Section of Odontology

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Meeting 25 February 1974

Paper

A Review of Recent Experimental Work on the Dental Cyst

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Experimental models of dental cysts are essential for our understanding of these lesions. Genvert *et al.* (1940) produced cysts in monkeys while studying periapical infection. Szabo (1954) noted that cysts with a keratinized epithelial lining consistently developed in the epithelium overlying the enamel organs of explanted molar teeth from albino mice. Szabo contrasted these with the nonkeratinizing cysts that developed in the enamel organs of his cultured teeth. However, the cysts were not apparently of primary interest in either of these series of experiments.

Cyst Epithelium

Follicular: The source and behaviour of cyst epithelia have been studied more recently by several workers; Bartlett *et al.* (1973) produced experimental odontogenic keratinizing cysts by transplanting maxillary first molar toothgerms from 2-day-old mice under the kidney capsules of isologous adult male mice. Nephrectomies were performed 1–180 days later.

The toothgerms continued to lay down enamel, dentine and cementum and also induced the formation of trabecular bone with hæmopoietic marrow in the surrounding connective tissue. Bartlett *et al.* noted that clefts arose in the intermediate area of the reduced enamel epithelium. This was usually after enamel formation had ceased at about 10 days post-transplantation. These clefts continued to enlarge until they enclosed the crown to produce the appearance of typical dentigerous cysts after about 50 days.

Epithelial buds and multiple cysts were not uncommonly seen in some specimens and all the cysts arose within the reduced enamel epithelium. In the older, larger cysts (*c.* 120 days) the lining epithelium appeared to be keratinized and keratin flakes stained by the Barnett-Seligman dihydroxyldinaphthyl-disulphide (DDD) method filled the lumen.

Riviere & Sabet (1973) carried out similar work with mice, transplanting 7-day-old 1st molar tooth germs into mammary fat pads for periods up to 21 days. They likewise demonstrated continued tooth growth with cementum, periodontal membrane and alveolar bone formation and the development of coronal cysts in the enamel organ, which did not show keratin formation.

It is interesting to compare these structural changes with those of tooth eruption (McHugh 1961, Ten Cate 1963). McHugh has shown that the outer layers of the reduced enamel epithelium proliferate and unite with a downward growing knot of mucosal basal cells. As this united epithelial mass encloses the erupting crown to form the gingival cuff a split occurs in the reduced enamel epithelial component of the cuff above the degenerating ameloblast layer. It is this split which becomes the gingival crevice when the tooth erupts into the mouth. The experimental follicular cyst appears to arise in the same way as this programmed crevicular separation, its enlargement being due to the proliferation of the unbreached outer reduced enamel epithelium away from the immobile or retarded erupting tooth, so explaining the recognized relationship between follicular cyst formation and delayed eruption.

Although heterotopic in the extreme, the important parameters of tooth formation seem to have been little disturbed in both series of experiments. This work then questions the concept of so-called postfunctional epithelium giving rise to simple unkeratinized cysts with its corollary that the keratocyst arises from prefunctional epithelium (Shear 1960). Furthermore Rud & Pindborg (1969) have said that true follicular cysts do not show keratinization. However, the cysts produced by Bartlett *et al.* (1973) appeared

to keratinize and they propose this to be a keratocyst model but admit that although they had a flat keratinized or parakeratinized surface epithelium with no rete pegs or inflammatory cells, the walls were not always as thin as the human keratocyst and the basal cells did not show the same collumnar appearance.

Browne (1972) has noted that there are 'keratocysts', and cysts which show keratinization. The latter can have complete thin walls and little subepithelial inflammation, and enlarge to a significant size but, like these experimental cysts, differ in certain respects from the classic type. It is recognized that freedom from inflammation may allow a cyst lining to keratinize but it is not known whether such a cyst may also become a true keratocyst. This is of particular importance in explaining those keratocysts which show a true dentigerous relationship to an unerupted tooth as opposed to the so-called secondary dentigerous cyst (Toller 1967) or pseudodentigerous cyst (Browne 1971).

Keratocysts: Few workers dispute the remnants of the dental lamina as being the origin of the keratocyst. Such remnants have been frequently described, especially in the third molar region overlying unerupted teeth. Gettinger (1940) found cell rests in 62% of 220 cases. Baden *et al.* (1968) noted odontogenic epithelial rests which they called hamartomas in the corium of the gingiva, and in some cases demonstrated a cellular connexion with the basal layer of the mucosa. These rests were occasionally associated with dentinal structures, keratin pearls or small cysts.

Stoelinga & Peters (1973) have proposed an interesting theory of origin and recurrence of the keratocyst based on similar observations.

