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Histoenzymological and ultrastructural study of a bifocal calcifying epithelial odontogenic tumor. Characteristics of epithelial cells and histogenesis of amyloid-like material

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Summary. A calcifying epithelial odontogenic tumor, simultaneously located in the two jaws (maxilla and mandible) was examined by histochemical and electron microscopic methods. Squamous tumor cells without secretory polarity were different from those of common ameloblastoma. High activities of alkaline phosphatase and ATPases were demonstrated by light and electron microscopy on the cytoplasmic membrane, findings similar to those in the stratum intermedium cells of the normal dental germ from which these tumor cells seem to arise. The tumor cells, like preameloblasts of the dental germ, also produce a granulofilamentous material in intracytoplasmic vesicles and discharge it into the stroma. This "pseudo-amyloid" substance represents an abnormal protein of the enamel matrix and calcification, mainly occurring in that substance, might be an attempt at mineralization.

Key words: Calcifying epithelial odontogenic tumor – Electron microscopy – Histoenzymology – Ameloblast – Amyloid-like substance

The calcifying epithelial odontogenic tumor (C.E.O.T.), firstly described by Pindborg (1958), is a rare entity. Krolls and Pindborg in 1974 reviewing 46 cases of the literature, reported 23 new cases.

In spite of some ultrastructural studies (Anderson et al. 1969; Chaudhry et al. 1972; Mainwaring et al. 1971; Matsumura et al. 1975; Page et al. 1975; Solomon et al. 1975), the histogenesis of this neoplasm is still debated and the signification of some of its histological features (amyloid substance and calcification) are still uncertain.

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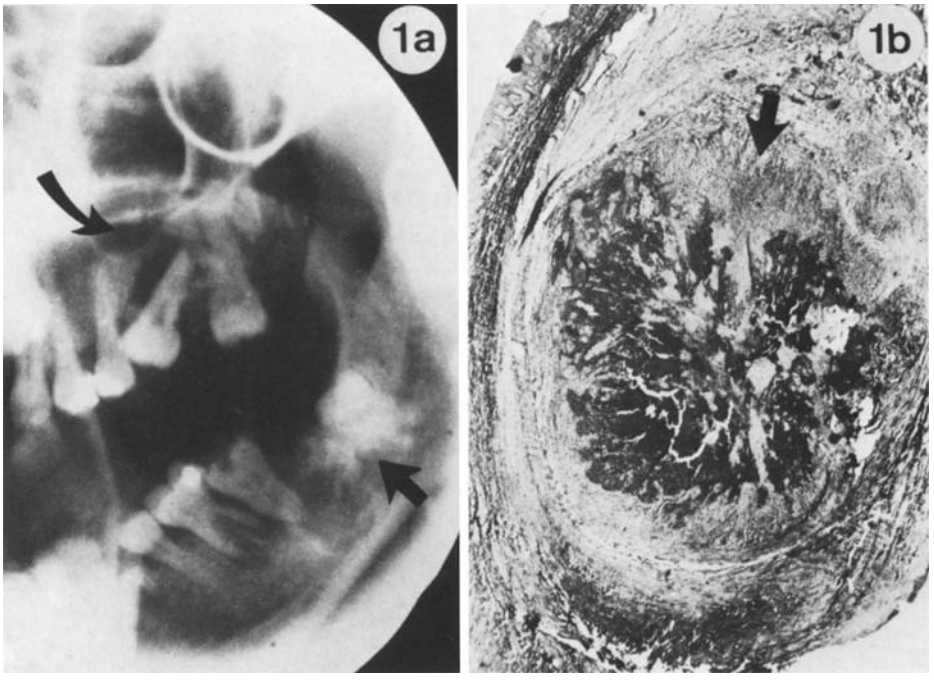

Fig. 1. a Roentgenogram. Bifocal radiolucent area in left maxilla (➘) and left mandible (➔). Numerous microopacities in the mandibular tumor. **b** Mandibular tumor. Huge dark calcifications with irregular outlines surrounded by epithelial sheets (➔). Haematoxylin and eosin. ×20

Case report

F.H., a 40-year old woman, presented in February 1983 to the Institute of Stomatology after a systematic roentgenogram of jaws which showed two radiological abnormalies.

Physical examination revealed two intraoral swellings:

– The first, located in the left maxilla, was a hard, painless bulging of the gingival and vestibular areas, opposite the first and second molars. The first molar was absent (previous extraction); the second was obliquely deviated.

