano et al., 1964; Poon et al., 1971). The cultures faithfully reproduce the features of pronounced interdigitation of adjacent cell processes, the presence of fine intracytoplasmic filaments, and the

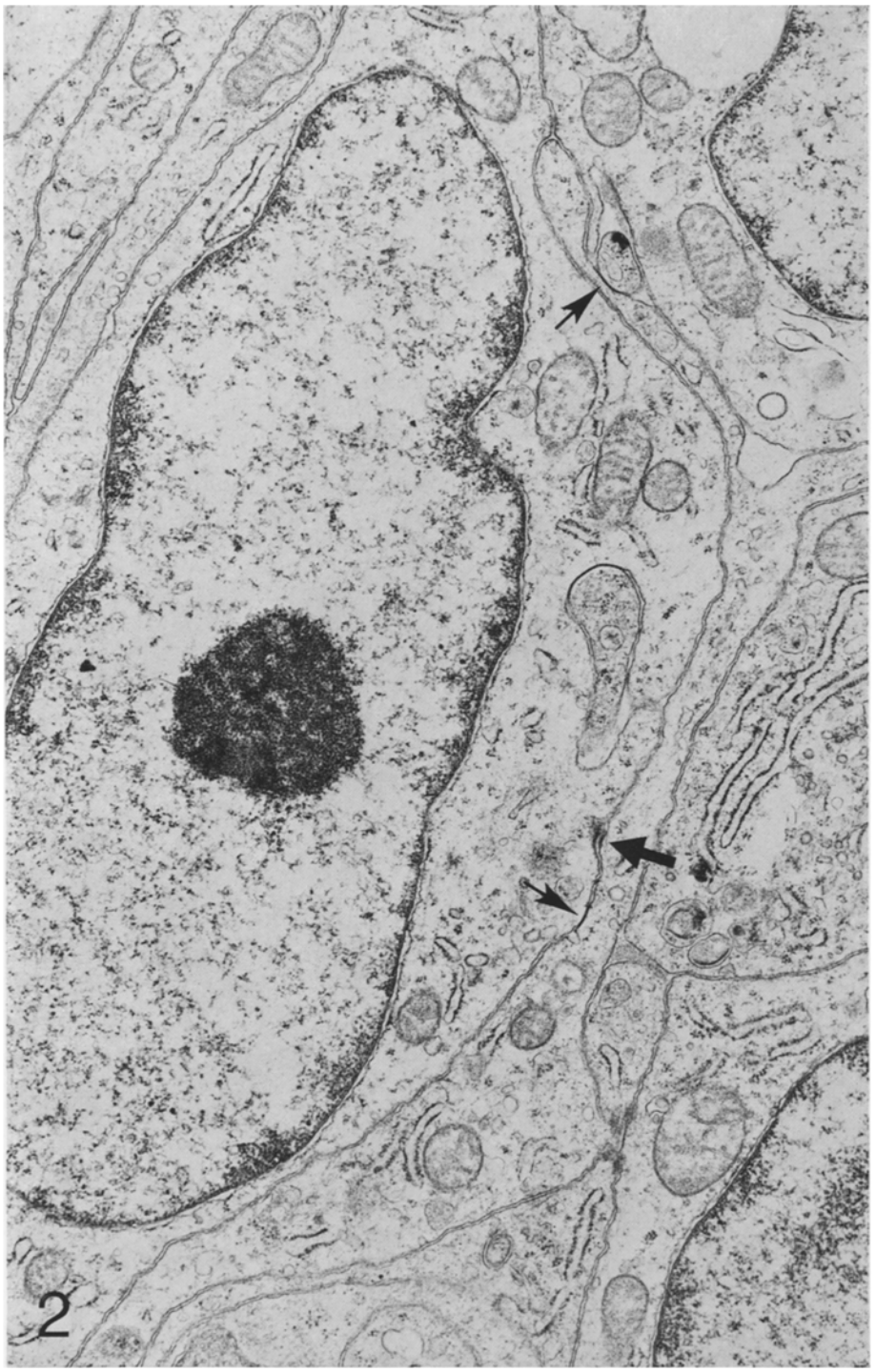


Fig. 2

appearance of specialized intercellular junctions. Specialized modifications of the plasma membranes in vitro include desmosomes, tight junctions, and gap junctions identical to those described in human meningiomas in vivo (Tani et al., 1974).

