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PERIODONTAL TISSUE CHANGES IN VITAMIN A DEFICIENT YOUNG RATS

by

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The influences of nutritional deficiencies upon the etiology and pathogenesis of periodontal diseases are poorly understood. Vitamin A deficiency, specifically, is one area where contradictory findings and considerable gaps in knowledge prevail. The present study was planned in an attempt to establish a clear-cut deficiency state by controlled animal experiment, and thereby elucidate some consequences of this deficiency. A secondary objective was to review the information at hand regarding the influence of vitamin A deficiency on human and animal periodontium.

Human periodontium

Observations of population groups from areas where nutritional deficiencies are endemic indicate a high incidence of periodontal disease. *Day* (1944) examined such a group from northern India, and was not able to establish any specific relationship of vitamin A deficiency to the periodontal conditions mainly because the diets were poor in vitamins A, C, and D as well as other nutrients.

Aided by grants from the California Dental Association, and the Regents of the University of California.

Recently more precise epidemiologic studies were made by *Russell, Consolazio, & White* (1961) and by *Waerhaug* (1962). By suitable scoring of the periodontal conditions and contributory factors such as soft and hard deposits on the teeth, these investigators could rationally relate periodontal disease to other observed phenomena, among which was vitamin A deficiency. The former authors, studying Canadian Eskimos, made biochemical determination of serum vitamin A and carotene, and found no increase in periodontal pathology in nine vitamin A deficient persons. The latter author, in his study of population groups in Ceylon, selected about 500 persons which were predominantly deficient in vitamin A. The criteria for this selection were accepted clinical deficiency symptoms of xerophthalmia, Bitot's spots, and nyctalopia. The conclusion was, that vitamin A deficiency should be considered not as a causative but probably as a conditioning factor.

Experimental vitamin A depletion of human subjects (*Hume & Krebs* 1949) revealed no oral changes within an observation period of 25 months, and indicated the long time required to exhaust the vitamin A stores of previously well nourished persons. A case of severe juvenile periodontitis was related to vitamin A deficiency (*Sud* 1958). However, in this instance a specific relationship may be questioned because the patient evidently suffered from multiple deficiencies, and secondly, most cases of juvenile periodontitis look alike, whether they have a contributory history or not.

Animal periodontium

As more exact knowledge of the effect of vitamin A deficiency on the periodontal tissues is desirable, various investigators have resorted to animal experimentation. In vitamin A deficient monkeys a transient gingivitis with localized recession and eventual necrosis of interdental papillae were noted (*Topping & Fraser* 1939). A histologic examination of the same material (*Tomlinson* 1939) revealed slight keratosis of the alveolar epithelium with occasional small inflammatory foci of the crevicular and attachment epithelium. There was thickening of the attachment epithelium, and, in one animal, necrosis of this area.

In vitamin A deficient dogs (*King* 1937, *Mellanby* 1930, *Mellanby & King* 1934) the gingiva exhibited hypertrophy of the epithelium and thickening of the corium. Gingivitis and formation of periodontal pockets occurred, and the periodontal membrane was irregularly thickened. It was not possible to produce periodontal lesions by resection of the inferior dental nerve (*King* 1937), and thus it was eliminated that the nerve degeneration associated with vitamin A deficiency caused the periodontal disease. In guinea pigs (*Boyle* 1941), vitamin A deficiency produced atrophy of the alveolar bone and retardation of new bone formation. No tendency towards gingival infection was noted.

Regarding the gingival findings in vitamin A deficient rats, two conflicting points of view have been expressed. Several groups of investigators (*Boyle* 1938, *Boyle & Bessey* 1941, *Burn, Orten, & Smith* 1941) described essentially normal conditions, whereas other groups (*King* 1935, 1940; *Marshall* 1927; *Mellanby* 1941; *Mellanby & King* 1934; *Miglani* 1959) maintained that vitamin A deficiency did cause gingival changes, consisting of epithelial hyperplasia and hyperkeratinization, cellular infiltration of the corium, and recession of the gingiva. However, *King* (1940) made the statement, that the changes were not severe, and "might not be generally accepted as evidence of parodontal disease". *Glickman & Stoller* (1948) found no histologic changes of the marginal tissues in the absence of local irritation. When local traumata were present, a higher incidence of gingivitis associated with epithelial proliferation and hyperkeratosis was noted in the deficient rats.

With regard to the alveolar bone of vitamin A deficient rats, various observations are on record. *Marshall* (1927) found portions of the alveolar bone replaced by fibrous connective tissue and abnormally large marrow spaces in the remaining bone. *King* (1935), on the basis of roentgenographic and gross observations, noted rarefaction of the alveolar bone and loosening of the teeth, *Boyle* (1938) described no differences in the alveolar bone of deficient and control rats. However, if the deficiency state was rendered acute by employing the offspring of deficient mothers (*Boyle* 1941), atrophy and resorption of the alveolar bone were noted. In rats, deficient from birth, *Mellanby* (1941)

found alveolar bone of increased density and containing fewer marrow spaces. Rats subjected to a mild chronic vitamin A deficiency (*Burn et al.* 1941), displayed pronounced osteoclastic activity and fibrous replacement of the trabeculae. *Schour, Hoffman, & Smith* (1941), in rats deficient from weaning, found a significantly lower rate of apposition of the molar alveolar bone and a thicker alveolar bone around the base of the incisor. *Irving* (1949, 1956), examining the incisal alveolar bone of rats deficient from weaning, maintained that "the chief change was an excess of osteoblastic activity". *Miglani* (1959) started the rats on the experimental diet when they were 40 days old. He noticed increased osteoclastic activity, little remodelling, and decreased formation of new bone. Further, in the deficient rats the mesial root surfaces exhibited the least amount of cementum resorption in contrast to the control rats, in which the distal root surfaces were the least affected.