– The other swelling was obvious in the left posterior mandible, opposite the second and third molars. Radiography detected an horizontally embedded third molar.

Roentgenograms revealed two well-defined radiolucent maxillary and mandibular areas (Fig. 1a) containing numerous small opacities. The surgical enucleation of the two lesions was easily performed.

Material and methods

For light microscopy, pieces of tumor were embedded in paraffin. Usual staining procedures included haematoxylin and eosin, periodic acid-Schiff reagent (P.A.S.), Alcian blue, Masson's trichrome and Vilder's techniques. In addition, staining procedures for calcium (alizarine, Von Kossa) and for amyloid substance (congo red examined with polarizing microscope, Crystal violet, thioflavin T examined with fluorescent microscope) were also used.

Other pieces were taken for histochemical investigations. They were immersed in liquid nitrogen and sections were tested according to Pearse's methods (1972): oxidative enzyme activities (lactic-dehydrogenase, enzymes of Krebs cycle, diaphorases, enzymes of pentose shunt

pathway); hydrolase activities (acid phosphatase, alkaline phosphatase-coupling azo dye method-), ATPases.

Specimens for electron microscopy were fixed in glutaraldehyde, postfixed in osmium tetroxide and embedded in Epon. Thin sections were cut on L.K.B. ultramicrotome, stained with uranyl acetate-lead citrate and with Silver-Methenamin (Movat's technique) for detection of glycoproteins, and were examined with a Hitachi H 300 electron microscope. An histochemical study to show the exact localisation of ATPases activities in the tumor was also performed, according to Wachstein and Meisel's technique (1957).

Results

I. Morphological study

The two tumors are quite similar, both measure 3 cm in diameter and are ill-defined, firm and grayish. Histologically, they are composed of epithelial sheets surrounding central amorphous substance and calcification (Fig. 1b and 2a, 2b).

1. Epithelial cells

Tumour cords and clusters are composed of polyedral epithelial cells with eosinophilic cytoplasm (Fig. 2a and 2b). Some cells, however, are degenerating (clear vacuolated cytoplasm, retracted nucleus).

In contrast to the cells of the common ameloblastoma, these cells never show any secretory polarity by electron microscopy (Fig. 2c). They all exhibit the characteristics of squamous epithelium: tonofilament bundles, numerous intercellular desmosomes (Fig. 2c-inset-). Many microvilli are present on cell peripheries, lining intercellular spaces filled with granular material (Fig. 2d). In addition some particular features are noted: numerous mitochondria and glycogen bodies; intracytoplasmic cisternae filled with a granular or filamentous substance; frequent autophagic lysosomes. In the degenerating cells the endoplasmis devoid of cytoplasmic structures and contains only some swelling mitochondria, sometimes showing round intramatricial calcifications and large vacuoles (Fig. 2c).

2. Connective substances

Near the epithelial clusters the connective tissue shows a distinctive feature which we shall describe before we study the amyloid deposits, calcifications and common connective stroma.

A. Perilobular connective tissue sheath. Some epithelial lobules are surrounded by an eosinophilic granulo-oedematous material (Fig. 2a). All the lobules are lined with an argyrophilic basal lamina covered with perpendicular spikes.

By electron microscopy, these sheaths are composed of the following structures:

– a thin, sometimes thickened or replicated continuous basal lamina (Fig. 3a–3b).