Although desmosomes and tight junctions are encountered with regularity, the gap or nexus junction is also an exceedingly frequent membrane specialization (Fig. 2). These junctions have the typical substructure produced by fusion of the outer leaflets of the apposed plasma membranes that form the narrow 20—30 Å gap (Fig. 3) seen in a wide variety of cell types (McNutt and Weinstein, 1973; Staehelin, 1974). Periodic central dense lines repeating at 80—90 Å intervals create the appearance of a ladder-like arrangement (Fig. 3). The overall width of the junctional specialization is 140—160 Å.

The distribution of gap junctions within the cultures follows no evident specialized pattern. Gap junctions are so common that many of the cells examined appear to have at least one or often several sites of coupling to adjacent meningioma cells or processes.

## DISCUSSION

The role of gap junctions as a possible intercellular pathway for cytoplasmic substances that influence growth and differentiation of cells is currently a subject of considerable interest (Lowenstein, 1973). Convincing evidence has been accumulated that gap junctions possess two main properties; intercellular adhesion like adherens and occludens junctions and the property of intercellular ionic and molecular transfer (Staehelin, 1974) associated with low resistance electrical communication between excitable cells (Bennett, 1966). In certain instances transcellular macromolecular transfer has been observed between cells connected by gap junctions (Lowenstein, 1973). The ease with which cellular metabolites undergo intercellular transfer by these transnexus channels has quite naturally led to the concept that cellular homeostasis and even regulation of cell growth and differentiation may be directly related to these intracytoplasmic pathways (Johnson et al., 1974).

A wide variety of neoplastic cell types exhibiting uncontrolled growth in vitro possess gap junctions. Among these, virus-transformed fibroblasts (Furshpan and Potter, 1968), Novikoff hepatoma cells (Johnson and Sheridan, 1971), and Morris hepatoma and sarcoma 180 cells (Sheridan, 1970) have been shown to possess abundant gap junctions. Coupling has also been observed between cells of different strains such as between normal mouse embryo cells (3T3) and transformed hamster cells (PY 19) (Furshpan and Potter, 1968). One in vitro approach to the problem of cell growth control has been to develop hybrid cell lines which are noncoupling and yet exhibit aggressive growth in vitro with the production of highly malignant tumors in animals (Lowenstein, 1973). When intercellular coupling is reestablished in these hybrids, the cell lines exhibit contact-inhibited growth and do not produce tumors when injected into animals (Azarnia and Lowenstein, 1971).

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Fig. 2. Human meningioma cultured on sponge foam, 3 days in vitro, demonstrating interdigitation of cell processes and two types of intercellular junctions: desmosome (large arrow) and gap junctions (small arrows). × 16200

