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HISTOCHEMICAL STUDIES OF THE INTERDENTAL EPITHELIUM IN THE ETIOLOGY OF PERIODONTAL DISEASE[1]

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ABSTRACT

The investigation was performed to show the role of interdental epithelium in the etiology of periodontal disease. Materials obtained from 10 *Cynemolgus* monkeys of mixed dentition stage were studied histochemically. The results disclosed that the DDD reaction and tyrosine test demonstrated the existence of nonkeratinization of the enamel epithelium of the col; that the alcian bule-PAS reaction showed corresponding evidence of low cellular activity of the degenerating enamel epithelium in terms of protective proliferation and keratinization (this fiinding is similar to that of other investigators studying the crevicular epithelium); that the distribution of alkaline phosphatase activity was higher in the inflammatory area beneath the col; and that acid phosphatase occurred only in the keratinized portion of the oral epithelium. The histochemical findings were evaluated in relation to the initiation of periodontal disease. The roles of oral and enamel epithelia, of bacteria, of toxic seepage, of enzymes and of other etiologic factors in the early stage of development of periodontal disease were discussed. It is postulated that the intrinsic weakness of the enamel epithelium may represent the most primary factor in the initiation of periodontal disease, and the interdental and crevicular epithelia may be the most vulnerable sites for the initial lesions of periodontal disease.

The etiology of periodontal disease has been a controversial problem for centuries. It is reported (1) that less than eight per cent of dental research in both Britain and the United States of America is devoted to periodontal disease, and there is a similar situation in Scandinavian and German literatures. As for studies concerning the etiological factors and pathogenesis of periodontal disease, relevant scientific literature is far from abundant.

To find out the primary etiological factors of periodontal disease, investigators (3-10) have recently focused their attention on the vulnerability of the interdental gingival tissues which are under suspicion as the primary sites of the interdental initial periodontal lesions. They and Fish (11) emphasized the need for further confirmation and further study in this field. The author confirmed morphologically that the intrinsic weakness of the interdental epithelium might

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be the most primary factor in the initiation of periodontal disease in his first part of the studies on interdental epithelium (2).

Since the vulnerability of the enamel epithelium of a young interdental col is the starting point from which the whole hypothesis is advanced, the second part of the studies on interdental epithelium was designed and performed to further check up the plausibility of this hypothesis by means of histochemical reactions.

MATERIALS AND METHODS

On account of the lack of regular supply of autopsy material of children, its inavailability of the immediate fixation necessary for histochemical studies, and the need for the great adequacy of close inspection, macroscopic or microscopic, of the interdental tissues, as is facilitated by the studies of experimental animals, the materials of the present study were restricted to the laboratory approaches. A specific phase in the eruption process, the mixed dentition stage was needed for this purpose. Ten monkeys of *Cynemolgus irus* (12) of mixed dentition stage were used to afford material representative of the consecutive period of dental development. In the monkey, the relatively close morphogenetic resemblance to the human dental pattern (13) allows for more conclusive deductions than does other animal material.

The monkeys were sacrificed successively from monkey A to monkey J by means of intraperitoneal injections of overdoses of nembutal sodium. Both jaws were dissected and the pertinent tissues cut into small blocks. The minimal amount of tissue desired for histochemical study consisted of two teeth, *in situ*, in each block.

All the blocks from monkey A to I and some blocks from monkey J were immediately fixed in 10% buffered neutral formalin for two weeks. After decalcification in buffered sodium citrate-citric acid solution, they were embedded in paraffin wax, cut into bucco-lingual serial sections of 8 microns through the center of the interdental septa between adjacent teeth. The plane of sectioning was, as nearly as possible, at right angle to the mesio-distal plane and perpendicular to the occlusal plane. Sections from the left half of the upper and lower jaws of monkey I were used for Gomori's modified alkaline phosphatase reaction (14). Three blocks of monkey I were used for periodic acid-Schiff's reaction (15). The blocks from monkey A to I were used for PAS reaction, Alcian blue-PAS reactions and hematoxylin and eosin stain. Of the remaining blocks from monkey J, six, from the left half of each jaw, were embedded in paraffin as above, and their sections were used for Alcian blue-PAS reaction for glycogen (15), and hematoxylin and eosin stain for comparison. Eight blocks from the right halves of the jaws of monkey J were freshly cut into buccolingual sections at six microns in cryostat after freezing with liquid nitrogen. They were used for the following stains and histochemical reactions: Barrnett-Seligman's DDD reaction for disulfide and sulfhydryl groups (16) Diazo-coupling method for tyrosine (17) Azo dye method for alkaline and acid phosphatases (16) and hematoxylin and eosin stain for comparison.

