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Product Identification of Ameloblastoma

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PRODUCT IDENTIFICATION OF AMELOBLASTOMA

BY

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DEDICATION

To my family and friends

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VITA

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CHAPTER I

INTRODUCTION

During the past-recent decades the views concerned with pathological sciences have been immensely evolved in many aspects. Among instrumentals for such accomplishments, immunohistology has been conferred a novel, reliable, and valid contribution for the enhancement of the knowledge of intricacies in diagnostic pathology. Founded on such disciplines, scientific attitudes in this field, to date, have started to learn how to translate static impressions, practiced since the inception of light optic microscopy into a more dynamic parameters and access beyond this precursive stage to explanations with greater causal explanatory power. The legacy for that, firstly, relates to the limited values inherent to the art of morphology also to the subjectivism, innate to the traditional surgical pathology disciplines. Histochemical devices, once attempted to exploit predictable chemical reactions between the components of the reagents and molecules to demonstrate these tissue elements.

Its restriction, however, soon manifested by the fact that many enzymes and chemical reactants are, indeed, common to different cells. Immunohistology, in contrast, by conjoining the specificity of serological probes, as complementary, offers an invaluable strength to anatomical pathology in that it delineates functional moieties as distinct antigenic determinants, and, as a consequence of interaction of antibodies relevant

to these biomolecules, illuminate the magnitude of expression relevant to the level of cell differentiation, the fact of prime concern in prognosis and treatment of modalities of any given neoplasm.

On the subject of ameloblastomas, i.e., growths derived from the misfunctioning epithelial anlage of the developing tooth, the objective of this research is formulated as:

1) To present tentative classification of ameloblastomas;

2) To trace immunohistopathologic profile for the given classes in terms of inter-, intracellular, and basement membrane product differences. Normal developing tooth at different stages of maturation is used as control.

3) To study the views relevant to proliferations of this nature, and to advance possible histogenetic concept for ameloblastomas.

CHAPTER II

LITERATURE REVIEW

A. TERMINOLOGY AND EVOLUTIONARY HISTORIC EVENTS

1) Early literature: Aside from the poorly documented examples as the one describing multilocular cystic mandible in an archaic fossil skull, Salama (1951), and the narration of vague entities under the names of "follicular cysts" and "odontomes," Sprawson (1937), the earliest authentic and in-detail clinical study of the ameloblastoma focusing on behavior of the neoplasm is credited to Cusack (1827), cited by McFarland (1931).

Dupuytren (1837), cited by Robinson (1937), described a lesion akin to ameloblastoma and coined the term "fibrous bodies of the jaws." He suggested infection as the causative etiologic factor. It was Forget (1840), cited by Robinson (1937), who proposed the curious name "cystic disease of the jaw" and cystosarcoma adenoides reported by Wedl (1853).

On December 30, 1867, Paul Broca presented the first comprehensive study of odontogenic neoplasms before the Academy of Science of Paris, Sprawson (1937). Broca used the general term "embryoplastic odontome" to those lesions arising during the stage of histodifferentiation of the dental organ. This included the ameloblastoma and ameloblastic fibroma.

Folkson (1879), narrated by McFarland (1931), made the earliest histopathologic observation of the neoplasm. He recognized follicular architecture, squamous metaplasia, degenerative changes of stellate

reticulum, and microcyst formation. The author proposed the names "follicular cystoid" and later on "cystoma proliferum folliculare." This was an inappropriate choice and a misleading nomenclature in that it was irrelevant to the known biological behavior of the neoplasm, at that time. Nonetheless, Falkson's contribution to the knowledge of pathology of the neoplasm remains significant.

2) Development of histopathological concepts: Malassez's extensive investigations (1885), cited by Bump (1927), was a turning point and, indeed, provided new insights into the concept. Since then, the ameloblastoma was considered a genuine neoplasm. Malassez called the lesion "adamantin epithelioma." The evolving term "adamatinom" was suggested by Drujinsky in 1890, and, finally, the word adamationoma was coined by Blum (1901) and Borst (1902), both cited by McFarland (1931).

Gallippe (1910), cited by Bump (1927), gave his commentary on the word adamantinoma. The terminology derived from the Greek "ADAMAS" meaning the hardness of stone, analogous to enamel. Ethymologically, adamantinoma indicates a growth composed of enamel rather than enamel-forming cells or adamantino-blasts. The term adamantinoma was thought to be inappropriate.

Krompecher (1917), introduced the word "epithelioma adamantinum malignum, and, later on he proposed the term "basalioma." Churchill and Evy (1934), then coined the term ameloblastoma and, at that time, it was predicted that the new term may generally be accepted in the future, an anticipation which has been fulfilled.

Other suggested names include: epithelioma of basal cell variety, Cahn (1938), adamantoblastoma, Thoma (1946), premeloblastoma Byars (1946),

carcinoma of the tooth germ residue, Willis (1948) and basal cell carcinoma of the jaw, Schulenburg (1951).

Yet, there remain other terminologies proposed for the ameloblastoma of which, central papilloma of the jaws, chorioblastoma, central epithelioma, adamantinosarcoma, adamantinocarcinoma, adenoma adamantinum, and proliferating cysts of the jaws may be mentioned, Masson (1959).

B. THE ORIGIN OF AMELOBLASTOMAS

Given the myriad of names and synonyms simply indicate that ambiguity overshadowed the origin and pathogenesis of ameloblastomas for many years.

The earliest authentic classification of odontogenic anomalies, including ameloblastoma, is entitled to Broca (1968), cited by Sonesson (1950). This author applied the term "odontome" to any type of neoplastically-derived odontogenic lesion. In his "Treatise on Tumors" analogous to periods in embryonal normal developing tooth, he advanced the following grouping: 1) Odontome related to the period of histodifferentiation. This category also covered "epithelial odontome" or "adamantinomata." 2) Odontome related to period of morphodifferentiation. This included calcified odontome or "petrified epulis." 3) Odontome related to the period of crown maturation. 4) Odontome related to the period of root formation and tooth eruption.

The "in-growth theory" of Buchtemann (1881) cited by Bump (1927), regarded adamantinoma to originate from the mucous membrane or mucous glands of the mouth, and to extend into sinuses or infiltrate along the

roots of the teeth into the bone. This author, evidently, failed to correlate the lesion to the developing enamel organ.

Folkson (1879), cited by Robinson (1937), believed that ameloblastomas originate from the supernumerary dental germ. However, it was Malassez in 1885, quoted by Bump (1927), who conceptualized that "adamantin epithelioma originates from the atrophied and isolated group of cells, known as "pardental epithelial debris." Malassez's contribution to the recognition of odontogenic lesions remains basic in that not only does it make clear the distinction between the cysts, neoplasms, and concise histopathologic description of ameloblastoma, but also provides a unified view for the histogenesis of such lesions from cell rests of Malassez.

The cell-rest theory was supported by Ewing. However, Bland-Sutton argued against that theory because the adamantin epithelioma, if stemmed from the embryonal rests, like other blastomata, it should have predilection for younger age, cited by McFarland (1931).

Kruse, (1891), cited by Bump (1927), mentioned three potential sources for ameloblastomas. These were: 1) epithelial anlages from the heterotopic tooth bud, 2) surface epithelium, and 3) cell rests vestigia. Krompecher (1918) in analogy with basal cell carcinoma of the skin inferred "basaliomata" to arise from the surface epitheliom of the mouth, cited by Simpson, et al, (1974).

Kronfeld (1930), determined adamantinomata as pathologic overgrowth of tooth anlage and outlined the spectrum of the neoplasm based on his morphologic observations. The author divided adamantinomas into: 1) solid variety, corresponding to the earlier stage of tooth development,

composed of strands and cords of dense spindle cells embedded in an embryonic, loosely-arranged, connective tissue stroma. 2) Solid adamantinoma, corresponding to the later stage of tooth development in which stellate reticulum, palisading basilar cells surrounding follicles (ganoblasts), well-defined basement membrane (membrana limitans) all embedded in a dense "ripe" and vascularized connective tissue stroma. 3) Cystic adamantinoma (multilocular cystoma) as the consequence of progressive cystic degeneration in solid type.

Solid variety: Equivalent to the earlier stage of odontogenic epithelium

Solid variety: Equivalent to the later stage, pre-ameloblastic maturation

Cystic variety: Multilocular cystoma

The Summary of KRONFELD'S VIEW OF AN AMELOBLASTOMA

In Churchill's classification of oral neoplasms in 1932, cited by Churchill (1934) and Robinson (1937) under the heading "Epidermal Blastomata", a subgroup of odontogenic neoplasms is mentioned. Within that subgroup, the ameloblastomas are divided under solid, cystic and melanotic variants, see below.

BLASTOMATA

I. Tumors of epidermis, its degenerated tissue and squamocellular mucosa.

II. Tumors of odontogenic apparatus (dentocytic tumors).

A. Benign

1. Ameloblastoma:

solid, cystic, melanotic

2. Dentigerous cyst:

simple, compound, eruptive, heterotopic

B. Malignant

1. Carcinoma ameloblasticum

2. Sarcoma ameloblasticum

Churchill's grouping of ameloblastoma (modified from Robinson, 1937).

Cahn, (1933), raised the concept of the dentigerous cyst as a potential source for the origin of the ameloblastoma. In his case presentation, he introduced the descriptive term "dentigerous cystic adamantinoma." He illustrated intraluminal nodules associated with dentigerous cyst wall, analogous to the currently used term plexiform unicystic ameloblastoma. Gardner (1981). Churchill, (1934) reported two cases under "invading dentigerous cysts," and made a distinction between these entities and the conventional ameloblastomas. Churchill observed marked vascularization of connective tissue enclavements associated with the invading epithelia in the cysts which has never been encountered in the stellate reticulum of ameloblastomas. Thoma and Goldman (1946) reiterated the solid/cystic concept in their classification, and also added "calcifying

adamanto-blastomata" as the third entity. Thoma's classification was accepted by the Fifth Annual Meeting of the American Academy of Oral Pathology, Robinson (1952).

Small and Waldron (1955) gave an account that the enamel organ, vestigium of enamel organs, epithelium of odontogenic cysts, surface epithelium, and displaced epithelium in the other parts, extramaxillary, are the potential sources for the neoplasm. These authors also mentioned that cystic changes in ameloblastomas occur as function of time. In their proposal, ameloblastomas are classified as:

1. Follicular, or follicular with cystic changes or squamous metaplasia.
2. Ameloblastoma composed of ramified cords and irregular masses.
3. Other variants: Adenoameloblastoma, hemangioameloblastoma.

Pindborg and Clausen (1958), presented functional classification of odontogenic neoplasms based on embryonic principles and experimental evidences obtained from transplantation of developing tooth and tissue culture studies as follows:

I. Mesenchymally derived neoplasms

II. Epithelially derived neoplasms

   A. With inductive effect

   B. Without inductive effect: (ameloblastoma)

1. Hemangioameloblastoma
2. Adenoameloblastoma
3. Simple ameloblastoma
4. Ameloblastic neurinoma

III. Mixed types of neoplasms

Masson (1959), in a clinicopathologic survey, stated that adamantinomas corresponded to very early stages of tooth development and that they might reveal solid epithelial strands embedded in loose connective tissue stroma. Similarly, in ameloblastomas relevant to a later stage of tooth development, stellate reticulum surrounded by palisading ganoblasts (preameloblasts) would predominate. In Masson's view, ameloblastomas lie within four categories as:

1. Plexiform
2. Squamous
3. Glandular
4. Sarcoma-like

Gorlin, Chaudhry and Pindborg (1961), proposed the subdivision of ameloblastomas based on their histopathologic patterns as

1. Follicular
2. Plexiform
3. Acanthomatous
4. Mucoepidermoid
5. Vascular (hemangioameloblastoma)

These investigators also determined epithelial lining of dentigerous

cysts, remnants of dental lamina, enamel organ, and the basal layer of oral mucous membrane as the sources of neoplastic growth.

Spouge (1967) in his extensive studies of odontogenic neoplasms considered ameloblastomas as a benign proliferation of dental lamina-like cells.

In World Health Organization's Classification of odontogenic neoplasms, Pindborg et al (1971), ameloblastomas are defined as benign but locally invasive neoplasms. WHO classification considers ameloblastomas as:

1. Follicular type
2. Plexiform type
3. Acanthomatous type
4. Basal cell type
5. Granular cell type
6. Other variants:
    a. Ameloblastic hemangioma
    b. ameloblastic neurinoma
7. Ameloblastomas arising in odontogenic cyst wall, i.e., dentigerous cyst

Unicystic and plexiform unicystic concept of ameloblastomas are described by Robinson, et al (1977). Within their conclusive survey, they pointed, once again, to the ameloblastomatous proliferating potential of epithelial linings of dentigerous cysts in young, 2nd, and 3rd decade of life, individuals. These investigators advanced morphological criteria

for ameloblastomatous epithelium (see Material and Methods). Shteyer et al (1978) collated the entity with the so-called mural ameloblastoma and arrived at a similar conclusion.

Gardner (1981), and Gardner, et al (1983) further rectified the concept. Gardner introduced the term plexiform unicystic ameloblastoma. He applied the term to those intraluminal or intracapsular patterns of plexiform epithelial proliferations occurring within the wall of dentigerous cyst which does not exhibit those histologic criteria proposed for the true ameloblastoma, Vickers, et al (1970). Gardner, et al (1983) concluded that plexiform unicystic ameloblastoma is an undifferentiated variant of conventional unicystic ameloblastomas. However, none of these studies document histogenetic assessment for ameloblastomas. Up to this point, the functional classification of ameloblastomas includes the following:

1. Central (intraosseous)
   Solid or multicystic (includes WHO variants)

   Unicystic

   Plexiform unicystic
II. Peripheral (extraosseous)

Odontogenesis: Current biological concepts

Ontogenetic relevance to odontogenesis, at best, is defined as an interdisciplinary science, integrating embryology, anatomy, biochemistry, genetic and immunology, Hall (1983). This field as a developing

subsection of biology, has motivated biologists, especially those extracellular matrix specialists, to investigate circumstances that provoke cells to activate or regulate their genomic apparatus. Among these growing concepts are those of cellular interactions and recognition of the "interphases" at the molecular level, Lash et al (1977).

Interphase may be defined as an interaction zone, chemical or physical, between similar, homotypic, or disparate, heterotypic, tissues through which communications, functional relationships, and, ultimately, morphogenesis are conducted, Lash (1977). With particular emphasis on odontogenic developmental events, interaction interphase is mediated as transduction events of the extracellular basement membrane matrix, Kollar (1983). In this context, the reciprocal theory of interdependent epithelial-ectomesenchymal interaction provides a unified view and is the best-fitted on the epigenetic influences implicated in tooth morphogenesis, Thesleff, et al (1981), and Slavkin, et al (1981).

Tissue recombination studies provide significant break-through, pertaining to these intermediary effects and controls exerted on de novo heterotypic genomic function and determination, Hall (1982). These techniques also highlight the pathway through which the extracellular microenvironment evokes irreversible differentiation and specific gene expression, through either cell to cell (direct) contact or extracellular surface-associated products and extracellular matricies, Hall, et al (1984).

For the specific tooth-forming organ, instructive tissue induction governing the determination and differentiation of odontoblasts are mediated by the presumed macromolecular signal substances. Such substances

are secreted by preameloblasts, the basement membrane integer, and the developmental information communicable between the two adjacent circuits are thus postulated to be through the functional properties of these heterogeneous products, Slavkin (1976), Thesleff, et al (1978), and Lesot et al (1981). Some of these macromolecules are now identified. These include fibronectin, laminin, type IV collagen varieties of proteoglycans, glycoproteins and in combination with certain inorganic ions, Kefalides, et al (1979), Bernfield, et al (1982), Thesleff, et al (1981) and Lesot, et al (1981).

