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Ameloblastoma

Light and electron microscopic study

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Summary. Light and electron microscopic studies of ameloblastoma were reviewed. The 21 cases of ameloblastoma examined were classified into 12 cases of the plexiform type and nine of the follicular type. The average age of the patients with the plexiform type was 25.3 years, while that of those with the follicular type was 54.4 years. Histologically, in the follicular type, the tumor cells consisted of two cell types, central polyhedral and star-shaped cells resembling the stellate reticulum and peripheral cuboidal and columnar cells similar to the inner enamel epithelium. The resemblance between the tumor follicle and enamel organ was confirmed electron microscopically. In the plexiform type, however the tumor cells did not show two cell types, but resembled squamous epithelium. Electron microscopically, all cells of the tumor strands had relatively numerous bundles of tonofilaments and were joined together by desmosomes. Differentiation of tumor cells to squamous epithelium less evident than in normal surface epithelium. We speculate that these histological differences between plexiform and follicular types represent the differentiation tendency of the remnant of the dental lamina at the time of neoplastic transformation. What decides the histological pattern is unknown but age may be a factor. Central epidermization with keratinization or microcytic changes was frequently seen in the follicular type. Keratinization and microcystic changes rarely occured in the plexiform type. We do not believe that these chagnes are a form of involution or result from multipotentiality of the tumor cells.

Key words: Ameloblastoma – Plexiform type – Follicular type – Ultrastructure

Ameloblastoma is a tumor that develops from the epithelium of the odnotogenic apparatus or from its derivative or remnant. Its microscopic appearance shows considerable variation

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and the tumour has been classified into several types. In the international histological classification of the WHO (Pindborg and Kramer 1971), follicular, plexiform, acanthomatous, basal-cell and granular-cell types are described. The first two types, follicular and plexiform, are classified on the basis of the arrangement of the tumor cells, and the latter three types, acanthomatous, basal-cell and granular-cell types, on the basis of the morphological appearance. The tumor mass of both the follicular and plexiform types is recognized to consist of angular cells resembling the stellate reticulum of the enamel organ and of cuboidal or columnar cells similar to the internal enamel epithelium. However, these two cell types are not always found in all cases of the plexiform type (Gardner 1981).

Many ultrastructural studies of ameloblastoma have been made (Kitamura 1958; Moe and Philipsen 1961; Matsuda 1967; Matsuo and Kuhara 1968; Lee et al. 1971; Mincer and Maginnis 1972; Kim et al. 1979). However, these ultrastructual studies have been mainly of the follicular type of ameloblastoma.

We observed the ultrastructure of 21 cases of ameloblastoma, excluding basal- and granular cell ameloblastoma. The purpose of this paper is to attempt to show morphological differences between the follicular and plexiform types of ameloblastoma on light and electron microscopy and discuss their histogenesis.

Materials and methods

The tumor tissues of 21 cases of ameloblastoma, which had been taken at the time of operation at the Oral Surgery Clinic of Tokyo Medical and Dental University between 1978 and 1982, were fixed in 10% neutral formalin for routine histological examination. Sections were stained with hematoxylin and eosin, PAS, mucicarmine and alcian blue.

For electron microscopy, the tumor tissues of all 21 cases were cut in 1-mm^3 or smaller sections and fixed in ice-cold 2.5% glutaraldehyde with 0.1 M phosphated buffer (pH 7.4) for two hours, then rinsed several times by 0.1 M phosphate buffer (pH 7.4) and kept overnight. Following postfixation of 2% osminum tetroxide in 0.1 M phosphate buffer for two hours, they were then dehydrated in grade ethanol, treated with prophylene oxide and embedded in Epon 812. Approximately 1 μ thick sections were then cut and stained with methylene blue for light microscopic examination. Ultrathin sections were double-stained with uranyl acetate and lead citrate. These sections were observed with an Hitachi HU-12 electron microscopy.

Results

Clinical findings and histological type

All 21 cases of ameloblastoma were classified into the plexiform and follicular types. However, most cases showed a mixed arrangement of plexiform and follicular structures and so the types were classified by their dominant structures. Data on the age and sex of the patients, location and histological type are summarized in Table 1.

The age range in this series was from 12 to 87 years; the over-all mean age was 37.8 years. The age range in the plexiform type was from 12 to 48 years with a mean of 25.3 years, while that in the follicular type was from 38 to 87 years, with a mean of 54.4 years. The average age of the

Table 1. Summary of clinical data for 21 patients with ameloblastoma

Case (no.)	Sex	Age (years)	Location
Plexiform type:			
1	M.	16	Left mandible, molar area
2	M.	13	Right mandible, premolar area
3	M.	20	Left mandible, molar area
4	F.	23	Left mandible, molar area
5	M.	29	Left mandible, premolar-molar area
6	M.	30	Left mandible, molar area
7	M.	18	Left mandible, molar area
8	M.	20	Left mandible, molar area
9	F.	44	Left maxilla, anterior-molar area
10	M.	14	Left-mandible, premolar-molar area
11	M.	29	Right mandible, molar area
12	M.	48	Left mandible, premolar area
Follicular type:			
1	M.	46	Right maxilla, premolar area
2	F.	45	Right mandible, molar area
3	M.	86	Right mandible, molar area
4	M.	50	Left mandible, molar area
5	F.	87	Right mandible, premolar-molar area
6	M.	48	Left mandible, molar area
7	M.	43	Right maxilla, molar area
8	M.	47	Left mandible, molar area
9	M.	38	Left mandible, anterior-molar area

patients at the time of surgery in the plexiform type was approximately 29 years younger than that in the follicular type of ameloblastoma. It appears unlikely that there is any significant sex or location differences between in the plexiform and follicular types.

