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Histological, ultrastructural and chromatographical discrimination of phospholipids in meningiomas

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Summary. Phospholipids in meningiomas were studied by light and electron microscopy, and by high-performance liquid chromatography. They were microscopically demonstrated in six of the ten cases by Sudan III staining after the fixation with potassium dichromate. However, the conventional ultrastructural fixation with glutaraldehyde and osmium tetroxide failed to confirm phospholipids, as most of them were dissolved during dehydration. In contrast, the specimens pretreated with tannic acid before osmication ultrastructurally retained phospholipids which were represented by multilamellar bodies or ribbon-like rings. Both were found in 23 of the 30 cases within the cytoplasm, among the plasma membranes and in the extracellular matrices. The outermost lamella or ribbon showed a direct continuity from the neighbouring plasma membranes of the cytoplasm or the mitochondria. The multilamellar bodies showed an overall distribution, while the ribbon-like rings were predominantly distributed around the psammoma bodies. Precipitation of hydroxyapatite crystals within the ribbon-like rings resulted in matrix minerals of psammoma bodies. Chromatographical analyses of meningiomas disclosed phospholipids including phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, sphingomyelin and phosphatidyl inositol in that order. Psammomatous meningiomas contained a higher percentage of phosphatidyl serine than non-psammomatous tumors. Ultrastructural study of synthetic phospholipids adequately treated with tannic acid showed that the multilamellar bodies were similar to phosphatidyl choline, while the ribbon-like rings were similar to phosphatidyl serine. The role of phospholipids in meningiomas is discussed.

Key words: Meningioma – Phospholipid – Psammoma – Ultrastructure – Chromatography

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As phospholipids are incompletely retained by the conventional ultrastructural fixation with glutaraldehyde and osmium tetroxide, most of them are extracted during subsequent dehydration. Accordingly, they can be hardly observed by the routine electron microscopy. Tannic acid (galloylglucose) was originally introduced as a supplementary fixative by Mizuhira and Futaesaku [7]. It interacts with the choline component of phosphatidyl choline, a major component of mammalian membranes, and is stabilized by post-fixation with osmium tetroxide [5]. By introducing tannic acid preceding osmication, Yamashima et al. [15] recently found pulmonary surfactant-like multilamellar bodies in human arachnoid villi. In this study we could, by the same procedure, demonstrate two forms of phospholipids: multilamellar bodies and ribbon-like rings in meningiomas. Furthermore, we could confirm phospholipids by high-performance liquid chromatography (HPLC). This report presents histological, ultrastructural and chromatographical data of phospholipids found in meningiomas.

Materials and methods

The specimens of meningiomas were obtained at surgery from the consecutive 30 patients of 24 women and 6 men aged 32 to 79. These meningiomas histologically comprised 9 cases of meningothelial type, 10 transitional type, 4 fibroblastic type, 2 psammomatous type, 3 angiomatous type and 2 malignant type.

Light microscopy

For the routine microscopic investigation, the blocks were fixed in 3.7% formaldehyde and embedded in paraffin. Thin section was made and stained with hematoxylin and eosin. For the demonstration of phospholipids, the blocks obtained from ten cases were fixed with the mixed solution of potassium dichromate, formaldehyde and acetic acid, and were embedded in paraffin. Thin section was made and stained with Sudan III and hematoxylin.

Electron microscopy

The specimens were fixed in 2.5% glutaraldehyde with or without 0.1% tannic acid (Wako Pure Chemical Industries Ltd. Osaka, Japan) in 0.1 M phosphate buffer, pH 7.4, for 6 h at 4°C. Subsequently, they were repeatedly rinsed in phosphate buffer to remove unreacted tannic acid. The specimens were then post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4, for 2 h at 4°C, dehydrated through graded concentrations of acetone and embedded in the Epon-Araldite mixture. Ultrathin sections were stained with uranyl acetate and lead citrate or the latter alone, and were examined with a Hitachi H-600 electron microscope.