DESCRIPTION OF PRODUCTS: AN OVERVIEW

Fibronectin

This component is a high molecular weight glycoprotein found in its insoluble phase within the connective tissues and in the early basement membrane framework (vide infra). Fibronectin also is found as well as in soluble phase circulating within plasma and other body fluids, Ruoslahti, et al (1982), Chen et al (1978), Mosher (1981), Pearlstein, et al (1986), Gordon, et al (1980) and Ekblom et al (1980). A plethora of names suggestive of its diverse histological activities have been specified for fibronectin. They include those among which Cold-Insoluble Globulin (CIG) and Large External Transformation Sensitive Protein (LETS) may be mentioned Pearlstein, et al (1980).

Structurally, fibronectin is composed of the two nearly identical disulfide-linked polypeptide chains with a combined molecular weight of 450,000 Dalton, Ruoslahti, et al (1982). The insoluble fibronectin

extracted from the cell layer in culture media is formed as disulfide - bonded dimers and multimers, Mosher, et al (1981), and Ruoslahti, et al (1982). The dimers and multimers are synthesized through the oxidation of cysteine residues. These disulfide-linked multimers secure their insolubility of fibronectin under normal physiologic condition, Pearlstein, et al (1981). In vitro expression of fibronectin has been demonstrated for varieties of human epithelial cells, among which are fetal intestine and adult bladder, Smith, et al (1979); and rat kidney cells, Ruoslahti et al, (1982-b).

The binding capacity of fibronectin to type IV collagen within the basement membrane, as well as enhanced substrate adhesion, is known to be due to the affinity of the macromolecule toward proteoglycans and the chondroitin sulfate moieties. Proteoglycans and the chondroitin sulfate moieties also enhance fibronectin incorporation within the constituent complex of the basement membrane, Engwall, et al (1978), Ruoslahti (1982-b), Carlsson, et al (1981) and Johnson, et al (1981).

Relevant to the synthesis and secretion of fibronectin by mesenchymal cells, there are some practical points that warrant clarification. Of them are the quantities of fibronectin proportionate to the levels of maturation of the producing cells as reported earlier, Thesleff, et al (1979). In the present research, it was substantiated that the less mature and less differentiated mesenchyma was found to be the more intensely reactive with monoclonal antibody to fibronectin. Also, appreciated is the key role this glycoprotein plays in the supportive stromal groundwork organization and in the presumed morphology of the parenchymal tissue, Hay

et al (1978), Bernfield, et al (1978). Moreover, the avidity of the basement membrane component for fibronectin, the alternate source for this glycoprotein, i.e., from plasma, and its loss from the basement membrane as a chronologic event, all in context must be viewed positively. This is particularly true when inflammatory process is a superimposed event, Hay (1978), Bernsfield, et al (1978), Stenmann, et al (1978), Yang, et al (1980) and Madri, et al (1980).

Laminin

This glycoprotein molecule while sharing several biological functions with fibronectin, differs substantially in shape and domain organization, Hynes et al (1982). It expresses complex conformational, Beta-pleating and Alpha helix, as well as aperiodic or undefined structural elements, mapped in an exotic cross-shaped, one long and three similar short polypeptide arms, arrangement, Timpl, et al (1983).

Clues to the biological role of laminin originate from different studies. This high molecular weight (MW 950,000) glycoprotein mediates, primarily, the attachment of epithelial cells to type IV collagen substrate, Terranova, et al (1980). It, also, displays strong affinity for the other glycoproteins and proteoglycan, fibronectin-proteoglycans and in combination with these elements, laminin incorporates within the insoluble phase of the basement membrane, Johansson, et al (1981), Foidart, et al (1980), Timpl (1979). Laminin is a universal product of all kinds of basement membrane so far studied, Timpl (1983). Also, it is known as the first extracellular matrix protein to appear during the embryonic development between cells at 16-cell (morula) embryo, Levio, et al (1980).

Embryonic ectoderm and ectodermally derived epithelium, as is the mature epithelium, does produce laminin, along with the other glycosaminoglycans and proteoglycans, Ekblom, et al (1980), Gordon, et al (1980). Neoplastic transformation and invasion of the epithelially derived tissues are accompanied by changes in the expression or incorporation of the macromolecule of laminin within the basement membrane, Hayman, et al (1981), Albrechtsen, et al (1981).

DENTAL ORGAN: MORPHOLOGY AND DIFFERENTIATION

For some vertebrates, such as the murine and rabbit, for example, preameloblastic differentiation from the evolving predetermined odontogenic epithelium to a terminally differentiated ameloblast have been extensively investigated, Kallenbach (1971), Saliva, et al (1972), Slavkin, et al (1976), Mosher, et al (1981), Bernfield, et al (1978), Osman, et al (1981) and Slavkin, et al (1983, 1984). These investigations unanimously show that when the dividing cuboidal cells in the inner enamel epithelium mature into columnar cells, preameloblasts with reverse polarization of their nuclei, there is simultaneous evidence of thickening of juxtapositional basement membrane. These are known as hypothetical signal substances which target their instructive, inductive effect action over the underlying ectomesenchyme and induce the preodontoblasts to differentiate (Grobstein (1967, 1975), Kallenbach (1971), Kollar (1972, 1983).

At the next step, simultaneous with late proliferative and early differentiative stage of inner enamel epithelium, the predontoblasts

extend their cytoplasmic processes crossing the basement membrane toward the preameloblasts with reversed polarized nuclei. This has been interpreted as the earliest evidence of direct, cell-to-cell, heterotopic interaction Slavkin, et al (1976).

In the mouse, terminal cytodifferentiation of the inner enamel epithelium is accompanied by a complete degradation of the basement membrane, Thesleff, et al (1981). In vitro studies, however, substantiate that such basement membrane degradation is not a prerequisite for epithelial cytodifferentiation and morphogenesis in tissue culture conditions Slavkin, et al (1983).

Extrapolating from the normal embryonic pathway of ameloblastic cytodifferentiation and, also, from Pierce's theory of neoplasia (1974), ameloblastomas, hypothetically, may recapitulate cellular morphology, ranging from cuboidal to predetermined columnar preameloblast prior to differentiating to secretory, mature ameloblasts.

Epithelial tonofilaments:

The cytoskeletal fibrillar proteins comprise heterogeneous groups of microtubules, microfilaments and, the recently discovered, intermediate-size tonofilaments, Gabbiani, et al (1981), and Osborne, et al (1981). These 7-11 nm diameter class of filaments are part of the major cytoplasmic organization of the cell and exert an enormous effect upon the dynamic function of cytoplasm in the cell, Franke, et al (1979).

These filaments are expressed either singly or in laterally aggregated bundles, Weber, et al (1982). Common functions pertaining to these filaments include: 1) nuclear anchorage, 2) mechanical integrator of

cellular space in muscle cells, 3) maintenance of configuration of columnar cells, by forming their underlying unique cytoskeleton, and 4) interdependency with microtubular expression such as depolymerization and rearrangement of tubulin, Letho et al (1978), Franke, et al (1978, 1979), Paulin, et al (1980) and Lazarides, et al (1980).

The polymorphic character of these medium-sized filaments, in terms of their peptide mappings and physicochemical properties, relevant to a given cell type, is a working hypothesis for the following five subclasses: ctyokeratin, vimentin, desmin (skeletin), glial fibrillary acidic proteins, and neurofilaments, Franke, et al (1978), Gabbiani (1981).

Cytokeratin filaments are incorporated by a variety of polypeptides having molecular weights ranging from 48,000 to 68,000 Franke, et al (1978). Using antisera for identification, these filaments act as reliable markers for those epithelia expressing true desmosomes, Weber (1982).

In the human epidermis and oral mucosa, antisera against total keratin labeled all keratinocytes in all structural layers in normal and pathological conditions, Loning et al (1980). The staining intensity for prekeratin was studied for the sections of normal epithelium and related neoplastic lesions. It was found that the intensity of fluorescent staining was proportional to the degree of differentiation of tumor cells, Sieinski, et al (1981).

Relevant to this research, the significance of prekeratin antibody lies, to a great extent, in their utility for differential diagnosis of those plexiform variants of the ameloblastomas in comparison with the other variants.

LECTINS:

The cell surface is the locus for performing distinct biological activities essential to the viability of the cell, Singer (1982). In terms of interaction with adjacent cell surfaces such as molecular recognition on neighboring cells, extracellular matrices and the functional motilities and attachment reactivities of cells, it requires certain components such as glycoproteins and glycolipids on cell membrane which impose definitive action, Roth (1980). The common denominators in all these events are the terminal carbohydrates, exposed on cell surfaces, Young et al (1974).

A few decades earlier, the specificity and innate affinity of certain plant proteins, lectins, toward these terminal sugar moieties or cell membranes was acknowledged. In this context, it was disclosed that the agglutinability of blood group antigens was due to the specificity of the reaction between the lectin and the cell's carbohydrate ligand, Boyd (1945).

Neoplastic transformation of cells, either benign or malignant, modifies the normal pathway of expression of the cell surface terminal carbohydrate components. Along with the other misfunctioning genomic products expressed on cell surfaces, the altered terminal carbohydrates enhance proliferation of cells beyond normal limits, apparently without being affected by contact inhibition mechanism, Rapin et al (1974).

The earliest research in lectin binding capacities delineated an increased quantity of membrane bound receptors for lectins on transformed

cancer cells. It was found that a remarkable avidity of the malignant phenotype for agglutination occurred with a lipase derivative of wheat germ agglutinin, Easty et al (1960). The cell surface carbohydrate ligand molecule was then recognized to be a glycoprotein-containing β -(1->4) di-N-acetyl glucosamine termini, Monsigny, et al (1978).

The immunohistochemical methods currently in use employ lectins as highly sensitive and specific reagents, to demonstrate differences in the terminal carbohydrates on the surfaces of cell populations, functions, and their level of differentiation. The immunohistochemical technique, however, when applied to the formalin-fixed tissues poses certain limitations, Rittman, et al (1982), Leathem, et al (1983).

Upon using lectins certain generalization could be made: 1) none of the lectins now known are exclusively tumor-specific, 2) that the binding capacity of the lectins with respect to differentiation may render results as positive, negative or, on occasion, of no difference. The latter is not an uncommon observation; on the other hand, the binding differences between neoplastic phenotype and the normal phenotype may not be extensive.

The following lectins are used for this research: Concanavalin A (Con A): the lectin was extracted from Jack bean, Canavalia ensiformis, Goldstein (1978). The lectin is composed of four identical protomer subunits, each of 26,000 molecular weight. Each subunit contains the cations of Ca^{++} and Mn^{++}, which are required for biological activity of the lectin, Goldstein (1974). Con-A binds and forms precipitates with

either of the polysaccharides containing a D-glucopyranosyl, a-D-arabino-furanosyl, a-D-mannopyranosyl, or B-D fructofuranosyl residues in their non-reducing termini, Nicholson (1979). Such capacity will provide for interaction with dextran, yeast mannan, D-fructan, glycogen and a variety of glycoproteins, Sharon (1972), Goldstein (1978) and Debray (1980).

The distribution pattern of Con-A in formalin-fixed materials for a variety of normal tissue entities, hyperplastic lesions and in some neoplasias have been studied. At the inception of such studies, it was a common concept that the cells binding Con A to be virtually restricted to malignancies. A recent immunofluorescence study considered clear cut-differences between normal and malignant cells in terms of Con A binding capacity, Louis (1981).

Studies on embryonic tissues disclosed features similar to the transformed phenotype, Moscona (1971). Distribution of Con A in the normal palatal and buccal mucosa was found intercellularly in basal and prickle cell layers and for the palatal epithelium, additionally, the cytoplasm was also positively decorated, Dabelsteen (1978).

Ricinus communis agglutinin I, (RCA-I, RCA 120). RCA is a disulfide-linked glycoprotein with a molecular weight of 120,000 Dalton extracted from the toxic castor bean, Goldstein (1978). The lectin binds oligosaccharides of (1 --4) galactopyranose configuration, in general, and, preferentially to B(1 --4) D galactopyranase-ending sugars, Debray (1980); it may also interact with N-acetylgalactosamine, Baenziger (1979).

Topographic distribution and the expression of the receptors for

RCA-I in histologically different epithelial cell surfaces have been studied. RCA-I was found deposited in keratinocytes of the skin epidermis, in different locations of the oral mucosa, and in the esophageal squamous epithelium where it was reactive intercellularly at prickle and basal cell layers, Dabelsteen (1978 b), Hyum, et al (1984). A similar pattern of reaction has been observed for keratoacanthomas; while actinic keratosis and Bowen's disease reportedly displayed a decreased reactivity toward the lectins, Graem (1983).

The agglutinin property of transformed malignant mouse fibroblasts in culture was found to substantially decreased by RCA-I, Nicholson, et al (1972).

RCA-I binding reactivities in formalin-fixed tissues of oral squamous cell carcinomas and in epithelial outgrowths from the edge of healing wounds have been shown to be accompanied by a heavy loss of the ligands specific for the lectin, Graem (1982) and Dabelsteen (1978-a).

Vedtofte, et al (1981), studied binding reactivity of solid ameloblastomas for RCA-I. They found distribution differences for RCA-I in the peripheral cells of ameloblastomas and related these features to the possible biologic behavior of differentiation and migration in the neoplasm.

Wheat germ agglutinin (WGA); Tritium vulgaris: The lectin as indicated was one of the earliest probes used for surface changes occurring in cancer cells, Easty (1960). It was the prevailing view, at that time, that the reactivity to the lectin ought to be relevant to the underlying neoplastic process, rather than growth and division, Aub (1963). The lectin is a 36,000 MW protein consisting of two identical, dimeric, subunits

each of 8000 MW, Goldstein (1978). Membrane bound receptors for this lectin are oligosaccharides containing terminal non-reducing N-acetyl glucosamine and N-acetylneuramininic acid, Debray (1980).

Embryonic ectodermal tissues and retina, dissociated with EDTA, were found to agglutinate with WGA, like the adult untransformed culture cells, only after treatment with trypsin to unmask the receptors, Moscona (1971).

In the oral mucosa, receptors for WGA were found on the basal and prickle cell layer, Vedtofte et al (1981). Also, by using WGA in immunofluorescence techniques to sections of ameloblastoma, the lectin was found to bind peripheral cuboidal or polyhedral cells and spindle cells; columnar cells were bound to be non-reactive, Vedtofte et al (1981).

CHAPTER III

MATERIAL AND METHODS

Materials:

Formalin-fixed paraffin embedded sections of forty-two cases of unequivocal ameloblastomas of different classes and subtypes which were courteously provided by the : 1) Department of Pathology, Division of Oral Pathology, Northwestern University; 2) University of Chicago, Billings Hospital, Zoller Dental Clinic; 3) Surgical Pathology Section, McGaw Hospital; and 4) the files of the Department of Oral/General Pathology, Loyola University School of Dentistry.

Antisera to laminin, fibronectin and prekeratin were supplied from the following companies:

Keratin: Dako Corp., Santa Barbara, Ca.

Laminin: Bethesda Research Lab, Bethesda, Md.

Fibronectin: Cappel Lab, Westchester, Pa.

The following biotinylated lectins all were supplied from Vector Laboratories, Burlingame, Ca.

