

**ADENOMATOID ODONTOGENIC TUMOUR –
INDUCTIVE TUMOUR OR HAMARTOMA WITH
METAPLASTIC MINERALISATION?**

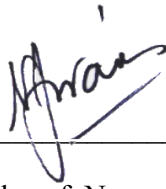
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A research report submitted to the Faculty of Health Sciences, University of the
Witwatersrand, in partial fulfilment of the requirements for the degree
Of
Master of Science in Dentistry

Johannesburg, 2005

DECLARATION

I, Vibha Jivan declare that this research report is my own work. It is being submitted for the degree of Master of Science in Dentistry to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

A handwritten signature in dark ink, appearing to read 'Vibha Jivan', is written over a horizontal line.

9th day of November, 2005

DEDICATION

In memory of my brother

Heeran Jivan

1972-1995

ABSTRACT

There is considerable confusion regarding the origin and classification of the AOT with the most recent WHO classification including the AOT as a non-inductive tumour or hamartoma showing metaplastic mineralisation. This study reviewed the clinical and epidemiological features of 51 AOTs retrieved from the archives of the Division of Oral Pathology, University of the Witwatersrand. In addition a detailed histological analysis, including histochemical and immunohistochemical investigations, was undertaken with a view to provide evidence for induction in AOTs.

4µ haematoxylin and eosin sections were examined. Selected cases were stained with PAS, alcian blue at pH 2.5, Congo red, reticulin, mucicarmine, von Gieson, Masson's trichrome and Prussian blue. Melanin bleach was performed on certain sections. Immunohistochemistry was performed in the presence of adequate preparations and controls with MNF 116 and Vimentin antisera.

Analysis of the clinical and epidemiologic data revealed that the AOT in our series had the same clinicopathological features as those reported from other parts of the world. This data will be included in a review article being prepared to commemorate the 100th anniversary of the description of this lesion.

AOTs occur in both follicular (64%) and extrafollicular forms (21%) most commonly in the anterior maxilla (62.7%) in females (63.6%) in the second decade (66.6%) where they are frequently associated with unerupted canines (42%). There is some evidence

suggesting that extrafollicular AOTs may originate in other odontogenic cysts and that this might explain why some AOTs grow to a large size and behave aggressively causing root resorption and expansion.

Histologically the unique and important presence of tall columnar cells resembling ameloblasts or odontoblasts were identified in 5 cases of AOT. These cells were arranged in a circular configuration and were actively secreting PAS positive material, which we have interpreted as dental matrix material. We have called these '*circular secretory units*'. The tall columnar cells did not always surround the entire secretory unit suggesting either that there was a variable rate of differentiation or that having completed their function these cells change shape and become unrecognisable. We regard these *circular secretory units* as providing definite evidence of induction. Further evidence of induction is provided by the presence of clusters or strands of odontogenic epithelium intimately associated with a lace-like pattern of dental matrix material. No evidence of residual ectomesenchyme was found, but this does not rule out the possibility that induction has indeed occurred.

We can also find no evidence linking the *circular secretory units* with the pseudo-ductular spaces, which characterise the AOT.

In conclusion, based on our observations, we recommend that the AOT be classified as a benign tumour with inductive capacity.

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TABLE OF CONTENTS

DECLARATION.....	ii
DEDICATION.....	iii
ABSTRACT.....	iv
ACKNOWLEDGEMENTS.....	vi
TABLE OF CONTENTS.....	vii
LIST OF FIGURES.....	ix
LIST OF TABLES.....	xiv
1.0 INTRODUCTION.....	1
1.1 Ectomesenchyme.....	2
1.2 Epithelio-Ectomesenchymal Interactions – Induction.....	2
2.0 AIMS.....	5
3.0 LITERATURE REVIEW.....	6
3.0 Subtypes of the AOT.....	6
3.1 Nature and Histogenesis.....	6
3.2 Epidemiology.....	8
3.3 Clinico-pathological features of the AOT.....	10
3.4 Special Investigations.....	15
3.5 Behaviour and Treatment.....	17

4.0	MATERIALS AND METHODS.....	19
4.0	Biopsy Material.....	19
4.1	Histology and Histochemistry.....	19
4.2	Immunohistochemistry.....	20
4.3	Evaluation.....	21
4.4	Ethical Considerations.....	21
5.0	RESULTS.....	22
5.0	Clinical and Epidemiological.....	22
5.1	Macroscopic.....	33
5.2	Histology.....	33
5.3	Histochemistry.....	48
5.4	Immunohistochemistry.....	55
6.0	DISCUSSION.....	57
7.0	CONCLUSION.....	66
8.0	REFERENCES.....	67

LIST OF FIGURES

Fig.1 Clinico-pathologic variants of the AOT (n = 51).....	23
Fig.2 Radiograph of a surgical specimen of a follicular AOT showing flocculent radiopacities.....	24
Fig. 3 Panoramic radiograph showing a large AOT in the symphyseal region of the mandible. An unerupted tooth is contained within the lesion which shows a ‘cotton wool’ type of opacification.....	24
Fig. 4 Occlusal plane radiograph of the lesion depicted in Fig. 3. Note the buccal and marked lingual expansion and ‘ground glass’ appearance of the radiopacification.....	25
Fig. 5 Panoramic radiograph of an extrafollicular variant of the AOT extending from tooth 13 to tooth 17 and causing root divergence and resorption.....	25
Fig. 6 Histogram showing the age distribution of the AOT.....	27
Fig. 7 Histogram showing the preponderance of females in nearly all age groups except in the 5 th decade.....	27
Fig. 8 Histogram depicting gender distribution of the follicular and extrafollicular variants of the AOT.....	29
Fig. 9 Site distribution of follicular and extrafollicular variants of the AOT.....	30
Fig. 10 Histogram showing the association of the AOT to different teeth.....	32
Fig. 11 Gross morphology of cystic follicular AOT which has an enveloped tooth within the lesion.....	35

Fig. 12 Cut surface of an AOT showing a thick well-defined capsule and contents which have a distinct likeness to tapioca. A tooth is embedded in the lesion.....	35
Fig. 13 Cut surface of a solid AOT. Note that the tumour is attached to the neck of an unerupted tooth and the resemblance of the contents to tapioca.....	35
Fig. 14 Early AOT formation in the wall of a large cyst. Nodules of tumour tissue appear to originate in the wall of the cyst before bulging into the lumen, where proliferation and coalescence probably occurs (H & E, original magnification X4).....	36
Fig. 15 Early AOT originating in the wall of a dentigerous cyst or dilated follicle. Much of the cyst is lined by reduced enamel-like epithelium. The newly formed tumour is bulging into the cyst lumen which may eventually become filled with tumour tissue (H & E, original magnification X4).....	36
Fig. 16 Low power view showing lobular architecture, large pseudo-ductular structures, cribriform lace-like areas and large PAS positive amorphous globular masses (PAS, original magnification X4).....	37
Fig. 17 Rosette showing PAS positive basement membrane-like material creating a layered appearance (PAS, original magnification X40).....	37
Fig. 18 Cribriform or lace-like area showing cords of cells surrounding loose, oedematous stroma containing dilated thin walled blood vessels (H & E, original magnification X40).....	38
Fig. 19 Numerous scattered PAS positive tumour droplets or globules in amongst the sheets of spindled epithelial cells (PAS, original magnification X20).....	38

Fig. 20 Large and small pseudo-ductular structures lined on the luminal aspect by PAS positive layer or rim. The rosettes show a prominent PAS positive basement membrane-like material resulting in a ‘layered’ appearance (PAS, original magnification X40).....	39
Fig. 21 Areas of what appear to be dystrophic calcification were a common feature. The tissue was not decalcified resulting in this “shattering” artefact. Areas of stromal hyalinisation (arrows) can also be seen at the one edge (PAS, original magnification X40).....	41
Fig. 22 Amorphous eosinophilic globular masses were present in only a few cases. Clusters of epithelial cells (arrowed) can be seen in amongst the cords of calcified material indicating an inductive influence (H & E, original magnification X10).....	42
Fig. 23 High power view showing clusters of epithelial cells intermingled with cords of dental matrix material in a lace-like pattern (H & E, original magnification, X40).....	42
Fig. 24 The dental matrix material stained positively with the PAS stain and frequently showed a calcospherite pattern of calcification reminiscent of normal dentine. Note the tall columnar cells (arrowed) actively secreting this product (PAS, original magnification X20).....	43
Fig. 25 Several circular structures (arrowed) consisting of a single layer of peripherally palisaded tall columnar cells surrounding a central lumen and secreting PAS positive dental matrix material (PAS, original magnification X20).....	43
Figs. 26a and 26b A partly intact <i>circular secretory unit</i> . Note the peripherally placed tall columnar cells (arrowed) and the secretion by these cells of PAS positive dental matrix material (PAS, original magnification X20 and X40 respectively).....	44

Fig. 27 Several <i>circular secretory units</i> lined in part by tall columnar cells showing secretory activity (arrowed). On the other side the columnar cells having completed their function have disappeared (PAS, original magnification X40).....	45
Fig. 28 Note how the cells on one side of the secretory unit are fully differentiated and actively secreting (arrowed) while on the other side they are less differentiated (bold arrows) and may already have completed their function (H & E, original magnification X40).....	45
Fig. 29a A different case similarly showing <i>circular secretory units</i> . In the larger unit (arrowed) the row of columnar cells is incomplete while in the smaller unit (bold arrows) the tall columnar cells completely surround the newly formed dental matrix material (H & E, original magnification X20).....	46
Fig. 29b Higher power view of previous case showing the basally placed nuclei, eosinophilic homogenous cytoplasm and resemblance of these cells (arrowed) to actively secreting odontoblasts (H & E, original magnification X40).....	46
Fig. 30 Another case showing a single large secretory unit. Note the secretory activity of the tall columnar cells (arrowed) (PAS, original magnification X40).....	47
Fig. 31 A smaller secretory unit. In this case the dental matrix material has calcified. Note the actively secreting tall columnar cells (arrowed) (PAS, original magnification X40).....	47
Fig. 32 The inner layer of the pseudo-ductular structures stained positively with a variety of histochemical stains. In this instance the ‘luminal rim’ is staining with alcian blue (Alcian blue, original magnification X20).....	51
Fig. 33 The von Gieson stain colouring the ‘luminal rims’ red in this instance (von Gieson, original magnification X40).....	51

Fig. 34 The globular masses showing scattered ‘salmon pink’ positivity with the Congo red stain (Congo red, original magnification X20).....	52
Fig. 35 With polarised light the Congo red positive areas showed characteristic apple-green birefringence (Congo red, original magnification X20).....	52
Fig. 36 Extensive melanin deposits in the epithelial cells are visible on this photomicrograph (H & E, original magnification X40).....	53
Fig. 37 Reticulin positivity on the inner aspect of the pseudo-ductular structures (Reticulin, original magnification X10).....	53
Fig. 38 The material on the inner aspect of the pseudo-ductular structures stained positively with mucicarmine (Mucicarmine, original magnification X40).....	54
Fig. 39 In this instance Masson’s trichrome stained the luminal rims a bright green colour. The basement membrane material in one of the rosettes (arrowed) stained a similar colour (Masson’s trichrome, original magnification X40).....	54
Fig. 40 Diffuse, moderately intense staining of both spindle epithelial cells and epithelioid cells in the rosettes and lining the pseudo-ductular structures (MNF 116, original magnification X20).....	56
Fig. 41 Staining with vimentin was largely restricted to stromal cells (Vimentin, original magnification X20).....	56

LIST OF TABLES

Table 1. Age and gender distribution of 51 cases of AOT.....	26
Table 2. Gender distribution of the follicular and extrafollicular variants of the AOT (n = 51).....	28
Table 3. Site distribution of the follicular and extrafollicular variants of the AOT.....	30
Table 4. Specific location of the AOTs in relation to teeth and adjacent anatomical structures.....	31
Table 5. Follicular AOTs in association with both deciduous and permanent teeth.....	32

CHAPTER 1

1.0 INTRODUCTION

The adenomatoid odontogenic tumour (AOT) is a benign epithelial odontogenic tumour that has long been the subject of debate with regard to its origin and terminology. It has variably been referred to as epithelioma adamantinum, adenoameloblastoma and odontogenic adenomatoid tumour. In 1969 Philipsen and Birn¹ introduced the term adenomatoid odontogenic tumour which was adopted by the WHO in 1971² in the first edition of their classification of odontogenic tumours. In the 1992 version of the WHO classification³ the AOT was classified as a tumour of “odontogenic epithelium with odontogenic mesenchyme with or without dental hard tissue formation”. Recently however, doubts have been expressed about the tumour’s inductive capacity. It is now suggested that this tumour is not a true neoplasm but a hamartoma instead, and that the presence of dental hard tissue within the tumour is not due to induction but is rather a metaplastically produced mineralisation.⁴ Thus, these authors recommended that its classification be revised and grouped it under the heading of odontogenic tumours “which arise from odontogenic epithelium with mature fibrous stroma, odontogenic ectomesenchyme not present”. They argued that the AOT was not characterised by having a stroma composed of odontogenic ectomesenchyme. The rather scant AOT stroma was that of a mature fibrous variety and thus was not of a type that could lead to inductive phenomena. Their recommendation was supported by the work of Gao *et al.*⁵ who had found no evidence of bone morphogenetic protein (BMP) in the AOT while tumours such

as ameloblastic fibrodentinoma and compound odontoma, amongst others, which do show inductive phenomena, were positive for BMP.

1.1 Ectomesenchyme

Beneath the epithelial lining of the primitive stomodeal cavity, the embryonic connective tissue is covered by a cellular band of approximately two to three cell layers in thickness. This layer is infiltrated by migrating neural crest cells hence it has been termed 'ectomesenchyme', which plays a vital role in odontogenesis.⁶

At first, the odontogenic potential is found in the epithelium of the first arch. If this epithelium is combined with caudal or cranial neural crest in the anterior chamber of the eye, teeth will form. After 12 days of development, the odontogenic potential is transferred from the first arch epithelium to the ectomesenchyme. Now tooth formation can occur from various epithelia. For example, combining the first arch ectomesenchyme with embryonic plantar (foot) epithelium will result in the formation of an enamel organ in the footpad. This is due to the expression pattern of the transcription and growth factors in these tissues.⁶

1.2 Epithelio-Ectomesenchymal Interactions – Induction

A series of complex epithelio-ectomesenchymal interactions are the key elements during tooth development. These interactions lead to one tissue inducing changes and initiating development of another tissue. The most basic example of the above is seen in the first stages of odontogenesis, when the ectomesenchyme induces the overlying oral ectoderm to change and form the primary epithelial band that thereafter forms tooth buds.⁶

Other examples include cells of the inner enamel epithelium undergoing morphological changes that induce the cells of the neighbouring dental papilla to differentiate from undifferentiated mesenchymal cells to large dentine-secreting odontoblasts.

It is well known that odontoblast development and dentine formation cannot occur without the inductive influence from the cells of the inner enamel epithelium. Prior to the formation of the first dentine layer, the differentiating cells of the inner enamel epithelium secrete enamel proteins. These combine with several growth factors and are responsible for the epithelio-mesenchymal signalling that leads to the terminal differentiation of the odontoblasts.⁶

Once dentine secretion begins, it induces the inner enamel epithelium to differentiate into ameloblasts. Enamel matrix is produced by the ameloblasts and is deposited adjacent to the newly formed dentine layers. The entire process depends on communication between the differentiating odontoblasts and ameloblasts. This is believed to occur via chemical signals between the two cell populations and is an example of reciprocal induction.⁶

What is the nature of these inductive signals? It has been found that the epithelial cells of the inner enamel epithelium express and secrete a variety of growth factors, viz. transforming growth factor- β 1 (TGF- β 1), BMP-2 and insulin-like growth factors. On the other hand, the ectomesenchymal cells of the dental papilla can only become competent once a precise number of cell divisions have occurred. Thereafter, they are able to express the specific cell surface receptors to enable them to respond to the growth factors.⁶

There is very little information available about the regulatory mechanisms of the signalling proteins and it would be a mammoth task to untangle the web of regulatory events, because there are at least 12 transcription factors expressed in odontogenic mesenchyme and at present more than 90 genes have been identified that control the oral epithelium, dental epithelium and dental mesenchyme during the actual initiation of tooth development only.⁶

Dental ontogeny is recapitulated by odontogenic tumours and they are replete with similar examples of epithelio-mesenchymal interactions and reciprocal induction. Ameloblastic fibromas and fibro-odontomas are perfect examples to demonstrate these inductive influences, which are probably under the control of similar signalling proteins.

CHAPTER 2

2.0 AIMS

The aim of this study is two-fold:

Firstly, to review the clinical and epidemiological features of a large series of cases of AOT from South Africa so as to contribute this data to an international collaborative study aimed at commemorating the 100th anniversary of the first description of this lesion by definitively defining its clinico-pathologic characteristics.

Secondly, to undertake a detailed histological analysis, using both histochemical and immunohistochemical techniques with the specific intention of identifying any features which might provide evidence of inductive processes occurring in the AOT.