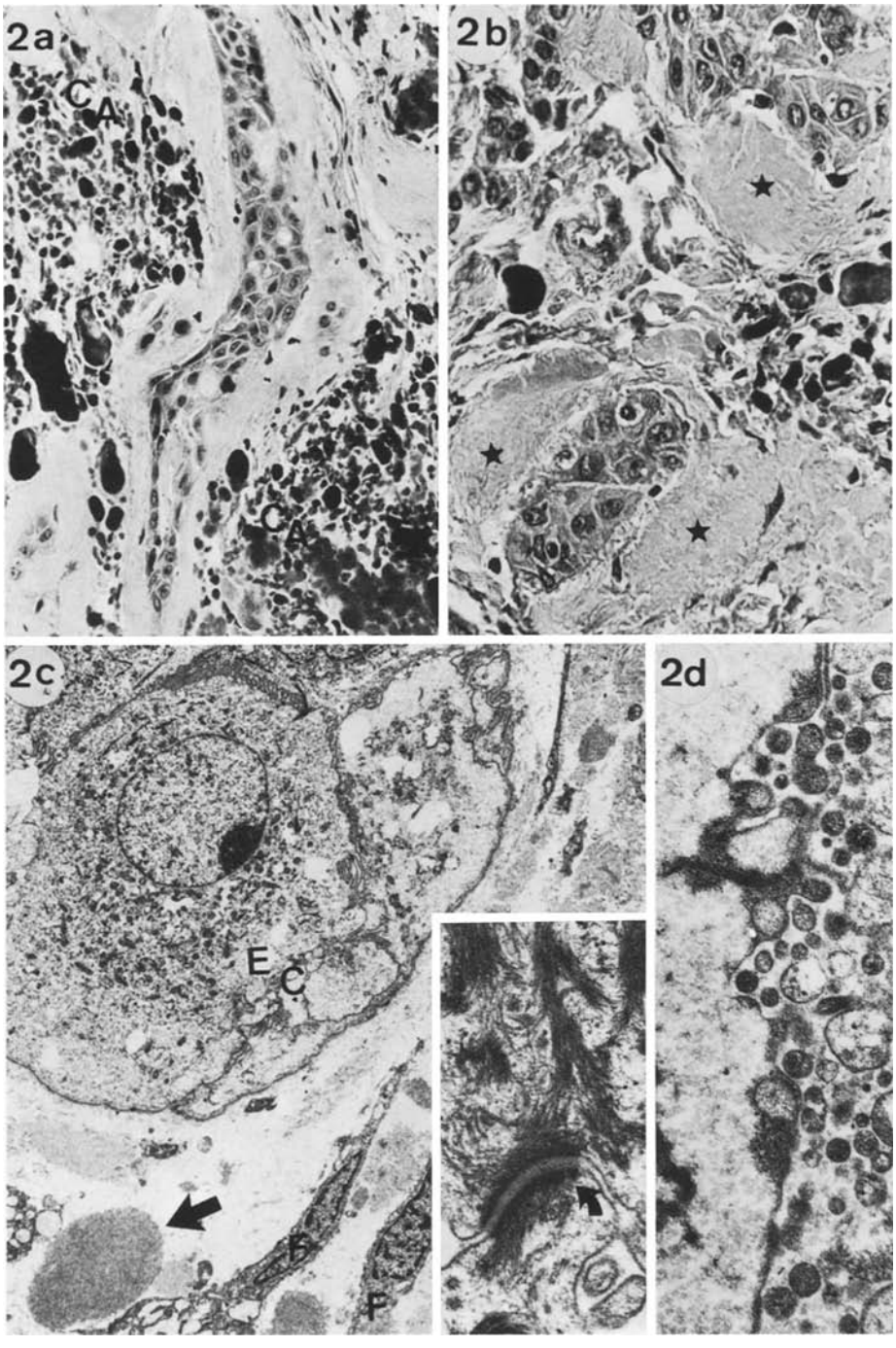

Fig. 2. **a** Epithelial cord penetrating among nodular dark calcifications (*CA*) (high magnification of Fig. 1b). Haematoxylin and eosin ×160. **b** Epithelial nests composed of polygonal cells and surrounded by sheats of amyloid substance (★). Nearby some nodular calcifications. Haematoxylin and eosin ×250. **c** Epithelial lobule (EC): squamous cell; on the right degenerating cell with cytoplasmic vacuoles. In the stroma, fibroblasts (*F*) and granular amyloid substance (➡). ×5,000. *Inset:* junction between 2 squamous cells. Numerous tonofilaments attached to a desmosome (➡). ×60,000. **d** Intercellular space filled with numerous microvilli of cytoplasmic membrane. ×50,000