Concanavalin-A (Con-A).

Ricinus communis agglutinin I (RCA-I).

Wheat germ agglutinin (WGA).

Methods:

Upon the established criteria for the ameloblastomas in the literature, Pindborg et al (1971), Cohinson et al (1977), Shtyer et al (1978), Gardner et al (1980), Gardner (1981), Nasu (1983) and Gardner et al (1983);

and, also, upon the current immunohistological proofs suggestive of product differences pertaining to the level of maturation of the neoplasm, observed in our laboratory and that which had been confirmed ultrastructurally by others, Nasu et al (1983), the following sub-grouping of the neoplasm was prepared for study:

A. Intraosseous solid, or multicystic, Gardner et al (1980). Of the twenty-nine specimens of ameloblastomas of this type, two distinct subgroups were recognized:

1) Solid or multicystic ameloblastoma, follicular type, 25 cases.

2) Solid ameloblastoma, plexiform type, 4 cases.

B. Unicystic and plexiform unicystic, Robinson et al (1977), Shteyer et al (1978), Gardner (1981) and Gardner et al (1983) - thirteen cases.

Ameloblastomas were considered follicular when these features in combination were present: The follicles were composed of central spindle or star-shaped cells resembling stellate reticulum surrounded by peripheral and clearly columnar or cuboidal, preameloblastic-like epithelia, which met the criteria of Vickers and Gorlin (1970). Neoplastic parenchymal cells also were found to be embedded within dense, ripe, mature, fibrous connective tissue stroma.

The plexiform variant was considered only when the following morphological observation were made: the plexiform network of anastomosing strands of epithelium in which the central cells did not reveal similarities to the stellate reticulum, rather, they presented a more flattened and squamoid appearance. Also, the peripheral palisade of the plexiform variant cells did not meet the criteria of Vickers et al (1970) for

ameloblastomas. Neoplastic cells of the plexiform variant were found to be embedded within primitive, loose, or at times, an unconspicuous connective tissue stroma.

The unicystic and plexiform unicystic ameloblastomas were regarded in a single category. The basis for this single category was that some of the cases in this study showed unicystic feature, primarily and, when such lesions clinically recurred, were found as plexiform unicystic variant and vice versa. In the unicystic ameloblastoma, the epithelium was considered to be neoplastic when the following criteria, singly or in combination, were present: 1) downgrowth of epithelium invading the connective tissue capsule; 2) columnar basal cells containing hyperchromatic nuclei.

The plexiform unicystic ameloblastoma was defined when, in addition to the above pattern, there was present evidence of intraluminal or capsular epithelial proliferation in a plexiform fashion. For such nodular plexiform pattern of epithelial proliferation, the criteria of Vickers et al (1970) was almost invariably absent.

One normal human developing tooth was also provided from the file of Surgical Pathology Division McGaw Hospital.

The following immunohistochemical technique was applied to the above group of neoplasms and normal developing human tooth as control. 1) Peroxidase anti-peroxidase (PAP) technique, Sternberger (1979) for the identification of the glycoproteins, laminin and fibronectin, within the substance of the basement membrane, and the prekeratin protein within the cytoplasm of epithelial cells. 2. Avidin-biotin-peroxidase complex (ABC) of Hsu, et al (1980) for lectins.

Avidin-biotin-peroxidase complex (ABC),technique: the high affinity lectins used in this study were: 1) Con-A, representative of the group of lectins with a specificity for α-mannose, α-glucose; 2) RCA-I, from the group of lectins with a specificity for galactose or N-acetyl galactosamine; 3) WGA, representative of the group of lectins with a specificity for N-acetylglucosamine.

Procedures:

A: 1) The specimens of ameloblastomas and the normal developing teeth were cut into 3 microns sections and mounted on glass slides, pretreated with 2% aqueous Elmers glue.

2) Following drying at 60°C, the sections were deparaffinized in two changes of xylene. The xylene was removed by immersion in absolute alcohol.

3) The sections were treated with 0.075% hydrochloric acid and absolute ethanol for 15 minutes to remove any pseudoperoxidase and endogenous peroxidase.

4) Following washing in tap-water, the sections were immersed in tris-buffered saline, pH 7.4, containing 0.2% bovine serum albumin, (TBS/BSA) for twenty minutes.

5) Biotinylated lectins in TBS with 10% (W/V)BSA were applied at concentrations: 10 ug/ml for Con A, and 50 ug/ml for RCA and WGA, for thirty minutes.

6) After washing in three changes, of TBS, each for five minutes, the section was treated with avidin-biotin-peroxidase complex in TBS for 60 minutes, and washed in three changes of TBS each for five minutes.

7. The sections then were treated with the chromogen diaminobenzidine tetrahydrochloride, 40 mg% in ammunium acetate-citrate buffer, pH 5.5 containing 0.0075% H_2O_2 for 2.5 minutes.

8) Following rinsing in de-ionized water, the sections were counterstained in hemotoxylin for one minute.

B. The peroxidase antiperoxidase (PAP) technique, Sternberger (1979), was used for definition of the basement membrane, laminin, fibronectin and for cytoskeleton prekaratin. The PAP method was applied as follows:

The sections were cut at 3 μm, mounted on glass slides, pretreated with 2% aqueous Elmers glue.

After drying and deparaffinization in two changes of xylene and alcohol, pseudoperoxidase and endogenous peroxidase were inactivated by the treatment with 0.075% hydrochloric acid in absolute ethanol for 15 min., Weir et al (1974). Duplicate sections to be stained for Keratin were trypsinized in prewarmed pancreatic trypsin, 0.1% in 0.01% M Tris buffer, pH 7.6 containing 0.1% calcium chloride at 37°C for 15 minutes, followed by thorough washing in cold tris buffer, Curran et al (1977) and Mepham, et al (1979). Sections to be stained for laminin or fibronectin were pretreated with pepsin, 0.1% in 0.1 N Hcl for 45 minutes at 37°C.

In a moist chamber, the sections were covered with a 3% solution in PBS with 0.2% bovine serum albumin (PBS/BSA), (of normal serum from the species in which the link antibody was raised). This was used to block non-specific tissue binding of the subsequent reagent, the primary specific immunoglobulin, Sternberger (1979). The sections were blotted on

filter paper and covered with primary antibodies in PBS/BSA at a dilution of 1:1000 containing 1% normal serum from the link antibody species, for keratin, laminins and fibronectin, respectively. Keratin antibodies were applied for 30 minutes at ambient temperature; antilaminin and antifibronectin were applied for 18-24 hours at 4°C. This was followed by washing in three changes of PBS to remove unreacted primary antibodies.

The link antibodies for prekeratin, laminin and fibronectin were diluted at 1:100 in PBS and applied to the sections for 30 minutes, 25°C. These reagents consist of whole antisera from which antihuman IgG cross reactivity had been removed by absorption onto CNBr - Sepharose linked human gamma globulins, antibodies. After washing in PBS, the sections were treated with PAP, of the appropriate species, diluted at 1:200 in PBS/BSA with 1% normal serum for 30 minutes. The slides were washed in PBS and rinsed with deionized water. The slides, were placed in peroxidase substrate solution for 2.5 minutes. This solution consists of ammonium acetate-citric acid buffer, pH 5.5, containing 0.05% of 3,3' diaminobenzidine tetrahydrochloride, prepared immediately before use, filtered to which 0.0075% hydrogen peroxide is added, Weir et al (1974), Vacca et al (1978). Finally, the sections were counterstained with hematoxylin for one minute and mounted with Permount.

CHAPTER IV

RESULTS

A. Normal developing tooth (control).

1) Hematoxylin and eosin sections of the dental organ, corresponding to early and late bell stage of differentiation, revealed a proliferative burst of ectomesenchymal and enamel organ epithelium both surrounded by the radially arranged fibrils of dental follicles. The dental lamina was seen to break into discrete islands of rosetting epithelial aggregates and showed a loss of continuity of dental organ with the surface epithelium, evidence of the late bell stage of odontogenesis. The enamel organ at bell stage showed four structurally distinct layers, indicative of histodifferentiation of the epithelium (Fig. 1). The outer enamel epithelium, one to two layers in thickness, was composed of cuboidal epithelium with high nuclear cytoplasmic ratio, numerous buddings and protruding of epithelial outgrowths, extending from outer enamel epithelium were also noted. The stellate reticulum was formed by a multilayer of cells, and delicate cytoplasmic extensions. No evidence of vascularization was noted at this stage of development of the enamel organ. The stratum intermedium, two to three cell layers in thickness, was located subjacent to the inner enamel epithelium. Within the inner enamel epithelium a distinct maturation pattern of the vestigium of enamel forming cells through the preameloblasts was noted. Structurally, this layer was composed of single small round cells with hyperchromatic nuclei in and around the cervical loop area. Assuming cuboidal and further columnar features and repositioning of the nuclei in reverse polarity to the basement membrane, characteristic for preameloblasts,

were found in the more centrally located inner enamel epithelium.

The epithelial-mesenchymal basement membrane interface at the presumed cuspal area showed an increased thickening, parallel with preodontoblastic differentiation. The earliest reciprocity of heterotypic tissue interaction was traced as preodontoblastic protoplasmic extensions, across the basement membrane. Direct, cell-to-cell contact, basement membrane degradation, and predentin matrix deposition were the observed features for late bell stage of the enamel organ (Fig. 2).

The developing tooth during matrix deposition and calcification, revealed amylase-resistant, secretory ameloblasts and odontoblasts and the absence of a basement membrane (Fig. 3).

2) Immunoperoxidase studies of the sections of normal developing tooth at the histodifferentiation stage and crown stage were performed as follows:

LAMININ: Monospecific antibody to laminin revealed a distinct linear deposition outlining the epithelial-mesenchymal interface and the associated outgrowth of blood vessels (Fig 4). Laminin was traced also around the vestigia of the dental lamina. However, laminin was absent at the site of organic matrix deposit (Fig. 5).

FIBRONECTIN: Monospecific antisera against this glycoprotein decorated fibronectin in a diffuse pattern with moderate intensity in the mesenchyme of the immature dental pulp tissue. Following histodifferentiation of the tooth germ, the staining intensity was substantially less than in previous stages, a feature which corresponded well with mesenchymal maturation. In the same background, the fibronectin reaction was fairly detectable in the

cytoplasm of odontoblasts. In contrast, the mesenchymal component was mildly reactive. In the basement membrane zone, fibronectin was found during histodifferentiation stage of tooth development. Ectodermally-derived epithelium, in general, was non-reactive at the inner and outer enamel epithelium. Blood vessel walls in all instances disclosed the greatest stainability (Fig. 6).

Cytokeratin: Monoclonal antibody to prekeratin revealed a positive cytoplasmic reaction in all four layers of enamel epithelium (Fig. 7).

Con A: In the enamel organ epithelium, the lectin was found to be intensely positive intercellularly, in and adjacent to the cervical loop.

Secretory ameloblasts were also found to be intensely positive and was amylase resistant (Fig. 8). Within the outer enamel epithelium there was variability in staining reaction and few cells were found to be more reactive. The epithelium in the stratum intermedium was diffusely positive. In the mesenchymal part of the tooth germ, preodontoblastic maturation was associated with an intense intracytoplasmic positive reaction, as also seen for the secretory ameloblasts (Fig. 3). In contrast, the pre-ameloblasts were reactive only intercellularly. The tissue of the pulp at this stage was entirely negative.

RCA-I: This lectin specified two groups of reacting and non-reacting population of cells within the enamel organ epithelium. The preameloblasts were reactive both intercellulary and intracellularly (Fig. 1), whereas the secretory ameloblasts and odontoblasts were non-reactive. Such reacting and non-reacting population within the outer enamel epithelium was also noted.

TABLE 1

IMMUNE LABELING INDECES FOR THE ELEMENTS OF NORMAL DEVELOPING TOOTH AT DIFFERENT STAGES OF MATURATION

CELL TYPE	CON A	RCA	WGA	FN	LN	KER
IEE, at cervical loop area	+/-	-/+	+/-	-	-	+
Preameloblasts	+	+	+	-	-	+
Secretory ameloblasts	+	-	+	-	-	+
Stellate reticulum	+	+	+	-	-	+
OEE	+/-	-/+	+/-	-	+	+
BMZ: Bell/crown	-	-	-	+/-	+/-	-
Ectomesenchume/Pulp	-	-	-	+	-	-
Predentin	-	-	-	+	-	-

WGA: For WGA, cells of the epithelial enamel organ at different stages of maturation through secretory ameloblasts were positive, whereas, connective tissue mesenchyme remained non-reactive.

PLEXIFORM AMELOBLASTOMA

Histopathology: Hematoxylin and eosin preparation of the sections of four cases in this group revealed heterogeneous strands of odontogenic epithelium, predominantly resembling dental lamina type showing hyperchromatic pleomorphic nuclei and, occasional, bipolar mitotic figures. The central part of the plexiform strands did not display the star-shaped or spindle stellate reticulum-like cells observed in the enamel organ, nor did it undergo cystic degeneration. Rather, they assumed a flattened or squamoid appearing features. Within epithelial strands, the peripheral cells did not exhibit preameloblastic maturation. In general, the Vicker's criteria for early ameloblastomatous epithelium, i.e., palisading with polarization of hyperchromatic nuclei and inter--, intracytoplasmic vacuolization, Vickers et al (1970) was found extremely rigid when applied to this group of neoplasms (Fig. 9). The connective tissue stroma appeared primitive, loose in texture and free from inflammation.

Laminin: All sections of plexiform ameloblastomas after treatment with pepsin, showed linear arrangement of laminin within their basement membrane (Fig. 10).

Fibronectin: The pattern of expression of this glycoprotein within the basement membrane of plexiform ameloblastoma was unremarkable and, for the most part, negative.

Con A: Receptor-lectin complexes were found positive for polyhedral and negative for those mature cells. The polyhedral cells with high N/C ratio were reactive to Con A in and around their cytoplasm and cell membrane.

RCA-I: The receptor sites for RCA-I in the plexiform ameloblastoma was found to be undetectable (Fig. 11), as indicated by very weak stainability of the sections.

WGA: Two distinct populations in the plexiform ameloblastoma were noted: polyhedral cells were found reactive; while the more mature, columnar, preameloblasts were unreactive.

Cytokeratin: All sections treated with antibody to keratin were positive in the plexiform ameloblastoma.

Immunohistological findings for plexiform ameloblastoma is summarized in Table 2.

FOLLICULAR AMELOBLASTOMA:

Histopathology: Hematoxylin and eosin sections of this group of neoplasms revealed islands, follicles or occasional strands of odontogenic epithelium with some resemblance to the enamel organ. Such features included palisading peripheral columnar cells with hyperchromatic nuclei and inter-, intracellular vacuolization (Fig. 12). The central epithelial follicular cells revealed stellate, spindle or, occasional, squamous changes. Marked vascularization, hemorrhage, cystic changes were also seen. In four cases, superimposed features of the aneurysmal bone cyst characterized by large blood-filled spaces lined by septae of vascularized

TABLE 2

IMMUNE LABELING INDICES FOR SOLID TYPE OF AMELOBLASTOMA, PLEXIFORM VARIANT

CELL TYPE	CON A	RCA	WGA	FN	LN	KER
Ameloblastomatous epith.	+/-	-	+/-	-	-	+
Basement membrane zone	-	-	-	-	+	-

connective tissue, osteoclast-like giant cells and osteoid or woven bone were noted.

Cytokeratin: Keratin antibody to all types of cells in the follicular ameloblastoma were found to be positively reactive (Fig. 13).