The microscopic examination of the materials prepared as above provided the basis for analysis and interpretation.

RESULTS

The Gomori's modified alkaline phosphatase reaction disclosed nothing. The failure was probably due to the too long period of decalcification in fifty days. The other histochemical reactions revealed the following findings:

(1) Barrnett and Seligman's DDD (2,2′-dihydroxy-6,6′-dinaphthyl disulfide) reaction for sulfhydryl and disulfide groups—This test showed positive reaction

in the superficial keratinized zone of the oral epithelium but was negative in the enamel epithelium of the col (*Fig. 1*).

(2) Diazo coupling method for tyrosine— was only positive in the keratinized layer of the oral epithelium but was negative in the enamel epithelium of the col (*Fig. 2*).

(3) Alcian blue-PAS reaction for acid mucopolysaccharides and glycogen— positive reaction was found only in oral epithelium at the tip of the interdental papillae (*Fig. 3*). In cases of partly replaced col, the keratinized oral epithelium at the ends of the col was also positive but in somewhat less intensity than the tip area (*Fig. 4*). The diastase digested slides showed a lesser area of weaker positive reaction (*Fig. 5*).

(4) Alkaline phosphatase activity — Alkaline phosphatase activity was found in the subepithelial layer of connective tissues and the border of the bony interdental septum. An interesting finding was that enzyme activity was almost evenly distributed over the whole region beneath the keratinized oral epithelium. However, the papillary zone near the basal layer of the epithelium showed higher alkaline phosphatase activity (*Fig. 6*). There was markedly increased activity under the thin enamel epithelium or in the inflamed area. *Fig. 7* shows this area of increased activity stained by the azo dye method.

(5) Acid phosphatase activity was found only in keratinized epithelial tissues (*Fig. 8*).

DISCUSSION

The histochemical findings of the present study showed some of the characteristics of the interdental epithelium.

(1) Barrnett and Seligman's DDD reaction for combined sulfhydryl and disulfide groups—According to Barrnett and Seligman (18–21), Barrnett (22), Pearse (23), Carruthers and Suntzeff (24), Eisen *et al.* (25), Schultz-Haudt and From (26), and many other investigators, sulfhydryl and disulfide groups of protein are widespread in tissues. They provide an intracellular oxidation-reduction system for metabolism (27–28). These groups may be found at identical sites but with the equilibrium shifted far to one side or the other. Keratins are one class of disulfide-containing proteins. In Keratinization sulfhydryl groups will be oxidized to form disulfide groups between two polypeptide chains. Therefore sulfhydryl groups increase just prior to keratinization and the keratinized zone contains a variable amount of disulfide groups and some sulfhydryl groups as well. The DDD reaction depends upon the specific reactivity of the reagent 2,2′-dihydroxy-6,6′-dinaphthyl disulfide with thiols. It should be strongest in the keratinized zone of the epithelium (18, 29). The present result of this reaction is paralled with that found by Baume recently (30). This indicates the nonkeratinization of the enamel epithelium in the col. Similar findings have also been found in the crevicular epithelium and in the enamel organ by Baume (31) and Dewar (32) respectively.