Light microscopic appearance

Plexiform type (Fig. 1A): The tumor epithelium is arranged in a network of anastomosing strands. In most parts, the outer cells of the strands were not always columnar but varied in shape, showing cuboidal, spindle, flat and polygonal forms, and their nuclei did not always show a palisade arrangement or well-regulated polarization. The inner cells of the strands were not always similar to the stellate reticulum of the enamel organ but consisted of a variety of shapes, such as star-shaped, spindle and polygonal cells. However, in some parts, the branching epithelial cord had two cell types, resembling the inner enamel epithelium and the stellate reticulum of the enamel organ, respectively. This is commonly recognized in the follicular type of ameloblastoma.

Follicular type (Fig. 1B): The tumor epithelium was in the form of discrete islands. These islands consisted of central areas of polyhedral or loosely connected angular cells resembling the stellate reticulum surrounded by

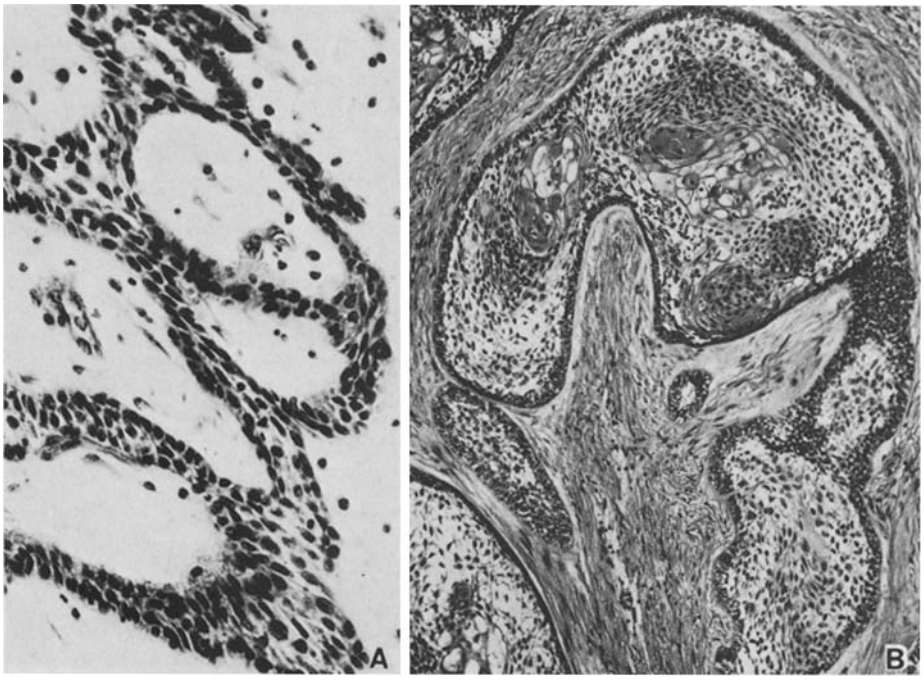

Fig. 1 A, B. Light microscopic histology of ameloblastoma. **A** *Plexiform type:* The epithelial strands form in an interlacing pattern, consist of spindle, cuboidal and oval cells and do not show distinct differences between the outer and inner cells of the strands Hematoxylin and eosin, ×252. **B** *Follicular type:* The tumor follicles are clearly shown and consist of a central portion of stellate cells and peripheral layer of columnar or cuboidal cells. Microcyst formation and squamous metaplasia occur amongst the stellate cells. Hematoxylin and eosin, ×33

layers of cuboidal or columnar cells resembling the internal enamel epithelium or pre-ameloblasts. The peripheral cells were generally well-polarized and showed a palisade arrangement. Central squamous metaplasia with keratinization and microcystic formation were frequently found in the follicles. Microcysts were occasionally stained with PAS, mucicarmine and alcian blue.

Electron microscopic appearance

Plexiform type. No basic ultrastructural differences existed between the outer and inner cells of the strands. The tumor strands were separated from the surrounding stroma by a continuous basal lamina 30–100 nm in thickness. The tumor cells of both the outer and inner cells in the strands were varied in shape as seen by light microscopy and occasionally revealed an irregular and invaginated contour with one or two nuclei (Fig. 2). The number and shape of the mitochondria were varied in each case, and profiles of rough endoplasmic reticulum were scarce. The Golgi complex was rudimentary, if present. Ribosomes were numerous (Figs. 3 and 4), and abundant glycogen particles were occasionally seen in the cytoplasm (Fig. 4).