Laminin: Within the basement membrane, laminin was uniformly positive (Fig. 14).

Fibronectin: Polyclonal monospecific antibody to fibronectin disclosed a kaleidoscopic distribution pattern in the follicular ameloblastoma. The neoplastic cells, when associated with dense, mature, fibrous tissue, were found to be invariably negative for fibronectin within their basement membrane (Fig. 15). However, neoplastic tissue revealed incorporation of fibronectin within their basement membrane, whenever inflammation and/or blood extravasation was noted in the stroma. In such events, the basement membrane within and subjacent to the follicular ameloblastomas was positive for fibronectin.

Con A: The cuboidal and polyhedral cells in the periphery of the follicles or strands were positively reactive to Con A on their cell surfaces; to a lesser extent columnar cells were positive on their cytoplasmic surfaces. However, foci of squamous metaplasia were associated with an intense intracytoplasmic staining reaction with Con A.

RCA I: The lectin revealed reactive and non-reactive patterns, in most of the sections of the follicular ameloblastomas. In general, columnar differentiation was accompanied by the expression of the lectin in the plasma membrane, whereas populations of polyhedral or oval cells were found alternatively positive or negative. In foci of squamous metaplasia,

however, RCA-I was shown found to be positive intercellularly (Fig. 16).

WGA: This lectin revealed columnar and the stellate appearing cells to be positive on surface. Squamous differentiation was found positive to WGA in their cytoplasm (Fig. 17). Table 3 is a summary of immunohistological findings for follicular ameloblastoma.

UNICYSTIC AND PLEXIFORM UNICYSTIC AMELOBLASTOMA:

Histopathology: Hematoxylin and eosin sections of this group of lesions revealed non-keratinized, cystic lining epithelium of various thickness and morphologic organization. In many instances, the unicystic ameloblastoma was composed of few layers of flattened squamous cells bearing little or no morphologic resemblance to ameloblastomatous epithelium described earlier, Vickers et al (1970). The overlying epithelium, in many instances, was discohesive and loosely texture, suggestive of intracellular edema. The latter feature was always accompanied by vascularization and intense edema of the underlying capsular fibrous connective tissue stroma. Numerous down-growth of epithelium into the connective tissue and/or intraluminal extensions composed of anastomosing cords of proliferating cells, forming plexiform pattern, were the prominent feature, in some cases. In one case, the primary lesion disclosed only a unicystic type of growth. However, as the clinical history revealed, it was a genuine plexiform unicystic type of ameloblastoma. Correspondingly, the cystic lining epithelium of the plexiform pattern of epithelial proliferation hardly, if any, revealed the criteria for ameloblastoma (Fig. 18).

Cytokeratin: Monospecific antibody to laminin delineated foci of sharp

TABLE 3

IMMUNE LABELING INDICES FOR SOLID AMELOBLASTOMA

FOLLICULAR VARIANT

CELL TYPE	CON A	RCA	WGA	FN	LN	KER
Peripheral Columnar	+	+/-	+	-	-	+
Central:						
1) Spindle type	+	-/+	+	-	-	+
2) Squamous metaplasia	+	+	+	-	-	+
Basement Membrane:						
1) In proximity with dense fibrous stroma	-	-	-	-	+	-
2) In proximity with ABC or hemorrhage	-	-	-	+	+	-
3) Stromal infiltration by inflammatory cells	-	-	-	+	+	-

and an evenly distributed reaction product at epithelial-connective tissue interface. However, laminin, at some sites was found to be discontinuous or absent at or around the tip of the epithelial ridges extending into the underlying connective tissue.

Fibronectin: In unicystic and plexiform unicystic ameloblastoma fibronectin distribution followed the same character cited earlier for solid, follicular variant (Fig. 19).

Con A: The reaction pattern for the cells having a spindle appearance were positive either around the nuclei or on their plasma membrane. In foci of squamous differentiation, cytoplasm of the cells were intensely positive for Con A (Fig. 20).

RCA-I: The epithelium lining the cystic cavity are plexiform epithelial proliferations, were positive for RCA-I.

WGA: This lectin intensely reacted with cytoplasm of the mature keratinocytes around the lumen, or those flattened cells found in plexiform strands of epithelium. However, the spindle cells were to a lesser extent reactive on their surface.

Cytokeratin: In trypsin pretreated sections keratin in squamous and flattened epithelial cells stained positively. However, basaloid cells in aggregates and proliferating nodules were occasionally negative.

TABLE 4

IMMUNE LABELING INDICES FOR (MURAL), UNICYSTIC/PLEXIFORM UNICYSTIC AMELOBLASTOMA

CELL TYPE	CON A	RCA	WGA	FN	LN	KER
Spindle and basal cells	+	+/-	+	-	-	+/-
Flattened cells or sq. metaplasia	+	+	+	-	-	+
Basement membrane:						
1) In proximity with dense fibrous stroma	-	-	-	-	+	-
2) In proximity with massive vascularization	-	-	-	+	+/-	-
3) Stromal infilt. by inflammatory cells	-	-	-	+	+/-	-

CHAPTER V

DISCUSSION

A. FIBRONECTIN:

The distribution pattern of this macromolecule in developing dental organ at different stages of maturation was studied. Using immunoperoxidase techniques and monoclonal antibody, fibronectin decorates within the substances of the epithelial-mesenchymal basement membrane at histodifferentiation - the Bell Stage. This feature is also accompanied by the diffuse deposit of fibronectin in the mesenchymal dental papilla. Also, there is evidence of fibronectin accumulation within the stellate reticulum. Such features, whenever present, suggests an absorption phenomenon of plasma from the blood circulation.

Calcification of the tooth germ modifies the preexisting feature in that mesenchymal dental papilla undergoes maturation, which is associated by a reduction in staining intensity of fibronectin. Highly differentiated secretory odontoblasts are absolutely non-reactive. The predentin matrix, however, is mildly positive, indicative of incorporation of fibronectin into the stromal matrix, similar to uncalcified bone matrix (osteoid). In contrast, ectodermally-derived enamel organ does not manifest fibronectin by the immunoperoxidase within its organic matrix. These findings are consistent with previous immunofluorescence studies of the enamel organ in different species, Thesleff et al (1979, 1981), and Lesot et al (1981). Plexiform ameloblastomas embedded within loose fibrous connective tissue, as well as those follicular variants embedded within dense, exudate-free

connective tissue stroma both were found negative for fibronectin.

There are several lines of evidence suggesting that ectoderm does not synthesize fibronectin, Vaheri et al (1978), Thesleff (1979), Wartiavaara et al (1980). Embryonic ectoderm or ectodermally-derived epithelium, including the tooth anlage, produces glycosaminoglycan, proteoglycan and laminin, Hay (1978), Wartiavaara et al (1980), Gordon et al (1980) and Ekblom et al (1980). Fibronectin, as part of embryonic ectodermal basement membrane, is never found within the embryonic ectoderm or ectodermally-derived tissues, Wartiavaara et al (1980), Yang et al (1980).

In vitro studies, however, display ectoderm or ectodermal-derivatives during their formative phase to incorporate fibronectin within their basment membrane in order to secure the presumed morphology of the tissues, Brownell et al (1981), Osman et al (1981). The disappearance and loss of fibronectin from the face of the basement membrane in subsequent stages of differentiation is a known chronologic functional event, Madri et al (1980).

The majority of ameloblastomas of different classes reveal fibronectin significantly reactive in their basement membranes by using specific antisera immunohistochemically. However, in such instances where fibronectin is present, the neoplasm invariably is associated with inflammation, massive exudation, extravasation or co-existing aneurysmal bone cyst. In such instances, the basement membrane positivity, therefore, is attributed to the blood borne plasma.

It is appreciated that fibronectin bears peculiar affinity for a number of basement membrane constituents, among which type IV collagen, proteoglycan and glycosaminoglycan should be mentioned, Yamada, et al (1978)

Birdwell et al (1978), Orly et al (1979) and Perkins et al (1979). Such affinity, indeed, has been applied for purification of fibronectin on collagen affinity column, Ruoslahti et al (1978).

In this context, it is significant to mention that ameloblastic fibromas reveal uniquivocal positive reaction for fibronectin at the epithelial-mesenchymal interface and is suggestive of a function attributed to the mesenchyme in elaboration of the glycoprotein. Accordingly, it is reasonable to apply the above explanation made for solid and multicystic ameloblastomas to unicystic and plexiform unicystic variants of ameloblastomas, as well.

B. LAMININ

Laminin is the first extracellular matrix protein to appear during the embryonic development at 16-cell, morula stage, Levio (1980). It then is incorporated into the basement membranes derivatives of each embryonal line. Laminin is generally accepted as an ubiquitous product of ectoderm or ectodermally-derived embryonal structures, Timpl et al (1979, 1982), Ekblom, et al (1980), Brownell (1981).

Within the sections of solid follicular or plexiform ameloblastomas laminin decorates the basement membrane in a continuous linear fashion. Also, in developing human tooth, prior to organic matrix deposition, the basement membrane is reactive for laminin. Earlier studies of tooth development also have shown that, at the time of reciprocal interaction and predentin deposition, laminin is absent along with the other constituents of the basement membrane, Kallenbach (1971), Slavkin et al (1976).

It is interesting to note that the cystic lining epithelium in the unicystic ameloblastomas is found, in some instances, to be non-reactive to laminin, suggestive of failure of synthesis or degradation.

Malignancy also is known to modify the expressional pattern of laminin. The normal distribution pattern of laminin, as exemplified in this study, when compared to an ameloblastic carcinoma (unpublished) laminin was found uneven in distribution and frequently absent.

Laminin, while serving as a biological marker of differentiation, may also be used as an indicator product for the differential diagnosis of ameloblastomas. Additionally, such aberrations in synthesis or incorporation of laminin within the integrity of the basement membrane for the other kinds of cancers also has been reported, Timpl, (1979), Albrechtsen, et al (1981) and Hayman, et al (1981).

C. KERATIN:

In the normal developing tooth, keratin is found in all enamel epithelial cell layers, including secretory ameloblasts.

In the ameloblastomas, solid or cystic variants, keratin is not detected in some basaloid-appearing cells, aggregating in proliferating nodules. Relevant to the possible immaturity of ameloblastomatous neoplastic cells, it is suggestive that the cellular expression of keratin polypeptides may correlate with their state of differentiation, in analogy with basal cell carcinomas, Loning, et al (1980).

D. LECTINS:

The lectins, RCA-I, Con -A, and WGA are useful in the study of

ameloblastomas. The distribution patterns of glycoconjugate receptors or specific free sugars on cell membrane and in the cytoplasm, pertaining to these lectins, in normal developing tooth and morphologically different ameloblastomas suggest the following proposed models:

1) Con-A: Con A has the specificity for α-mannose and α-glucose, which displays two distinct patterns of decoration. The developing tooth germ of all stages of differentiation reveals the cells within proliferative compartment. This includes those, yet, capable of division and multiplication, morphologically characterized as cuboidal, polyhedral cells, columnar preameloblasts, and some flattened cells within the outer enamel epithelium. All such cells express mannose and glucose on their cytoplasmic membranes. Whereas, in the secretory ameloblasts Con-A intensely decorates their cytoplasmic mannose and glucose.

Cell membrane binding reaction to mannose and glucose by Con A in cells of this nature in the developing tooth is similar to the previous findings on EDTA-dissociated embryonal cells when agglutinated by Con-A, Moscona (1971).

In ameloblastomas, in general, proliferative and less differentiated, dental lamina-like cells, and ameloblastomatous columnar cells reveal the same features of their analogous normal counterparts. Squamous metaplasia, however, is associated with intense cytoplasmic reaction. These observations suggest that at earlier, embryonic or proliferative, stages, Con-A reaction with mannose and glucose lies on cell surface. However, with maturation the cytoplasm of the odontogenic epithelia becomes intensely reactive.

RCA I:

Studies on sections of normal developing tooth, and the follicular, plexiform, unicystic and plexiform unicystic ameloblastomas reveal binding differences by galactosyl residues by RCA-I, among different cell populations in each given section. Cuboidal and polyhedral cells of the inner enamel epithelium, proliferative compartment, close to the cervical loop area with high nuclear/cytoplasmic ratio are found non-reactive. Cells with similar morphology identifiable within the outer enamel epithelium are non-reactive, suggestive of the absence from failure of synthesis or low concentrattion.

Earlier studies with lectins, Nicolson et al (1973), on non-epithelial tissues, show a cell-contact dependent increase of membrane D-galactopyranosyl-like residues in normal murine fibroblasts, but not in the transformed phenotype. Subsequent findings of the RCA-ligand complex on cell surfaces of normal flattened keratinocytes in the upper prickle and granular layer, well differentiated keratinocytes in squamous cell carcinomas, as well as squamous metaplasia in follicular ameloblastomas, all are in perfect analogy with the suggestion of a disorder of galactosyl residues synthesis or loss as indicated by RCA-I, Dabelsteen et al (1978 a,b). Nicolson, et al (1972) found decreased agglutinability of dissociated malignant cells by RCA-I.

Recent studies on formalin fixed tissue materials disclose further clues about RCA-I receptors. It has been shown in squamous cell carcinoma and healing epithelial wounds, that some population of cells are less reactive with RCA-I, Dabelsteen, et al (1978 b), Graem (1982). This event

has been attributed to an increase physiologic or pathologic motility of the cells.

RCA-I binding profiles in variants of ameloblastomas and the developing tooth germ are in agreement with the above explanation. On this context, some cuboidal or polyhedral cells with high nuclear cytoplasmic ratio and negative for RCA are virtually from the same compartment of the cells previously found reactive for Con A. RCA-I binding receptor intensity on metaplastic squamous cells in ameloblastomas and all flattened keratinocytes, in general, may, on the other hand, be interpreted as a maturation event on cell surfaces.

Applying immunofluorescence techniques, RCA-binding receptors were studies in solid ameloblastomas. Vedtofte et al (1981) found preameloblastic columnar cells to be negative. In contrast, in this study preameloblasts react positively on their cell surfaces. This may indicate a greater sensitivity of the immunoperoxidase technique over that of immunofluorescence.

WGA

By the immunoperoxidase techniques and using biotinylated wheat germ agglutinin, the lectin decorates B-D-N-acetylglucosamine and sialic acid. In this study it was found WGA to react with preameloblasts on their surface. Suqamous differentiation in follicular ameloblastoma was found to be positive intracellularly, the pattern more or less similar to that of Con A. This observation is also consistent with ultrastructural localization of WGA binding sites on mouse myocardium, Gros et al (1982).

WGA bound-fluorescent isothiocyanate-labeled sections of

ameloblastomas, Vedtofte, et al (1981), demonstrated barely positive reaction of preameloblasts. However, by the immunoperoxidase technique applied in this study, these populations of cells are frankly reactive to WGA.

DISTURBED TISSUE INTERACTIONS: THEIR POSSIBLE IMPACT ON MORPHOGENETIC MALDEVELOPMENT.

In epithelial-mesenchymal interacting systems, e.g. exocrine glands, embryonic tubuli and glomeruli, lower respiratory system, and feather-forming organs, organization of the interacting tissues and their subsequent differentiation require participant heterotopic tissues to be "aware" of each other. This enables them to communicate in a developmentally significant way, Saxen (1977). In all epithelial-mesenchymal interacting circumstances, the eventual informative and transmissible signal substances act on the genome of the target cells. The precise mode of action of these "organizers" and the current state of knowledge about their molecular function is fragmentary and, to a greater extent, is speculative, Lash, et al (1977). However, the principles of these interdisciplinary orders between hetrotopic cells have been known for decades under the epithet "induction."