High-performance liquid chromatography [6, 10]

Blocks of 1×3 cm obtained from three cases including two meningothelial type and one psammomatous type, were rinsed in 2 ml distilled water and homogenized. Two milliliters of the sample was mixed with 4 ml of chloroform, and was centrifuged at 3,000 rpm for 10 min. The chloroform layer was membrane-filtered and 1 ml of the filtrate evaporated. The pellet was rinsed in 100 µl chloroform and was dissolved in ethylalcohol. A Shimadzu LC-6A HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a SPD-6A UV detector was used. The phospholipid classes were separated on a silica HPLC column of shim-pack CLS-SIL (Shimadzu) with UV light detection at 205 nm. The mobile phase was 5:1 mixed sodium perchlorate anhydrate (pH 2.6). The flow rate was 1.0 ml/min and the temperature was 40°C.

Electron microscopy of synthetic phospholipids

Twenty-five milligrams of synthetic phospholipids purchased from Sigma Chemical Co. (St. Louis, Mo) was suspended in 1.5 ml distilled water and shaken in the water bath at 60°C for 2 h. The suspension was fixed for 3 h at 4°C with an equal volume of 2.5% glutaraldehyde containing 0.1% tannic acid in 0.1 M phosphate buffer. Suspensions were then centrifuged at 4,000 rpm for 30 min. The pellets were washed with 0.1 M phosphate buffer, post-fixed with 1% osmium tetroxide, dehydrated with acetone containing 1% paraphenelenediamine and were embedded in the Epon-Araldite mixture.

Results

Light microscopy

Six of the ten meningiomas studied microscopically exhibited vesicular or lamellar phospholipids not only in the cytoplasm but also in the extracellular matrices (Fig. 1).

Electron microscopy

With the conventional fixation with glutaraldehyde and osmium tetroxide there was extensive extraction of phospholipids and their adequate preservation was quite difficult. In contrast, pretreatment with tannic acid before osmication exhibited such phospholipids as multilamellar bodies or ribbon-like rings in 23 of the 30 meningiomas including 5 of the 6 cases in which phospholipids were microscopically demonstrated (Fig. 2). These ultrastructural elements were assumed to represent accumulations of particular phospholipids, as they were rarely seen with the conventional method. The multilamellar bodies showed an overall distribution while the ribbon-like rings were predominantly found around the psammoma bodies.

The multilamellar bodies varied considerably in overall shape but often showed ordered, fingerprint-like appearance (Figs. 3, 5). They were found among the plasma membrane (Fig. 3), in the extracellular matrices (Fig. 4) and within the mitochondria (Fig. 5) or the cytoplasm of meningioma cells. The multilamellar bodies were often surrounded by clusters of glycogen particles in the cytoplasm. Multilamellar bodies sometimes surrounded the matrix minerals [13, 14], which might serve as major calcification nidi of psammoma bodies. The periodical width of lamella was approximately 5.0 nm, of which the electron-dense zone measured 3.0 nm and the electron-lucent zone measured 2.0 nm (Fig. 5).

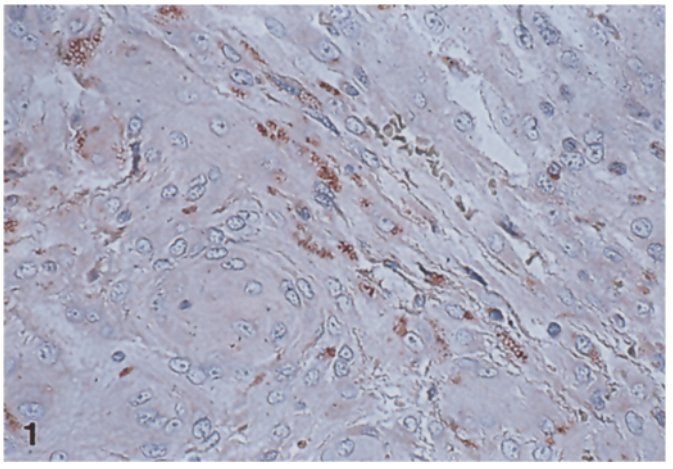
The ribbon-like rings were characterized by single or several arrays of irregular thickness which were similar to ribbons. They sometimes showed a direct continuity from the plasma membranes of disintegrating mitochondria (Fig. 6). A number of ribbon-like rings were, not infrequently, seen in the interstitial tissue especially around the mature psammoma bodies (Fig. 7). Hydroxyapatite crystals were frequently precipitated within or over the rings (Fig. 8) with resultant matrix minerals. Furthermore, a number of glycogen granules were precipitated over the ribbon-like rings.