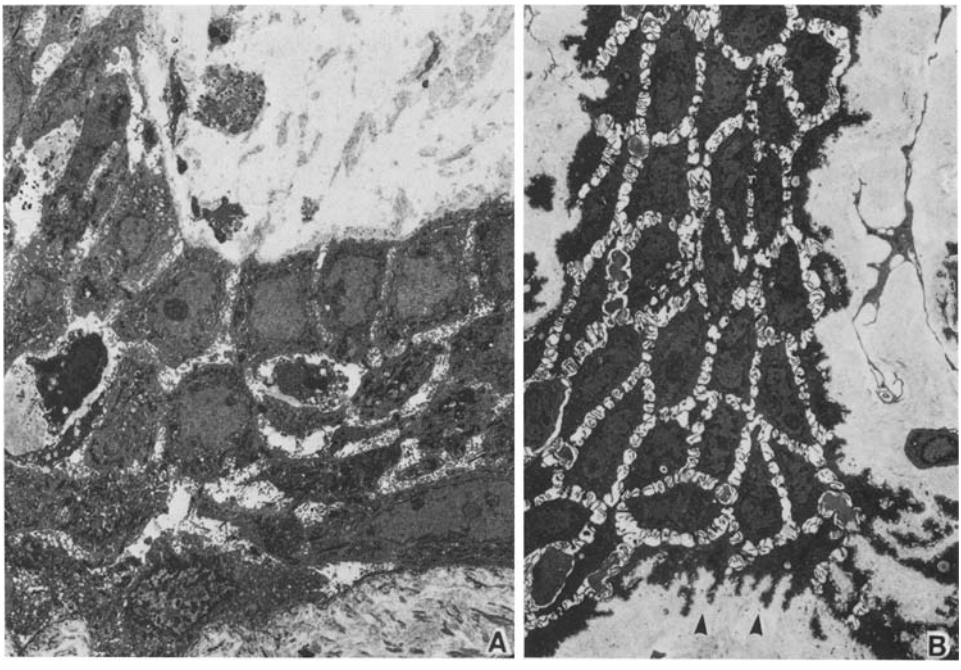

Fig. 2A, B. The strand of the plexiform type in two cases. Both outer and inner cells in strands show various shapes, such as cuboidal, spindle and polygonal. Intercellular spaces are wide with numerous microvillic projections of tumor cells. **A** Relatively smooth base of the outer cells. × 1,800. **B** Very irregular base of the outer cells (*arrow*). × 2,100

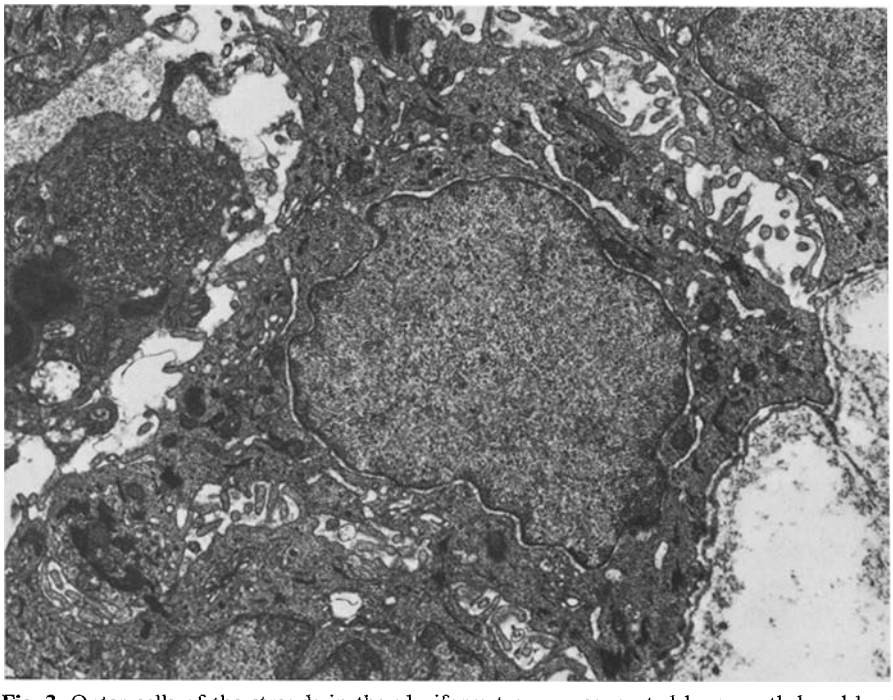

Fig. 3. Outer cells of the strands in the plexiform type are separated by smooth basal lamina from the connective tissue. Numerous ribosomes, scattered small mitochondria and bundles of tonofilaments are seen throughout the cytoplasm. Many microvillic processes project into the intercellular spaces. × 9,100