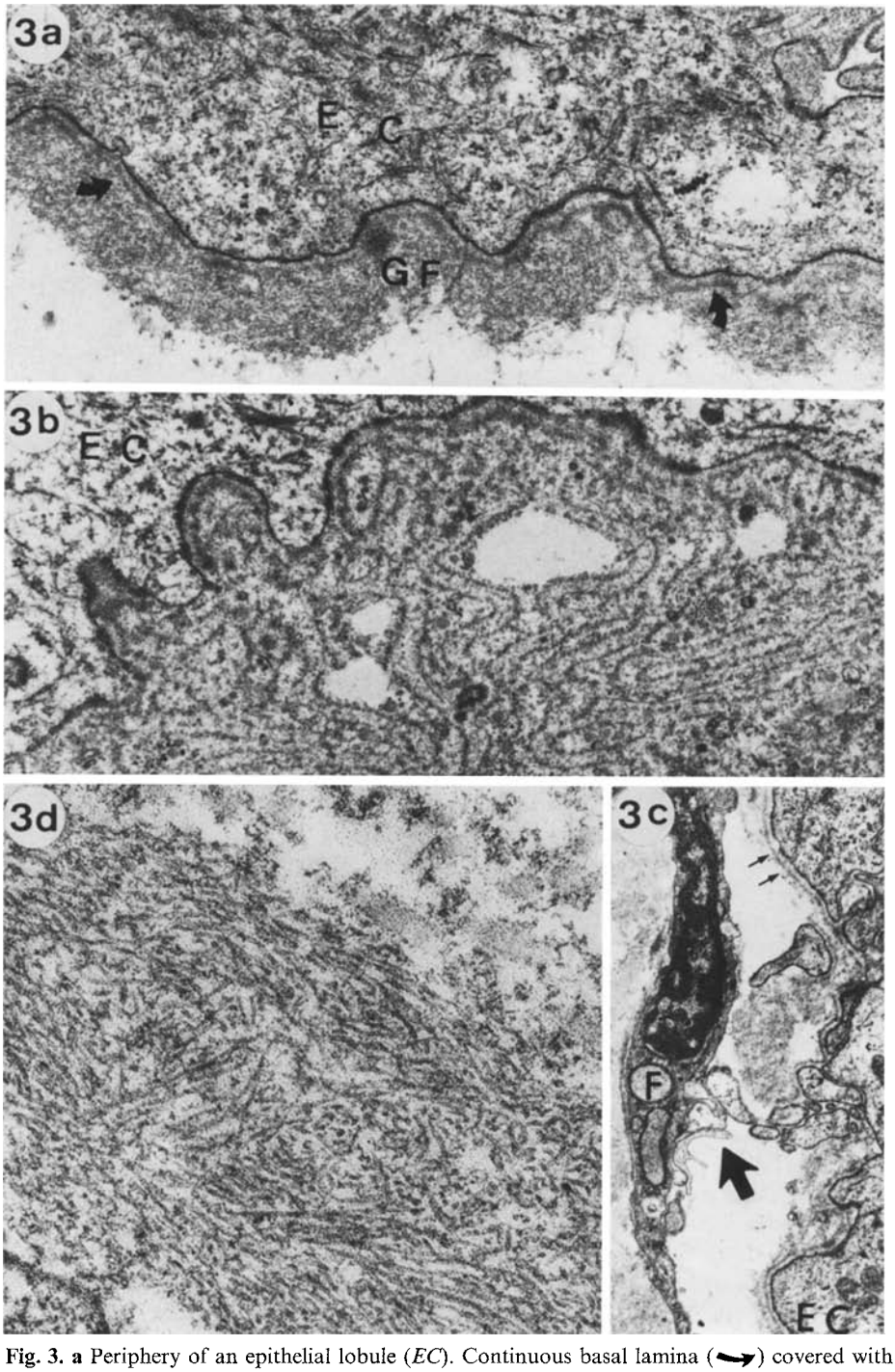

Fig. 3. a Periphery of an epithelial lobule (*EC*). Continuous basal lamina (➘) covered with granulo-filamentous deposits (*GF*). ×40,000. **b** Replicated basal lamina round a peripheral epithelial cell (EC). ×40,000. **c** Peripheral epithelial cell (*EC*) lined with a basal lamina (⇉). Digitiform cytoplasmic processes connecting (➔) with stromal fibroblast (*F*). ×20,000. **d** Amyloid-like substance. Thin fibrils (100–150 A). Longitudinal and transverse sections. ×120,000

– a granulofilamentous material (Fig. 3a) composed of thin fibrils, 50 A in diameter and of more thicker microfibrils, 150 A in diameter. Rarely disposed in a continuous rim shading off the basal lamina this material is absent in some areas where the basal lamina is in close contact with stromal collagen fibrils.
– numerous cytoplasmic processes of peripheral epithelial cells are seen (Fig. 3c). Connected with basal lamina by hemidesmosomes, they are either finger-like or more often rounded or triangular. They contain many vesicles, 10–15 manometers in diameter, which fuse with the cell membrane and discharge into the external environment.

B. The "amyloid" substance. Large areas of an eosinophilic and P.A.S. positive material are present near epithelial nests (Fig. 2b). They are variably stained by the usual procedures for amyloid substance: low positivity with Congo red but high green birefringence in polarized light; high red metachromatism with crystal violet but low fluorescence with thioflavin T. Ultrastructurally, these deposits are quite similar to those of the perilobular sheaths (Fig. 3d). They are composed of thin fibrils 100–150 A in diameter. Some collagen fibrils and fibroblastic processes are also present.