CHAPTER 3

3.0 LITERATURE REVIEW

3.1 Subtypes of the AOT

There are 2 subtypes of the AOT, namely, central and peripheral. The central (intra-osseous) type is further found in either a follicular (tooth associated/embedded) or an extrafollicular (no tooth associated/embedded) form. The peripheral type is found extra-osseously i.e. in the gingiva.

Central AOTs are the most common type (97.2%) with the follicular variant comprising 73% of these. The follicular variant is associated with an impacted tooth (sometimes several teeth) and the maxillary canine is most often involved.⁷ Any tooth may be involved including permanent molars,⁸ third molars,⁹ deciduous teeth¹⁰ and even supernumerary teeth.¹¹ The extrafollicular variant most commonly occurs in the maxilla either above the roots, in between the roots, or superimposed on the roots of the teeth. The peripheral variant occurs almost exclusively in the anterior maxillary gingiva.¹²

3.2 Nature and Histogenesis

The early literature regarded the AOT as a variant of the ameloblastoma, hence the term adeno-ameloblastoma. However, in 1971, the WHO² classified the AOT as a separate entity. Some authors believe that it originates as a developmental or hamartomatous process,^{13, 14} while others support the concept that it is a true neoplasm.^{8, 15, 16}

Regardless of the true nature of the AOT, it is agreed that the tumour is derived from odontogenic epithelium. This is evident from the fact that it occurs only in tooth-bearing areas of the jaws, is frequently related to embedded teeth and histologically shows features which have histological resemblance to parts of the enamel organ.¹⁵

Various odontogenic epithelia have been suggested as potential sources of origin of the AOT ranging from the dental lamina, reduced enamel epithelium, the enamel organ and the stratum intermedium.¹⁷⁻¹⁹

Philipsen *et al.*⁸ postulated origin from the dental lamina since it is the only odontogenic epithelium which satisfies the requirements of widespread distribution necessary to conceptualise a unified source of origin for the diverse locations of the AOT. On completion of odontogenesis, the dental lamina complex disintegrates, but its epithelial remnants can still be found in the jaw bones and gingiva. These remnants are located only in the gubernaculum dentis, which is connected from the bony crypts of developing tooth buds to the lamina propria of the gingiva via a series of gubernacular canals. They proposed that the follicular variant originates from remnants of the successional dental lamina in the gubernacular canals and that as the tooth erupts fusion of the tumour with the follicle occurs – but that if the tumour develops from epithelial remnants situated at the periphery of the gubernacular canals the erupting tooth may bypass the developing tumour resulting in an extrafollicular variant. The peripheral variant similarly arises from epithelial remnants of the successional dental lamina which are found in the gingiva.⁸

3.3 Epidemiology

3.3.1 Incidence

The incidence and prevalence of AOTs is difficult to estimate because there is insufficient data available. Most research has been based on numbers of cases in oral pathology biopsy services.¹⁵ In biopsy material, the AOT is ranked as the fourth most common odontogenic tumour but it is nevertheless rare as it constitutes a mere 2.2% to 6.8% of all odontogenic tumours.^{7, 12, 16} The only available data on the incidence of the AOT comes from South Africa where Shear & Rachanis²⁰ reported an annual incidence of 0.11 cases per million population per year in the Witwatersrand area.

3.3.2 Geographical Distribution

In a review of 499 literature cases, Philipsen *et al.*¹⁵ showed that these cases were reported virtually from all parts of the globe. Almost half of these cases were from Asia. A relatively high frequency has also been reported from Nigeria.¹² This is probably coincidental and does not represent a racial predilection.

3.3.3 Age Distribution

The age of occurrence of the AOT is wide, ranging from 3 to 82 years,^{10, 12} but there is a marked preponderance of cases in the second decade of life with 68% of cases occurring during this time period. The tumour can remain undetected for many years possibly explaining the occurrence in older patients.

A relationship between the tumour variants and age has also been determined with follicular variants being diagnosed at an earlier age. The reason for this might be that the

patients are more likely to present to a dentist at an earlier age regarding the absence of a particular tooth, for example; a missing canine, than if a full complement of teeth were present. The peripheral type is also diagnosed early as it often presents on the gingiva of the anterior maxilla as an epulis resulting in the patient seeking dental advice.¹⁵

3.3.4 Gender Distribution

There is a definite predilection for women with an overall F:M ratio of 1.9:1.^{7, 12, 15-18} In Asia, the female predominance is even more marked with a F:M ratio of 3:1.^{12, 21} This female predominance is most marked in patients in their second decade (3.9:1), while a male predominance of 0.6:1 was observed in the third decade.¹⁵ The possible reason for this male predominance remains unknown. It is interesting to note that in a series reported from Africa there was an equal distribution in males and females.²²

Once again, Philipsen *et al.*¹⁵ could determine a link between gender and tumour variants, as follows:

- Follicular type: female: male = 1.9:1
- Extrafollicular type: female: male = 1.7:1
- Peripheral type: Generally females are more frequently affected but statistics are not available as too few cases have been documented.

3.3.5 Site/Location

The maxilla is affected twice as often as the mandible.^{7, 15-18} Again a relationship between location and age can be established. Lesions occur more frequently in the maxilla in the first, second and third decades, but in the fourth decade, the mandible is more frequently

affected.¹⁵ Lesions tend to occur more often in the anterior part of the jaws. The canine region is involved in almost 60% of all cases. In a series of cases reported from Africa an equal occurrence of cases in the maxilla and mandible as well as a preponderance of cases from the posterior regions was reported.²²

3.3.6 Race

No link between race and occurrence of AOTs has been found. Philipsen & Reichart¹² and Philipsen *et al.*¹⁵ have found that there was a widespread distribution of the tumour globally.

3.4 Clinico-pathological features of the AOT

3.4.1 Clinical Features

The tumour usually presents as a slow growing painless mass and expansion of the cortical bone is common. There is usually concern related to a missing or unerupted tooth. Altered nerve sensation is not a feature. Size of the lesions usually range from 1-3cm in diameter for the central variants but larger AOTs can occur.^{7, 15-17} Two cases, both measuring in excess of 7cm have been reported by Raubenheimer *et al.*²³ Occasionally, AOTs can be characterised by advanced root resorption, displacement of teeth, displacement of the mandibular nerve, hyperaesthesia, extensive destruction of bone and extension into adjacent structures such as maxillary antrum, nasal passage, orbital floor and even the intracranial space.²⁴ In the presence of such behaviour malignancy must be considered, although, only one case of a truly malignant lesion has been reported.²⁴

3.4.2 Radiographic Features

The central variants usually present as a well-defined unilocular radiolucency but multilocular lesions have been reported by Tsaknis *et al.*¹⁰ and Giansanti *et al.*²⁵ A possible explanation for this could be that two AOTs may have developed concurrently and then fused. In the follicular variant, the AOT resembles a dentigerous cyst as it contains an embedded tooth within the lesion. Sometimes there may be more than one tooth involved. The radiolucency may surround only the crown of the tooth or both the crown and part of the roots. The tooth is frequently displaced. Radiopaque foci may be seen due to the calcifications present in the lesion. The opacities are usually small and are described as ‘flocculent’.²⁶ Much larger coarse opacities may occur. Mild erosion of the cortical bone may be present in the peripheral variant, which usually shows very little radiographic change.^{7, 15-17}

3.4.3 Macroscopic Features

The AOT is circular in shape and has a fibrous capsule that is rather thick.¹⁵ The lumen is filled with grey/white tissue but may be partly or wholly cystic. Tiny calcifications may also be present, thus upon sectioning, it is gritty. The cut section often has a strong likeness to tapioca.⁷ If the lesion is cystic then the macroscopic appearance changes: the lumen would contain modest amounts of fluid, usually straw-coloured. The capsule would still be thick and well-defined.^{7, 17, 27} In the case of the follicular variant, an unerupted tooth will also be present with the lesion frequently being attached to the neck of the tooth.^{8, 15}

3.4.4 Microscopic Features

The tumour has a lobular architecture and the histology remains the same in all variants.

The following cell types can be observed:

- 1) Solid nodules of spindle or cuboidal epithelial cells which vary in size but are arranged in rosette-like formations. There are hardly any traces of stromal connective tissue. Eosinophilic material in the form of droplets or globules may be seen within the rosette-like structures while basement membrane-like material creates a layered appearance.¹⁵
- 2) Sheets of spindle cells or polygonal cells with dark eosinophilic cytoplasm and round vesicular nuclei fill in the spaces between the rosettes. The eosinophilic globules are also widely scattered in and amongst the sheets of epithelial cells.¹⁵
- 3) Tubular or duct-like spaces can usually be seen within the sheets of spindle cells. They are lined by a single palisaded layer of cuboidal or low columnar epithelium. A distinguishing feature to note is the polarised ovoid nuclei orientated away from the lumen. Eosinophilic material and cell debris may be present in the lumen or the lumen may be empty. These duct-like spaces may differ considerably in diameter and may sometimes be absent, hence their presence is not pathognomic for AOTs.¹⁵
- 4) Islands of squamoid appearing epithelial cells that are polygonal and eosinophilic may be present and may contain abundant amorphous material that is said to resemble amyloid. The cells have well-demarcated boundaries and intercellular bridges. This particular histological appearance has resulted in the concept of a 'combined AOT-CEOT lesion', but this concept is not tenable as the so-called hybrid lesions do not differ in behaviour to conventional AOTs. This appearance is regarded as part of the histomorphologic spectrum of the AOT.^{15, 28}

- 5) A trabecular, plexiform or cribriform epithelial pattern is present between the nodules and connecting the nodules. This pattern is created by strands of epithelium 1-2 cell layers thick that are intertwined, and separated by a haemorrhagic oedematous stroma. The cells may be round or flattened and their nuclei are tiny, hyperchromatic and round.¹⁵ A scanty loose connective tissue stroma consisting of several small blood vessels, usually thin-walled and congested may be visible between the epithelial cells.^{7, 15-17, 29, 30}
- 6) Calcified material is present in many of the lesions. These can take on a variety of configurations namely; irregular dystrophic deposits, concentric layers or amorphous globular eosinophilic masses.

In addition to these classical cell types, Philipsen & Reichart¹² mention the presence of tall columnar cells resembling ameloblasts adjacent to small and large masses of calcified bodies or globules but do not provide any further details nor do they reference the source of this description. Reference to similar tall columnar cells is made by Allen *et al.*⁹ and by Nomura *et al.*²⁴ but they appear to be describing odontomes associated with AOTs or hybrid lesions, which the latter authors termed adenomatoid dentinoma. Two single case reports of AOTs showing induction are of special interest. In the first, the authors report the presence of tubular dentine within typical pseudo-ductular structures which characterise the AOT although the photomicrographs are not clear. They do also illustrate a row of very tall columnar cells with eosinophilic cytoplasm and polarised nuclei abutting on enamel matrix.⁵³

In the second, in addition to characteristic histologic findings of AOT calcified dysplastic dentine closely associated with a row of tall columnar cells and mesenchymal cells is illustrated. A small amount of immature enamel is formed between the columnar epithelial cells and the osteodentine.⁵⁴

3.4.5 Ultrastructural Features

The ultrastructure of the AOT can be divided into 4 main components, viz. the three cell types seen histologically and the calcified or unmineralised deposits. Type I cells, which are the main constituents of the nodules and rosettes, have few organelles, well developed gap junctions and interdigitate with each other via desmosomes. Type II cells, which are the cells constituting the duct-like structures, have more organelles and cytoplasm as well as lysosomes and glycogen. Type III cells, which represent the CEOT-like areas, are similar to the spinous layer of stratified squamous epithelium with bundles of tonofilaments and well-developed desmosomes.²⁹

The eosinophilic tumour droplets are only found extracellularly. The size, shape and periphery of the tumour droplets are variable. The borders are tightly adherent to the epithelial cell membrane. The nature of the tumour droplets can be either homogenous or granulo-tubular. It has been suggested that the tumour droplets most probably represent some form of enamel matrix. If this is indeed the case then the fact that enameloid secretion has occurred distant from typical secretory epithelium establishes the concept of hamartomatous odontogenesis or metaplastic formation of dental matrix material.²⁹

The exact nature of the amorphous globular masses has not been adequately determined. Several theories exist regarding their origin and composition. Some authors believe that the non-calcified deposits represent an 'abortive form of pre-enamel',²⁹ while others relate the eosinophilic masses to a mesenchymal derivative like dentine or cementum or even a form of dystrophic calcification.^{16, 31}

Lee³¹ suggested that the eosinophilic amorphous masses of the AOT could be divided into 3 components i.e. 1) amyloid-like material, 2) dystrophic calcification, and 3) degenerate tumour cells with dentine-like material. El-Labban³² expanded on this work in an electron microscopic and histochemical study. She found that the tumour was positive for reticulin, especially on the inside of the duct-like structures and at the periphery. The amyloid stains showed positive reactions in the majority of sections. El-Labban³² further reported that the eosinophilic amorphous masses were ultrastructurally heterogenous and consisted of three types of fibrils: thin collagen fibrils, electron dense fibrils probably resulting from degradation of collagen and masses of amyloid filaments. In all areas there was a peripheral layer of fine filaments perpendicular to the epithelial basal lamina similar to that found in early dentine formation. She speculated that this might be responsible for the reticulin staining found in this tumour. On the other hand the great majority of the laminated masses represented calcified amyloid. In an earlier study El-Labban and Lee³³ studied the blood vessels in 3 cases of AOT ultrastructurally. They found that 70-90% of the vessels in the stroma showed degenerative changes and concluded that this degeneration affected the structure of the collagen fibrils.

Several authors^{19, 31, 34, 35} have commented on the ultrastructure of the pseudo-ductular structures. All have shown that the 'luminal' surface of the ducts contain a granulo-fibrillar material which is separated from the adjacent tumour cells by a basal lamina-like zone, a finding which lends support to earlier theories³⁶ which suggested that the 'ducts' were formed as a result of degeneration of the stromal tissue. However, other authors have taken a different point of view and have concluded that ultrastructurally the duct lining cells have the features of pre-ameloblasts and that the ducts are a result of secretory activity.^{19, 34}

3.5 Special Investigations

3.5.1 Histochemical Findings

The central focus has been on the eosinophilic droplets, amorphous globular masses and duct-like structures. The eosinophilic droplets are reticulin and Periodic-acid-Schiff (PAS) positive but stain negatively for amyloid^{19, 36} while the amorphous masses are also PAS positive but stain positively for amyloid and show apple-green birefringence.³¹ The duct-like spaces show a rim of material which stains positively with haematoxylin and eosin (H & E), reticulin, PAS, alcian blue, toluidine blue, mucicarmine, von Gieson and Masson's trichrome. This array of positive stains led Shear³⁶ to postulate that the material in the ducts represented ectomesenchyme and induced pre dentine.

3.5.2 Immunohistochemical Findings

There have been relatively few immunohistochemical studies on the AOT. Tatemoto *et al.*³⁷ demonstrated co-expression of keratin and vimentin in the tumour cells at the periphery of the ductal and rosette structures. In studying the expression of cytokeratins in

AOT, Crivelini *et al.*³⁸ showed that the antibody against CK14 was the only one to label cytokeratin polypeptides in all tumoral epithelial cells. Vimentin stained some fusiform or ovoid cells close to calcified bodies. Saku *et al.*³⁹ and Mori *et al.*⁴⁰ demonstrated the presence of amelogenin and enamel in within the rosettes, in the hyaline droplets, at the periphery of the small mineralised deposits and in the tumour cells. Intraluminal hyaline material was negative for enamel and the calcified material was negative for keratin. Enamelysin (MMP-20) has also been demonstrated in AOT around the calcified droplets and in the mineralised deposits themselves, but not in the dysplastic dentine.⁴¹ All cell types except the polygonal cells stained positively for transferrin, ferritin and α 1-antitrypsin (α 1-AT). Lactoferrin, S-100 protein and α 1-antichymotrypsin (α 1-ACT) were absent in all cases. Transferrin and α 1-AT were also present in the calcified material but the amorphous masses reacted only with α 1-AT.¹⁶ Carcinoembryonic Antigen (CEA) has been shown to be absent in AOTs confirming the benign nature of this tumour as has p53 protein.^{42, 43}

BMP could not be demonstrated in AOTs suggesting that the calcified substances and amorphous materials were not related to dental hard tissues.⁵

3.6 Behaviour and Treatment

The AOT is a benign lesion characterised by slow expansile growth. Root resorption of neighbouring teeth is very rare and very seldom are adjacent teeth mobile as a result of the lesion. Few cases of AOT show somewhat aggressive behaviour extending into the maxillary antrum, the orbital floor, the ethmoid sinuses and the mandibular canal.^{24, 25, 44-47}

The tumour hardly ever recurs following enucleation.^{7, 16, 17} The recurrence rate of AOTs has been estimated as 1 in 500 (0.2%).⁴⁷ Toida *et al.*²¹ reported 2 cases of recurrences from a total of 750 cases in the Japanese literature. One of these cases was exceptional as it showed extension into the intracranial space. A recent case of multiple recurring AOT-like lesions has also been reported, but no consensus has been reached whether it is indeed a classical AOT or a new odontogenic tumour.⁴⁸

The tumour is usually removed via complete surgical enucleation. Due to its thick capsule, enucleation is relatively uncomplicated and successful. If a tooth, especially a canine is impacted in the AOT, the tumour can be successfully enucleated without removal of the impacted tooth. Subsequent orthodontic repositioning of the tooth may then be required.⁴⁹ Very rarely large mandibular AOTs may even require a partial en-bloc resection with reconstruction via an iliac bone graft.²⁴

CHAPTER 4

4.0 MATERIALS AND METHODS

4.1 Biopsy Material

Fifty-one cases of AOT were retrieved from the archives of the Department of Oral Pathology – University of the Witwatersrand. Clinical parameters of each case, viz. age, gender, site, race, clinical and radiographic features were obtained from the pathology request forms, where available and were recorded and analysed.

