A Study of Epithelial Odontogenic Residues in the Pig

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The formation of the junctional epithelium was studied by routine light microscopy in the molar teeth of commercially slaughtered pigs. It was found that the process whereby the reduced enamel epithelium (REE) contributed to the junctional epithelium formation corresponded largely with changes in man previously described by Schroeder and Listgarten (Monographs in Developmental Biology, Vol. II, Basel, S. Karger, 1971).

The configuration of the marginal rests of Malassez (ROM) and their relationship to the junctional epithelium and REE was similarly studied by means of true serial sections. It was found that the ROM in that portion of the periodontium below the alveolar crest formed a well defined network of epithelial strands, but the network became more poorly defined and diverged away from the surface of the cementum as the cementoenamel junction was approached. The coronal border of this network ultimately became continuous with the REE by means of a relatively few vertical strands of epithelium.

The possibility that the presence of epithelial residues in the periodontium may contribute to pocket formation and the potential effects of its configuration and continuity with the junctional epithelium are speculatively considered.

This study is concerned with the changes which occur in the coronal epithelial odontogenic residues, principally the contribution which the reduced enamel epithelium makes to formation of the junctional epithelium, and with the anatomic relationship of these coronal epithelial residues to their radicular counterparts, the rests of Malassez, in the permanent molar teeth of the pig.

Concerning the coronal residues, a comprehensive review of the studies which previously have been carried out to determine the manner in which the reduced enamel epithelium (REE) contributes to formation of the junctional epithelium (JE) has been made by Schroeder and Listgarten. The majority of the early works which they review are of historic interest only, and present day concepts are based mainly upon the light microscopy studies of Engler et al., supplemented by the ultrastructural studies of Stern, and later by the work of Schroeder and Listgarten themselves.

This latter work provides the basis for modern terminology relating to these events, as well as giving a description of equivalent changes which are seen in the human dentition. They show that, initially, increased mitotic activity occurs both in the basal layer of the oral epithelium and also in the outer cells of the REE directly overlying the tip of the erupting tooth approaches the surface. With continued eruption, these two epithelial proliferations fuse to form a solid cylindrical 'plug' of cells, the central portion of which soon afterward is shed. As a result, the tip of the tooth is permitted to penetrate into the oral cavity and crevice formation is initiated at the same time. Deepening of the crevice appears subsequently to result from a continuing loss of epithelial cells from the upper portion of the JE which forms the base of the crevice.

Initial formation of the JE occurs concurrently with initial formation of the crevice. The same mitotic activity which originally was seen in the outer cells of the REE overlying the tip of the erupting tooth spreads slowly and progressively in an apical direction within that tissue. Shortly afterward, the epithelial cells involved in this proliferation undergo a gradual change which renders them indistinguishable from ordinary squamous epithelium, and the innermost of them eventually replace the reduced ameloblast layer. The squamous epithelial cells become adherent to the tooth by means of a hemidesmosomal union to a basement lamina which previously had been elaborated onto the surface of the enamel by the reduced ameloblasts (the 'secondary' epithelial attachment). The reduced ameloblasts themselves previously had been united to this same basement lamina by a similar hemidesmosomal union (the 'primary' epithelial attachment). In summary, final formation of the JE in the mature tooth involves a sequence consisting of proliferation of the outer cells of the REE, squamous conversion, and substitution for the reduced ameloblasts on the surface of the enamel. Apical progression of this sequence down...
the crown of the tooth which would tend to result in elongation of the JE is offset by crevicular deepening and active tooth eruption, so that the length of the JE remains approximately constant until activity ceases when the cementoenamel junction is reached and the situation becomes static.

Concerning the radicular residues, the anatomic configuration of the rests of Malassez (ROM) has never finally been determined in man. It has generally been assumed to be in the form of a network although Scott and Symons in 1977 were still describing this network as being incomplete. The relationship of the REE and JE to the ROM also has not been decided in man despite exhaustive studies of the JE by Listgarten, Siem, Garant, and Schroeder and Listgarten. This could be of crucial importance when considering the development of the periodontal pocket.