(2) Diazo-coupling metod for tyrosine— No available literature describes the tyrosine test for the interdental epithelium. Tyrosine is an important precusor of melanin. It is also a component of keratin but only in very small amounts (27–33). However, Sammartino (33) found in keratins an unusually large percentage of tyrosine and some other aromatic amino acids, but relatively low percentages of aliphatic mono-amino acids. "This condition" he explained, "shows that prior to, or simultaneously with, keratinization there is hydrolytic splitting off of amino acids which do not contain sulfur; that aliphatic mono-amino acids are split off from the protein molecule during keratinization and that there is a pseudo-increase in the percentage of the rest." This explanation might be applied to the findings under consideration. The result of both positive

thiols and tyrosine reactions would be the signs of keratinization in oral epithelium and the negative reactions, nonkeratinization in enamel epithelium; and this was found true in the present experiment.

(3) Alcian blue-PAS reaction for mucopolysaccharides and glycogen—There are many PAS-positive compounds in various tissues. The diastase digestion before this reaction is one of the methods used to differentiate glycogen from other PAS-positive substances (34). PAS reaction is widely used for the detection of glycogen and epithelial mucopolysaccharides (10, 26, 30, 32 and 35–47). The presence of glycogen in gingival epithelium is controversial. None of the possible explanation seems to be entirely satisfactory. However, all have agreed that in undamaged skin, the epidermis contains no glycogen (40). In the case of normal gingiva, some suggest that normal epithelium is devoid of glycogen (32, 42). Others described the presence of glycogen in gingival epithelial strata (10, 30, 35, 36, 37, 43, and 46) usually in its marginal part. Since glycogen provides energy for the functions of epithelial cells, such as keratinization and repair, through glycolysis and Krebs cycle reactions, many authors (10, 26, 30, 35, 36, 37, 42, 43 and 48) correlate glycogen deposition in epithelium with keratinization and inflammation. They explain this correlation as follows: Serving as a source of energy, glycogen deposition might have a close relationship with cellular activity. Glycogen may provide energy for keratinization and inflammatory repair. In inflammation, capillary permeability increases and the diffusion of glucose out of capillaries for repair also increases. If this diffused glucose is in excess or the epithelial cells cannot utilize it, it will be deposited as glycogen. Therefore, as inflammation increases the tendency of glycogen deposition also increases in proportion to its severity. In active keratinization, glycogen will be used up, thus keratinization and glycogen are inversely related. No investigator has found any accumulation of glycogen in enamel epithelium.

Fig. 1. Bucco-lingual cryostat section of the col between the two maxillary right permanent incisors of monkey J. Barrnett and Seligman's DDD reaction for sulfhydryl and disulfide groups is positive only in the superficial layer of the oral epithelium, but not in the enamel epithelium of the col. × 40.

Fig. 2. Bucco-lingual cryostat section of the col between the two mandibular right permanent incisors of monkey J. The diazo-coupling method for tyrosine shows positive results only in the keratin layer of the oral epithelium but not in the enamel epithelium. × 22.

Fig. 3. Bucco-lingual paraffin section of the col between the two mandibular left deciduous molars of monkey J. Non-digested Alcian blue-PAS reaction is positive at the tips of both interdental papillae. × 24.