4.2 Histology and Histochemistry

For each case representative H & E stained sections were reviewed to confirm the diagnoses, to identify cases with calcified tissue and to seek histological evidence of induction of dental matrix material. The nature, distribution and relationship of the calcified tissue and dental matrix material to the odontogenic epithelium was studied and recorded.

Thirty cases were selected and stained with PAS, alcian blue at pH of 2.5 and Congo red. Occasional selected cases were also stained with reticulin, mucicarmine, von Gieson, Masson's trichrome, Prussian blue and subjected to a melanin bleach where deemed appropriate. Standard routine histochemical staining procedures were used with appropriate negative and positive controls. The sections were examined, using conventional light microscopy and polarised light in the case of sections stained with

Congo red. A multi-headed microscope was used allowing two examiners to view the sections simultaneously.

4.3 Immunohistochemistry

Sections were cut from paraffin wax embedded archived blocks and were mounted on 3-aminopropyltriethoxysilane-coated glass slides. They were stained immunohistochemically with the following antisera:

- a) Vimentin (Novocastra Ltd, Newcastle) dilution = 1:200; in order to assess the stromal component.
- b) Anti-human cytokeratin, clone MNF 116 (Dako, Glostrup, Denmark), dilution = 1:300; in order to confirm the epithelial nature of the cells closely associated with the dental matrix material. The Envision detection system (Dako, Glostrup, Denmark) was used for the streptavidin-biotin complex immunoperoxidase technique.

Deparaffinised sections (xylene, 2 x 5 minutes) were microwaved (800W, medium power) in 0.01M citrate buffer (pH 6.0) for 2 x 5 minutes. When cool (after 20 minutes), they were quenched in 3% methanol-hydrogen peroxide for 30 minutes, washed in water and rinsed in phosphate-buffered saline (pH 7.6), incubated with normal goat serum (1:5) for 20 minutes and then treated with the primary antiserum at room temperature for 1 hour. They were then incubated with biotinylated secondary antibody for 30 minutes. After washing with phosphate-buffered saline, the sections were further treated with streptavidin-biotin complex for 30 minutes at room temperature. They were then washed with phosphate-buffered saline. Bound peroxidase was visualised with 3,3'-diaminobenzidine hydrochloride (Sigma – Aldrich, South Africa) for 5 minutes, resulting

in a brown reaction product. Sections were counterstained with Meyer's haematoxylin (5 minutes). All reagent dilutions were prepared with 3% bovine serum albumin factor V (Boehringer Mannheim, Germany). Positive controls were used throughout the study. Negative controls were achieved by substituting the primary specific antibodies with non-immune serum.

4.4 Evaluation

The presence and distribution of immunohistochemically stained cells and the intensity, pattern and localisation of their staining were determined using conventional light microscopy. When immunostaining was detected, a descriptive grading of weak, moderate or intense was used. No statistical analysis of the histochemical and immunohistochemical results was deemed to be necessary and the results are presented in a descriptive manner.

4.5 Ethical Considerations

Blanket approval has been granted to the Department of Anatomical Pathology of which Oral Pathology is a Division, by the University of the Witwatersrand Human Ethics Committee (Clearance Certificate no: M00/08/29) for the use of archival tissue in immunohistochemical studies.

CHAPTER 5

5.0 RESULTS

5.1 Clinical and Epidemiological

5.1.1. Number of Cases

A review of the AOT cases accessioned in the archives of the Division of Oral Pathology, University of the Witwatersrand, yielded 51 cases from 1959 to 2004 representing 0.88 cases per year. There were no recurrences. The AOT was the 4th most frequent odontogenic tumour accessioned in the departmental archives after ameloblastoma, cemento-osseous dysplasia and myxoma.

5.1.2 Variants

No peripheral variants were found. All of the cases (n = 51) were centrally located. The follicular variant was most frequent with 33 cases (64%) identified. These cases were varied in respect of their relationship to the unerupted teeth, as follows: 1 related to the apex, 11 to the crown, 10 associated with the entire tooth, 1 involving only the root and 10 cases where the exact relationship to the tooth was not stated. Eleven cases (21%) were extrafollicular in type. Thus, central: 51 cases (100%), follicular: 33 cases (64%), extrafollicular: 11 cases (21%), unknown: 7 cases (14%), peripheral: 0 cases (0%) (Fig. 1).

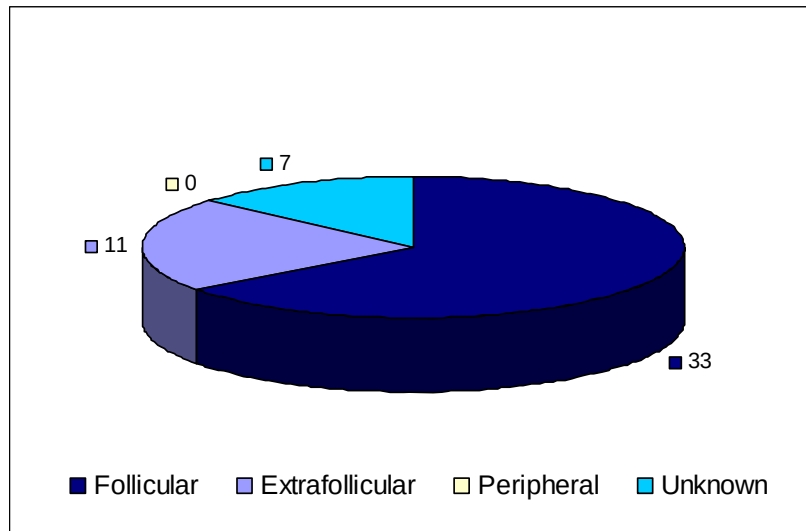


Fig. 1 Clinico-pathologic variants of the AOT (n = 51).

5.1.3 Radiographic

The radiographic appearance of the AOTs was variable. The usual presentation was that of a well-defined radiolucency with or without an impacted tooth/teeth. Very often, the AOT resembled a dentigerous cyst where the lesion was associated with the crown of an impacted tooth. Flocculent opacifications could sometimes be observed especially in intra-oral radiographs (Fig. 2), while in a few cases the calcifications could be described as ‘cotton wool-like’ or ‘ground glass’ (Figs. 3 and 4). Six cases recorded root resorption of neighbouring teeth (Fig. 5). Only 6 cases reported expansion of the cortex, i.e. 12%. Expansion in these cases was mainly buccal, although 2 cases each showed either buccal and lingual or buccal and palatal expansion (Fig. 4). A total of 8 cases showed displacement of adjacent or affected teeth. Five of these were of the follicular variant whilst the other 3 were extrafollicular AOTs. In 2 instances, there was displacement of the entire tooth/teeth to the floor of the nose. Likewise, in 3 other cases the associated teeth were displaced into the maxillary sinus.



Fig. 2 Radiograph of a surgical specimen of a follicular AOT showing flocculent radiopacities.



Fig. 3 Panoramic radiograph showing a large AOT in the symphyseal region of the mandible. An unerupted tooth is contained within the lesion which shows a 'cotton wool' type of opacification.

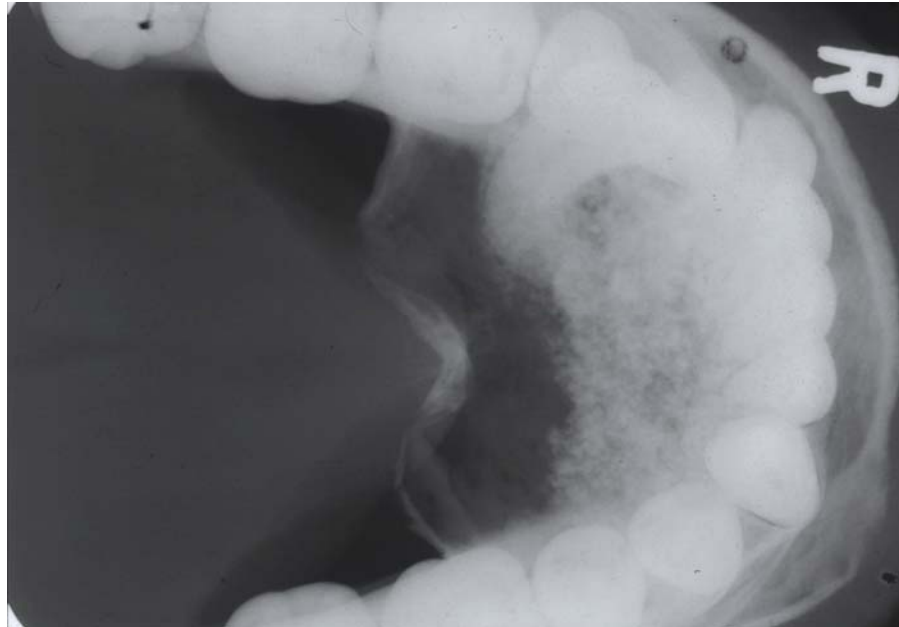


Fig. 4 Occlusal plane view of the lesion depicted in Fig 3. Note the buccal and marked lingual expansion and 'ground glass' appearance of the radiopacification.



Fig. 5 Panoramic radiograph of an extrafollicular variant of the AOT extending from tooth 13 to tooth 17 and causing root divergence and resorption.

5.1.4 Age

The age of the patients was recorded in only 42 cases. The mean age was calculated as 18.5 years; the median age was 15 years; the mode was 13 and 14 years as there was an equal occurrence of both. The age range was from 8 to 47 years. Most cases occurred in the second decade followed by the third and fourth decades (Table 1, Fig. 6).

Table 1. Age and gender distribution of 51 cases of AOT

Decade	Number	Female	Male	Unknown
1 st	1	1	0	0
2 nd	28	18	9	1
3 rd	8	5	3	0
4 th	4	2	2	0
5 th	1	0	1	0
Unknown	9	2	1	6
Total	51	28	16	7

The mean age for males was 20.35 years and for females 17.38 years. Both males and females showed a peak frequency in the second decade (Fig. 7). The mean age for the follicular variant was 18.2 years and for the extrafollicular variant 18.9 years.

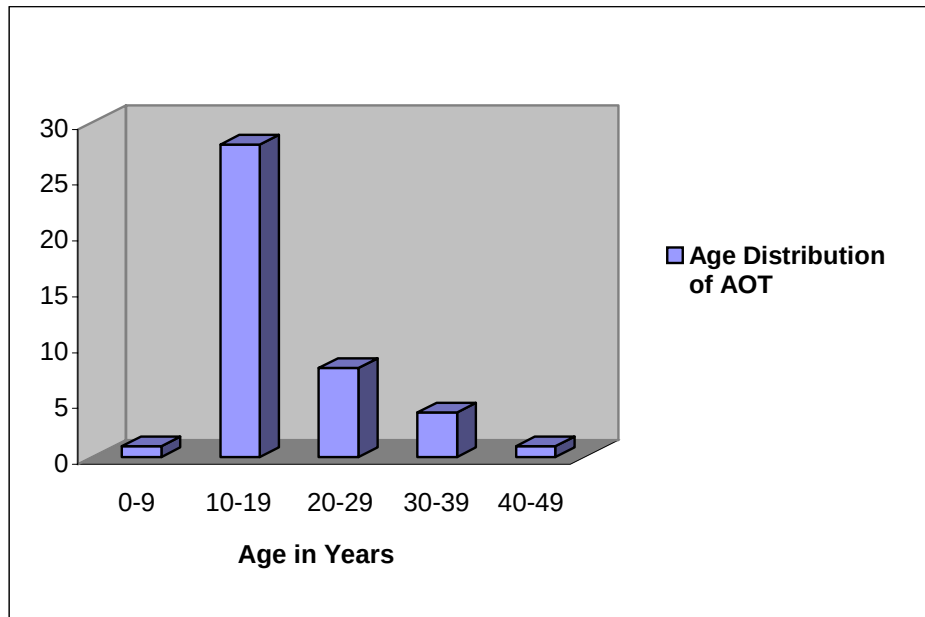


Fig. 6 Histogram showing the age distribution of the AOT

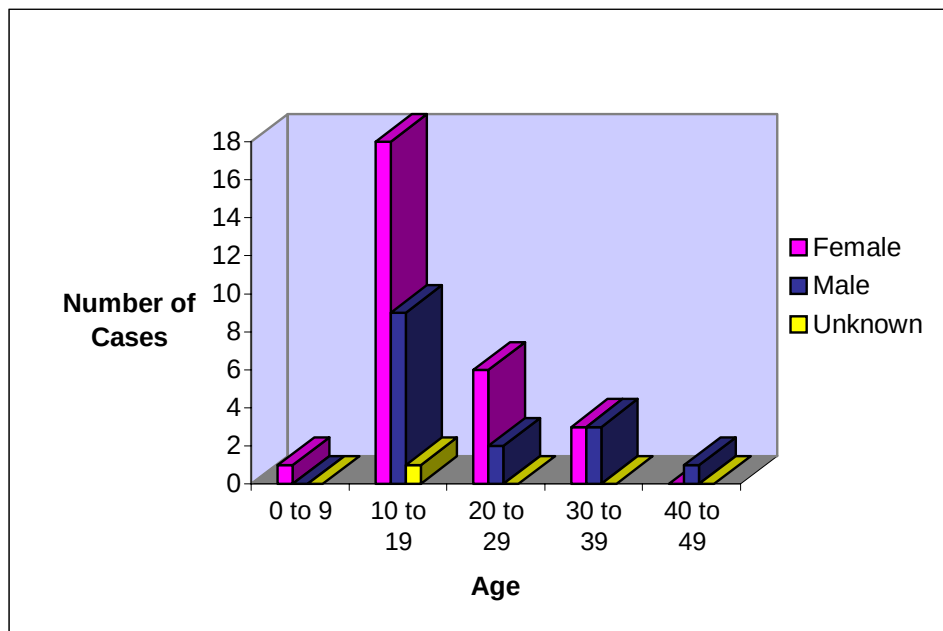


Fig. 7 Histogram showing the preponderance of females in nearly all age groups except in the 5th decade

5.1.5 Gender

The total number of females was 28 and of males 16. In seven cases the gender of the patient was not recorded (Female:Male 1.75:1). If gender was analysed according to the tumour variants, the F:M ratio for the follicular variant was 2:1 and for the extrafollicular variant was 1.6:1 (Table 2, Fig. 8).

Table 2. Gender distribution of the follicular and extrafollicular variants of the AOT (n = 51)

	Follicular	Extrafollicular	Unknown	Total
Female				
Count	18	8	2	28
Row %	64.3	28.6	7.1	100
Male				
Count	9	5	2	16
Row %	56.2	31.3	12.5	100
Unknown				
Count	4	0	3	7
Row %	57	0	43	100
Total	31	13	7	51
F:M Ratio	2:1	1.6:1		1.75:1 (n = 44)

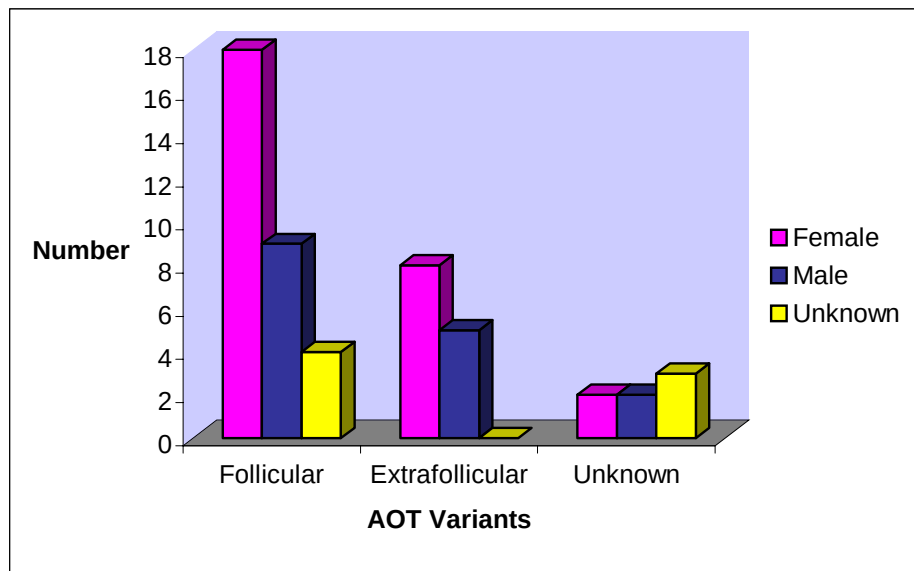


Fig. 8 Histogram depicting gender distribution of the follicular and extrafollicular variants of the AOT

5.1.6 Location

In this series of cases, 62.7% (32 cases) occurred in the maxilla and 21.5% (11 cases) in the mandible. In 8 cases the location was not stated. A ratio of 2.9:1 was calculated for the maxilla:mandible. The follicular variant occurred relatively more frequently in the maxilla than the mandible (max:mand = 3.9:1) whereas the extrafollicular variant was more evenly distributed between maxilla and mandible (max:mand = 1.6:1) (Table 3, Fig. 9).