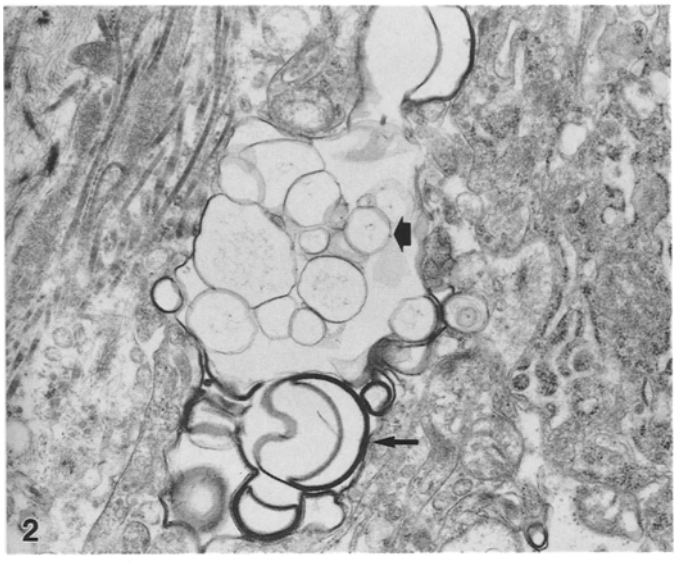
High-performance liquid chromatography

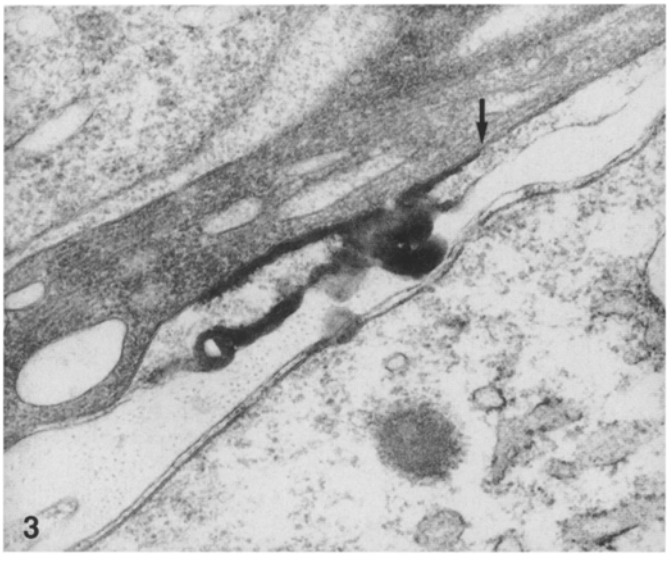
HPLC analysis of meningiomas disclosed five types of phospholipids regardless of the histological subtypes, including phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, sphingomyelin and phosphatidyl inositol. Two meningothelial meningiomas contained 96.0% and 96.4% phosphatidyl ethanolamine, 3.16% and 2.79% phosphatidyl choline, 0.45% and 0.26% phosphatidyl serine, 0.21% and 0.42% sphingomyelin as well as 0.18% and 0.13% phosphatidyl inositol. In contrast, psammomatous meningioma contained 93.5% phosphatidyl ethanolamine, 3.52% phosphatidyl choline, 1.97% phosphatidyl serine, 0.71% sphingomyelin and 0.3% phosphatidyl inositol. Psammomatous meningioma contained a higher percentage (1.97%) of phosphatidyl serine than meningothelial meningiomas without psammoma bodies (0.26% – 0.45%; Fig. 9).