Among the many definitions for inductions, Brill's contention is the most succint one in the English literature. Accordingly, induction implies "evocative action of one tissue over another." Embryonic induction operates when one population of cells act upon the other to induce changes in the behavioristic biologic character of the latter, and the outcome is known as determination.

Theoretically, two forms of inductive influences have been discussed: directive influences imply when an embryonic cells yet possessing more than one developmental option and the choice between the options is affected, largely, by the extracellular factors. Permissive influences, on the other hand, refer to a level of development in which the cells have become committed to a certain pathway, but yet require an exogenous stimulus to express their ultimate phenotypic character, Saxen, (1977). These two types of influences may alternate during progressive differentiation and gradual restriction of the number of developmental options of the already determined embryonal tissue, Gluecksohn-Wealsch (1977).

The determination relevant to the developing dental organ may be defined as those transcriptional and translational events at final steps of differentiation in which all other somatic possibilities are excluded, and the only stable option, i.e., "amelogenetic repertoire" remains available to the regulatory instructive effects of the subjacent mesenchyme. Developmental programming and sequential amoloblastic maturation, thereafter, operates through transduction effect of the signals, receiving and translation of such signals into a specific phenotypic product resulting in morphologic differentiation.

Experiments on developing limbs and investigations on disturbed interaction and teratogenesis in this system have led to some interesting speculations. There are some sorts of analogies between the developing tooth and limb in terms of sequences in morphogenetic tissue interaction and functional reciprocity. In the latter system, it is known under the circumstances where the inductor's contact is disrupted, or mesenchymal

blastoma when absent or the inducer when defective, there are abnormalities in apical ectodermal ridge (AER) development. Hypothetically, AER acts under a maintenance factor (MF) secreted by the mesenchymal blastoma. Derangement in secretion of this mesenchymal product led to the oversized and elongated AER. According to this model, maintenance factor also acted on the process of programmed ectodermal degeneration and setting of the "death clock" in ectodermal cells via its interactive effect Saunders, et al (1966).

Similarly, defective ectodermal responses to mesenchymal inductive influences, and also, faults in homotypic interactions may affect differentiation and morphogenesis, Sawyer (1972). In this connection, it has been suggested that altered adhesive property of the cell surface may interfere with the aggregative capacity and, thereby, normal morphogenetic movement of the cells, Sawyer (1972).

For odontogenic anomalies, it is a permissive influence of ectomesenchyme upon the already committed cells of dental lamina. By todays available knowledge, molecular specification of these inductive influences are not yet practical. Monoclonal antibodies with their innate property selectively make fine distinctions between minute molecular constituents of the cells and their products, however, by virtue of their unique potential, are promising to the investigator of this field.

AMELOBLASTOMA: PATHOGENETIC CONCEPT

Ameloblastoma may be defined as a developmental pathological entity, characterized by a unique cellular phenotype indicative of its embryonal

origin. The neoplasm is derived from the predetermined and proliferative cells of dental lamina in its embryonal phase. The cells of dental lamina are proliferating actively and continuously for a minimum of at least four years in man, prior to the formation of third molar tooth bud, Manley (1954), Moe et al (1961) and Spouge (1967).

Ameloblastomas, phenotypically speaking, do not meet the criteria for a genuine adult life neoplasm in several respects, Willis (1962), Bolande (1967, 1979). Adult neoplasms, firstly, benign or malignant, are induced as a regressive mutation of the cells within mature tissues, whereas, ameloblastomas arise from the embryonal laminal tissue and sustain invasive properties of their progenitor cells of dental lamina without any change. Secondly, adult neoplasms, despite ameloblastomas, may arise in cells retaining their ability to multiply and regenerate in adulthood. The dental lamina does not possess such a function.

Ameloblastomas, however, display pathogenetic characters as follows:

1) They arise in a primitive and transient tissue of dental lamina.
2) They are virtually restricted to the specific organ of the jaw.
3) They are composed of predetermined cells for a specific type of histogenesis.
4) The growth is composed of odontogenic cells retaining many features of a more primitive stage of their development. These features all together are shared by the entity called embryomas, Willis (1962).

It is already appreciated that higher incidences of proliferative growth arise in developmental vestiges while being kept in the body as

embryonal rests or heterotopias, Willis (1962). Examples include: craniopharyngiomas, developing from the epithelial vestigia of Rathke's pouch; chondromas, where notochord remnants are persisting within the nucleus pulposus after birth; papillary adenocarcinoma of cervix, uterus, and ovary in young girls as a developmental anomaly in mesonephric ducts or Wolffian ducts; carcinomas, though rare, arising in thyroglossal duct or branchial cleft cyst; high incidence of seminoma in undecendent testes, cryptorchidism, Willis (1962) Warkany (1971).

For the ameloblastomas, being in analogy with some of the above mentioned entities, the challenge for proof or disproof of their embryonic concept and malfunctioning heterotypic tissue interaction are in prospect and mandate further studies.

Aneurysmal bone cysts (ABC) secondary to a ameloblastomas: ABC is one of the most rapidly growing, solitary non-neoplastic and expansile lesion of the bone, Mira (1980). The true nature of the anomaly since its original description has been controversial, Jaffe, et al (1942), Lichtenstein (1950). It has been variably thought to be neoplastic, localized reaction to a precursor lesion or, alternatively, a developmental error, Sherman et al (1957).

In co-existence with the other bony lesions, non-ossifying fibroma, giant cell tumor, giant cell granuloma, osteoblastoma, chondroblastoma, fibromyxoma, solitary bone cyst, fibrous dysplasia and low grade osteosarcoma have been included, Levy at al (1975), Buraczewski, et al (1971), Biesecker, et al (1970), Mira (1980). These others have observed an incidence of 32% to 60% of ABC's associated with other lesions.

Theories on the evolution and pathogenesis of ABC now follow the contention that ABC is essentially an arteriovenous anomaly engrafted upon a precursor benign or low grade malignant lesion. These precursor benign or low grade malignant lesions have that potential to initiate an intraosseous arteriovenous malformation (fistula) and, subsequently, via hemodynamic forces within the induced cavernous spaces create further resorption of the adjacent bone. These events would manifest, histologically, as blood filled labyrinths bounded by the non-endothelial lined fibrous trabeculae, reparative osteoid and/or woven bone, and reactive multinucleated giant cells.

In this study, secondary ABC was discovered in intimate association with 4 (16%) of multicystic, follicular type, of ameloblastomas, (Fig. 21). With respect to ameloblastomas, the phenomenon has generally been referred under: adamantinohemangioma, Aisenberg (1950), hemangioblastoma, Grover, et al (1971), hemangioameloblastoma, Pindborg, et al (1971), and vascular ameloblastoma, Lucas (1975). No conclusive explanation for cause and effect relationship of coexisting ameloblastomas with aneurysmal bone cyst is known in the literature. It is believed that the blood-filled spaces result from dilation of vessels caused by stromal degeneration, Pindborg et al (1971), Lucas (1975).

In follicular ameloblastomas, the process of central cell necrosis, cystic changes of the follicles, and their continuous expansile growth may exert mechanical pressure upon the stromal vasculature. These hemodynamic alterations, due to local destructive effect of ameloblastomas, may cause formation of ABC within the vicinity of the precursor neoplastic growth.

In considering modality of treatment, finding ABC associated with precursor ameloblastoma is significant in that the anomalous vascular communication has also to be eliminated, Ruiter et al (1976).

SUMMARY

Immunohistochemical studies using the peroxidase-antiperoxidase method employed to specific antibodies for laminin and fibronectin within the basement membrane and for the three plant lectins WGA, Con A and RCA-I for the cell surface oligosaccharide receptors on 42 cases of ameloblastomas were performed.

Based upon the classification resulting from recent findings on the ameloblastomas, the neoplasms were tentatively categorized into: 1) follicular solid and multicystic, 2) plexiform-solid, and 3) unicystic and plexiform unicystic variants.

Sections of normal developing teeth at different stages of maturation, also, were used as normal control.

For the lectins, ameloblastomas of plexiform class, under the same conditions, compared to the other variants, were found to be very weakly reactive to RCA-I.

Con-A and WGA showed more or less similar reaction patterns as RCA-I. These were found, predominantly, on the periphery, cytoplasmic membranes of those less mature population of cells in different compartments. Whereas, terminally differentiated cells in both neoplastic proliferation and normal developing teeth were reactive intracellularly.

Laminin was an ubiquitous product found in the basement membrane of ameloblastomas of different classes, as well as that of the developing tooth. Odontoblasts producing a predentin matrix secretion, however,

revealed the complete loss of the basement membrane including laminin.

Fibronectin was found to be invariably absent in the basement membrane zone of ameloblastomas of the different classes. Additionally, however, in the presence of edema, inflammation or hemorrhage, fibronectin was easily detectable in the basement membrane. The latter observation was interpreted as an absorption phenomenon from the blood circulatory source.

CONCLUSION

Product identification of ameloblastomas in terms of their lectin and glycoprotein-antisera binding reactivities disclose subtle clues for advancing a biologically, as well as prognostically, more valuable classification of this group of odontogenic neoplasms. Despite the currently used groupings of the neoplasm, WHO 1971, here, the differences between the two classes of plexiform and the follicular variants are substantial and characteristic for each class, morphologically, and in terms of their behavior.

Innate to such retrospective studies of odontogenic tissues are the question of the suitability of formalin fixed-paraffin embedded materials. Such disadvantage, naturally, provides some limitations in degrees of scientific accuracy. Therefore, similar studies on fresh tissues of ameloblastomas are mandate to be performed.

There are several aspects on the pathogenesis of ameloblastomas and many basic biologic questions raised in this study which, yet, have remained unanswered. The findings reported in this work need to be supported through new and additional scientific experimentations.

Finally, the issue of uncoordinated and invasive growth relevant to ameloblastomas should be thoroughly revisited, in order to determine whether such invasive and proliferative character is simply reflective of the innate capacity of dental lamina or to a true neoplastic clonal proliferation.

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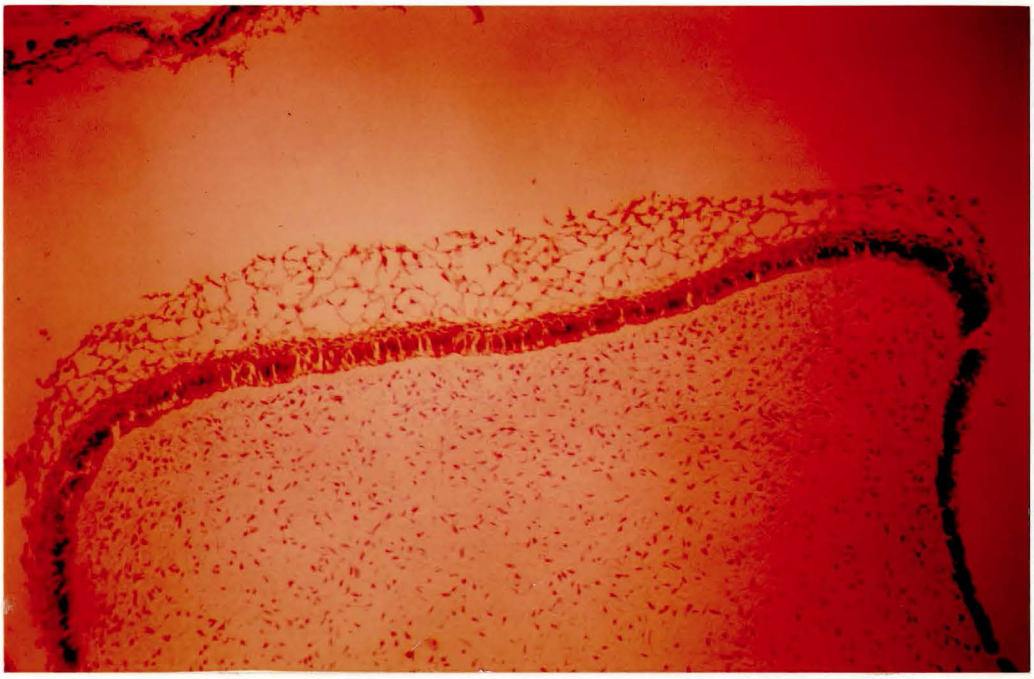

Figure 1. Normal developing tooth at histodifferentiation, Bell, stage. Note four structurally distinct layers within enamel organ epithelium (PAP staining for RCA-I, X125)

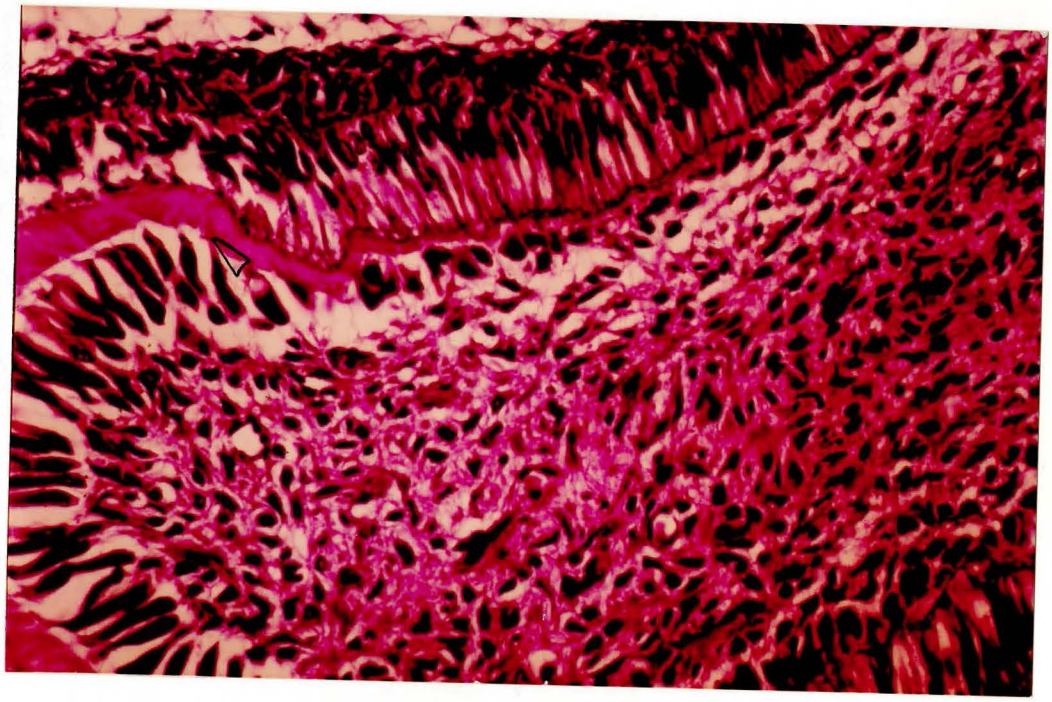

Figure 2. Normal developing tooth at late stage of histo-differentiation. Note degradation of the basement membrane and odontoblastic protoplasmic extensions (arrow) toward ameloblasts as the earliest evidence of epithelial mesenchymal cell-to-cell contact H.E. stain (X250)