Table 3. Site distribution of the follicular and extrafollicular variants of the AOT

	Follicular	Extrafollicular	Unknown	Total
Maxilla				
Count	24	7	1	32
Row %	75	21	3	100
Mandible				
Count	7	4	0	11
Row %	63	36	0	100
Unknown				
Count	2	0	6	8
Row %	25	0	75	100
Total	33	11	7	51
				N=44
				Max:Mand=2.9:1

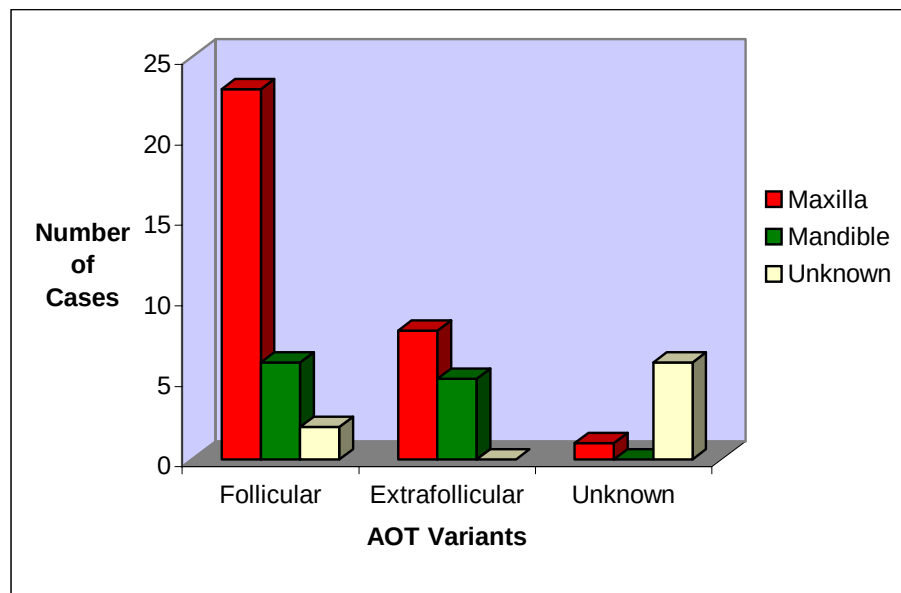


Fig. 9 Site distribution of follicular and extrafollicular variants of the AOT

Table 4. Specific location of the AOTs in relation to teeth and adjacent anatomical structures

Site	Exact Location	Number	Percentage
Maxilla	Anteriors : Incisors	6	11.8
	Canines	9	17.7
	Posteriors: Premolars	2	3.9
	Molars	1	1.9
	Anterior – Posterior	5	9.8
	Maxillary Sinus	1	1.9
	Unknown	8	15.7
Mandible	Anterior	2	3.9
	Body	6	11.8
	Angle	0	0
	Ramus	0	0
	Anterior – Posterior	0	0
	Unknown	3	5.9
Unknown		8	15.7
Total		51	100

From Table 4 it can be seen that most AOTs were found in the incisor-canine region of the maxilla and in the body of the mandible. Occasionally, the cases were so large that they extended from the incisor to the molar region of the maxilla.

5.1.7 Associated Teeth

In the follicular variant the canine was the most commonly associated tooth followed by the incisors in the maxilla and the premolars in the mandible. The posterior teeth were less commonly affected. Occasionally, deciduous teeth were involved (Table 5, Fig. 10).

Table 5. Follicular AOTs in association with both deciduous and permanent teeth

	Teeth	Maxilla	Mandible	Unknown	Total
Permanent	Canines	12	3	0	15
	Incisors	4	0	0	4
	Premolars	3	2	0	5
	Molars	1	0	0	1
Deciduous		1	0	0	1
Unknown		4	0	6	11
Total		25	5	6	36

Although 31 cases were of the follicular variant, some cases had 2 teeth present within the lesion, hence the total number of teeth depicted in the above Table is 36.

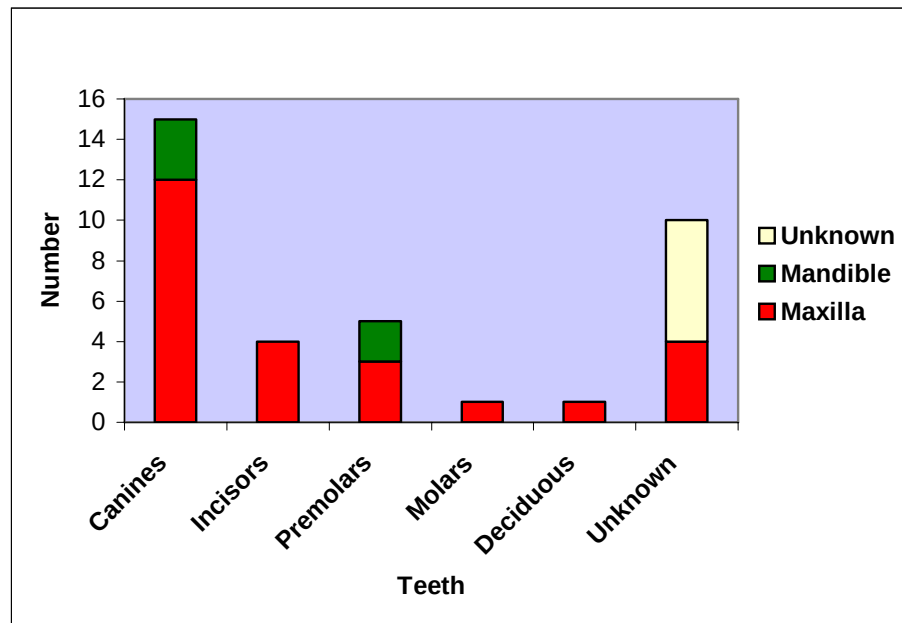


Fig. 10 Histogram showing the association of the AOT to different teeth

5.2 Macroscopic

The cut surface of the AOT was either cystic, solid or partly cystic. When cystic, focal thickenings of the cyst wall could be palpated and the cyst was filled with straw-coloured fluid. The cut surfaces of the solid lesions often had a circinate appearance and resembled tapioca. Embedded crowns of teeth or whole teeth were frequently present with the tumour often being attached to the necks of the teeth (Figs. 11-13).

5.3 Histology

Many lesions were cystic and were surrounded by a thick fibrous capsule. The cystic areas were surfaced by a reduced enamel-like epithelium and there was substantial histological evidence to show that the tumours originated from this lining epithelium with some cases demonstrating early proliferation (Figs. 14 and 15) to form small tumour nodules which later proliferate and coalesce to obliterate the cystic or follicular spaces. In the case of the extrafollicular AOTs it was interesting to note that some cases demonstrated a cystic structure, while others were solid in nature.

The histological structure of the AOT is very well described and the lesion is easily recognisable even to the inexperienced observer. There is little if any variation in the epithelial configuration with a nodular arrangement of sheets of epithelial cells containing scattered tumour droplets, layered rosettes, pseudo-ductular structures of varying sizes lined by a rim of amorphous material and lace-like areas of thin cords of epithelial cells in all cases (Figs. 16-20). In addition many cases contain variable amounts of extracellular deposits consisting of either a dystrophic calcification or calcified or uncalcified amorphous globular material which are generally interpreted as being dental matrix

material of undetermined type. The stroma was very scanty in all cases with hyalinisation thereof sometimes being a prominent feature.

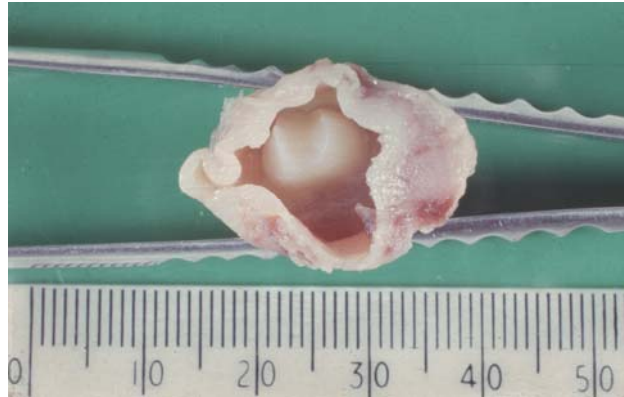


Fig. 11 Gross morphology of a cystic follicular AOT which has an enveloped tooth within the lesion.

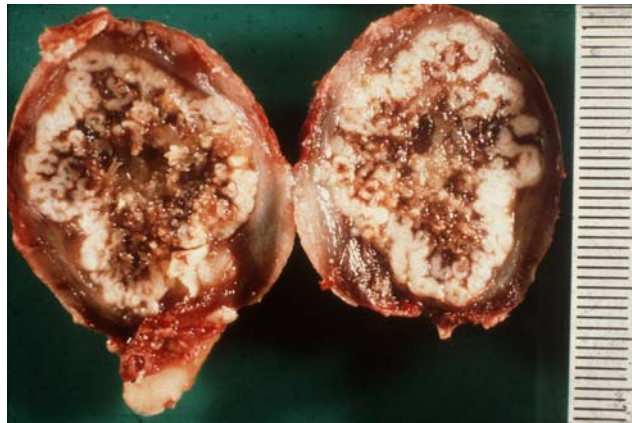


Fig. 12 Cut surface of an AOT showing a thick well-defined capsule and contents which have a distinct likeness to tapioca. A tooth is embedded in the lesion.



Fig. 13 Cut surface of a solid AOT. Note that the tumour is attached to the neck of an unerupted tooth and the resemblance of the contents to tapioca.

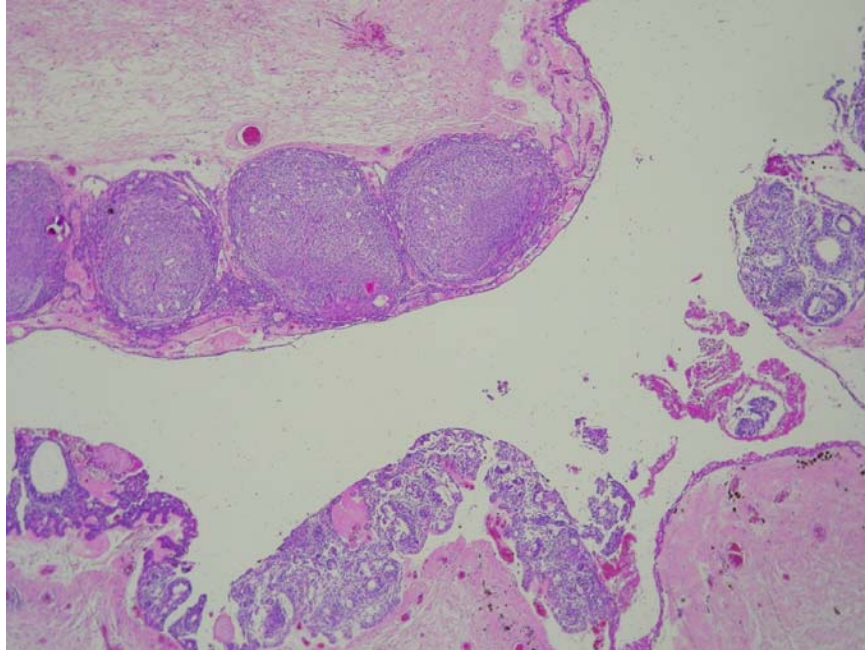


Fig. 14 Early AOT formation in the wall of a large cyst. Nodules of tumour tissue appear to originate in the wall of the cyst before bulging into the lumen, where proliferation and coalescence probably occurs (H & E, original magnification X4).

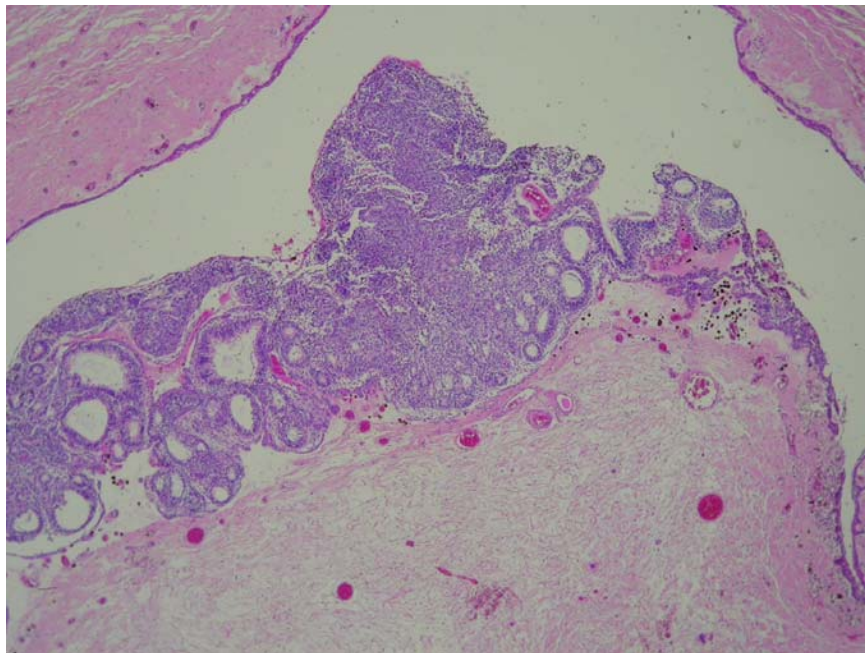


Fig. 15 Early AOT originating in the wall of a dentigerous cyst or dilated follicle. Much of the cyst is lined by reduced enamel-like epithelium. The newly formed tumour is bulging into the cyst lumen which may eventually become filled with tumour tissue (H & E, original magnification X4).

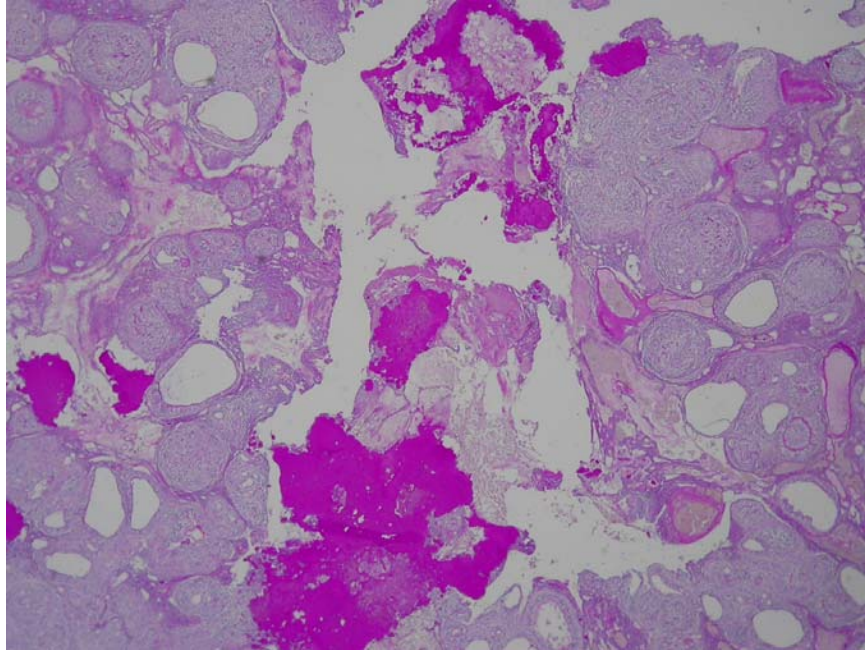


Fig. 16 Low power view showing lobular architecture, large pseudo-ductular structures, cribriform lace-like areas and large PAS positive amorphous globular masses (PAS, original magnification X4).

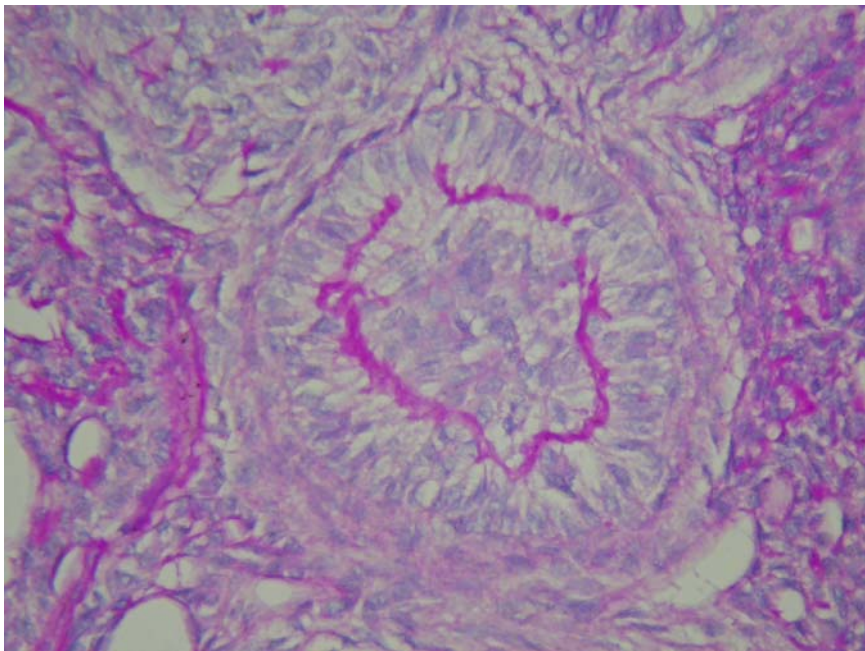


Fig. 17 Rosette showing PAS positive basement membrane-like material creating a layered appearance (PAS, original magnification X40).