C. Calcification. The calcification (Fig. 4a) consists of homogeneous or dark-rim circumscribed little spherules, or of large incrustations. By electron microscopy, they occasionally form a deposit on necrotic epithelial cells but usually consist of huge masses in the "amyloid" substance (Fig. 4b). Exceptionally, crystals of apatite lie along collagen fibrils.

D. The connective tissue stroma. Generally, the stroma contains few fibroblasts. Near the epithelial nests, some safranophilic zones with thin tubules are quite similar to dysplastic dentin (Fig. 4c).

Ultrastructurally, most cells have the usual feature of fibroblasts (ovoid cells with abundant rough endoplasmic reticulum). However, near epithelial lobules, some cells filled with many microfibrils (Fig. 4d), exhibit high ATPase activities on their cytoplasmic membrane and sometimes lie close to processes of epithelial cells (Fig. 3c).

II. Histochemical study

With the exception of degenerating cells, the epithelial cells show high activities of all oxidative enzymes (aerobic and anaerobic). The activity of acid phosphatases is also high.

On all cytoplasmic membranes, the very high activity of alkaline phosphatase and ATPases (Fig. 4e) is obvious (black continuous rim with thin granules). In the stromal tissue, the same activities are present in vascular walls and round some "fibroblasts" near epithelial nests. The high positivity of ATPase activity was also demonstrated by electron microscopy on the cytoplasmic membrane of epithelial cells (Fig. 4-f) and of some fibroblasts (Fig. 4d).