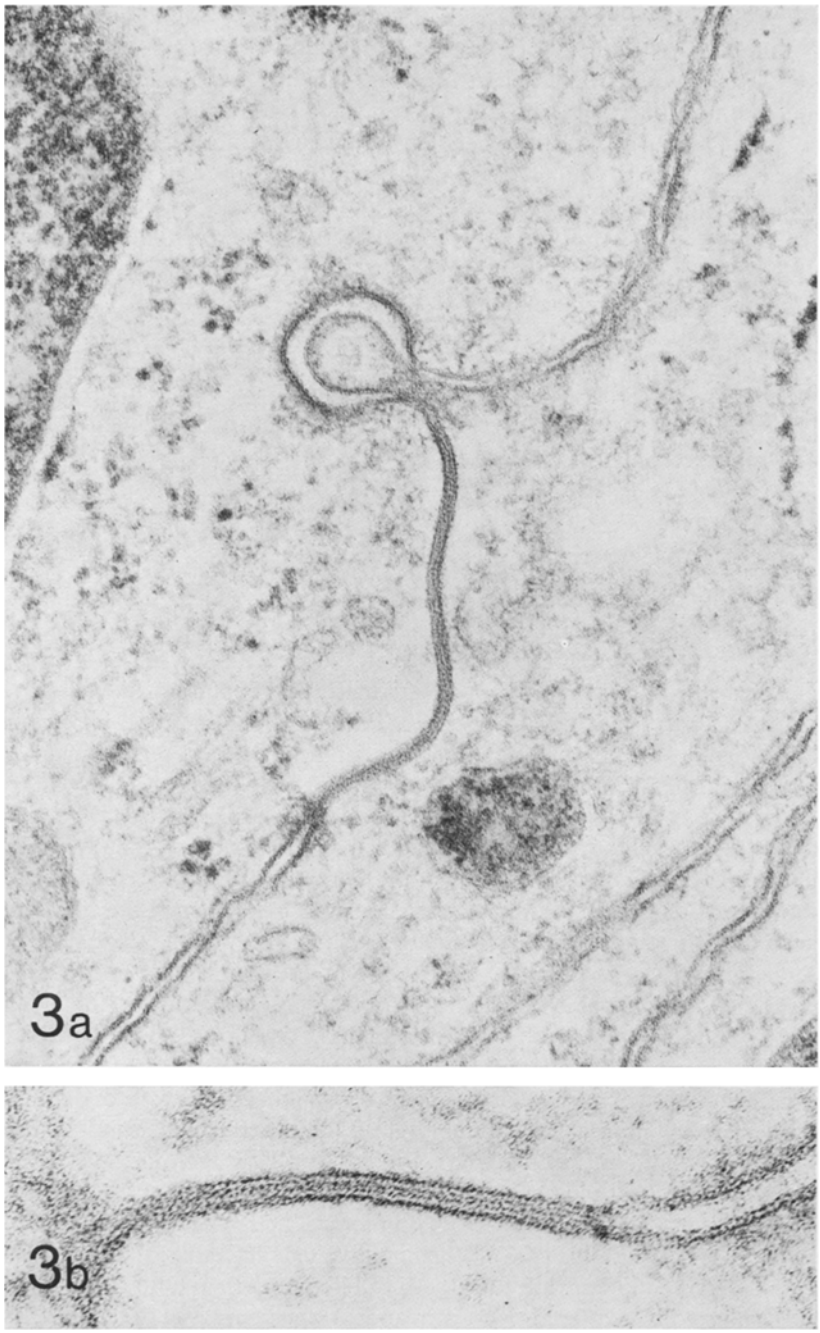


Fig. 3. (a) Same culture as Fig. 2. A gap junction (center) couples the cytoplasm of two adjacent meningioma cells. × 91 800 (b) Same culture as Fig. 2. The septalaminar gap junction is bisected by a median gap of constant width that is traversed by periodic slender dense lines suggesting a ladder-like arrangement. × 218 000

Although several neoplastic cell lines of mesenchymal origin have been used as models of cell coupling in vitro, there have apparently been no studies of central nervous system tumors connected by gap junctions in cell culture. Despite the presence of gap junctions between human astrocytoma cells in vivo (Tani and Ametani,1971), organ cultures of human glioblastomas and astrocytomas examined recently appear to lack the specialized intercellular junctions (Sipe et al., 1973). As reported here, human meningiomas, a common but typically noninvasive neoplasm, form abundant gap junctions between cells and processes in vivo and in vitro. Whether the obvious differences between human gliomas and meningiomas in intercellular communication by transnexus channels are in any way related to the growth characteristics of the tumors remains, as yet, unstudied.

In this study, organ cultures of a human meningioma faithfully reproduce the complex architectonic and ultratructural relationships of meningioma cells in vivo. There is the same fine structural appearance of the cell cytoplasm together with the preservation of a solid, three dimensional tumor mass (Fig. 1). Within the tumor all three types of intercellular junctions known to exist in meningiomas in vivo are clearly evident in organ culture. It is of particular interest that gap junctions are numerous and exhibit the characteristic substructure of such junctions described in the normal central nervous system (Brightman and Reese, 1969) and in normal arachnoid cells (Tani et al., 1974).

This study demonstrates that complex structural intercellular relationships are maintained in human meningiomas grown in organ culture systems. The presence of gap junctions between meningioma cells in vitro is of considerable interest. A three dimensional, solid mass of meningioma cells in culture offers the opportunity to examine not only the morphological characteristics of these junctions but also their physiological and biochemical properties. In turn, meningiomas in organ culture may provide a favorable environment in which to examine the role of intercellular transnexus channels in cellular homeostasis and regulation of cell growth in human brain neoplasms.

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