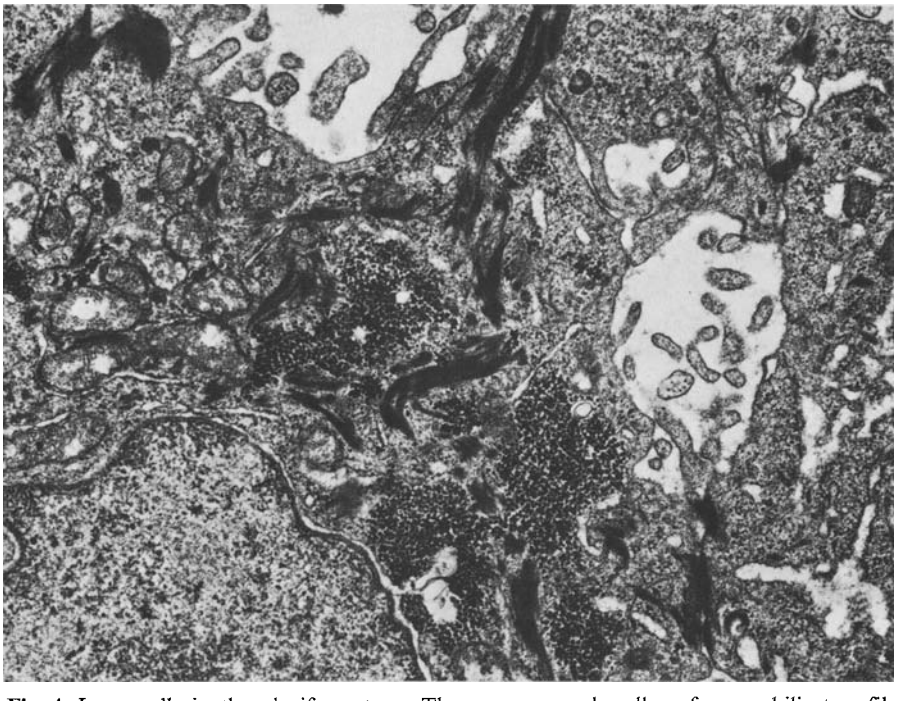

Fig. 4. Inner cells in the plexiform type. There are many bundles of osmophilic tonofibrils and accumulation of glycogen particles. Intercellular spaces contain many cell processes with their associated desmosomes and cytoplasmic tonofilaments. × 19,500

Bundles of tonofilaments were scattered throughout all tumor cells, forming tonofibrils (Figs. 3 and 4). Lipids, lysosomal granules and single cilia were occasionally contained in the tumor cells. The base of the basal cell was relatively smooth (Figs. 2A and 3), and the cell membrane facing the basal lamina contained many hemidesmosomes (Fig. 5A). In some strands, however, the base was made extremely irregular by the projection of cytoplasmic processes into the connective tissue (Figs. 2B and 5A), rarely accompanied by breaking of the basal lamina (Fig. 5B). The basal lamina was occasionally separated from the basal cells and broken in to fragments, which spread into the connective tissue (Fig. 5C). In some areas, anchoring fibrils were seen attached to the lamina densa of the basal lamina (Fig. 5C). Intercellular spaces were relatively wide, contained numerous microvilli sprouting from the tumor cells (Figs. 2 and 3) and were intermittently sealed by broad cell processes with apical desmosomes (Fig. 4).

Follicular type. A continuous basal lamina, 30–160 nm in thickness, separated the basal cells from the connective tissue. The basal lamina occasionally showed reduplication. Neither fragmentation nor disappearance of the basal lamina was demonstrated. Ultrastructural differences were recognized between the peripheral and central cells in the follicle. In the peripheral columnar cells (Fig. 6), the nuclei were spherical and longitudinally oriented