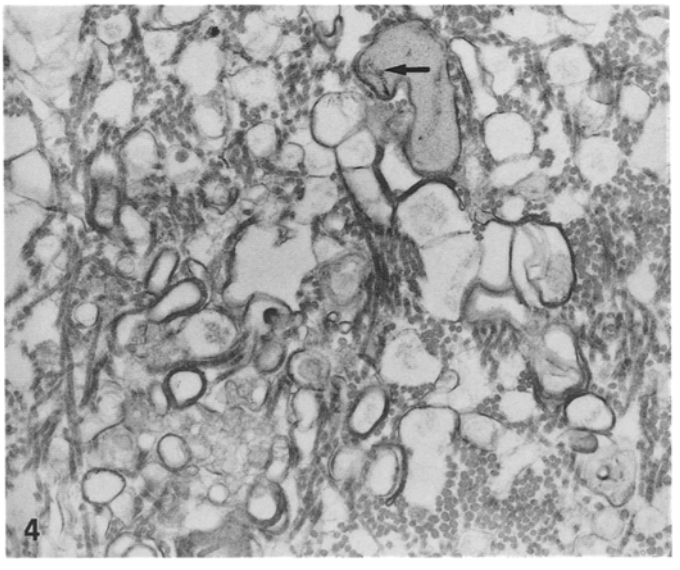
Electron microscopy of synthetic phospholipids

Among the five types of synthetic phospholipids investigated, phosphatidyl choline (Fig. 10 – Left), phosphatidyl inositol, sphingomyelin and phosphatidyl ethanolamine ultrastructurally showed lamellar structure with an alternation of electron-dense and -lucent zones. However, these phospholipids showed different characteristics in size, overall shape and electron density. Among them, phosphadityl choline was most similar to multilamellar bodies in meningiomas. In contrast, phosphatidyl serine (Fig. 10 – Right) comprised networks of ribbon-like structures with an irregular thickness.









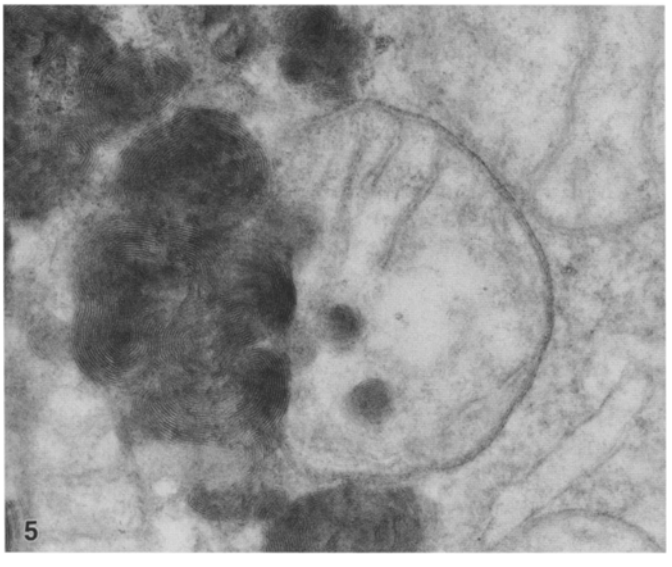


Fig. 1. Counterstain with Sudan III and hematoxylin reveals vesicular or lamellar phospholipids not only in the cytoplasm but also in the extracellular matrices. × 510

Fig. 2. Multilamellar bodies (*arrow*) and ribbon-like rings (*arrowhead*) partly showing a direct continuity, are seen among interstitial collagen fibers and cytoplasmic processes. × 14,850