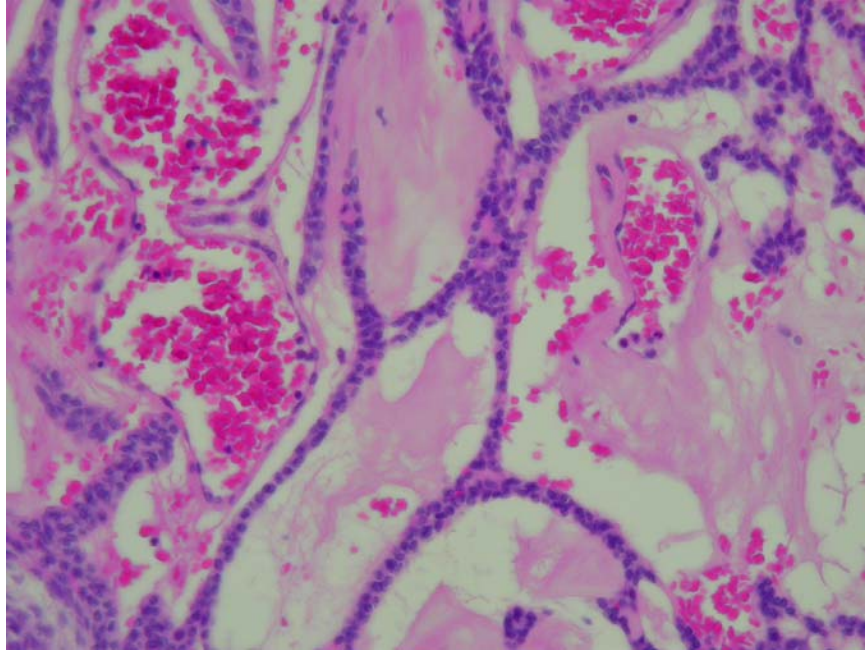


Fig. 18 Cribriform or lace-like area showing cords of cells surrounding loose, oedematous stroma containing dilated thin walled blood vessels (H & E, original magnification X40).

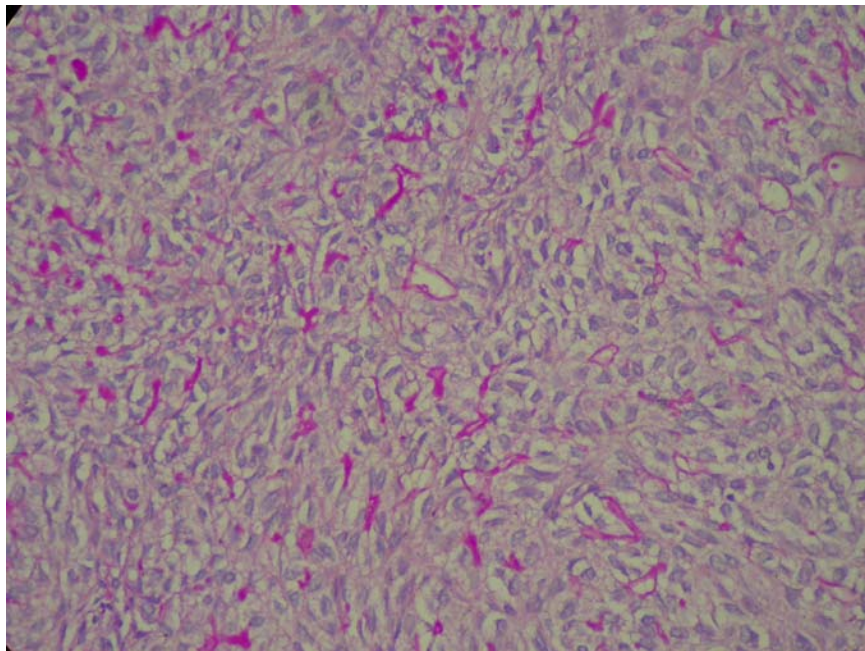


Fig. 19 Numerous scattered PAS positive tumour droplets or globules in amongst the sheets of spindled epithelial cells (PAS, original magnification X20).

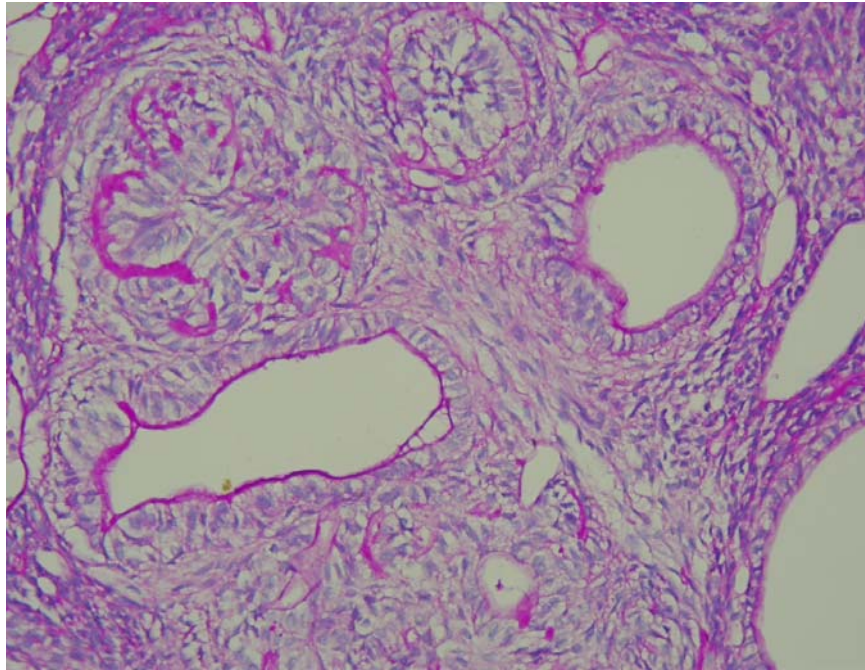


Fig. 20 Large and small pseudo-ductular structures lined on the luminal aspect by amorphous material which stains positively with the PAS stain. The rosettes show a prominent PAS positive basement membrane-like material resulting in a 'layered' appearance (PAS, original magnification X40).

Depending on the presence and nature of the extracellular deposits, the AOTs could be divided into 3 groups.

- a) Those showing only uncalcified tumour droplets or globules.
- b) Those showing tumour droplets and a dystrophic form of mineralisation either in the connective tissue wall or in the epithelium itself (Fig. 21).
- c) Those showing tumour droplets and the presence of eosinophilic globular masses which we also interpreted as representing some form of dental matrix material. A few cases also showed a lace-work of similar eosinophilic material which surrounded and was intimately associated with small islets of odontogenic epithelium (Figs. 22 and 23). Some of the larger globules showed a calcospherite pattern of calcification resembling the pattern seen in normal dentine (Fig. 24). In addition, in five cases a single row of tall columnar cells with abundant eosinophilic cytoplasm and with basally located nuclei were arranged in a circular or duct-like configuration. These cells appeared to be secreting globules of eosinophilic, PAS positive dental matrix material. These circular structures were abundant in one case and fairly scanty in the other 4 cases. The columnar cells resembled odontoblasts although no cytoplasmic processes were observed. We have termed these structures *circular secretory units*. The layer of columnar cells was not always complete indicating that either the cells differentiate at varying rates or having completed their function change shape or disappear (Figs 25-31).

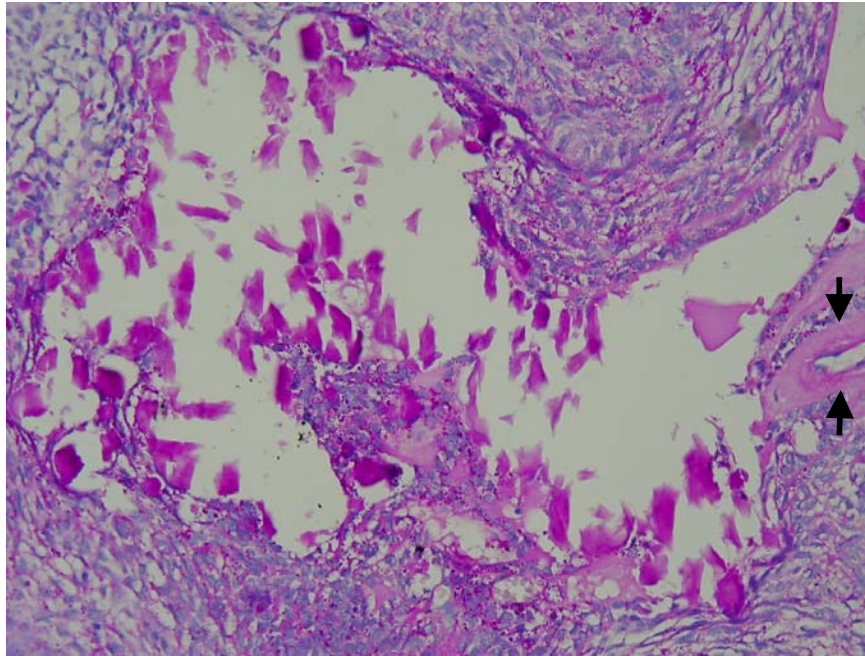


Fig. 21 Areas of what appear to be dystrophic calcification were a common feature. The tissue was not decalcified resulting in this “shattering” artefact. Areas of stromal hyalinisation (arrows) can also be seen at the one edge (PAS, original magnification X40).

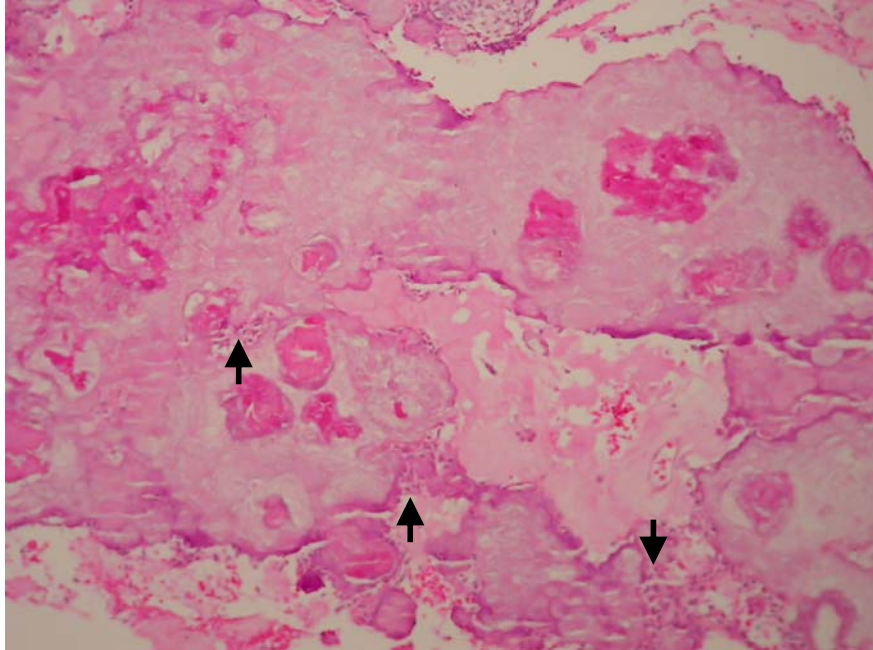


Fig. 22 Amorphous eosinophilic globular masses were present in only a few cases. Clusters of epithelial cells (arrowed) can be seen in amongst the cords of calcified material indicating an inductive influence (H & E, original magnification X10).

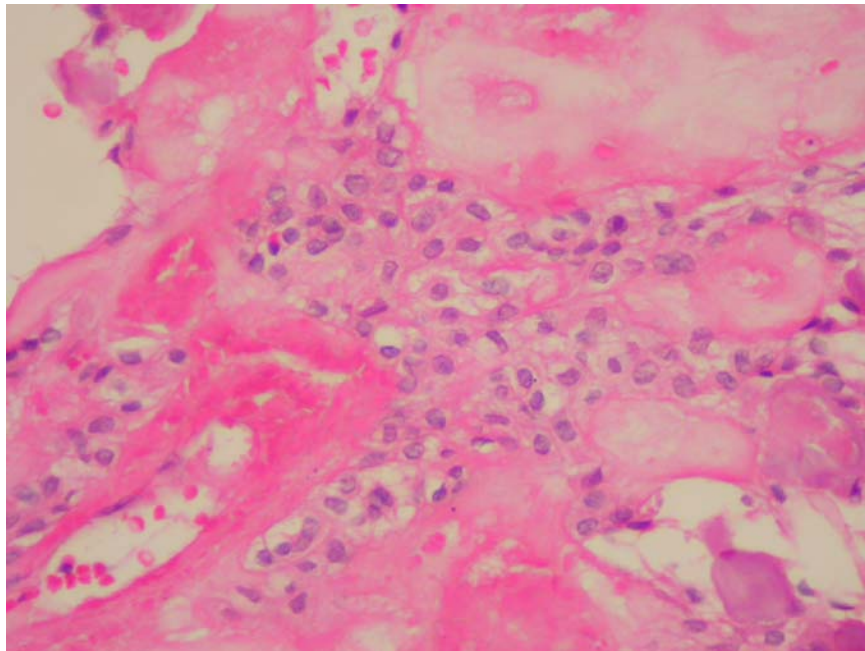


Fig. 23 High power view showing clusters of epithelial cells intermingled with cords of dental matrix material in a lace-like pattern (H & E, original magnification X40).

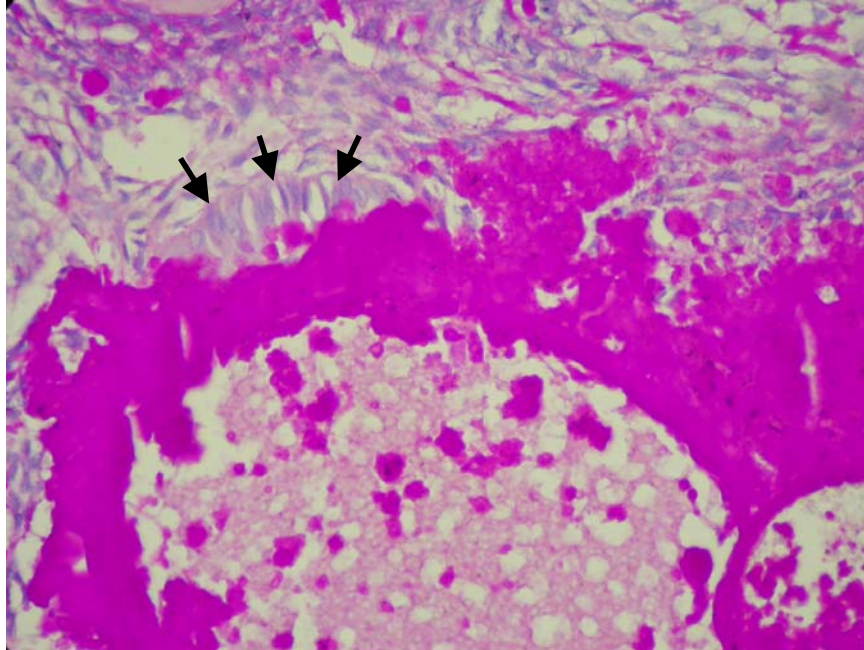


Fig. 24 The dental matrix material stained positively with the PAS stain and frequently showed a calcospherite pattern of calcification reminiscent of normal dentine. Note the tall columnar cells (arrowed) actively secreting this product. (PAS, original magnification X20)

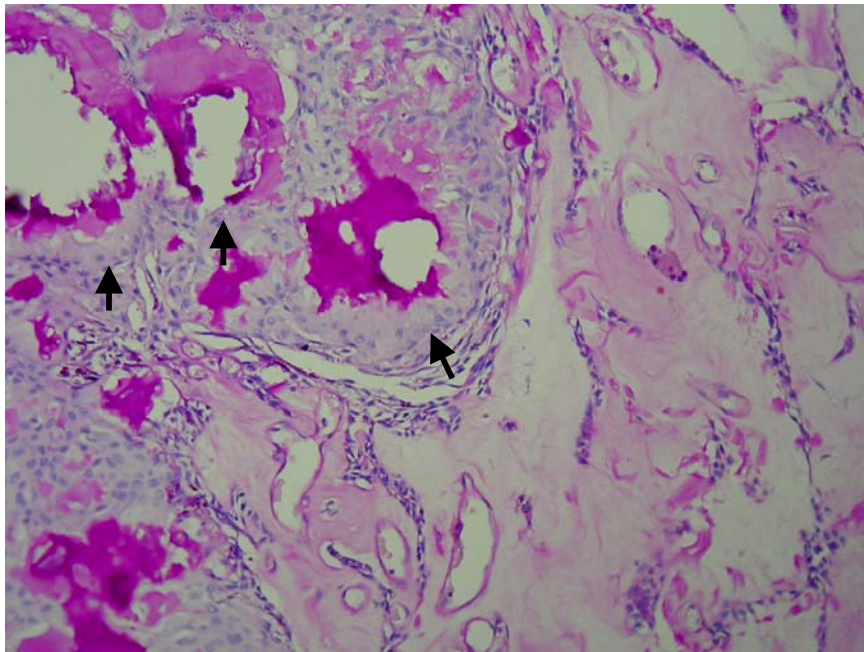


Fig. 25 Several circular structures (arrowed) consisting of a single layer of peripherally palisaded tall columnar cells surrounding a central lumen and secreting PAS positive dental matrix material (PAS, original magnification X20).