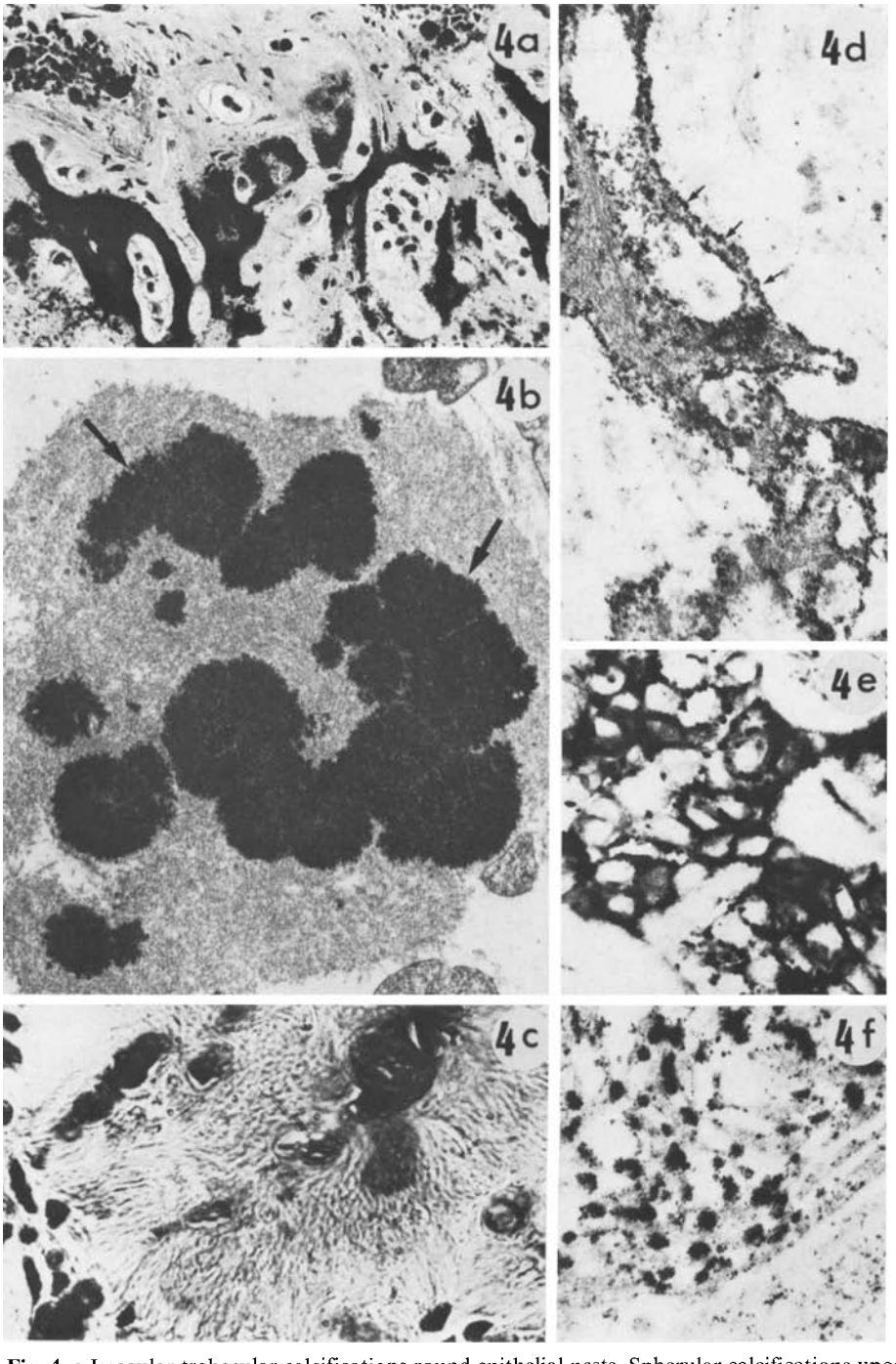

Fig. 4. a Irregular trabecular calcifications round epithelial nests. Spherular calcifications upon "amyloid" deposits. Haematoxylin and eosin. × 120. **b** Black calcifications with apatite crystals (➞) upon amyloid-like substance. × 25,000. **c** Dysplastic dentin with microtubules in periphery of calcifications. Haematoxylin and eosin × 360. **d** Stromal fibroblast. Numerous microfibrils in the cytoplasm. ATPase activity on the cytoplasmic membrane (→) × 75,000. **e** Optic microscopy. Histoenzymology. ATPase activity round epithelial cells of a lobule. × 400. **f** Electron microscopy. ATPase activity upon cytoplasmic membranes of epithelial cells. × 75,000

Discussion

The main characteristics of the C.E.O.T. are well known (Krolls and Pindborg 1974; Pindborg et al. 1972; Vap et al. 1970; Vickers et al. 1965). This tumor is observed with the same frequency in male and female. The average age of patients is 45 years. The usual localization of the neoplasm is the mandible (especially the premolar area). More rarely, it is located in the maxilla. Often associated with an unerupted tooth, the neoplasm is possibly derived from an epithelial-cell rest of the periodontal ligament or of a follicular sac. It might also arise from the basal layer of the oral epithelium and such an origin might explain the exceptional extra-osseous gingival and labial locations (Abrams and Howell 1967; Krolls and Pindborg 1974; Pindborg 1966). Usually latent, it is detected by a systematic radiography which shows an osteolytic focus filled with opacities. After surgical removal the prognosis is good and recurrences occur rarely (Krolls and Pindborg 1974; Vap et al. 1970). Our case, similar to those related in the literature, is original by virtue of its double localization in the maxilla and the mandible.

The structure of this tumor suggests a histogenesis different from that of the common ameloblastoma. Firstly, ultrastructural and histochemical differences between ameloblastoma and C.E.O.T. are obvious. Whereas lobular peripheral cells of the ameloblastoma are columnar with polarity of their organelles, all the cells of the C.E.O.T. resemble squamous cells (Anderson et al. 1969; Mainwaring et al. 1971; Matsumura et al. 1975; Page et al. 1975). Whereas in ameloblastoma alkaline phosphatases and ATPases are only present on apical poles of peripheral cells, in C.E.O.T. these enzymes show a high activity on the cytoplasmic membranes of all the epithelial cells, peculiarly on long interdigitating microvilli. Curiously, both these two cellular types show some similarities with two varieties of cells of the normal dental germ. Thus, ameloblasts of this organ have the same secretory polarity and enzyme activities (alkaline phosphatase – Deporter and Ten Cate 1976) as those of common ameloblastoma. However, the ultrastructural feature and enzymatic activity of the stratum intermedium cells (alkaline phosphatase and ATPase) (Kurahashi and Yoshiki 1972; Mjor and Pindborg 1973; Seibel et al. 1979) are the same as those of C.E.O.T. Thus, it seems logical to think that C.E.O.T. arises either from true cells of the stratum intermedium or perhaps from more differentiated cells (Page et al. 1975) transitional between stratum intermedium cells and ameloblasts and so able to secrete a granular material in intercellular spaces similar to that described by Frank and Nalbandian (1967) in the normal enamel organ.