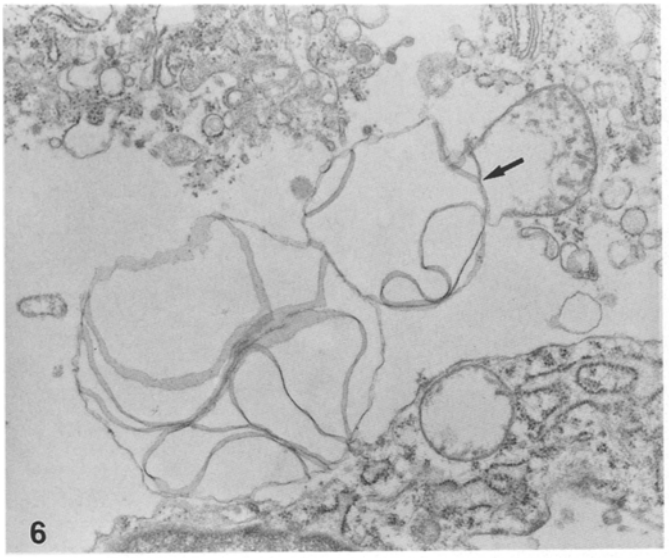
Fig. 3. The intercellular space contains fingerprint-like multilamellar bodies, the outermost lamella of which is in continuity (*arrow*) with the adjacent plasma membrane. Lead stain, × 39,600

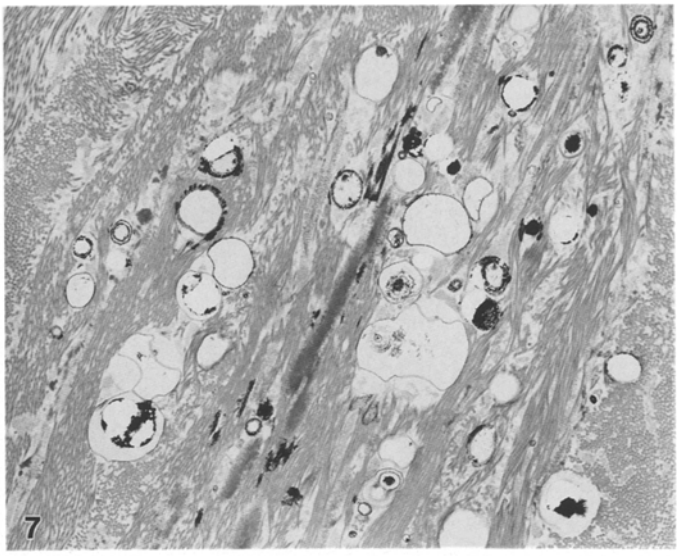
Fig. 4. Numerous multilamellar bodies are intermingled with interstitial collagen fibers, some of which show precipitation of hydroxyapatites (*arrow*). × 19,800

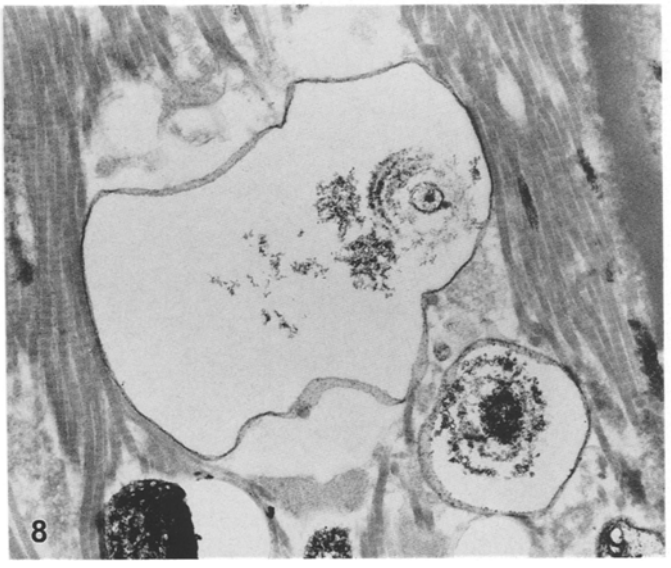
Fig. 5. Fingerprint-like multilamellar bodies are formed within and around the mitochondria in the cytoplasm of meningioma cells. × 49,500

Discussion

Cellular and intracellular interfaces are known to contain appreciable quantities of phospholipids. Phosphatidyl choline accounts for over 50% of the total phospholipids present in membranes of most animal tissues [11]. Glutaraldehyde interacts with phospholipids such as phosphatidyl serine and phosphatidyl ethanolamine by cross-linking at the primary amines in the polar head group [3, 8]. As the polar heads of phosphatidyl cholines lack primary amines, they are unable to react directly with either glutaraldehyde [3, 8] or osmium tetroxide