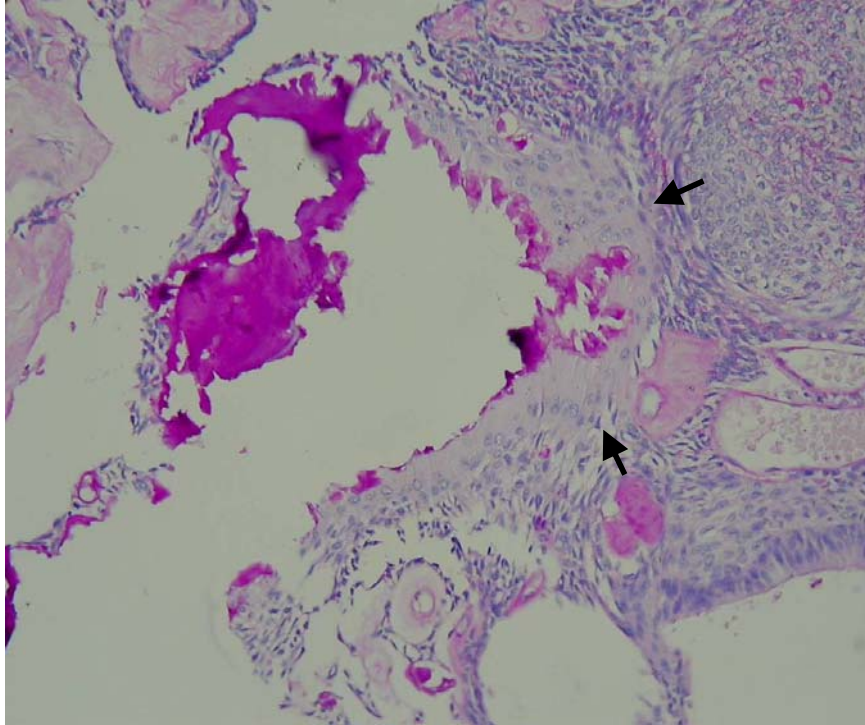


Fig. 26a

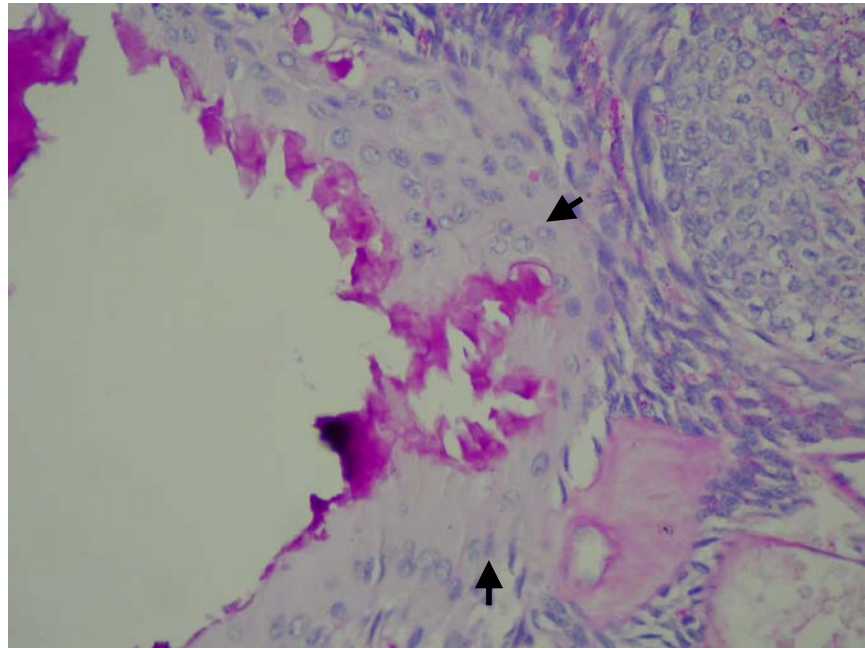


Fig. 26b

Figs. 26a and 26b A partly intact *circular secretory unit*. Note the peripherally placed tall columnar cells (arrowed) and the secretion by these cells of PAS positive dental matrix material (PAS, original magnification X20 and X40 respectively).

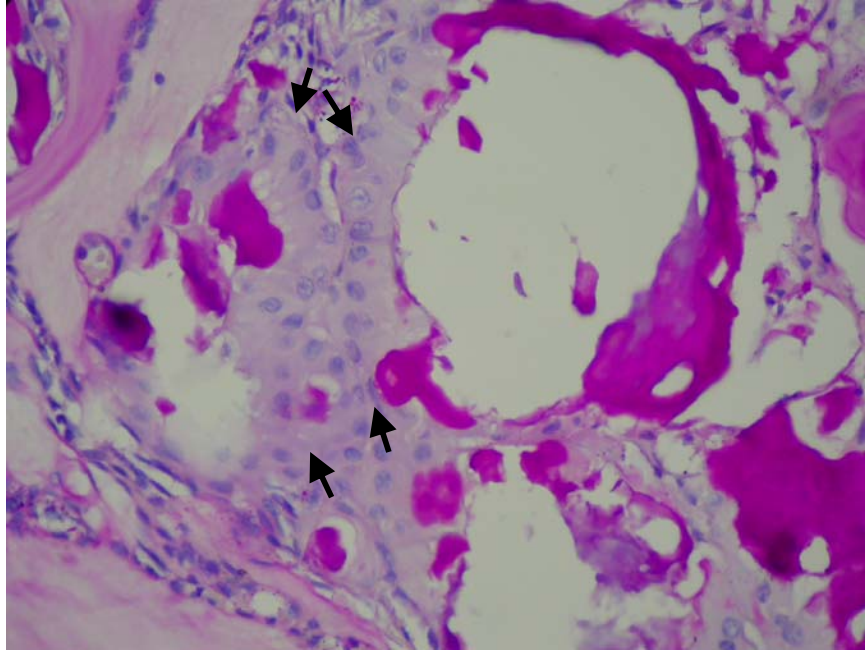


Fig. 27 Several *circular secretory units* lined in part by tall columnar cells showing secretory activity (arrowed). On the other side the columnar cells having completed their function have disappeared (PAS, original magnification X40).

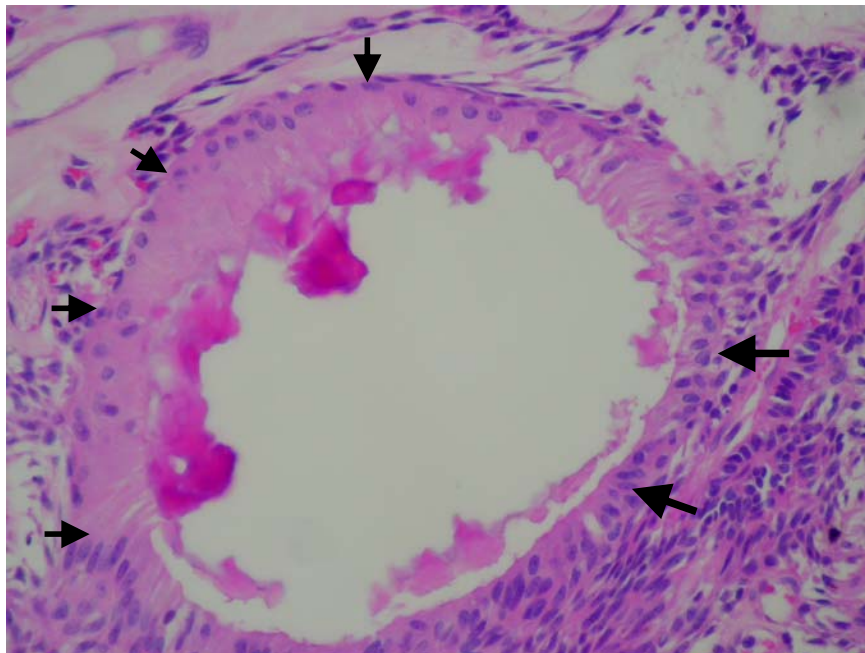


Fig. 28 Note how the cells on one side of the secretory unit are fully differentiated and actively secreting (arrowed) while on the other side they are less differentiated (bold arrows) and may already have completed their function (H & E, original magnification X40).

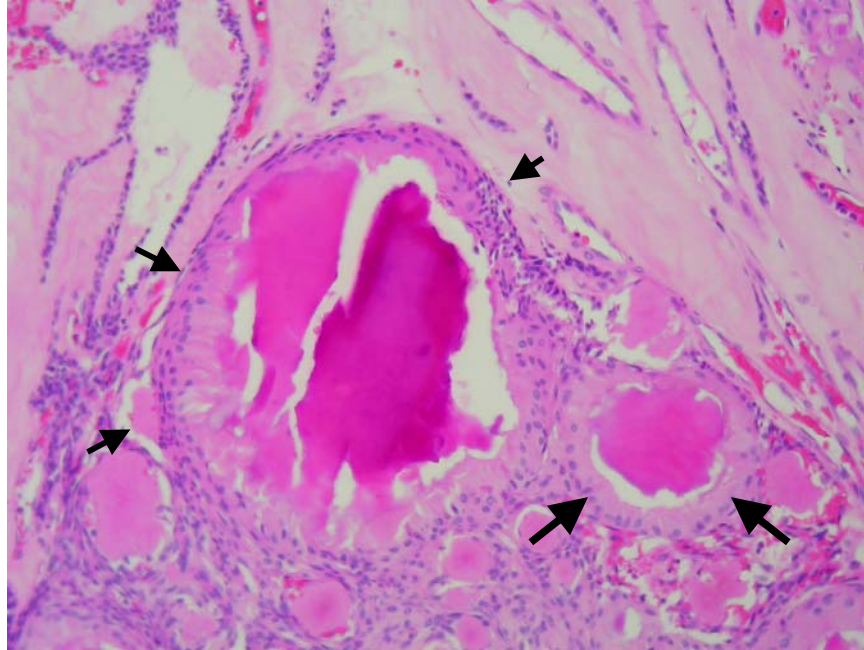


Fig. 29a A different case similarly showing *circular secretory units*. In the larger unit (arrowed) the row of columnar cells is incomplete while in the smaller unit (bold arrows) the tall columnar cells completely surround the newly formed dental matrix material (H & E, original magnification X20).

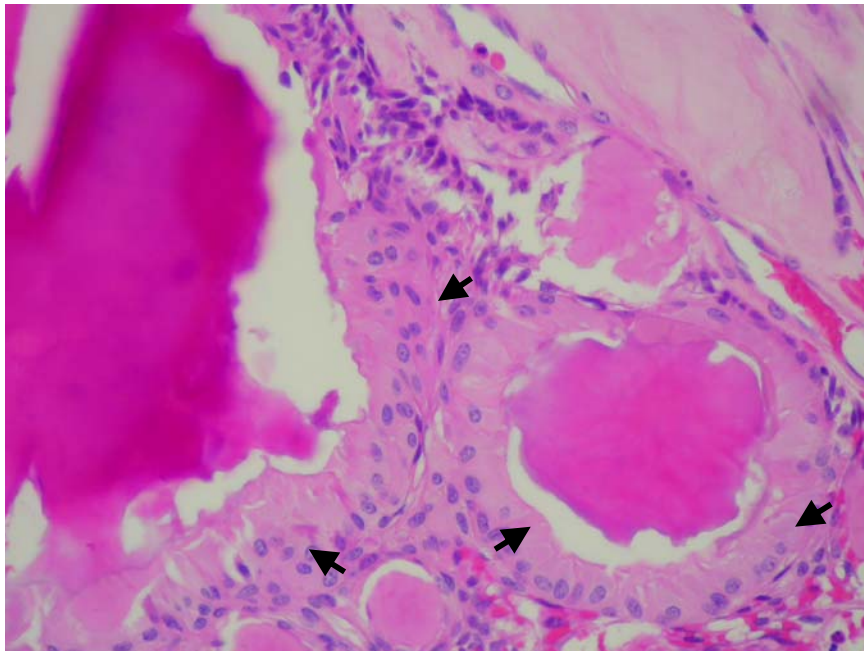


Fig. 29b Higher power view of previous case showing the basally placed nuclei, eosinophilic homogenous cytoplasm and resemblance of these cells (arrowed) to actively secreting odontoblasts (H & E, original magnification X40).

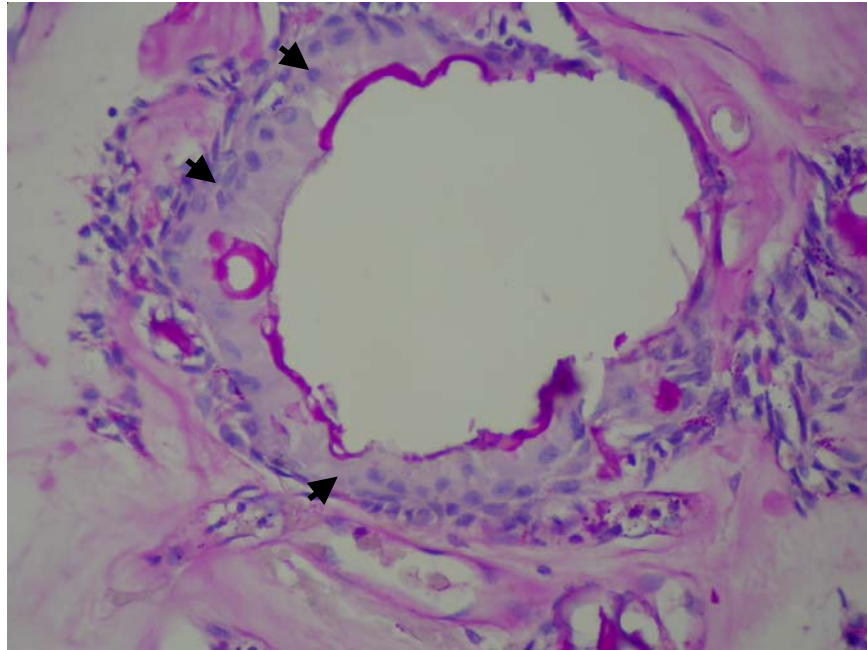


Fig. 30 Another case showing a single large *secretory unit*. Note the secretory activity of the tall columnar cells (arrowed) (PAS, original magnification X40).

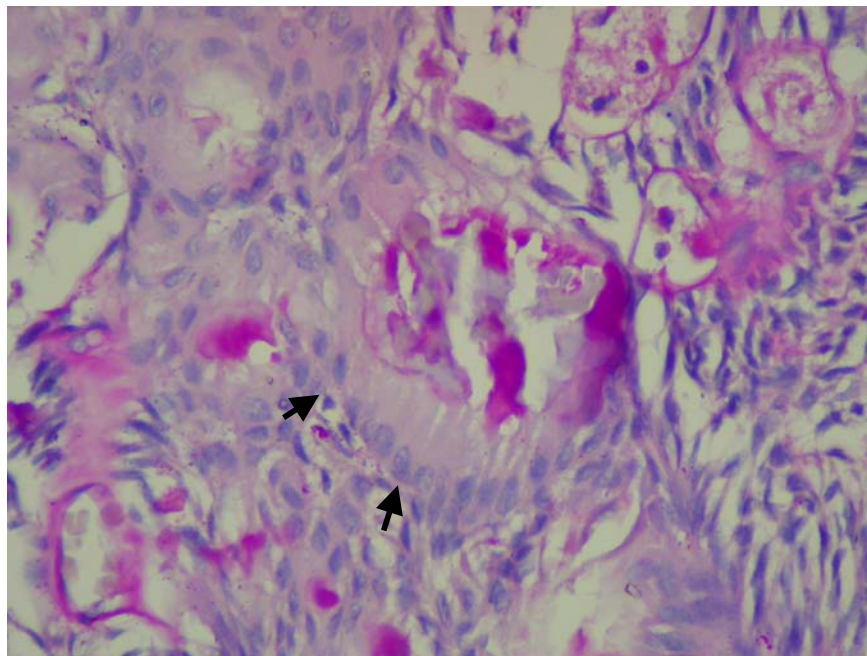


Fig. 31 A smaller secretory unit. In this case the dental matrix material has calcified. Note the actively secreting tall columnar cells (arrowed) (PAS, original magnification X40).