This peculiar type of cell is not only found in C.E.O.T. but also in the adenomatoid odontogenic tumor (Ishikawa and Mori 1961; Mori et al. 1969; Moro et al 1982; Smith et al 1979; Sponge and Spruyt 1968). Thus, it is not surprising that the association of both these tumors, adenomatoid odontogenic tumor and C.E.O.T. has been recently reported (Damm et al. 1983).

The special nature and biological capacities of these epithelial cells ex-

plain some peculiarities of the stroma. As in ameloblastoma, the epithelial-mesenchymal area of C.E.O.T., suggesting an odontogenic induction, exhibits changes quite similar to those of early amelogenesis (Kallenbach 1971; Orams 1978) with accumulation of granulo-filamentous substance, Tomes processes and more rarely connections between mesenchymal and epithelial cells. Also, as seen in ameloblastoma, most of the changes are somewhat defective: basal lamina is never disrupted by epithelial processes and is sometimes replicated; granulofilamentous materialis irregularly distributed. Nevertheless, here as in other conditions (calcifying odontogenic cysts – Chen and Miller 1975; Sapp and Gardner 1977; Donath et al. 1979; ameloblastic fibroodontomas – Josephsen et al 1980; Mohamed and Waterhouse 1973; Slootweg 1980; adenomatoid odontogenic tumors – Hatakeyama and Suzuki 1978; Moro et al. 1982), the abundance of that granulo-filamentous substance is surprising. This feature demonstrates the aptitude of C.E.O.T. for abnormal amelogenesis. In such a conception, deposits previously considered to be true amyloid (Ranlov and Pindborg 1966; Vickers et al. 1965) might be a pathological type of protein of the enamel matrix (Chaudhry et al. 1972; Moro et al. 1982; Page et al. 1975). This material whose, structure is doubtless similar to amyloid protein (Smith et al. 1979) is present in vesicles of epithelial cells (Chaudhry et al. 1972) and then discharged into the stroma. Nevertheless, calcification also has an odontogenic significance. Accumulating on amyloid microfibrils, it might signify an early mineralization induced by the high activity of calcium metabolism in this special tumor cell.

References

Abrams AM, Howell FV (1967) Calcifying epithelial odontogenic tumors. Report of four cases (including one case of extraosseous origin). J Am Dent Assoc 74:1231–1240

Anderson HC, Kim B, Minkowitz S (1969) Calcifying epithelial odontogenic tumor of Pindborg. Cancer, 24:585–596

Chaudhry AP, Hanks CT, Leifer C, Gargiulo EA (1972) Calcifying epithelial odontogenic tumor. A histochemical and ultrastructural study. Cancer 30:519–529

Chen SY, Miller AS (1975) Ultrastructure of the keratinizing and calcifying odontogenic cyst. Oral Med Oral Pathol 39:769–780

Chomette G, Auriol M, Vaillant JM (1981) Induction odontogène et améloblastome. Données histoenzymologiques et ultrastructurales. Ann Pathol 1:221–231

Damm DD, White DK, Drummond JF, Poindexter JB, Henry BB (1983) Combined epithelial odontogenic tumor: adenomatoid odontogenic tumor and calcifying epithelial odontogenic tumor. Oral Surg 55:487–496

Deporter DA, Ten Cate AR (1976) Fine structural localisation of alkaline phosphatase in relation to enamel formation in the mouse molar. Arch Oral Biol 21:7–12

Donath K, Kleinhans V, Gundlach KKH (1979) Zur Pathogenese der calcifizierenden odontogenen Cyste (Gorlin Cyste). Virchows Archiv A [Pathol Anat] 384:307–324

Frank RM, Nalbandian J (1967) Ultrastructure of amelogenesis. In: AEW Miles (ed) Structural and chemical organization of teeth, vol. 1 New York Academic Press, pp 399–462

Hatakeyama S, Suzuki A (1978) Ultrastructural study of adenomatoid odontogenic tumor. J Oral Pathol 7:295–310

Ishikawa G, Mori K (1961) Histochemical study of adenoameloblastoma. J Dent Res 40:230 (Abstract)

Josephsen KAJ, Larsson AKE, Fejerskov O (1980) Ultrastructural features of the epithelial-mesenchymal interface in an ameloblastic fibro-odontoma. Scand J Dent Res 88:79–95