They suggest that true keratocysts arise from basal cell hamartomas of the type described by Baden *et al.* Stoelinga's evidence to support this is that keratocysts are commonly found in the third molar region with an overlying fenestration of the bone implying a submucosal connexion, and that cell rests and microcysts are also found in this submucosal area.

Stoelinga, however, emphasizes the difference between such epithelial hamartomas and dental lamina remnants which he suggests would be deeper and intra-osseous.

The implications of this work are interesting in that: (1) It becomes comprehensible that keratocysts derived from basal cell hamartomas, odontogenic or otherwise, should be associated with basal cell cutaneous nævi in the complete basal cell nævus syndrome. (2) Recurrences after careful enucleation by experienced surgeons may merely indicate their skill in separating the mucoperiosteal flap with its contained epithelial islands from the underlying cyst. Also, subperiosteal dissections of massive keratocysts followed by grafting would, of course, replace these cells back on to the graft and lead to a recurrence. (3) It also fits with Browne's analysis (1971) that the tendency for keratocysts to recur was not related to the presence of satellite cysts or epithelial islands within the capsule of the original specimen. Browne in fact suggested that many of these microcysts appeared to undergo involution, and commented on the possibility of the troublesome epithelial rests having been left in the patient. He also noted melanocytes in the basal layers of two keratocysts from a young West Indian.

Perhaps Stoelinga's submucosal hamartomas provide a reason for these troublesome cysts arising principally in the third molar region. Such epithelial islands would in the normal course of events be carried to the surface by erupting teeth to be harmlessly incorporated in or destroyed amongst the overlying mucosa. It is also possible that their true role was to provide the submucosal component of the gingival cuff, whereas delayed eruption or the absence of the wisdom tooth leaves them to proliferate, producing instead a solitary or pseudodentigerous cyst. Szabo's observation (1954) of keratinizing cysts developing submucosally over explanted tooth germs might support this theory.

This could deny the need for a primordial cyst (Kramer 1974) and explain why sites of exuberant lamina growth producing supernumeraries, e.g. the midline in the maxilla, are not the principal sites of keratocyst formation (Stoelinga 1973).

It would certainly seem worth excising the mucosa overlying such cysts and removing it in continuity with the cyst as recommended by Stoelinga.

Periapical cysts: The pattern of epithelial growth giving rise to the periapical cyst has been a continued source of debate. Valderhaug (1972) has produced periapical cysts in monkeys by pulp extirpation and leaving the root canals open to the mouth for up to 360 days. Cysts developed in 11 out of 16 teeth left more than 200 days, but only did so if no vital tissue was left in the canals.

Valderhaug showed a transition from the periapical granuloma apparently without epithelium, revealing firstly round or ovoid islands then long strands of cells at the junction of the uninflamed connective tissue and granulation tissue. These arcades and rings appeared to surround the necrotic part of the granuloma.

Proliferation gradually created a continuous three-dimensional network or sponge throughout the periphery of the granuloma. Consolidation takes place in the epithelium until the inner surface becomes the lining to a periapical cavity which has been separated from the alveolar bone.

The overall picture is that of a protective or reparative role being played by activated epithelium isolating irritation or infection from the surrounding tissues.

One notable feature was the absence of degeneration within masses of cells producing small isolated cysts, although oblique sections through the sponge could presumably give the impression of daughter cysts. Valderhaug appeared to show, as has been seen in the skin, that younger recently divided epithelial cells push their way towards the vascular connective tissue (Ryan 1966) from the level of the immobile mitotic cell whereas the older degenerate cells are displaced towards the surface or, as in this case, centrally to line the cyst cavity.

Support for this concept of an epithelial repair process is that the very same proliferative changes were seen by Seltzer *et al.* (1969) after they extirpated vital and uninfected pulps and reamed the canals through the apices.

It might therefore be appropriate here to consider the role of Malassez's cell rests generally. Many of the earlier studies which are reviewed by Reitan (1961) have said that these epithelial rests have no function or merely an inconvenient role. This view has been supported more recently by Toller (1967). It has been suggested that the polymorph cell infiltrates (Shear 1963) and lymphocytic aggregates associated with cyst linings are an attempt by the body defence mechanism to eliminate proliferating cell rests (Toller 1972). However, Waerhaug (1958) and Loe & Waerhaug (1961) concluded from their tooth transplant experiments in dogs and monkeys that the rests appear to prevent root resorption. In fact Robinsohn had suggested in 1926 that they maintain the width of the periodontal membrane. Stahl & Slavkin (1972) have reviewed the evidence that the cell rests of Malassez may not only be responsible for the laying down of cementum but are also concerned with its repair. Furthermore, the disappearance of the cell rests or their absence *ab initio* would lead to a loss of periodontal attachment.