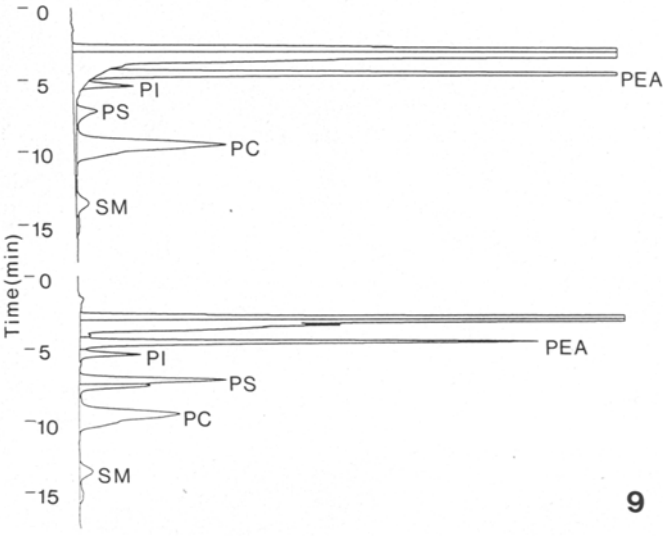


Fig. 6. Ribbon-like rings are characterized by single or several arrays of ribbons, being partly in continuity (*arrow*) with disintegrating mitochondria. ×9,900

Fig. 7. The interstitium around mature psammoma bodies contains a number of ribbon-like rings with a variable precipitation of hydroxyapatites. ×4,950

Fig. 8. Hydroxyapatite deposits are precipitated within or over the ribbon-like rings which presumably result in matrix minerals. ×19,800

Fig. 9. High-performance liquid chromatograms of meningothelial (*upper*) and psammomatous (*lower*) meningiomas show that the latter contain more phosphatidyl serine than the former. Vertical line represents retention time while horizontal line represents fluorescence intensity. *PEA*: phosphatidyl ethanolamine; *PI*: phosphatidyl inositol; *PS*: phosphatidyl serine; *PC*: phosphatidyl choline; *SM*: sphingomyelin

[1]. Tannic acid interacts with the choline component of phosphatidyl choline or sphingomyelin to form a complex, which can then be stabilized by osmium tetroxide [5]. By introducing tannic acid treatment before osmication, Kalina and Pease [5] could demonstrate highly ordered, preserved multilamellar bodies of phosphatidyl cholines in both model systems and type II alveolar pneumocytes. Human arachnoid villi adequately treated with tannic acid before osmication consistently disclosed highly ordered multilamellar bodies. As the latter were ultrastructurally quite similar to pulmonary surfactant, they were assumed to lubricate the surfaces of arachnoid cells for the smooth flow or absorption of the cerebrospinal fluid [15]. In this report, we could demonstrate that cellular and intracellular interfaces in meningiomas contain abundant multilamellar bodies which presumably have a chemical make-up for attaining a flexible boundary.

The phospholipid components of meningiomas showed a diverse class of molecules with a wide range of functions. It is widely accepted that phospholipids are involved in calcification at sites of primary mineralization. Epiphyseal cartilage contained such phospholipids as phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, phosphatidyl inositol and phosphatidyl serine in that order. In contrast, matrix vesicles isolated from the epiphyses contained significantly more