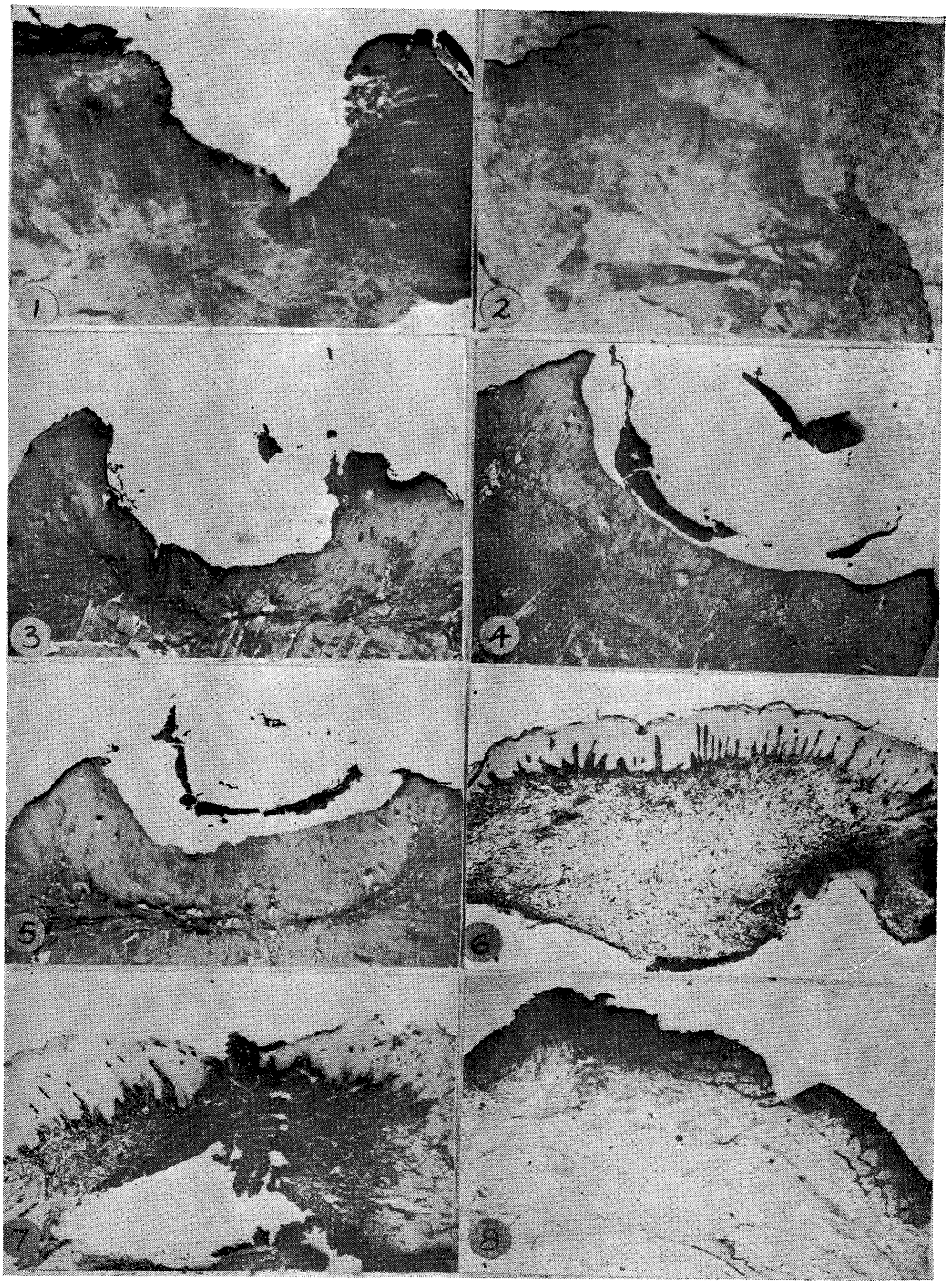
Fig. 4. Bucco-lingual paraffin section of the col between the two mandibular right bicuspids of monkey I. Non-digested Alcian blue-PAS reaction is positive at the tips of both papillae. The surface layer of the extreme ends of the col slopes, replaced by oral epithelium, is also positive. × 18.

Fig. 5. Digested Alcian blue-PAS reaction of the col seen in *Fig. 4.* Note the weakening or disappearance of the positive reaction of the end of both col slopes. × 19.

Fig. 6. Bucco-lingual cryostat section of the oral epithelium overlying unerupted mandibular right permanent second molar of monkey J. The azo dye method for alkaline phosphatase shows evenly distributed enzyme activity in the corium but the connective tissue papillae and the bone border present higher activity. × 18.

Fig. 7. Bucco-lingual cryostat section of the col between the two mandibular right deciduous incisors of monkey J. The azo dye method shows high activity of the enzyme under the erosion at the center of the col. × 24.