The Aisenbergs previously had described thin fingerlike projections of epithelial cells protruding from the JE between bundles of seemingly normal connective tissue fibers and mentioned that other workers thought that the ROM may be involved. Grant and Bernick suggested that a possible continuity may exist between the JE and the ROM in miniature swine, but their observations were made upon teeth prior to eruption and they made no endeavor to show conclusive supportive evidence. Valderhaug and Zander assessed the overall position of the rests in relation to adjacent structures within the periodontium and noted that groups of epithelial cells sometimes were found very close to the JE. Cutress and Crigger described cell rests as being present in the region of the JE in the incisor teeth of sheep, but Cutress subsequently was unable to substantiate continuity between the two structures.

MATERIALS AND METHODS

The specimens used in these studies were permanently erupted molar teeth taken from commercially slaughtered pigs. Approximately 1 hour after death, the mandibles were dissected out of two pig's heads, fixed for 48 hours in buffered formalin and then subsequently immersed in a solution containing 10% formic acid. When the bone had become partially softened, six specimens of clinically normal permanent molar teeth were carefully removed, along with a block of the surrounding tissue. This was done by means of vertical interdental saw cuts, and the blocks of tissue containing the teeth were freed by means of a horizontal cut made by an osteotome below their apices. Since significant color changes could not be accurately assessed in the post mortem tissue, a major criterion for clinical normality was the presence of a normal gingival contour and with the base of the crevice at or above the cementoenamel junction. Final decalcification was carried out on these smaller blocks of tissue and, when this was complete, each tooth was thinned by carefully using a sharp scalpel to pare away the mesial and distal surfaces, leaving merely a thin vertical buccal-lingual section of its central 5 mm or so. These specimens were then serially sectioned at a thickness of 10 μm, sequentially numbered and stained routinely with hematoxylin and eosin.

Sequential changes in the coronal epithelial odontogenic residues of each specimen were assessed by means of routine light microscopy. The relationship which these coronal residues (REE and JE) bore to the ROM, and the configuration of the latter in the marginal region was studied subsequently by means of a series of black and white photomicrographs made individually from each of the numbered specimens at a magnification × 80. By mounting these side by side in the appropriate vertical orientation, a picture of the ROM could be visualized and if necessary their 'third dimensional' pattern could be built up schematically, using methods which have previously been described.

RESULTS

Changes in Coronal Epithelial Odontogenic Residues

Since specimens of erupted teeth were selected in which the crevice was located on the lower portion of the crown, our study of the coronal epithelial odontogenic residues was restricted to those events occurring during the later stages of JE and crevice formation. The reduced enamel epithelium had been totally replaced in two specimens. In the other four it was 3 or 4 cells thick in its unchanged state with most of the cells, including the reduced ameloblasts, appearing somewhat flattened. Coronally the REE merged with a transitional cone (Fig. 1) in which the epithelial layer became gradually widened and increasingly squamous in its appearance, to merge at its coronal end with the JE. The junctional epithelium was typically squamous with a well defined hematoxyphobic layer of cubical basal cells. Prickle cells showing characteristic intercellular 'bridging' were frequently prominent within it. In several specimens the upper border of the JE appeared to divide and to extend coronally for a short distance so that a small portion formed part of the inner crevice wall, while the outer portion joined with the oral epithelium, to contribute to the outer wall of the crevice. The oral epithelium consisted of a typically squamous epithelium with marked papillary and rete ridge formations. Its relationship to the JE at their point of union was a variable one. In some specimens the two tissues 'butted-end'; in others it appeared to overlap the JE (Fig. 2) while in others the wall of the crevice was covered only by a thin layer of cells, or even showed micro-ulceration at the site of union (Fig. 3a).
Changes in the Coronal Connective Tissues

The connective tissues immediately surrounding the coronal epithelial odontogenic residues were of interest. Adjacent to that portion of the REE which was still unchanged, the surrounding connective tissue appeared to be completely normal. It was characterized mainly by the presence of prominent vessels, spaced intermittently at periodic intervals adjacent to the REE (Fig. 1). These probably represented a legacy from the rich vasculature which serves the outer surface of the enamel organ in many species. As one proceeded coronally...
as to comprise a chronic marginal gingivitis. This aspect will be discussed subsequently, but it was notable that it was present to a varying degree in all of our specimens despite the fact that, clinically, they had all appeared to be 'normal.'

**Relationship of Coronal to Radicular Odontogenic Epithelial Residues**

In all specimens the ROM were easily demonstrable in that deeper portion of the periodontal ligament which lay below the crest of the alveolar bone. Cross-sections through them in this region usually took the form of well-defined acinar-like aggregates of four or more epithelial cells. However, when photomicrographs of adjacent sections were examined, it could be seen that their overall format was essentially that of tortuous but continuous spirals of strands of epithelial cells running around the long axis of the tooth, joined at intervals by vertical connections to form a loose network.