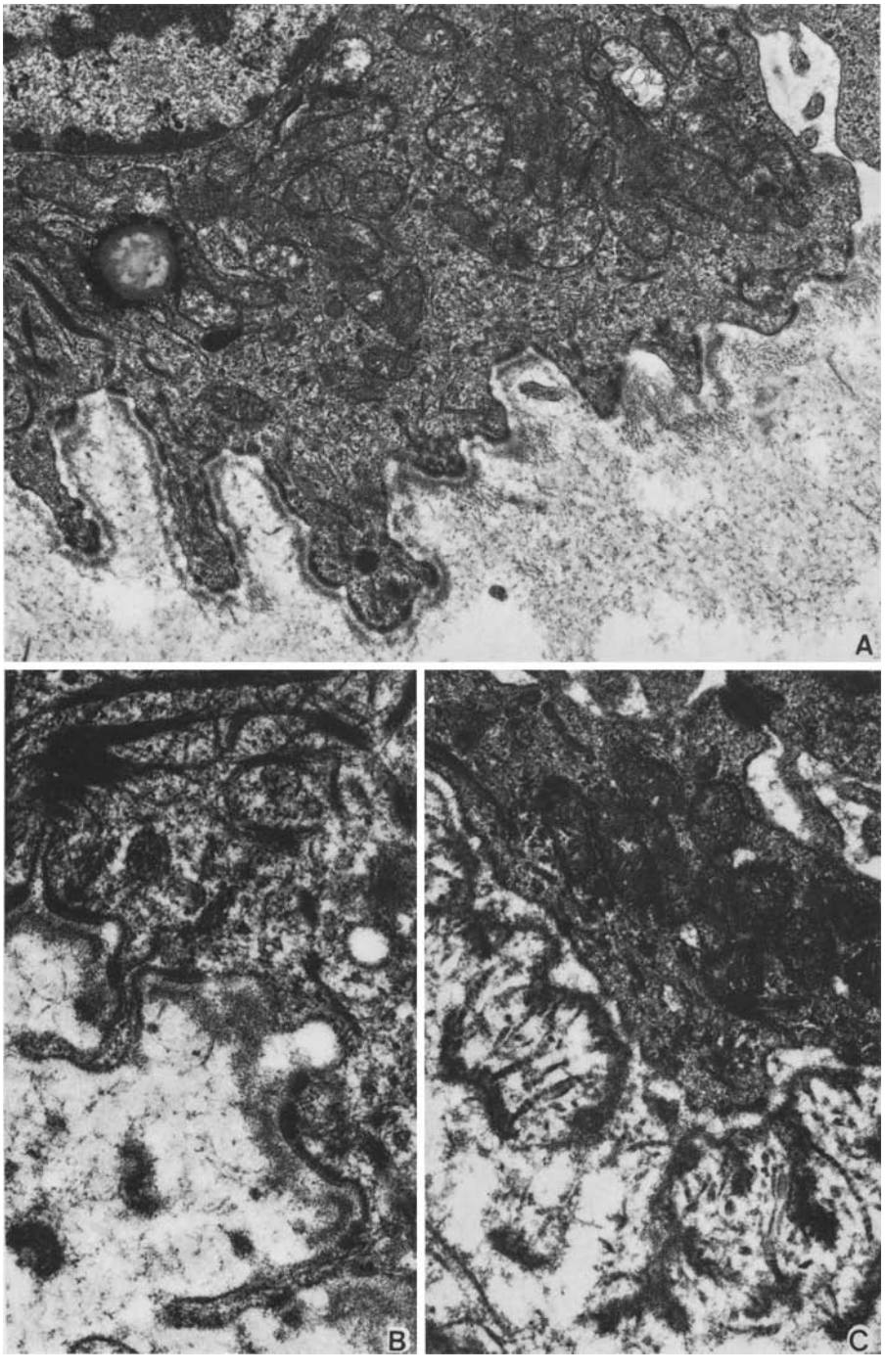

Fig. 5A–C. Some variants of the base of the outer cells in the plexiform type. **A** Irregular cytoplasmic membrane by projection of cell processes into the connective tissue. Numerous hemidesmosomes attach to the basal cytoplasmic membrane. × 20,400. **B** Two blunt extensions of the cell process are seen transversing a break in the basal lamina. × 35,100. **C** The basal lamina is separated from the basal cell and broken into fragments. Anchoring fibrils attach to the fragmentary basal lamina. × 27,300

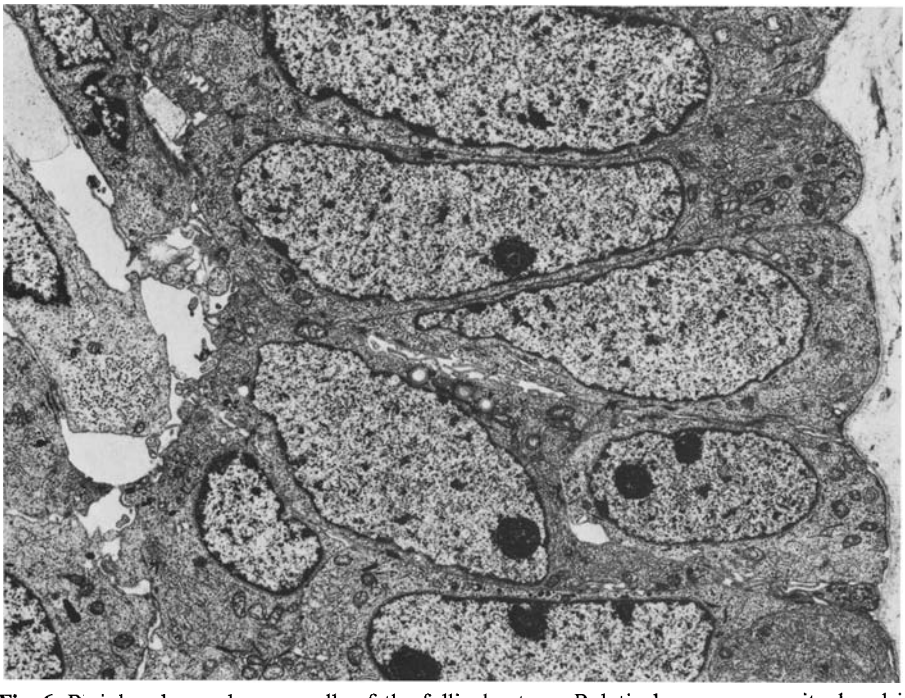

Fig. 6. Peripheral or columnar cells of the follicular type. Relatively numerous mitochondria and rough endoplasmic reticulum are mainly located in the basal portion. Tonofilaments are few. The basal cell membrane is smooth, and intercellular spaces are narrow and straight. × 3,900