5.4 Histochemistry

5.4.1 PAS

PAS positive material was extensively distributed extracellularly throughout the epithelium in most cases. This material which appeared to be an epithelial secretion was small and irregular in outline and is referred to as tumour droplets (Fig.19). PAS positive material was also present in many of the pseudo-ductular structures as a luminal rim (Fig. 20) and as basement membrane material outlining the layers in the rosettes (Fig. 17). The large eosinophilic globules of dental matrix material also stained intensely positively with the PAS stain (Fig. 16) as did the areas showing a calcospherite-like pattern of mineralisation (Fig. 24).

5.4.2 Alcian blue

A few cases showed extensive alcianophilia but this was largely restricted to the fibrous capsule. Faint foci of alcianophilic ground substance were also identified closely associated with the dental matrix material. This material could not be interpreted as representing residual ectomesenchyme. A rim of alcian blue positive material was present on the luminal surface of the duct-like structures (Fig. 32).

5.4.3 von Gieson

The von Gieson stain confirmed the absence of a significant collagenous framework in all cases. A rim of von Gieson positive material was also present on the luminal surface of the pseudo-ductular structures (Fig. 33).

5.4.4 Congo red

Most cases showed no Congo red staining. However, those few cases which were characterised by the large globules of dental matrix material showed isolated foci of Congo red positivity (Fig. 34). When viewed with polarised light this material showed characteristic apple-green birefringence (Fig. 35). This amyloid or amyloid-like material was fairly abundant in 2 cases while being relatively sparse in others but it was always intimately intermingled with the dental matrix material.

5.4.5 Prussian blue

Many cases contained foci of haemosiderin pigment which was confirmed using iron stains.

5.4.6 Melanin bleach

One of the cases contained what appeared to be extensive melanin pigmentation. This was confirmed using a peroxide bleach. The melanin was widely distributed in the epithelial cells (Fig. 36).

5.4.7 Reticulin

Reticulin fibres could be demonstrated in all cases where they were observed forming a luminal rim in nearly all the duct-like structures regardless of the size of the ducts. Some fibres could also be seen randomly distributed in and amongst the epithelial cells and the tumour droplets stained positively with the reticulin stain as did the basement membrane-like material in the rosettes (Fig. 37).

5.4.8 Mucicarmine

Positive staining in the form of a luminal rim was observed on the luminal aspect of the duct-like structures both big and small (Fig. 38).

5.4.9 Masson's trichrome

Once again the inner aspect of the duct-like structures stained positively with this stain, forming a luminal rim (Fig. 39).

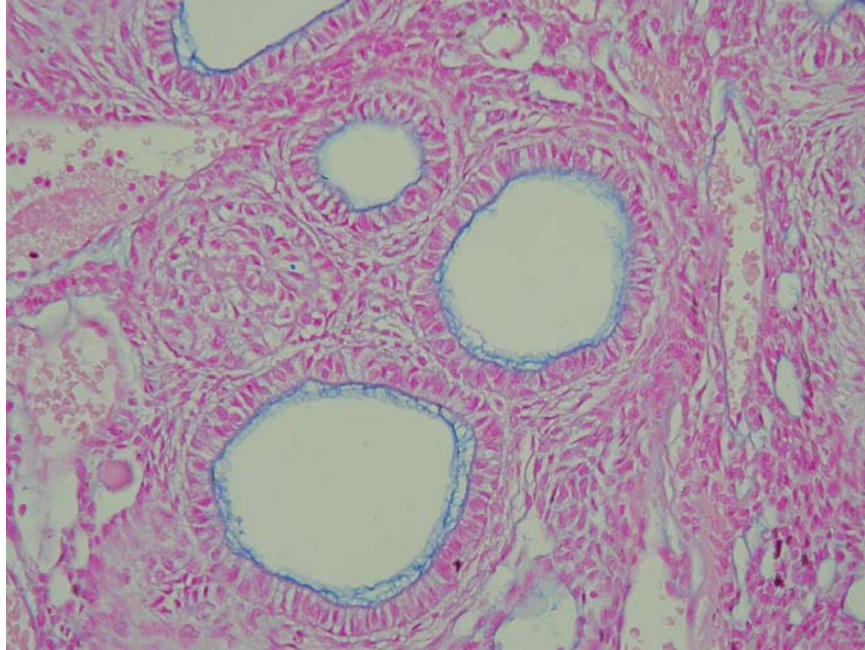


Fig. 32 The inner layer of the pseudo-ductular structure stained positively with a variety of histochemical stains. In this instance the 'luminal rim' is staining with alcian blue (Alcian blue, original magnification X20).

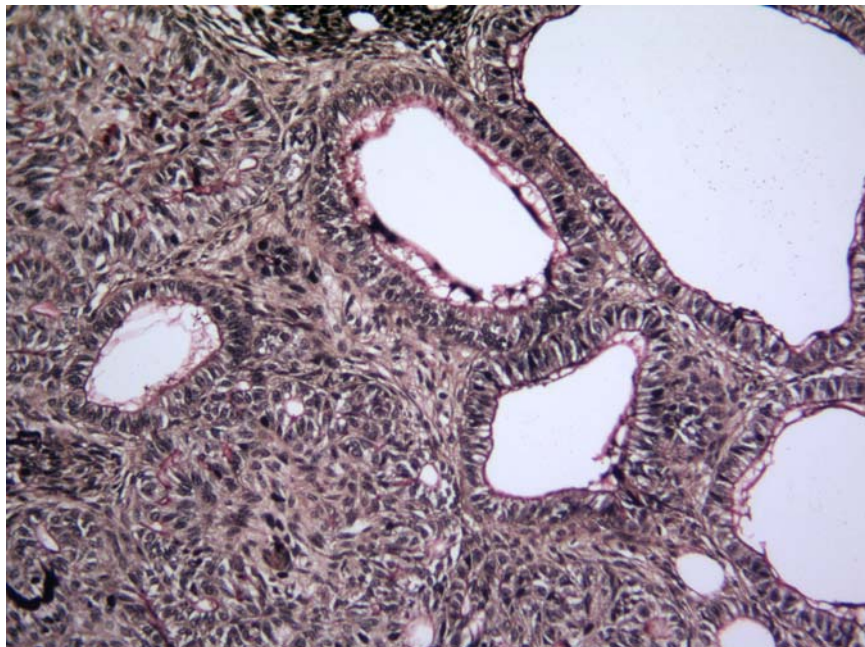


Fig. 33 The von Gieson stain colouring the 'luminal rims' red in this instance (von Gieson, original magnification X40).

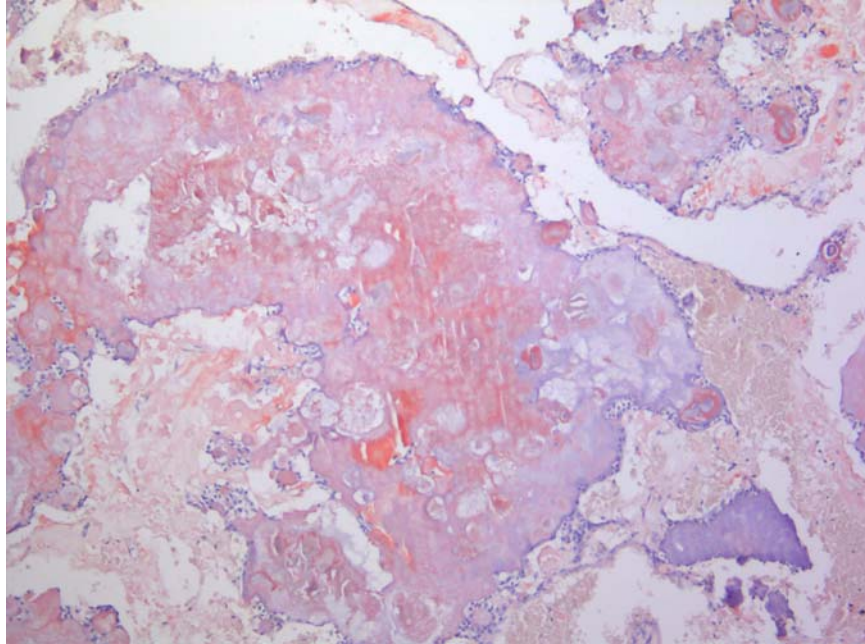


Fig. 34 The globular masses showing scattered 'salmon pink' positivity with the Congo red stain (Congo red, original magnification X20).

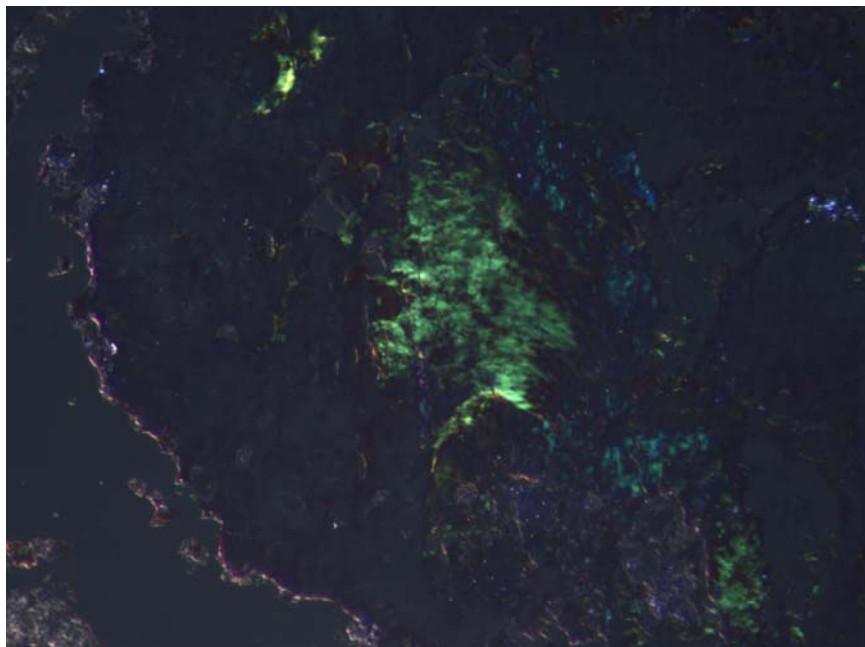


Fig. 35 With polarised light the Congo red positive areas showed characteristic apple-green birefringence (Congo red, original magnification X20).

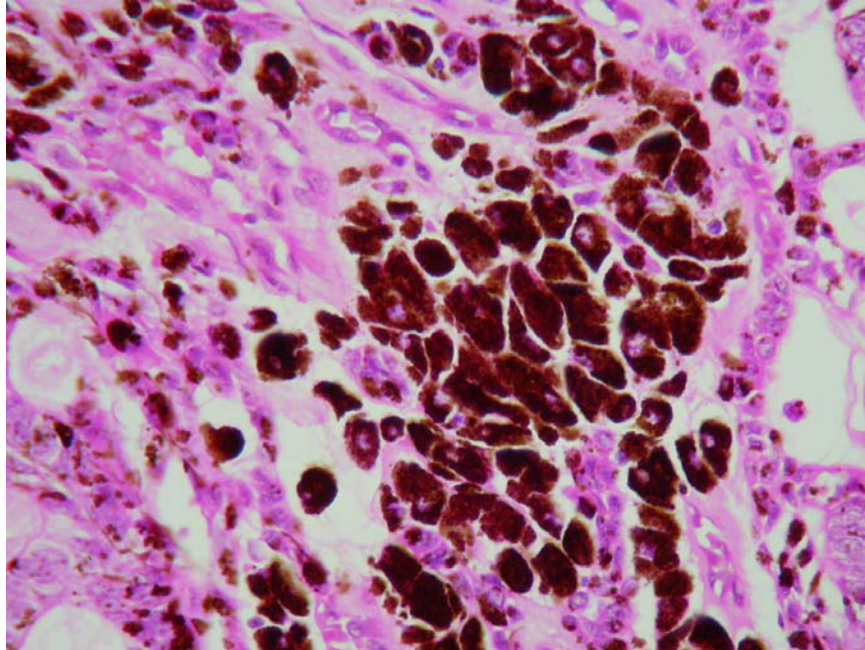


Fig. 36 Extensive melanin deposits in the epithelial cells are visible on this photomicrograph (H & E, original magnification X40).

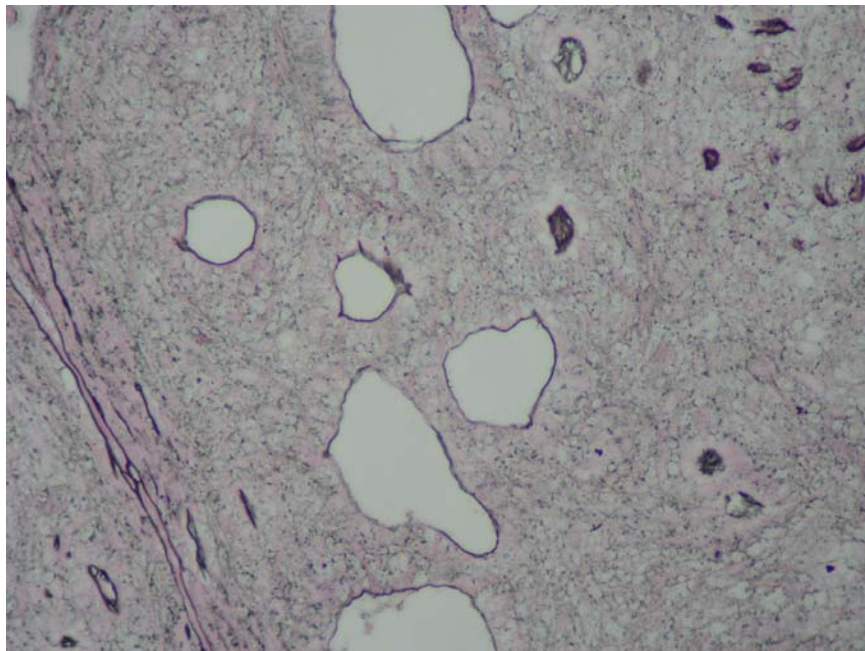


Fig. 37 Reticulin positivity on the inner aspect of the pseudo-ductular structures (Reticulin, no counterstain, original magnification X10).

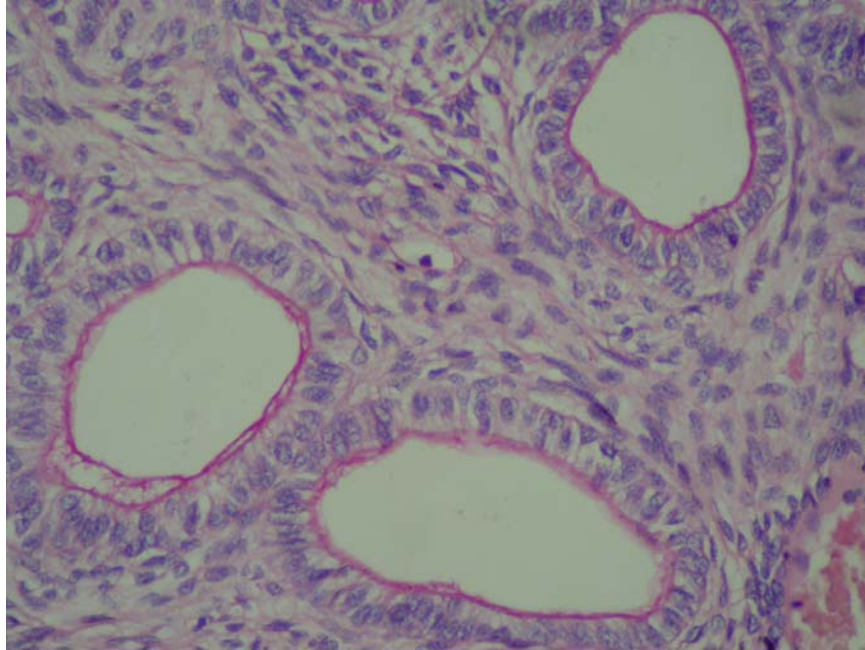


Fig. 38 The material on the inner aspect of the pseudo-ductular structures also stained positively with mucicarmine (Mucicarmine, original magnification X40).

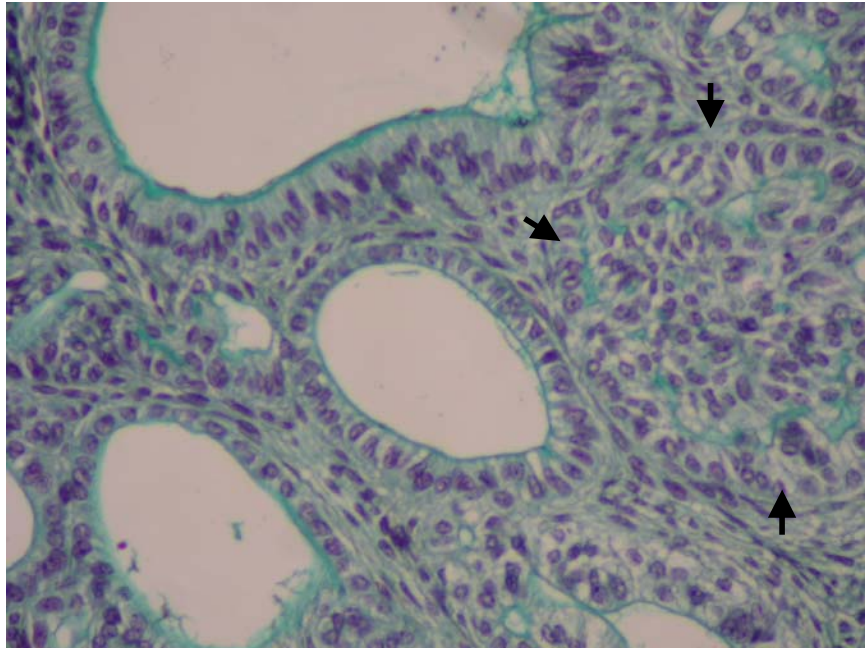


Fig. 39 In this instance Masson's trichrome stained the luminal rims a bright green colour. The basement membrane material in one of the rosettes (arrowed) stained a similar colour (Masson's trichrome, original magnification X40).