The relationship of this network to the coronal odontogenic epithelial residues was also examined. The well defined network, which was present deeper within the periodontal ligament proper, underwent a marked change in the more superficial gingival tissues as the cementoenamel junction was approached. The loops formed by the epithelial spirals tended initially to diverge away from the surface of the tooth and their strands became thinner and more scarce. In addition, the reduced number of strands which were present appeared to be oriented in a more vertical direction. As a result their presence and their continuity became more difficult to demonstrate in longitudinal sections taken through the tooth. However, it could be shown by comparing adjacent serial sections not only that they were continuous but also that they eventually joined up with the JE or REE on the crown (Fig. 4). This was in contrast to the easily demonstrated continuity which has been shown to exist between ROM and JE in pigs' teeth when the latter was affected by chronic marginal periodontitis and the JE was situated further down the root of the tooth.

**DISCUSSION**

Use of pig tissue in this study posed a number of 'pros and cons.' Advantages included the fact that the odontogenic epithelial residues (including the ROM) were readily identifiable in routine hematoxylin and eosin specimens, unlike many other species such as the smaller rodents. Pig molars also showed many similarities to the permanent teeth of man, including their general structure and a susceptibility to develop both chronic marginal gingivitis and periodontitis. These features, would appear to make the pig a particularly suitable model for investigating the role of the ROM in chronic marginal periodontitis and, despite the considerable size of even the "miniature" breed of swine, this
would appear to be an important subject for future radioactive isotope experimentation in those laboratories which are equipped and funded sufficiently to maintain these animals.

The most obvious disadvantages stemmed from the use of commercially processed animals. While this meant that the specimens were cheap and readily available, it inevitably involved some delay in fixation as well as a lack of precise knowledge concerning their previous general health, their diet, and their age. Even
more important, it precluded a precise clinical assessment of the health of the marginal gingiva using the criteria of color, consistency, and tendency to bleed in the living animal. With these limitations in mind these features seem worthy of comment.

First, the changes seen in the coronal odontogenic epithelial residues in the permanent molar teeth of pigs corresponded largely with the changes in man described by Schroeder and Listgarten. On the strength of their findings, they concluded that the outer cells of the REE were "... the progenitor cells which provide the germinative layer of the junctional epithelium." They also regarded cervicovaginal deepening as resulting from loss of epithelial cells from the upper surface of the JE which formed the base of the crevice. The results of this study do not conflict with either of these conclusions. The second factor of concern in the commercial specimens was the invariable presence of some degree of histologic chronic gingival inflammation. This may be peculiar to the species or it may be the result of the nature of the diet on which the pigs were raised, but it is disturbing that this aspect has not received mention or consideration by workers using other species. It is notable for example, that some degree of inflammation appears to be present in the one low power light microscopy illustration shown by Schroeder and Listgarten. Certainly, in the human, it is well recognized that normal tooth eruption frequently is accompanied by marginal inflammation. This is often sufficiently severe and prolonged as to have resulted in the recognition of "eruptive" gingivitis as a clinical entity.

It was assumed that the presence of perivascular "cuffing" probably resulted from vascular drainage of toxic material from the crevice area. In keeping with this it was minimal apically and became increasingly prominent as it proceeded coronally toward the crevice. The fact that it was accompanied by progressive changes in the adjacent epithelium may or may not have been fortuitous, although it is generally recognized throughout the body tissues that chronic inflammation is frequently accompanied by epithelial hyperplasia, squamous metaplasia, and connective tissue fibrosis. This association raises the question as to what portion of the changes seen in the adjacent epithelium was due to properties inherently "programmed" within that tissue and how much, if any, the adjacent chronic inflammation had contributed toward them.

A third observation which may be of potential significance concerned the finding that continuity existed between the epithelial residues on the crown and the main network of ROM which was present in the deeper periodontium. The fact that in the recently erupted tooth, this epithelial continuity comprised a relatively few, vertically oriented strands of epithelium may have clinical implications. Grant et al. have observed that, when JE formation reaches the cementoenamel junction "... there seems to be some arrest in the apical movement." The presence of this epithelial "cuff" could well be one important factor halting continued apical movement of the JE. However, such a concept might lead one to the supposition that once the bottleneck had been overcome (as by inflammatory hyperplasia) and contact made by the main bulk of the JE with the more profuse network of epithelial residues which are present in the deeper periodontium, this same continuity might well facilitate continuing apical movement of the JE and progression of the crevice to form a periodontal pocket.