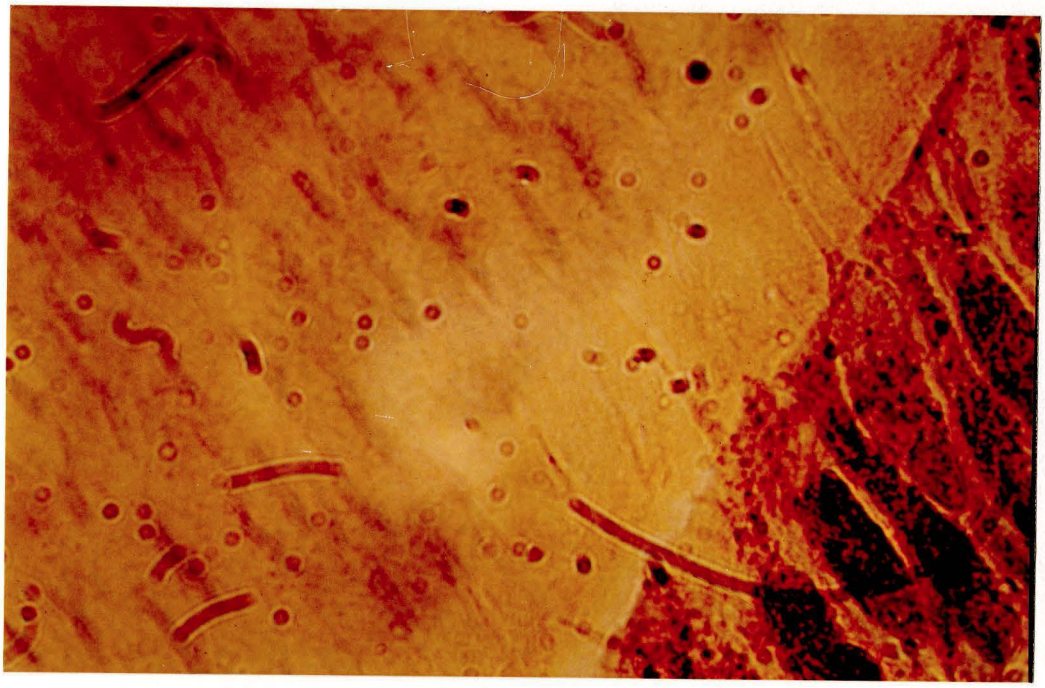

Figure 3. Normal developing tooth at late crownal stage. Note odontoblastic protoplasmic extensions across the calcified matrix, IPO-Con A (X400)

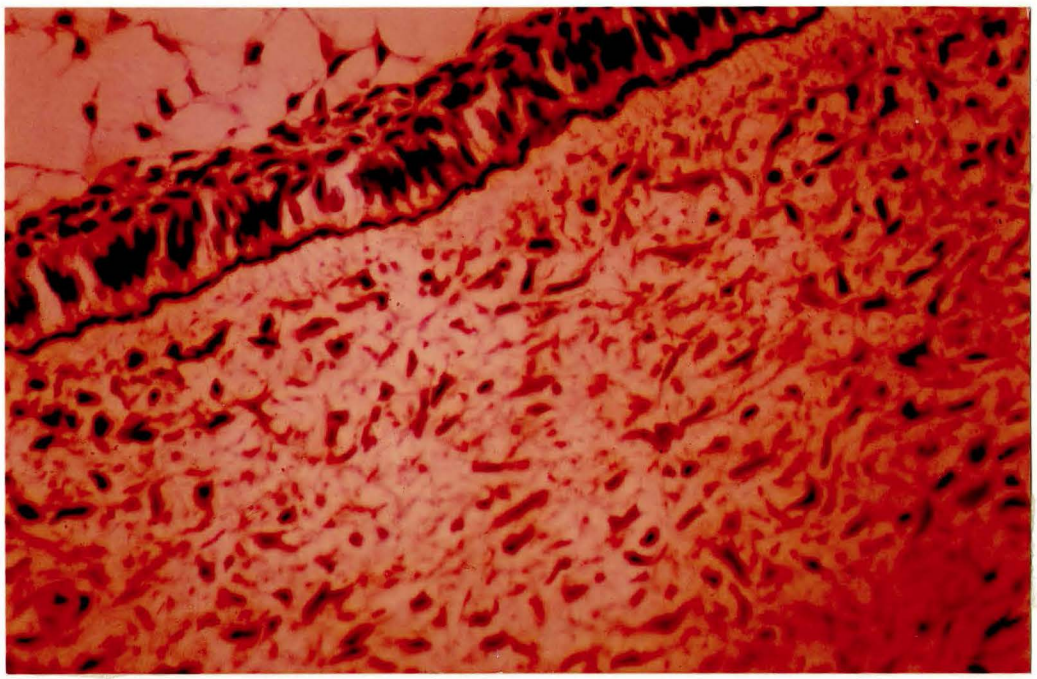

Figure 4. Normal developing tooth at early bell stage. Note linear deposition of laminin at the epithelial-mesenchymal basement membrane interface, (IPO X 250)

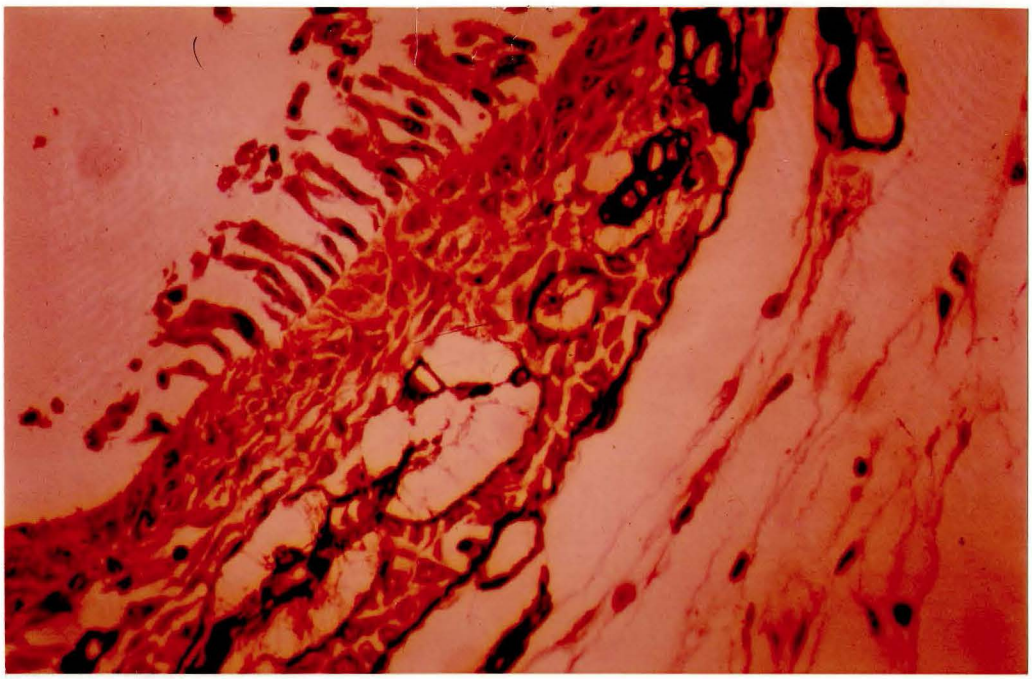

Figure 5. Normal developing tooth at the stage of calcified matrix depositon. Laminin is absent at the site of calcified enamel matrix deposition (IPO X 250)

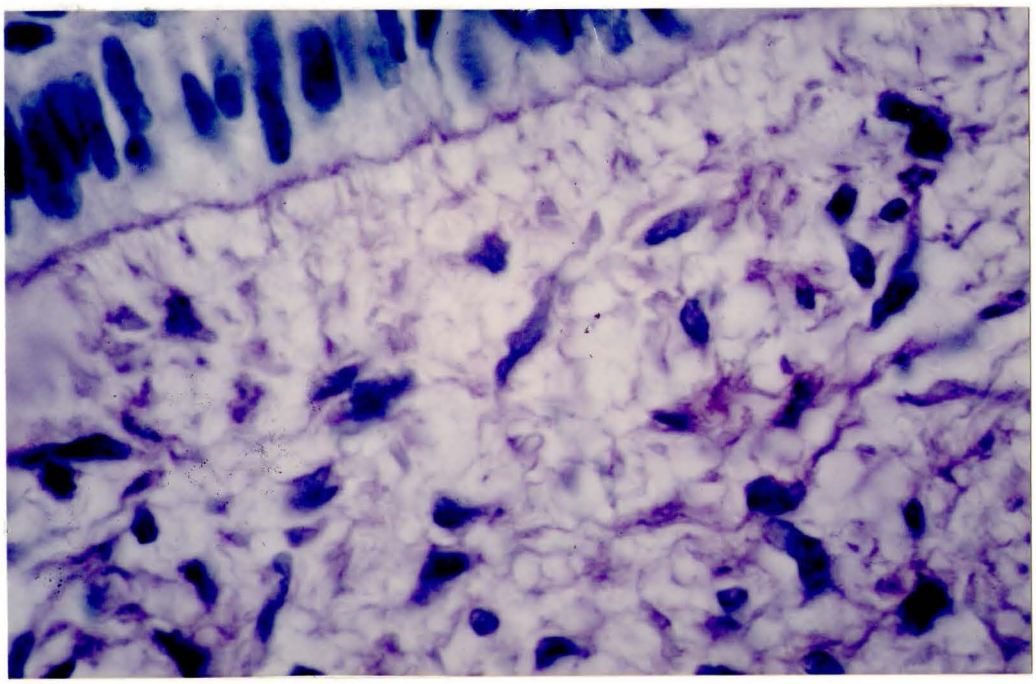

Figure 6. Normal developing tooth at bell stage. Fibronectin was undetectable at the site of basement membrane epithelial-mesenchymal interface (IPO X 250)

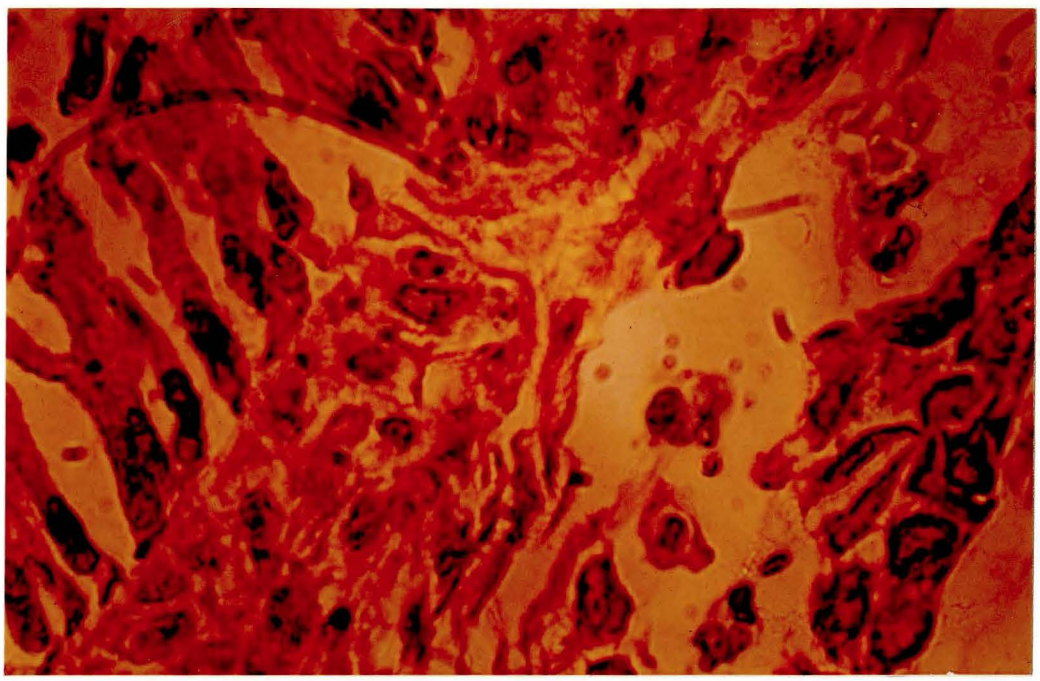

Figure 7. Normal developing tooth at the stage of matrix deposition. Note enamel epithelium in all four layers are reactive for Keratin (IPO X 400)

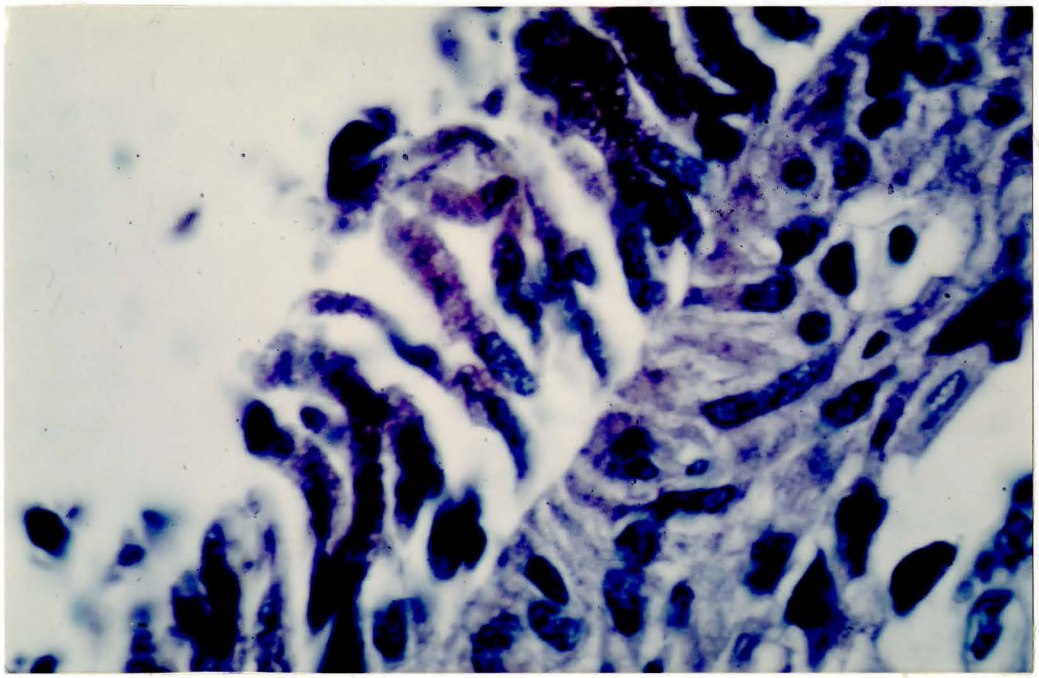

Figure 8. Normal developing tooth at the stage of calcified matrix deposition. Secretory odontoblasts are intensely reactive to Con A (PAP, X 400)