5.5 IMMUNOHISTOCHEMISTRY

5.5.1 MNF 116 (Cytokeratins)

Staining of both spindle and pseudo-ductular epithelial cells was observed with this anti-cytokeratin antibody (Fig. 40). Staining was cytoplasmic, focal or diffuse and moderate in intensity. The small clusters or strands of cells intermingled with the dental matrix material also stained positively.

5.5.2 Vimentin

Only few epithelial cells stained with vimentin. In addition, scattered stromal cells showed moderate focal positivity (Fig 41). The relative paucity of stromal cells in the AOT was confirmed.

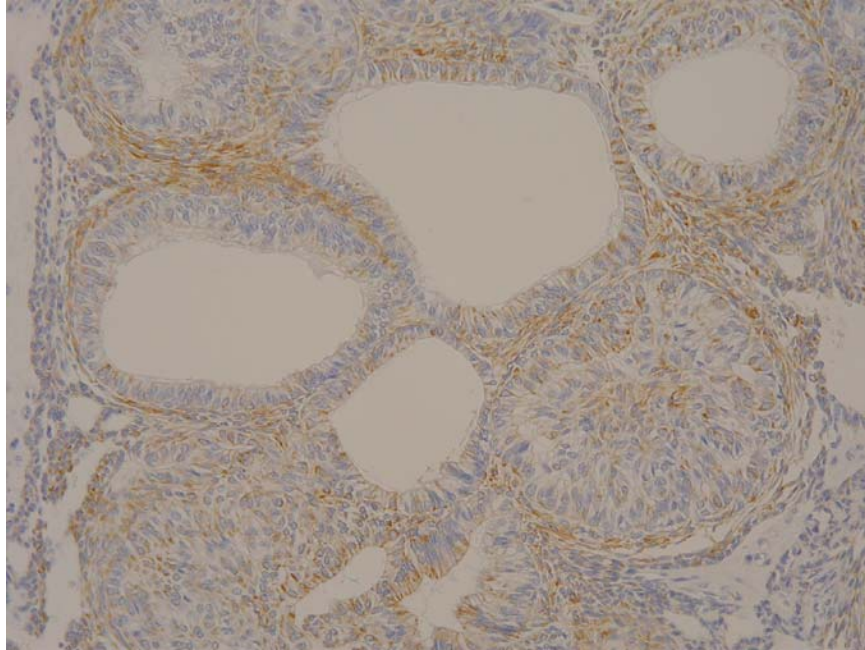


Fig. 40 Diffuse, moderately intense staining of both spindled epithelial cells and epithelial cells in the rosettes and lining the pseudo-ductular structures (MNF 116, original magnification X20).

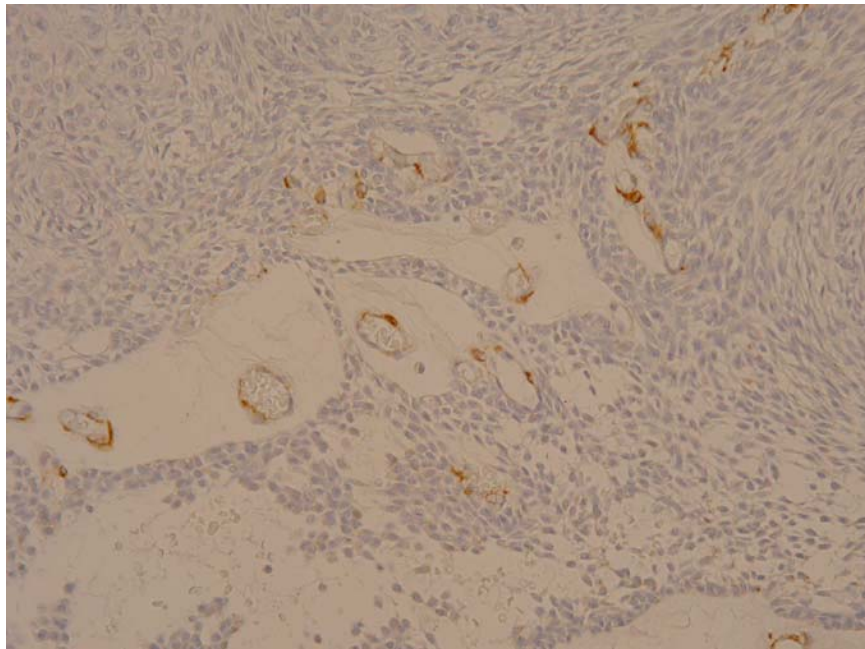


Fig. 41 Staining with vimentin was largely restricted to stromal cells (Vimentin, original magnification X20).

CHAPTER 6

6.0 DISCUSSION

The first part of this study consisted of a detailed analysis of the epidemiologic and clinico-radiographic characteristics of a large series of cases of AOTs. As was expected, this data did not differ from that previously published and which was reviewed by Philipsen & Reichart in 1998¹² and no further specific comment is necessary.

Our data has been combined with data derived from similar studies from all over the world as part of an international collaborative study and will be published to commemorate the centenary of the original description of the AOT in 2005.

The focus of our attention is with the histological structure of this lesion and whether histological evidence of induction having occurred exists.

Odontogenic tumours are said to recapitulate dental ontogeny and many show histologic evidence of reciprocal induction. Such complex epithelio-mesenchymal interactions lead in some instances to proliferation of epithelium and ectomesenchyme only e.g. ameloblastic fibroma, while in others the inductive process is taken one step further with deposition of dentinoid and even tubular dentine, e.g. ameloblastic fibro-dentinoma and finally production of enamel or enameloid matrix in the ameloblastic fibro-odontome. The newly formed dental matrix material always has a very close relationship to the odontogenic epithelium which may be characterised by tall columnar cells resembling

ameloblasts or odontoblasts. Such tall, columnar cells are not always present and in some instances the epithelium remains in the form of small nests or clusters of cells intermingled with the dental matrix material in a lace-like pattern.

In addition it is widely accepted that the inductive process in odontogenic tumours is governed by the same principles established for normal odontogenesis viz. induction of ectomesenchyme must precede epithelial proliferation and dentine/dentinoid deposition which in turn must precede formation of enameloid.

In the AOT, the presence of dental matrix material is acknowledged by most authorities but there is no consensus as to the exact nature of this material. In the 1992 WHO classification of Odontogenic Tumours³ the AOT was classified together with tumours consisting of “odontogenic epithelium with odontogenic ectomesenchyme with or without dental hard tissue formation”. It was defined as showing “varying degrees of inductive changes in the connective tissue”. Recent work has cast doubt on the existence of inductive influences in the AOT. Condensation or proliferation of ectomesenchyme has never been demonstrated. In fact the mesenchymal component in the AOT is extremely scanty especially where the epithelium is present in solid sheets with rosette and pseudo-ductular structures. In addition, Gao and colleagues have failed to demonstrate the presence of bone morphogenetic protein (BMP) in the AOT citing this as evidence of the lack of an inductive influence.⁵ The 1992 WHO³ definition of AOT did allude to the presence of small islets of odontogenic epithelium intermingled with the strands of dental matrix material but this has not been widely accepted as providing histological evidence of induction. These doubts as to the existence of an inductive influence has led Philipsen &

Reichart⁴ to argue for a re-classification of the AOT as an epithelial odontogenic tumour without induction. They have postulated that the dental matrix material is manifested not as an inductive but as a metaplastic process but have not been able to provide evidence to support this contention.

It was not our intention to describe in detail, the histological structure of the AOT, this has been done repeatedly. Instead, the focus of our study was by careful histological observation to seek evidence of an inductive process having occurred. This evidence could take the form of condensation or proliferation of ecto-mesenchyme, presence of small islets of epithelium in close association or intermingled with strands of dental matrix material, or the presence of tall ameloblast-like or odontoblast-like cells depositing dental matrix material. To this end we used mainly routinely stained haematoxylin and eosin stained sections and in selected cases various histochemical and immunohistochemical stains as detailed in the material and methods.

There was considerable histological evidence that the AOT originates from reduced enamel epithelium in the follicular cases, with small strands of tumour tissue first appearing in the wall of the follicle or dentigerous cyst below the lining epithelium then proliferating into discrete nodules before enlarging and bulging into the lumen where further proliferation and coalescence probably takes place, eventually filling the follicular cystic space (Figs. 14 and 15).

In the case of the extrafollicular lesions no differences in histologic structures could be determined and the exact tissue of origin remains uncertain. A cystic component was also

observed in these cases and it is possible that the AOT originates in the wall of some other odontogenic cyst. In fact we have observed an early AOT commencing in the wall of a unicystic ameloblastoma. Such hybrid lesions might explain the aggressive expansile nature of some AOTs. This case will be reported separately.

The presence of large amorphous globular masses that we interpreted as being dental matrix material were observed in relatively few cases of AOT. Other cases show dystrophic calcification but the majority do not show any form of mineralised or dental matrix material other than the tumour droplets which occur in virtually all cases. Hence the inductive influence, if there is one, is clearly not expressed in all cases. The amorphous globular masses, when present, were abundant, scattered throughout the epithelium and some showed a calcospherite pattern of calcification similar to that seen in normal dentinogenesis. This was particularly evident with the PAS stain which stained the globular material a bright magenta colour (Fig. 24).

In five cases we were able to identify '*circular secretory units*' consisting of rows of tall columnar secretory cells with eosinophilic cytoplasm and with basally located nuclei arranged in a circular or duct-like fashion. These cells could clearly be seen to be depositing dental matrix material and resembled odontoblasts or ameloblasts (Figs. 25-31). They should not be confused with the shorter columnar or cuboidal cells lining the pseudo-ductular or gland-like spaces which are usually not as tall, have a looser basophilic cytoplasm and which are never associated with dental matrix material deposition. Although there is only one case reported in which the authors illustrate the presence of tubular dentine within the lumen of what appears to be pseudo-ductular structures but we

remain unconvinced as the quality of the photomicrographs is not clear.⁵³ Tall columnar cells similar to the secretory cells we have described have only rarely been mentioned in AOTs and never in a duct-like arrangement. In fact very little attention has been paid to them. As mentioned in the literature review there are 2 reports in which AOTs are described which demonstrate tall columnar cells actively secreting dental matrix material and which in our opinion constitute evidence of an inductive process but this has not been widely accepted nor reported.^{53, 54} Morphologically they appear very similar to the cells we have illustrated in Fig. 34 but were not arranged in a circular or duct-like configuration as was mostly the case in this study. We are not certain whether this difference is significant or merely represents a part of a morphological spectrum in which the secretory cells can be arranged in a linear or circular configuration or both. These cells appear only to be present in the AOTs in which dental matrix material is present. The scanty numbers of these cells in some cases and absence in others where globular material was present suggests that having completed their function they change in shape making them indistinguishable from other epithelial cells in the AOT. This is supported by the fact that in the *circular secretory units* the tall columnar secretory cells frequently did not surround the entire unit but were present on one side only either suggesting that they were still to differentiate or that having completed their function they have changed shape.

In addition in several cases characterised by the amorphous globular PAS positive masses, small islets of epithelium were found in-between strands of dental matrix material (Fig. 23). The epithelial nature of these cells was confirmed using the anti-cytokeratin antibody MNF 116.

In our opinion the *circular secretory units* consisting of the tall columnar cells secreting what appears to be dental matrix material constitutes unequivocal histologic evidence of induction. We believe further histologic evidence of induction is provided by the intimate association between small clusters and strands of epithelial cells and the lace-like strands or sheets of dental matrix material.

The AOT is characterised histologically by numerous pseudo-ductular structures of varying size which consist of a single layer of palisaded columnar non-secreting cells surrounding a central lumen or microcystic space of varying size. The odontoblast or ameloblast-like secretory cells we have described were almost always arranged in a similar single palisaded layer in a round duct-like configuration with the induced dental matrix material deposited in the lumen of the 'duct'.

The question must be asked as to whether these *circular secretory units* might be derived from the columnar cells which line the pseudo-ductular structures. While on the one hand the circular or duct-like arrangement around a central lumen is virtually identical for the two cell types, on the other hand, the cells are morphologically quite different with the pseudo-ductular lining cells being shorter and having a more centrally placed nucleus with a loose basophilic cytoplasm while the secretory cells are taller with a more basally placed nucleus and homogenous eosinophilic cytoplasm. It could be argued that this is simply a morphological change due to the inductive process.

In considering the histogenesis of the pseudo-ductular structures, Shear³⁶ wrote that the so called 'ducts' represented invaginations of odontogenic epithelium that carried with them

ectomesenchymal stroma and that much of the stroma cut off from its blood supply underwent atrophy or necrosis while the surviving stroma, mostly at the periphery of the pseudo-ductular structures, retained its inductive capacity and induced the columnar cells to lay down pre-dentine matrix. In support of this hypothesis Shear demonstrated reticulin fibres as well as toluidene blue metachromasia on the inner aspect of the duct-like structures concluding that this constituted evidence of the presence of ectomesenchyme. Ultrastructurally it has been shown that the inner surface of the duct-like structures contains basal lamina-like material and a granular deposit which has been regarded as a product of secretory activity by these cells lining the duct. Shear³⁶ suggested that the secretory product was pre-dentine.

Against this hypothesis is that many other histochemical stains such as PAS, alcian blue, reticulin, mucicarmine, von Gieson and Masson's trichrome also stain the inner or luminal aspect of the duct-like structures forming a luminal rim. It is therefore possible that the 'deposits' on the inner aspects of the pseudo-ductular structures represent an 'edge' artefact. It is highly unlikely that one substance, regardless of what it maybe will stain positively with such a diversity of histochemical stains.

Be that as it may, as far as the luminal rims are concerned the investigative techniques used in this study cannot be used to substantiate Shear's³⁶ residual stroma theory nor our suggestion of an 'edge' artefact. Further investigations are necessary to elucidate the true nature of the luminal rims and indeed we are already planning a new study using IHC with antisera to laminin, fibronectin, collagen IV and tenascin.

While Shear's³⁶ early writings on this question have considerable merit we must conclude that a relationship between the pseudo-ductular structures and our secretory cells has not been established and it seems unlikely to us that the cells lining the duct-like structures are precursors to the secretory cells which we have described.

The alcian-blue stain failed to demonstrate any alcianophilic material that could have been interpreted as representing induced ectomesenchyme. Lack of a mesenchymal component in-between the epithelial cells was confirmed with vimentin immunohistochemistry when no positive cells were identified in these areas.

Lack of visible ectomesenchymal component does not mean that induction has not occurred. It is possible that having completed its function the ectomesenchyme disappears. Certainly there are other examples of odontogenic tumours characterised by induction of dentinoid in which no ectomesenchymal presence can be determined. A case of ameloblastoma with dentinoid induction has been described⁵⁰ and in the COC the induced dentinoid is associated with small islets of odontogenic epithelium but not with a visible ectomesenchymal component.¹⁸

The presence of amyloid-like material in the AOT was confirmed in this study. The amyloid was present in variable amounts intermingled with the dental matrix material. It showed the characteristic apple green-birefringence when Congo red stained sections were viewed with polarised light. Its origin and significance remain unknown.

One of our cases showed extensive melanin pigmentation throughout the epithelial cell component. The presence of melanin has previously been demonstrated in AOTs and is not surprising given the origin of ectomesenchyme from neural crest which also gives origin to melanocytes.^{51, 52}

CHAPTER 7

7.0 CONCLUSION

In conclusion, we have provided histological evidence in the form of tall columnar cells actively secreting dental matrix material, that an inductive process does indeed occur in AOTs and suggest that the absence of a visible ectomesenchymal presence does not negate this possibility. Similar tall columnar secretory cells have only rarely been mentioned in the literature and no special attention has been paid to them. The circular arrangement of these cells and the presence of a secretory product in the centre of the duct-like configuration have not previously been reported. These secretory cells possibly disappear once their function is complete. Further studies will be necessary to confirm the nature of these secretory cells and of the product they are producing. It seems unlikely that these secretory cells could have originated from the non-secretory columnar cells which characteristically line the pseudo-ductular structures although this cannot be entirely excluded. In addition we have reaffirmed the presence of small clusters of epithelial cells in close association and intermingled with cords of dental matrix material strongly suggesting further evidence of an inductive influence.

These findings lead us to the conclusion that induction does indeed play an important role in the histogenesis of the AOT and that re-classification of the AOT as an inductive tumour will once again be necessary.

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