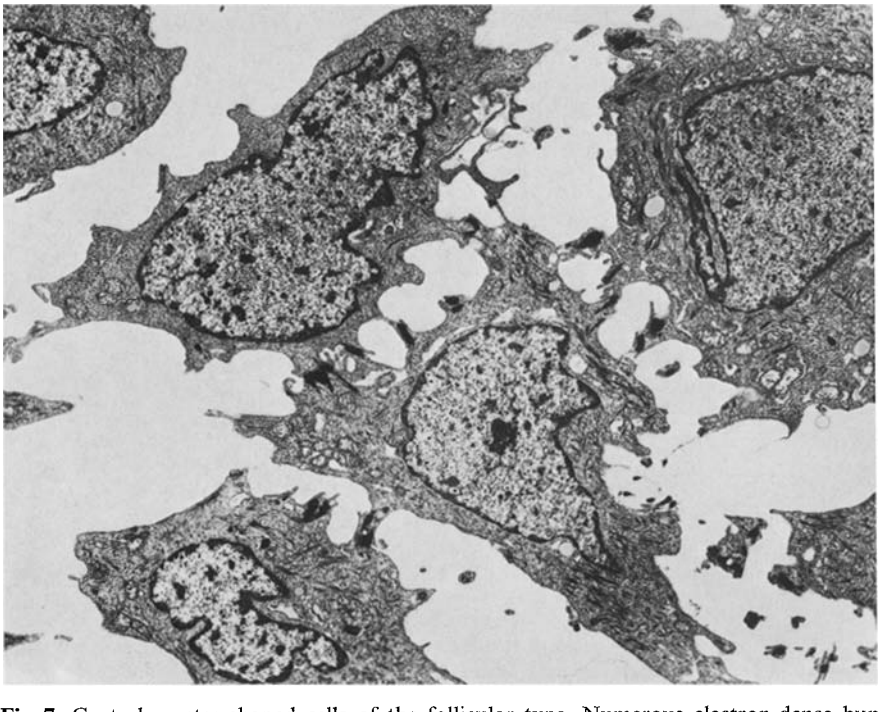

Fig. 7. Central or star-shaped cells of the follicular type. Numerous electron-dense bundles of tonofilaments and ribosomes are apparent. Intercellular spaces are irregularly wide and contain many long or short cell processes with their associated desmosomes and tonofilaments. x3,900

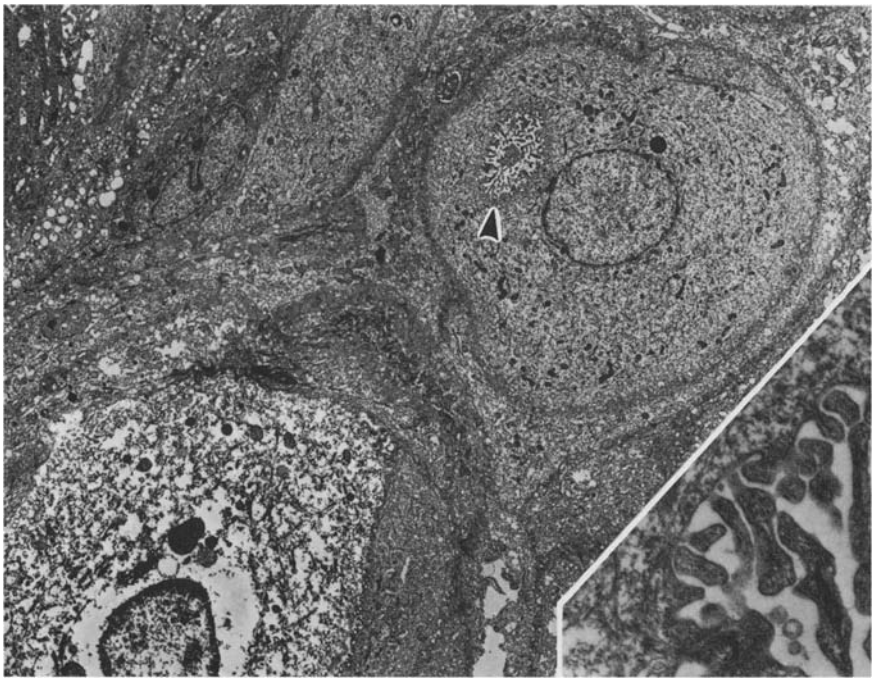

Fig. 8. Central cells of the follicular type. Two enlarged cells are seen. The cell at the *lower left* is disintegrating and that at the *upper right contains* numerous tonofibrils, *small dark* mitochondria and an intracytoplasmic lumen (*arrow*). ×2,340. *Inset:* The lumen is lined with microvilli. ×21,000