There have been many detailed observations of both stasis and regression of dental cysts (Molyneux 1964, Ohlers 1970) after removal of the irritant stimulus by surgery or following endodontic therapy. The belief that cyst epithelial discontinuities are due to inflammatory destruction was probably best discounted by Toller (1966) who showed an equal incidence in uninflamed and in inflamed cyst walls. Such epithelial loss could well be evidence of regression in which case one might find them more prevalent in residual cysts as suggested by Molyneux (1964).

Toller & Holborow (1969) have emphasized the presence of lymphocyte and plasma cell aggregations within the walls of cysts even in the absence of overt infection. By immunoelectrophoresis and fluorescent techniques they have elegantly shown the latter cells to be capable of producing gammaglobulins (IgA, IgG, and IgM) and carrying them into the cyst lumen. Rather than seek to establish the concept that it is the epithelial cells which are antigenically responsible for these lymphocytic foci, i.e. an autoimmune situation, it would seem equally plausible to consider them as simply part of the cyst repair-defence structure. A more refined organization of the epithelial network and associated inflammatory cells is seen at the periphery of granulomas.

The Cyst Capsule and Bone Resorption

The bulk of any dental cyst wall is the connective tissue capsule.

The induction of a connective tissue structure by epithelial cells is well recognized. Folkmann (1971) has shown that tumour cells can secrete a vascular connective tissue induction factor which appears to create an appropriate amount of capsule for the neoplastic epithelium. The cyst connective tissue wall must also be actively maintained by the epithelium or it would become attenuated or incomplete as the cyst enlarged.

The role of the capsule in bringing about the dissolution of the surrounding bone is obscure. This was investigated by culturing cyst explants with mouse calvaria (Harris & Goldhaber 1973). All the bones cultured with the vital cyst fragments showed significant resorption: one interesting exception in this series was an enlarged dental follicle. The technique did not reveal any difference between keratinizing and nonkeratinizing cysts or a cystic ameloblastoma.

This was further investigated by incubating cyst fragments for 30 minutes at 37°C in a 4% solution of elastase. The epithelium was then carefully separated from the capsule using a dissecting microscope and the fragments were then washed and cultured with calvaria as before. Whole cyst and muscle incubated in elastase were used as controls. The results suggested that whereas epithelium could alone produce little more resorption than the controls, capsule could produce significant resorption.

The activity of this bone resorbing agent is comparable to the action of parathyroid hormone and would be equivalent to 0.1 u/ml of parathyroid extract in the media. However, its identification is made difficult by the knowledge that many endogenous substances produce bone resorption in tissue culture (Raisz 1970). Furthermore, the action of a cyst-bone-resorbing agent may not be necessarily confined to the stimulation of osteoclasts. The role of the osteocyte at sites of bone resorption has been well reviewed by Belanger (1969) and Rasmussen & Bordier (1973).

Fell & Weiss (1965) have demonstrated antisera which appear to act on bone cell lysosomes to release destructive hydrolytic enzymes.

More recently Horton *et al.* (1972) have demonstrated stimulated lymphocytes releasing a potent bone resorbing factor in tissue culture. Toller & Holborow (1969) showed aggregates of lymphocytes including plasma cells in cyst walls actively producing immunoglobulins which are therefore available for promoting the release of osteolytic enzymes.

Two principal enzymes of bone cell origin compete for the role of destroying intercellular bone structure: (1) The lysosomal acid hydrolases, notably cathepsin D and cathepsin B1, whose role in matrix dissolution appears to be the degradation of proteoglycan as well as the splitting of polymeric collagen to tropocollagen molecules and their aggregates. Their optimum pH is 4.0 and so they may work intracellularly complementing the action of neutral collagenase. (2) Neutral collagenase which is probably not lysosomal.

Donoff *et al.* (1972) have shown that whole keratocysts have biologically active collagenase in their walls, whereas nonkeratinizing dentigerous cysts do not.

The collagenases, apart from their possible role in digesting bone matrix, are considered to be responsible for collagen remodelling. Collagenolytic activity in the follicle is also thought to facilitate tooth eruption (Ten Cate 1969). Recently Golub *et al.* (1973) have shown that partially erupted wisdom teeth have more activity in their overlying follicles than unerupted ones. Hence the isolation of active collagenase from whole keratocyst is intriguing, particularly if one considers the possibility that these cysts arise from epithelium intended to participate in the eruption process. Donoff *et al.* suggested that the presence of the enzyme may account for the ready separation of the epithelium from capsule, but it may also determine the exceedingly thin and friable nature of the capsule. However, one cannot be sure that the collagenase if released by the cyst wall would have access to the adjacent bone. It is perhaps possible that the factor promoting its release could diffuse from cyst to bone and so promote the release of bone collagenase.

Finally there is evidence that some component of the bone resorbing factor might be a prostaglandin (Harris *et al.* 1973). Cyst homogenized in Krebs solution releases significant quantities of material which when assayed on the rat fundus muscle strip behaves like a prostaglandin. Homogenization of cyst samples in acid/alcohol Krebs to inactivate enzyme activity shows a marked reduction in this activity.