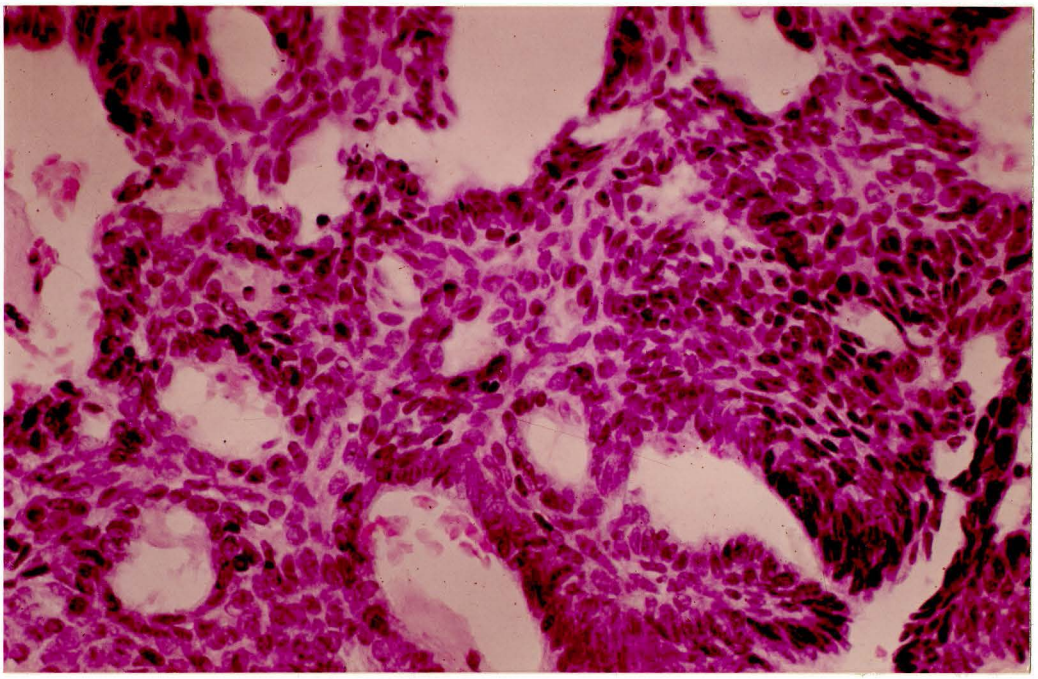

Figure 9. Plexiform Ameloblastoma (H&E, X 250)

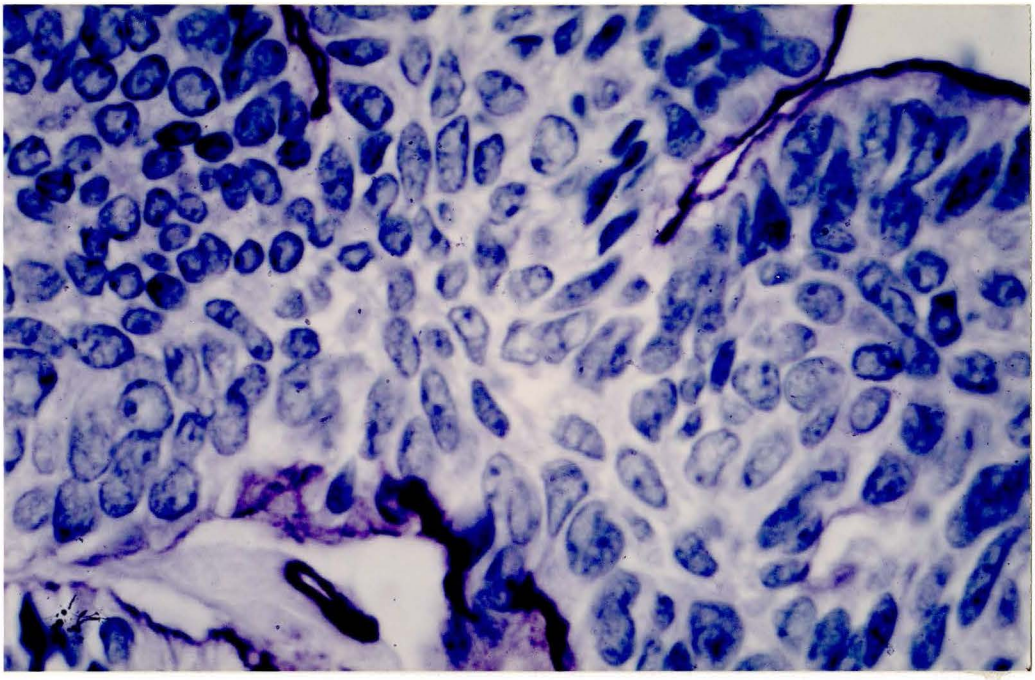

Figure 10. Plexiform ameloblastoma: Note linear deposit of laminin at the site of epithelial-mesehchymal interface and blood vessels (IPO, X 400)

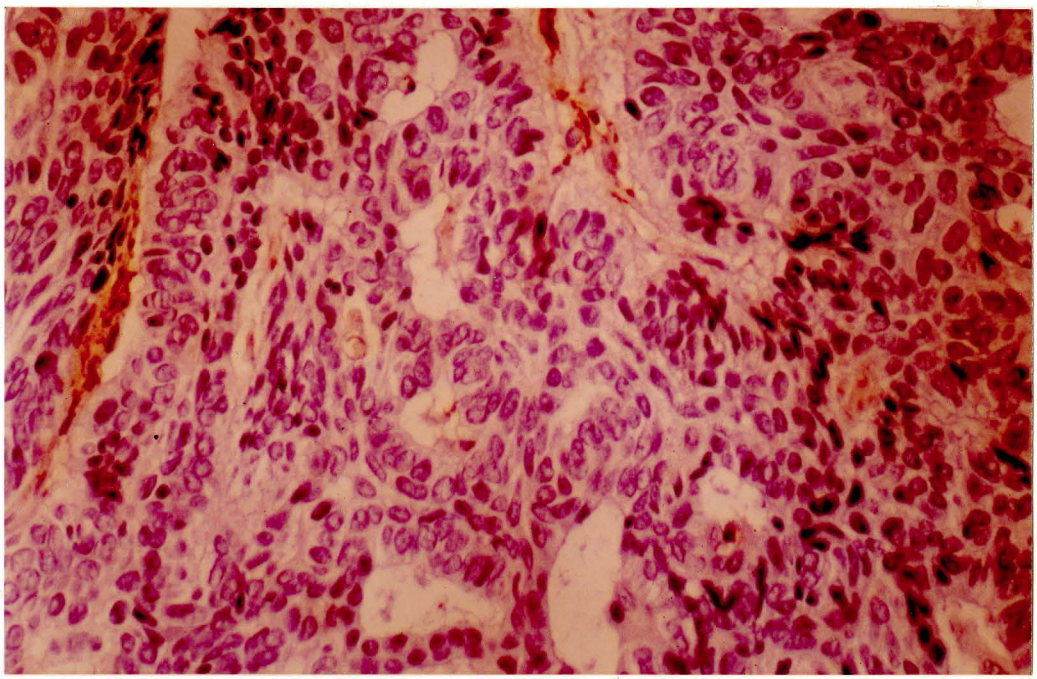

Figure 11. Plexiform ameloblastoma: receptor sites for RCA are undetectable for this group of ameloblastomas (PAP - X 250)

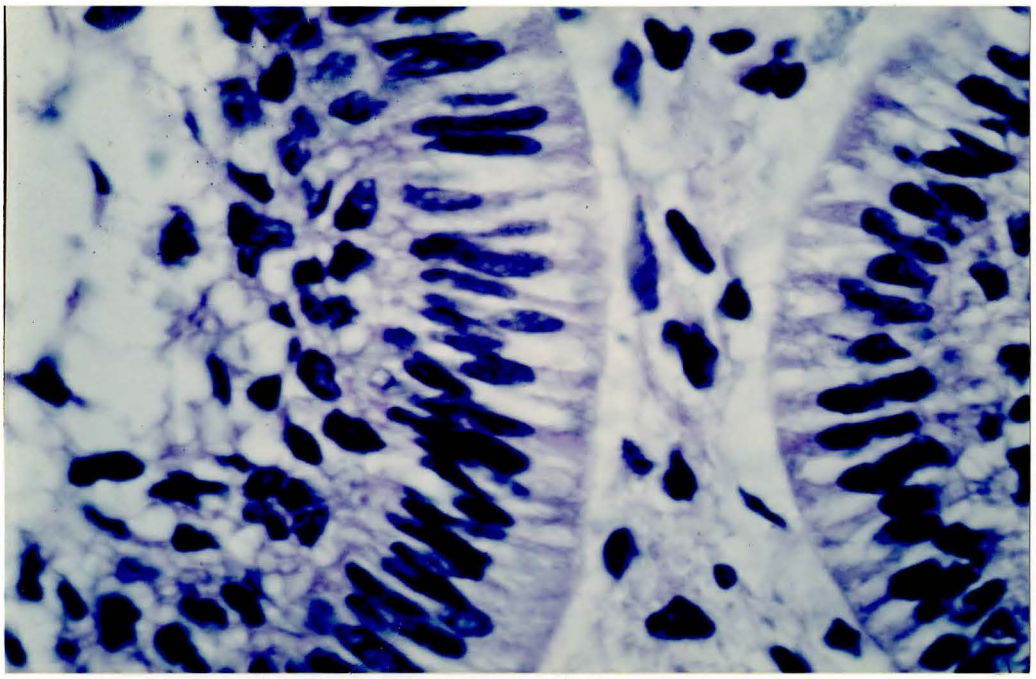

Figure 12. Follicular ameloblastoma (H & E X 400)

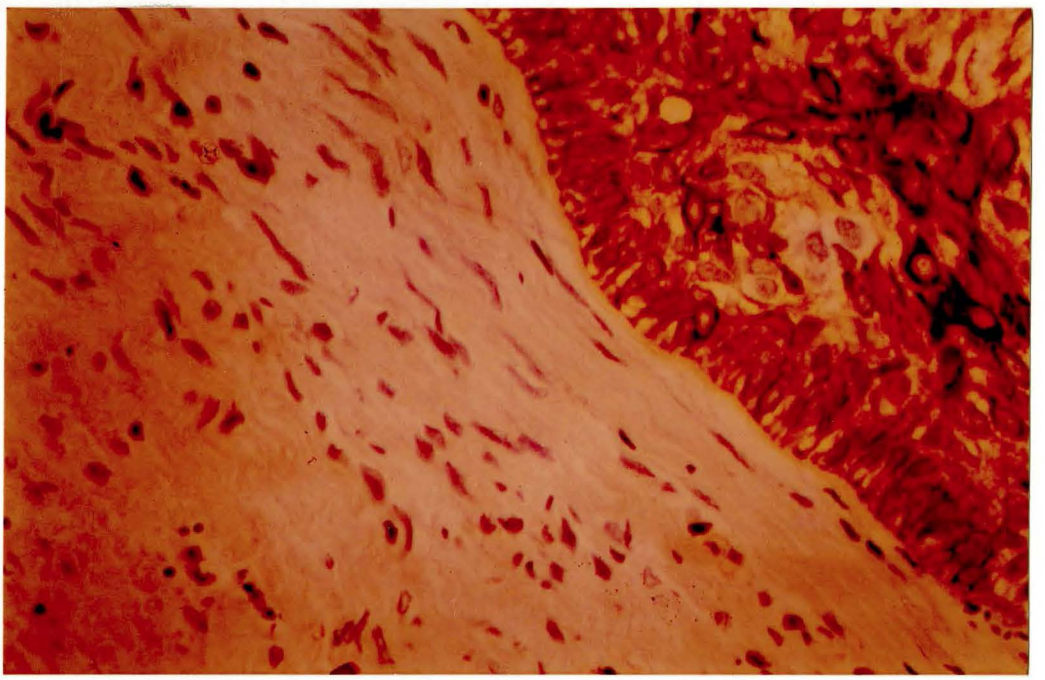

Figure 13. Follicular ameloblastoma. Keratin antibody is reactive with neoplastic cells. (IPO, X 250)

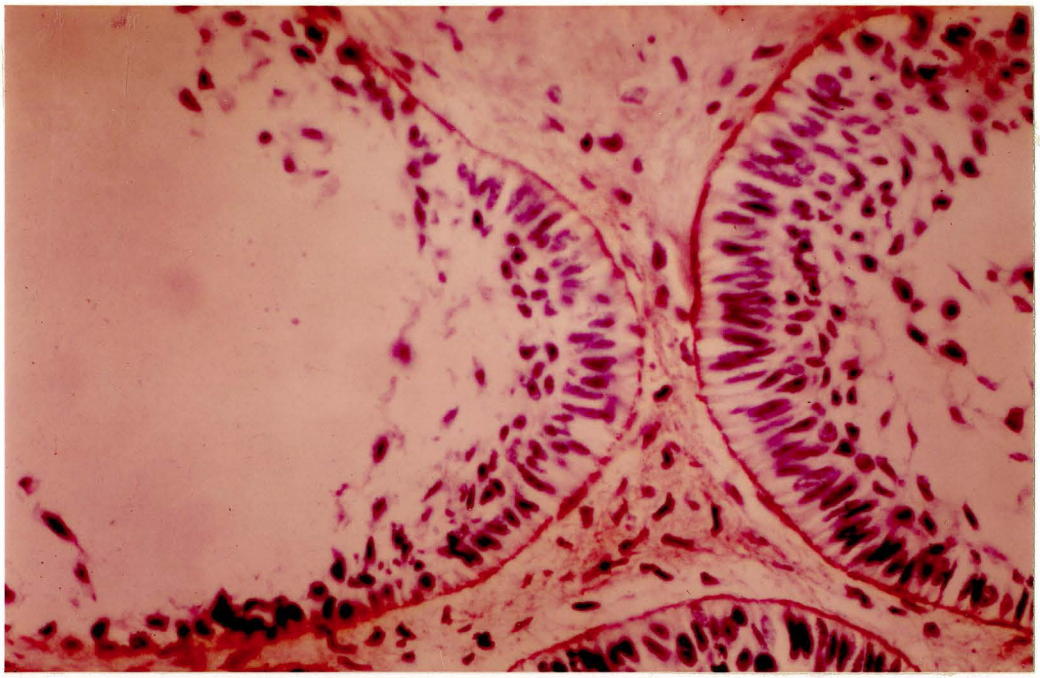

Figure 14. Follicular ameloblastoma. Laminin antibody is reactive at the basement membrane zone. (IPO X 250)

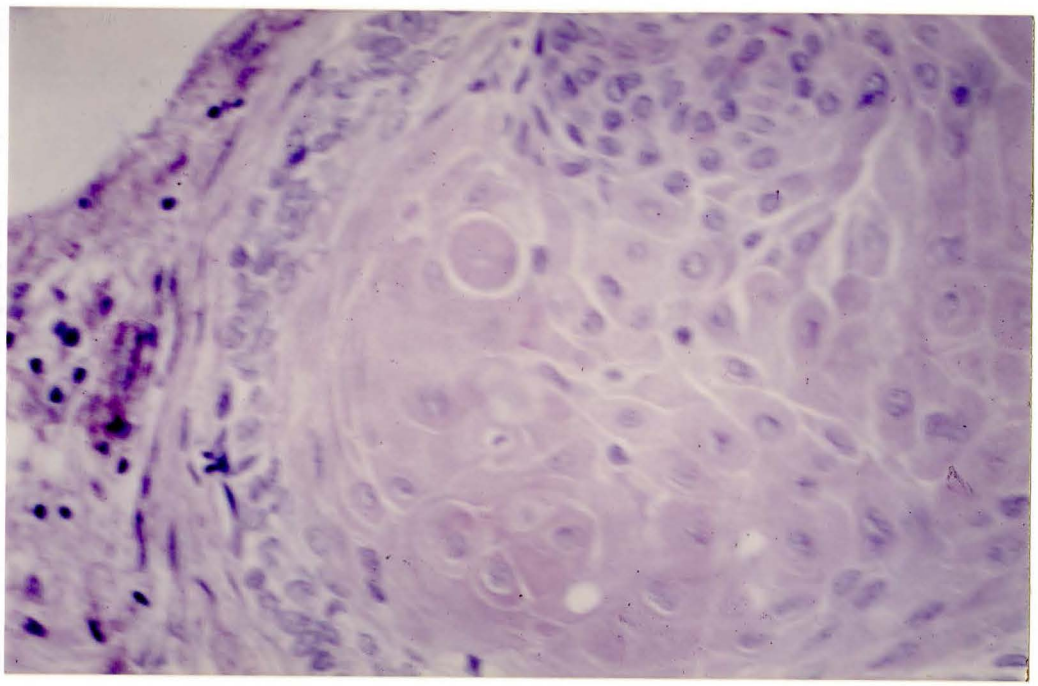

Figure 15. Follicular ameloblastoma with acanthomatous changes. Fibronectin appears to be undetectable at the site of basement membrane (IPO X 250)