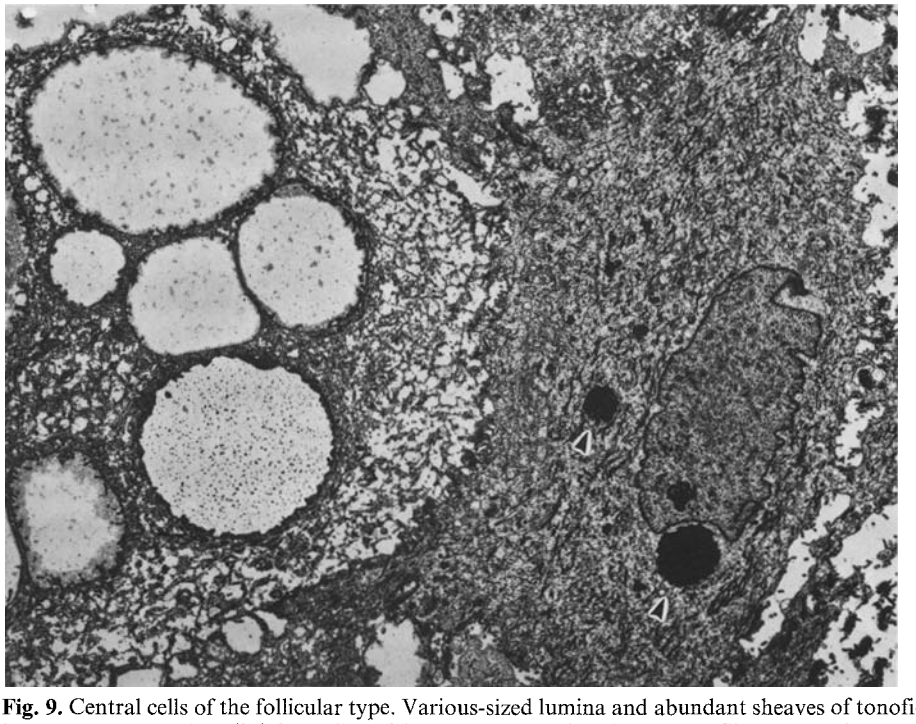

Fig. 9. Central cells of the follicular type. Various-sized lumina and abundant sheaves of tonofilaments are seen. The adjoining cell (*right*) contains many bundles of tonofilaments and several osmophilic keratohyaline granules (*arrow*). ×2,730

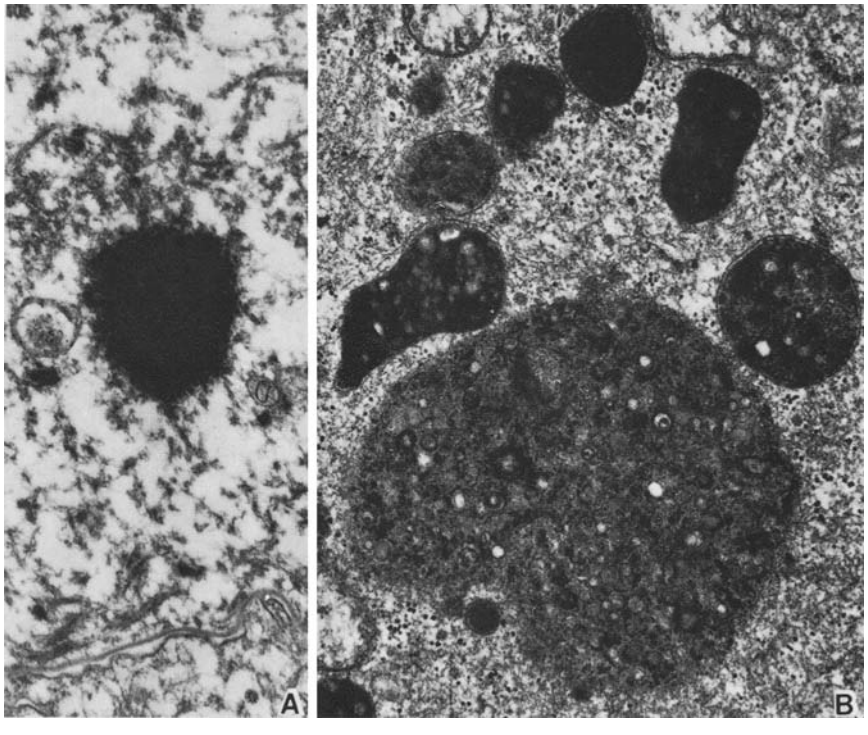

Fig. 10A, B. Keratohyaline granule **A** and mutivesicular dense bodies **B** in the centralmost cells. Multivesicular dense bodies contain many vesicles. **A** and **B**. ×25,200

in middle or inner portion of the cells with one or two nucleoli. Mitochondria and rough endoplasmic reticulum were mainly located at the basal portion. Golgi complexes with coated vesicles or primary lyososomes were seen mainly in the lateral or inner portion. Free ribosomes were numerous, glycogen and microtubules were preserved in varying amounts and tonofilaments were rather scarce. The cell membrane was relatively smooth and joined by scattered small desmosomes. The intercellular spaces were narrow and straight. The central cells of the follicles (Fig. 7) consisted mainly of star-shaped cells connected by long or short processes and separated by irregularly wide intercellular spaces. The cell process were linked by desmosomes. A few mitochondria and rough endoplasmic reticulum and many ribosomes were seen in the cytoplasm. The Golgi complex was not well developed. Many bundles of tonofilaments were found and extented to the cell processes, where the tonofilaments connected with the desmosomes. In some tumor follicles, the cytoplasm in the central cells was enlarged and filled with numerous bundles of tonofilaments (Figs. 8 and 9) and occasionally contained keratohyaline granules (Fig. 10A), lysosomal granules and large multivesicular dense bodies (Fig. 10B). Within these central cells, intracellular lumina were frequently found (Figs. 8 and 9). These lumina were varied size and bounded by many long or short microvilli, but secretory

granules were not recognized in the periluminar cytoplasm. Other central cells occasionally disintegrated (Fig. 8), were keratinizing and were shedding.