Kallenbach E (1971) Electron microscopy of the differentiating rat incisor ameloblast. J Ultrast Res 35:508–531
Krolls O, Pindborg JJ (1974) Calcifying epithelial odontogenic tumor. A survey of 23 cases and discussion of histomorphologic variations. Arch Pathol 98:206–210
Kurahashi Y, Yoshiki S (1972) Electron microscopic localization of alkaline phosphatase in the enamel organ of the young rat. Arch Oral Biol 17:155–163
Mainwaring AR, Ahmed A, Hopkinson JM, Anderson P (1971) A clinical and electronmicroscopic study of a calcifying epithelial odontogenic tumor. J Clin Pathol 24:152–158
Matsumura T, Matsumura H, Mori M, Kawakatzu K (1971) Calcifying epithelial odontogenic tumor (enzyme-histochemical findings). J Jpn Stomatol Soc 20:274
Matsumura T, Matsumura H, Hirose I, Sugahara T, Yuki M, Fujita K, Mori M, Kawakatsu K (1975) Calcifying epithelial odontogenic tumor. Enzyme histochemical and electronmicroscopic considerations. J Osaka Univ Dent. 15:25–36
Mjor IA, Pindborg JJ (1973) Histology of the human tooth. Munksgaard ed. Copenhague p. 23
Mohamed AH, Waterhouse JP (1973) A light and electron microscopic study of an atypical calcifying odontogenic tumor containing amyloid. J Oral Pathol 2:150–164
Mori M, Tamura K, Kawakatsu K (1970) Histochemical observations of enzymes in adenoameloblastoma. Oral Surg 30:659–669
Moro I, Okamura S, Okuda S, Komiyama K, Umemura S (1982) The eosinophilic and amyloid-like materials in adenomatoid odontogenis tumor. J Oral Pathol 11:138–150
Orams HJ (1978) The ultrastructure of tissues at the epithelial-mesenchymal interface in developing rat incisors. Archs Oral Biol 23:39–44
Page D, Weiss SE, Eggleston JC (1975) Ultrastructural study of amyloid material in the calcifying epithelial odontogenic tumor. Cancer, 36:1426–1435
Pearse AGE (1972) Histochemistry theoretical and applied. 3d ed. Churchill livingstone London
Pindborg JJ (1958) A calcifying epithelial odontogenic tumor. Cancer 2:838–843
Pindborg JJ (1966) The calcifying epithelial odontogenic tumor. Review of literature and report of an extraosseous case. Acta Odont Scand 24:419–430
Pindborg JJ, Kramer IRH (1972) Types histologiques des tumeurs odontogènes, kystes et lésions apparentées des maxillaires. N° 5 Classification histologique internationale des tumeurs. OMS Genève.
Ranlov P, Pindborg JJ (1966) Amyloid nature of the homogeneous substance in the calcifying epithelial odontogenic tumor. Acta Pathol Microbiol Scand 68:169–174
Sapp JP, Gardner DG (1977) An ultrastructural study of the calcifications in calcifying odontogenic cysts and odontomas. Oral Surg, Oral Med, Oral Pathol 44:754–766
Seibel W, Gartner LP, Hiatt JL, Provenza DV (1979) Ultrastructural localization of adenosine triphosphatase in the stellate reticulum, stratum intermedium and ameloblasts of the mouse molar. Histochemical J 11:435–445
Slootweg PJ (1980) Epithelio-mesenchymal morphology in ameloblastic fibroodontoma: a light and electron microscopic study. J Oral Pathol 9:29–40
Smith RRL, Olson JL, Hutchins GM, Crawley WA, Levin LS (1979) Adenomatoid odontogenic tumor: ultrastructural demonstration of two cell types and amyloid. Cancer 43:505–511
Solomon MP, Vuletin JC, Pertshuk LP, Gormley MB, Rosen Y (1975) Calcifying epithelial odontogenic tumor. A histologic, histochemical, fluorescent and ultrastructural study. Oral Surg 40:522–530
Spouge JD, Spruyt CL (1968) Odontogenic tumors: histochemical comparison of the adenoameloblastoma and developing tooth. Oral Surg 25:447–456
Vap DR, Dahlin DC, Turlington EG (1970) Pindborg tumor: the so-called calcifying epithelial odontogenic tumor. Cancer 25:629–636
Vickers RA, Dahlin DC, Gorlin RJ (1965) Amyloid (containing odontogenic tumors. Oral Surg 20:476–580
Wachstein M, Meisel E (1957) Histochemistry of hepatic phosphatase at a physiologic pH with special reference to the demonstration of bile canaliculi. Am J Clin Pathol 27:13–23

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