Further assays of media in which cyst fragments were cultured showed significantly larger amounts of prostaglandin-like material than produced by gingiva cultured as a control. Thin layer chromatography of these supernatants revealed a mixture of prostaglandin-like material which was predominantly like PGE_2, and a slower running material which could be PGE_3 or $PGF_{3\alpha}$. Both PGE_2 and PGE_3 are potent bone resorbers *in vitro*.

Summary

(1) Experimental evidence suggests that follicular cysts may be created by the frustrated reduced enamel epithelial component of the eruption process.

(2) Mucosal basal cell rests probably dental lamina in origin could be the source of the keratocyst. Like follicular cysts the origin of keratocyst may be related to a failure of eruption.

(3) The cell rests of Malassez appear to have a reparative role even when giving rise to a periodontal cyst. This is supported by the recognized ability of the cyst to regress once its provoking stimulus has been removed.

(4) There is experimental evidence that the dental cyst releases prostaglandins as its principal bone resorption agent.

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Meeting 20 May 1974

Bacteriological Problems in General Practice

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Should the Mucous Membrane be Sterilized Before Injection?

A potential endogenous source of infection in the mouth is the implantation of oral organisms into the subcutaneous tissues by the local anæsthetic needle. Since the number of implanted organisms can be reduced by the preoperative application of an antiseptic agent (Blake & Forman 1967, Streitfeld & Zinner 1958, Zinner *et al.* 1961) it has been suggested that such a procedure should be adopted when giving a local anæsthetic. However, the necessity for such a procedure may be questioned and the purpose of this paper is to examine the validity of this concept by assessing the extent and nature of the contamination encountered and the magnitude of the hazard involved.

The oral mucosa is continuously bathed in saliva which contains large numbers of diverse organisms. Although the majority of salivary organisms persist only transiently and are quickly washed away, certain bacteria adhere to the superficial epithelial cells of the oral mucosa and constitute the indigenous or resident population. Recently the ability of different oral bacteria to adhere to epithelial cells has been determined. Standardized suspensions of bacteria and epithelial cells were mixed together and incubated at 35°C for 30 min and the epithelial cells then washed free of unattached bacteria by membrane filtration (Gibbons & van Houte 1971). The bacteria attached to the epithelial cells were then counted. Strains of *Streptococcus salivarius, S. sanguis, S. mitis, Actinomyces næslundii* and *Fusobacterium* adhered to epithelial cells, whilst strains of *Neisseria, A. viscosus, Nocardia, Rothia dentocariosa, Escherichia coli* and *S. fæcalis* did not. These findings are of interest since the relative adherence of the organisms to epithelial cells *in vitro* correlates with their distribution on the mucosa *in vivo* (van Houte *et al.* 1971). Although strictly quantitative data relating to specific groups of indigenous organisms on the mucosa are lacking, the predominant bacterial species colonizing the cheek is *S. mitis* since it comprised 65% of the total anaerobically cultivable flora and 76% of the streptococci (Liljemark & Gibbons 1972).

The location of the resident flora on the mucosa was studied by two techniques: either successive layers of epithelium were removed from the cheek and palate with Sellotape and subsequently cultured, or small biopsies of mucosa were examined by electronmicroscopy (Dr D K Whittaker, Cardiff Dental School). Both investigations demonstrated that, in the absence of disease, bacteria were present only on the superficial layer of oral epithelium. There was, however, considerable variation in the number of bacteria attached to epithelial cells in different areas of the same mouth. Cheek and palatal epithelium retained, on average, 28 and 62 bacteria per cell.

It is not surprising, therefore, that the passage of a needle to secure local analgesia invariably implants organisms (Geary & Gavin 1963) consisting predominantly of viridans type streptococci into the subcutaneous tissues (Blake & Forman 1967). Implantation of organisms, however, is not always synonymous with infection (defined as the multiplication of organisms within the tissues) and infection is not always synonymous with disease. A number of factors will determine whether an organism will establish a foothold and produce an adequate reaction for a recognizable lesion to occur. Important in this context is the number and pathogenicity of the implanted organisms and the adequacy of the host defence mechanisms. Previous work has shown that a minimal number of organisms is usually necessary to initiate an infection. Elek & Conen (1957) observed that when fewer than 10^6 staphylococci were inoculated subcutaneously into humans no lesion developed. Clearly this number of bacteria is rarely, if ever, implanted beneath the oral mucosa by a local anæsthetic needle. However, the critical dose must be viewed as a dynamic event and under certain circumstances a smaller inoculum, or a non-pathogenic organism, may achieve the critical level. Particularly susceptible to infection are