Discussion

The histological appearance of ameloblastoma reveals considerable variation and is classified by WHO (Pindborg and Kramer 1971) into 6 types: follicular, plexiform, acanthomatous, granular-cell type, basal-cell type and other variations. The main histological pattern is comprised of the follicular and plexiform types. The former grows by the forming of discrete islands of tumor cells and the latter with continuous anastomosing strands. According to the histological description of ameloblastoma by several authors (Gorlin 1970; Shafer et al.; Lucus 1976), tumor epithelium consists of two cell types, namely columnar cells resembling the internal enamel epithelium and angular cells similar to the stellate reticulum.

The present studies by light and electron microscopy showed the histological difference between the plexiform and follicular types. The follicular type showed light and electron microscopic findings similar to those previously described. The central angular cells, which resembled the stellate reticulum, were bounded by columnar cells similar to the internal enamel epithelium or early preameloblasts (Matthiessen and Romert 1980). The light microscopical findings in the tumors satisfy the histological criteria of Vickers and Gorlin (1970), that is, hyperchromatism and palisading with polarization of basal cell nuclei. Cytoplasmic vacuolization was frequently observed in the basal cells. In other parts, the epithelial strands of the plexiform type hardly showed similarity to the enamel organs with differences between the outer or inner layers, as seen in the follicular type. All tumor cells contained relatively numerous bundles of tonofilaments and ribosomes, and were joined by many desmosomes. The basal cell membrane of the outer cells extended to the connective tissue, had many hemidesmosomes and were separated from the basal lamina, with occasional adhesion of anchoring fibrils. These changes in tumor cells resemble normal or pathological squamous epithelium (Schoreder and Theilade 1966; Silverman 1967; Frithiof 1969), but the degree of squamous differentiation seen in the tumour is less than that seen in normal mucosa. We speculate that two tumor growth patterns can exist in the transformed remnant of the dental lamina which has persisted as an epithelial island within the jaw. In one pattern, neoplastic transformation is accompanied by differentiation to enamel organs and this takes the follicular form. In the other, neoplastic transformation with differentiation to the squamous epithelium produces the plexiform type. It is unknown what decides the growth pattern, but the age of the patients is probably a factor. The average age of patients with the plexiform type is approximately 29 years less than that of those with the follicular type.

Some cases of ameloblastoma showed plexiform arrangement with two cell types (columnar and star-shaped cells) resembling the enamel organ.

However, such a plexiform arrangement is usually recognized as a part of the follicular type.

Central epidermization is a very common change in the follicular type and is frequently accompanied by varying degrees of keratinization or microcystic formations. However, these changes are very rare in the plexiform type. The characteristic electron microscopic finding of the stellate reticulum cell of the human enamel organ is the presence of many bundles of tonofilaments, which have a supporting function similar to those of the stratum spinosum of the epidermis (Matthiessen and Romert 1980). In addition, during the involution of the stellate reticulum, its cells apparently undergo a process that resembles the keratinization of the surface epithelium (Pannes 1961). Pannes (1961) described that the involution of the stellate reticulum is a process of incomplete keratinization, and the stellate reticulum cells retain the ability to keratinize, another basic biological characteristic of epithelia. The central cells of the tumor follicles also had many bundles of tonofilaments and were keratinaized in the central portion. Such central keratinization of tumor follicles seems to correspond to the involution of the stellate reticulum in the enamel organ. Thus, these changes seems not to be squamous metaplasia, but to be an essential property of tumor cells. It is thought that the acanthomatous type of ameloblastoma could not be classified as variant of ameloblastoma and is naturally included in follicular-type ameloblastoma.

Intracytoplasmic lumina were occasionally seen in the central cells of the follicular type, but were not found in the stellate reticulum of the normal enamel organ. The cytoplasm of the central cells frequently contained multivesicular dense bodies and lysosomal granules, so these cystic changes might result from intracellular autolysis by lyososomal activity. The lumina enlarge, coalesce and break through the cell membrane. Through the uniting of similarly produced lumina from adjoining central cells, patent excellular cysts are formed. On the other hand, the central layer of cells in the follicles might be keratinized or disintegrated and shed into the cyst. Parenchymal cysts of follicular ameloblastoma might be formed on the basis of such processes. The process of this cyst formation is very similar to that of the formation of the intraepidermal eccrine sweat ducts in the human embryo (Hashimoto et al. 1965). We do not believe these microcystic changes are a consequence of tumor involution, nor that they result from the multipotentiality of tumor cells.

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