Fig. 8. Bucco-lingual cryostat section of the tissue between the maxillary right deciduous lateral incisor and cuspid of monkey J, showing the positive acid phosphatase reaction which is found only in the epithelial layers. × 18.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In the present investigation, the findings of PAS reaction agree with the above description. At the ends of the col, the oral epithelium, which is incompletely keratinized with high cellular activity and is exposed to external irritants, tends to cause glycogen deposition. But enamel epithelium, as a degenerating tissue with low cellular activity in terms of both keratinization and mitotic regeneration, would not be expected to show deposition of glycogen even though there is always some degree of inflammation beneath it. As Trott (37) describes, there is no correlation between the glycogen deposition in the crevicular (enamel) epithelium and the degree of inflammatory changes in adjacent connective tissue. Trott (37) and Dewar (32) assume that glycogen may be a natural protective agent against protein degradation, and therefore, its depletion would allow a more rapid proteolytic breakdown in periodontal disease.

(4) Phosphatases—Phosphatases are the hydrolytic enzymes responsible for the breakdown of phosphate esters and the transfer of phosphate groups from one compound to another (34). Two major categories of phosphatase are catalysing different reactions *in vivo*. They are alkaline and acid phosphatases.

A. Alkaline phosphatases — These have been found in a great varity of cells and tissues, such as the osteoblast, ameloblast, (49–55) odontoblast (56, 57), outer layers of reduced enamel epithelium (58), endothelial cells of the blood vessels and their surrounding connective tissues, inflammatory cells, newly formed collageneous fibers and the basal cells of the epithelium (32, 41, 42, 59 and 60), The functions of this group of enzymes are multiple. Its basic function is the transfer of nutrients (phosphorylated glucose) across cell membrane or a cell barrier (like external enamel epithelium) and the utilization of carbohydrates (49, 55, 61–67). Besides the well-known role in calcification, alkaline phosphatases were found playing important roles in the following processes: mineralization of hard tissues, keratinization (68, 69), collagen synthesis and fibrilogenesis (42, 48, and 61). In these studies, the location of alkaline phosphatase activity fully coincides with the above descriptions. The alkaline phosphatase activity in the corium refects active cellular differentiation in the repair process in the inflamed zone. The negetive findings of enzyme activity in enamel epithelium indicates the extremely low activity of the reduced enamel epithelial cells in keratinization and regeneration.

B. Acid phosphatase — On the other hand, this enzyme is localized especially in epithelial tissues with the greatest concentration immediately beneath the keratinized layer (70). It was found related to the process of keratinization (48, 71), and bone resorption (72). In the present studies, the negative reaction for acid phosphatase in the enamel epithelium may be an additional sign of the lack of keratinization of this epithelium.

This nonkeratinized reduced enamel epithelium with low cellular activity is apparently not as strong in protecting the underlying tissue from the external irritants as the oral epithelium. Such external irritants might be trauma, mechanical irritation, calculus, bacteria and its metabolic by-products or toxins, toxic products from food or tissue breakdown and a great variety of other factors. As it was described in the first part of the studies on interdental epithelium (2), it may be assumed that bacteria and toxin products seem to be the most important "external" irritants in the initiation of periodontal disease. So it may be reasonably postulated that the earliest abnormal deviation in periodontal disease is caused by the toxic seepage through the vulnerable enamel epithelium of the interdental col. These toxic substances would further injure the integrity of the enamel epithelium to

increase its permeability and to decrease its protective cellular activity which is necessary for wound healing (73, 74). Some enzymes in seeping fluid (74–78) would then cause hydrolysis of the proteins in the intercellular substance. This proteolysis, its end product (79–81) along with other toxic substances already in the seeping fluid would be chemotaxic to cause constant infiltration of inflammatory cells beneath the enamel epithelium especially at its junctions with oral epithelium (2). The intrinsically weak enamel epithelium fails to undergo protective or healing reactions and thus becomes more and more thin and vulnerable to extrinsic irritants. Infection and ulceration may then follow, leading to gross manifestations and deeper invasion.

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