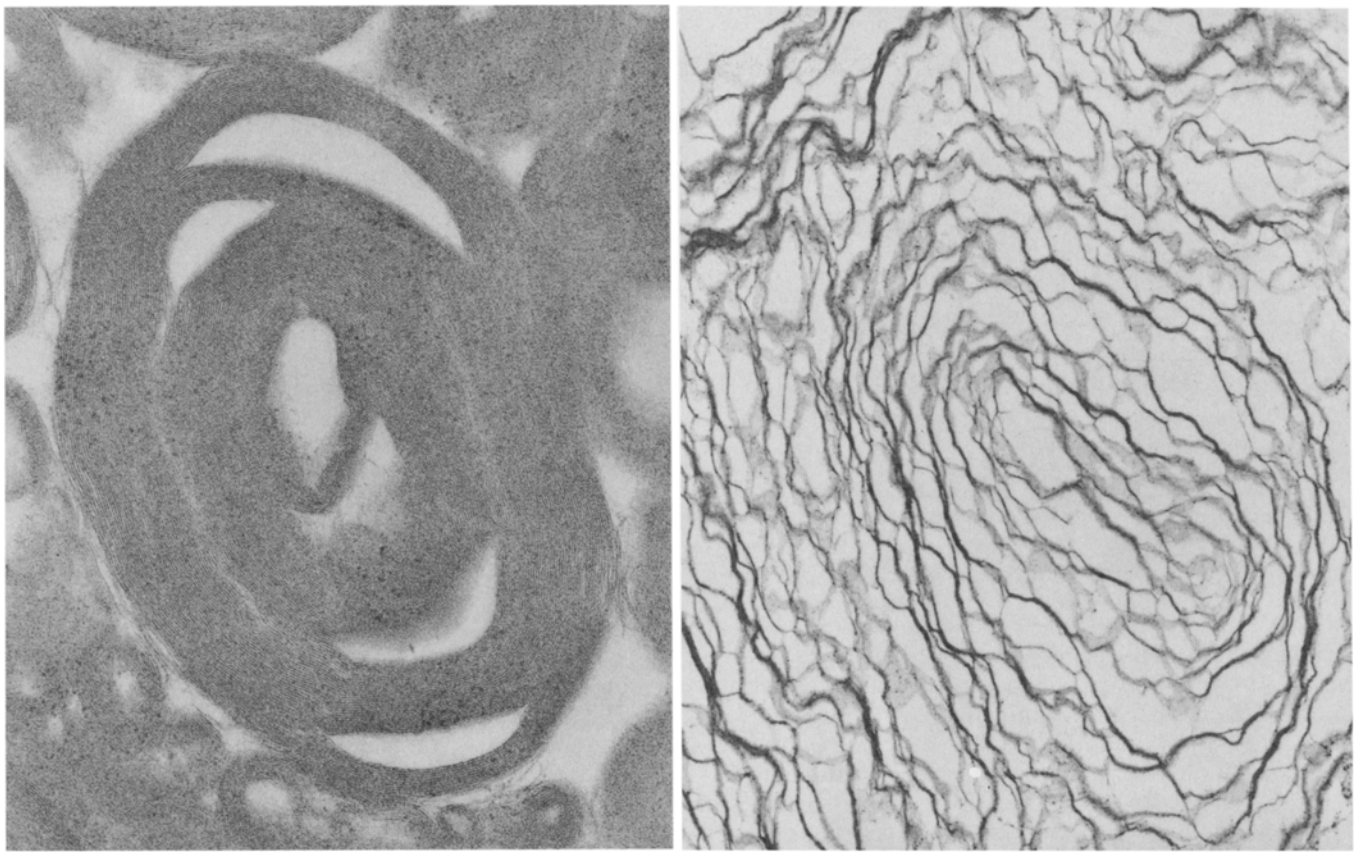


Fig. 10. *Left* Synthetic phosphatidyl choline was ultrastructurally similar to the multilamellar bodies. × 75,000. *Right* Synthetic phosphatidyl serine was ultrastructurally similar to the ribbon-like rings, × 18,000

phosphatidyl serine and sphingomyelin than cellular fractions [9, 12]. Phosphatidyl serine has the capacity of both binding to calcium ions [4] and stabilizing non-crystalline calcium phosphates [2]. In this study, we could confirm precipitation of hydroxyapatite crystals within the ribbon-like rings which were ultrastructurally similar to phosphatidyl serine. As psammomatous meningioma was chromatographically demonstrated to contain more phosphatidyl serine than meningiomas without psammoma bodies, it is suggested that phosphatidyl serine might be an indispensable constituent of matrix substances of psammoma bodies. Furthermore, meningiomas containing more phosphatidyl serine might show a tendency to form psammoma bodies.

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