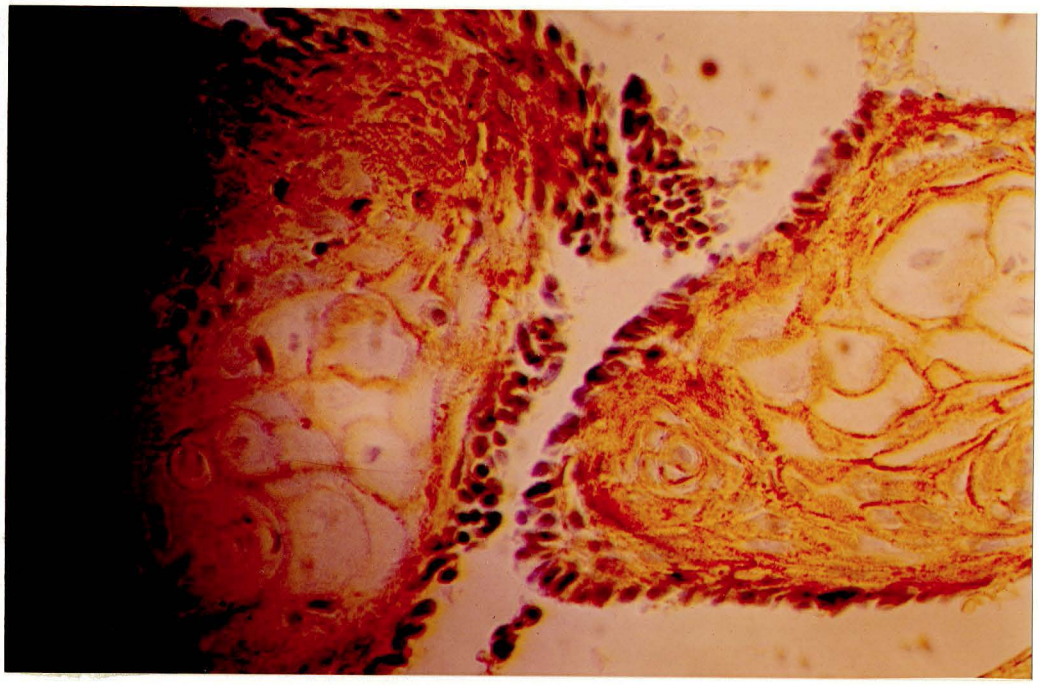

Figure 16. Follicular ameloblastoma with acanthomatous changes. RCA-I being sharply delineated intercellularly (PAP X 250)

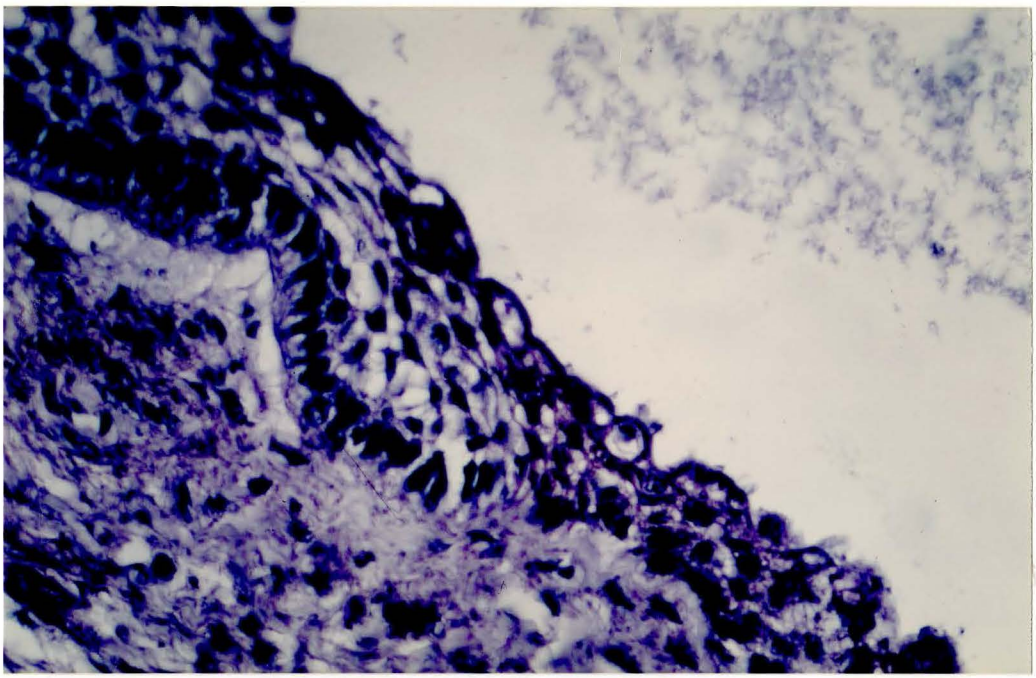

Figure 17. Follicular ameloblastoma. The lectin WGA is reactive within cytoplasm (PAP X 250)

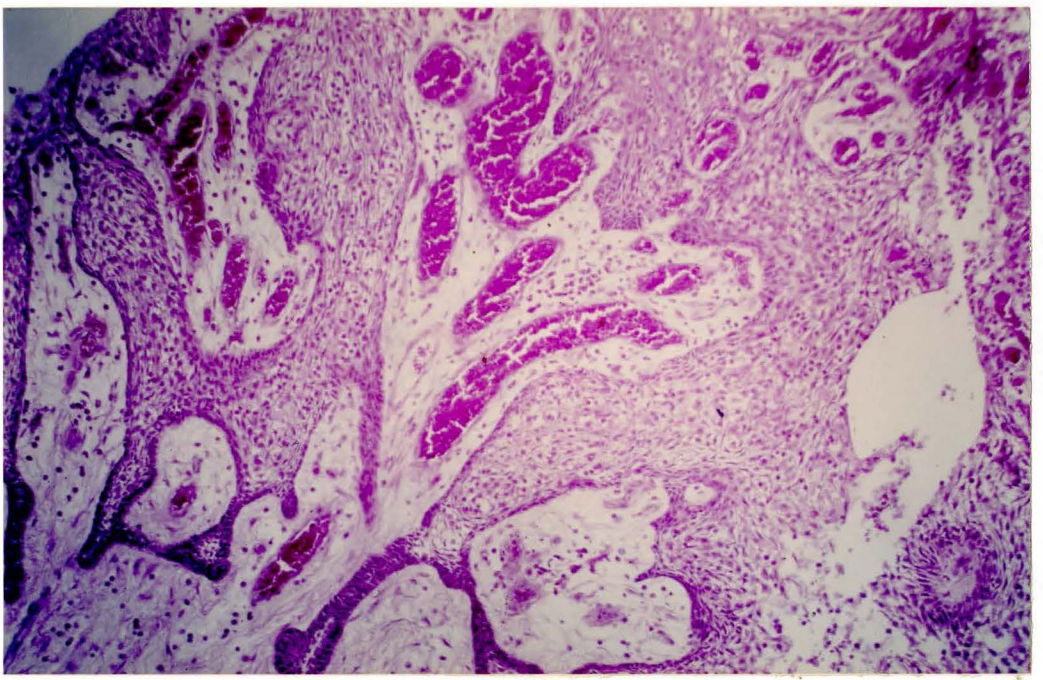

Figure 18. Unicystic ameloblastoma (H&E X 250)

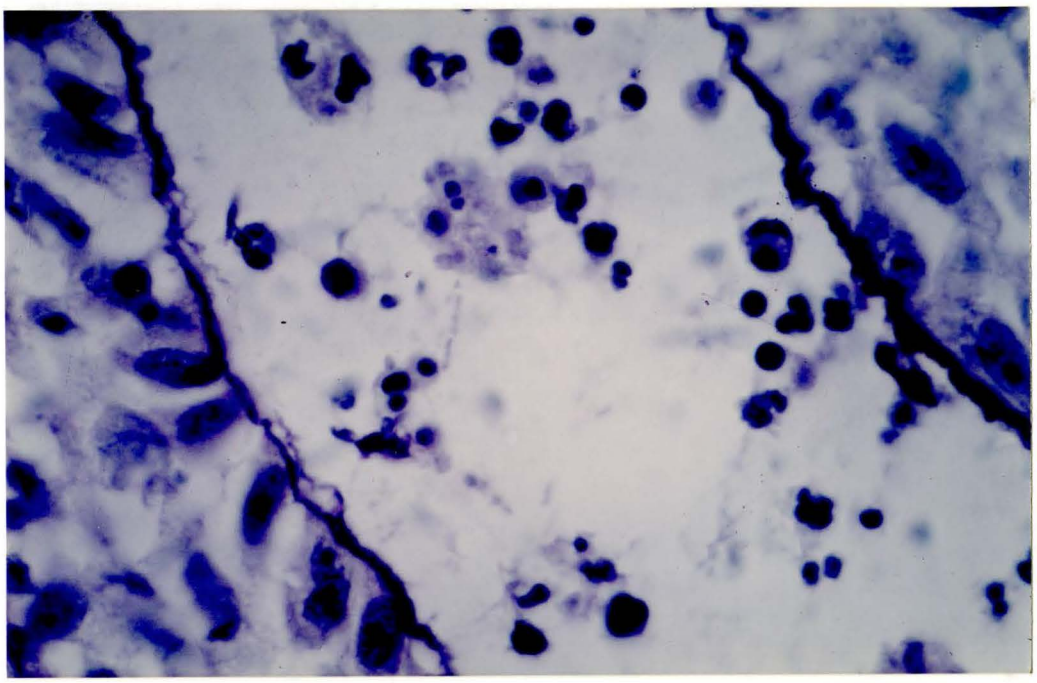

Figure 19. Plexiform unicystic ameloblastoma. Monospecific antibody to fibronectin is reactive at the site of basement membrane. Note inflammatory infiltrates. (PAP X 400)

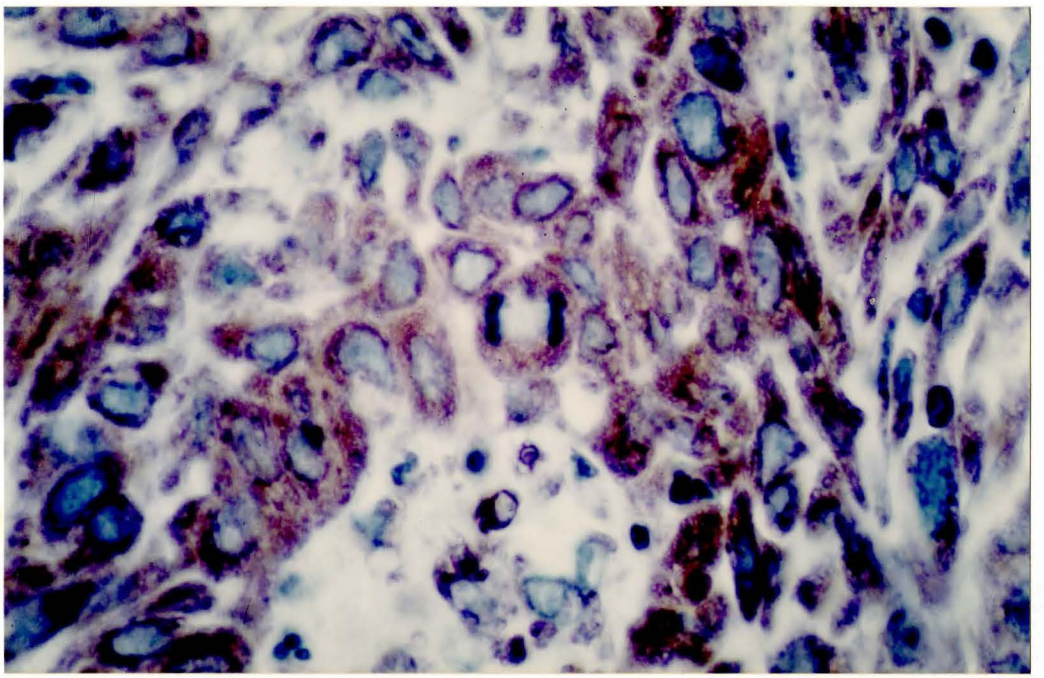

Figure 20. Plexiform unicystic ameloblastoma. The lectin Con A is positively reactive within the cytoplasm of the cells. (PAP X 400)

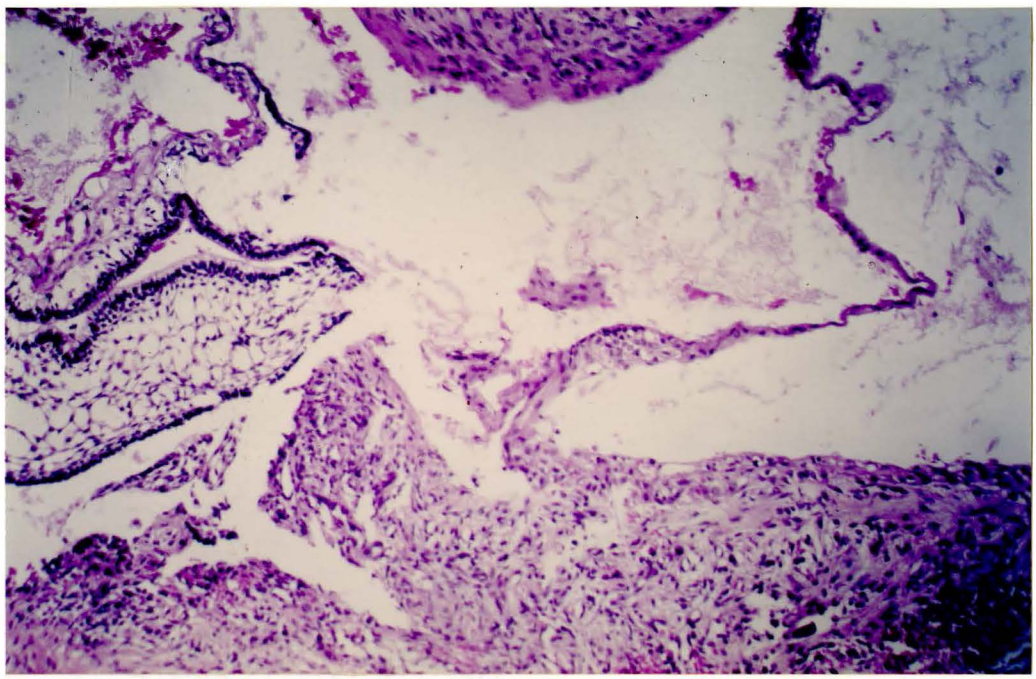

Figure 21. Coexisting follicular ameloblastoma and aneurysmal bone cyst (H & E X 125)

APPROVAL SHEET

The thesis submitted by Hasan Nadimi has been read and approved by the following committee:

Dr. Patrick Toto, Director
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Dr. Anthony Gargiulo
Professor, Periodontics Loyola

Dr. Hal McReynolds
Associate Professor, Histology, Loyola

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

June 14, 1985
Date

Patrick D Toto
Director's Signature