Early workers originally assumed, without too much evidence, that crevicular deepening and pocket formation resulted from reactive proliferation of the JE in response to an adjacent focus of inflammation. It was Gottlieb who first pointed out that epithelium could not migrate apically unless the coronal fibers of the periodontal ligament first had become compromised. Goldman subsequently identified the dentogingival bundles as forming an even earlier barrier. At the present time it is generally considered that pocket formation results due to damage in the subjacent connective tissues leading to a breakdown of the basement lamina in the JE. As a result, the epithelial cells of the
JE are enabled to grow down passively into the 'vacuum' and the basement lamina reforms at a lower level. Certainly, connective tissue changes have been shown to occur very early in the disease and Deporter and Brown have demonstrated that collagen loss does occur immediately below the JE.

None of these findings however would appear to preclude the possibility that active epithelial proliferation may be a contributory or even the principal factor in pocket formation. Ten Cate has demonstrated that the ROM have a potential for activity and possess the necessary enzymes for glycogen metabolism. Birk et al. and Limeback and Brunette have shown that the ROM possess the capacity to break down collagen, and so a potential for active encroachment upon adjacent connective tissue would not be surprising. In clinical practice it is readily accepted that proliferation of the ROM occurs frequently in the periradicular area. A survey of biopsy specimens taken from the apical periodontium of chronically inflamed teeth in our laboratory has shown that proliferation of the ROM could be demonstrated in approximately 88% of these specimens. In the marginal region some potential for the ROM to proliferate must also exist as inflammatory lateral periodontal cysts do occasionally occur, both in association with auxiliary lateral canals and also (apparently) spontaneously.

The presence of this epithelial 'bottleneck' between coronal and radicular residues in a newly erupted tooth therefore could explain not only why apical progression of the normal JE halts when the cementoenamel junction is reached but also why, despite the frequent presence of persistent and severe chronic marginal inflammation, the condition often remains as a superficial gingivitis for many years. A significant time lag, often lasting throughout the 'teen' years into the early 20s, usually precedes any eventual onset of chronic marginal periodontitis and pocket formation. However, once this epithelial bottleneck has been crossed, then Reese and Wentz could be correct in their assumption that the constant chronic inflammation around the gingival sulcus may cause proliferation of the epithelial rests in the area so that they contribute to its width. Certainly, in the pig, examples were seen which appeared to make this hypothesis worthy of consideration or, conversely, worthy of disproving. In addition, the fact that it has been shown that, in the pig, the ROMs form a network which is in continuity with the JE (Fig. 5) may also mean that any reactive proliferation within them must result in their forming the thin end of a wedge predisposing to the predominantly apical direction of the resulting JE encroachment.

Put another way, if these radicular epithelial residues are indeed capable of significant proliferation, then the concept of Goldmann, that intact dento-gingival fibers would act as a barrier to any primary epithelial proliferation would no longer hold good. In the pig, since they form a continuum with the JE, reactive proliferation of the ROM in response to a chronic inflammatory focus in the adjacent connective tissues would enable them to act as a 'fifth column' already in situ within the intact periodontal ligament, outflanking and bypassing potential connective tissue obstruction, regardless of whether or not the dento-gingival fiber bundles had remained intact.

REFERENCES


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Announcement

PERIODONTICS 1986: COLUMBIA PERIODONTICS
ALUMNI HOMECOMING DAY

DATE: Saturday, April 26, 1986
TIME: 8:30 a.m.-5:00 pm
PLACE: Columbia University School of Dental & Oral Surgery,
Hammer Health Sciences Center, Room 401. Corner
166th St & Ft. Washington Ave, New York, NY 10032
COST: $150 (includes coffee and luncheon)
SPEAKER: Dr. Sture Nyman, Associate Professor and Chairman, Department of Periodontology, University of Gothenburg, Sweden. Visiting Professor, University of Pennsylvania, School of Dental Medicine.
SEMINAR: Reattachment, New Attachment: Update on Basic and Clinical Research.
SPEAKER: Dr. Norton Talisman, Professor and Chairman, Department of Oral Pathology, University of Pennsylvania, School of Dental Medicine.