In a previously published report (*Frandsen & Becks* 1962), socket healing and endochondral ossification were investigated in young rats subjected to hypovitaminosis A. The main changes observed were a pronounced suppression of the activity of the osteoclasts and a decreased activity of the osteoblasts. In the same material, a phase contrast microscope study of the undenatured or only briefly formalin-fixed interdental papilla between the upper incisors (*Baume & Frandsen* 1953) revealed aplasia of the basement membrane and precocious disintegration of the tonofibrils of the stratum spinosum.

From this perusal of the literature, a part of which has been ably reviewed by *Boyle* (1947), it is evident that serious divergencies exist regarding the behavior of the soft and hard periodontal tissues in vitamin A deficiency. In the authors' rat material referred to above, changes of the gingiva and the molar alveolar bone not previously reported were noted. Therefore, a detailed study of the periodontal tissues of these rats was considered of interest.

MATERIAL AND METHODS

Forty-five male rats of the Long-Evans strain were divided into three groups: Eighteen rats received the vitamin A-free diet from the age of 14 days (seven days before weaning), 18 rats

served as pair-fed controls, and nine rats were given the control diet *ad libitum*. The technique of pair-feeding control animals was employed in order to rule out that changes produced by inanition might be interpreted as being caused by vitamin A deficiency. The rats were kept individually in wire bottom cages, and their weights and food uptake were recorded every second day. In order to produce a relatively standardized trauma of the interdental tissues, a small piece of freshly prepared amalgam was inserted between the right lower first and second molars while the rats were under general anesthesia. This took place when the rats were 60 ± 2 days old, and they were autopsied at ages ranging from 60 to 119 days. The right lower jaws were preserved for the present study, and central sagittal sections of the molar regions were prepared and stained with hematoxylin and eosin. A detailed description of the experimental conditions and the gross changes may be found in the preceding paper (*Frandsen & Becks* 1962).

RESULTS

The rats exhibited clinical symptoms of vitamin A depletion after approximately 30 days on the deficient diet. Shortly thereafter one or more suboptimal maintenance doses (3 μg vitamin A alcohol in oil orally) were required to ensure survival of the animals throughout the experiment. A comparison of the periodontal tissues of the *ad libitum* fed and the pair-fed rats revealed no differences. This indicated that the dietary restriction imposed on the pair-fed controls had no effect on the periodontal tissues. Therefore, differences between the deficient animals and their pair-fed controls may justly be ascribed to vitamin A deficiency.

Because of the amalgam impaction between the first and second molars, the only chance of observing undisturbed interdental papillae would be between the second and third molars. However, most of the rats displayed hair impaction in this area. In the few instances where undisturbed papillae were observed, no differences between control and deficient rats could be seen.

Since the degree of hair impaction varied greatly, deficient and control animals with similar degrees of hair impaction were

selected for comparison. The deficient rats exhibited an altered response to the impaction of hair by displaying increased epithelial proliferation, pocket formation, and decreased cellular infiltration of the lamina propria (Plate 1, Figs. A and B).

At the autopsy it was noted that the amalgam had disappeared from most of the rats. No record was made of when this happened. However, the histologic examination indicated a pronounced traumatic response in the interdental tissues between the first and second molars. Here too, the reaction of the deficient rats deviated from the controls. Most conspicuous was the decreased repair of the interdental tissues. This was demonstrated in nine of the deficient rats by severe pocket formation, abscesses, or denudation of the interdental septum. Similar changes were only seen in one control rat. The cellular infiltration and the pronounced bone resorption in the other control rats were indications of the previous trauma, but repair was well advanced (Plate 1, Figs. C and D). In the deficient rats, the bone resorption normally associated with inflammation and bone necrosis was suppressed.

Deposits of calculus and debris on the mesial surfaces of the first molars were frequent findings in control as well as deficient animals. Hair impaction in this area was only observed in one animal. While these deposits elicited a mild inflammatory reaction of the adjacent tissues, the outer gingival surfaces of the control rats were always free from pathologic changes (Plate 2, Fig. A). In the deficient rats, characteristic deviations from the normal condition included the pocket epithelium which displayed an increase in thickness and proliferated along the cementum; and the epithelium covering the outer surface, which showed focal interruptions of the basement membrane (Plate 2, Fig. B). Higher magnification (Plate 2, Fig. C) indicated that the atrophy of the epithelium was associated with liquefaction of the stratum germinativum making a distinction between epithelium and connective tissue difficult. In the control rats, the transitional epithelium of the gingiva to mucosa was composed of a few layers of cells distinctly corresponding in morphology to the epithelial layers. In the deficient rats, pronounced atrophy of this epithelium was observed (Plate 2, Fig. D). While areas of atrophy of the gingival and mucous membrane epithelium were seen in

most of the deficient animals, liquefaction zones were only observed in a third of them.

When central sagittal sections of the alveolar bone of control and deficient animals were compared (Plate 3), a difference in the architecture of the interradicular bone was noted. While large marrow cavities were present in the control rats (Fig. A), the corresponding areas in the deficient rats were characterized by the presence of bone trabeculae (Fig. B). The alveolar bone of the control rats displayed evidence of the physiologic distal movement of the molars, i.e. bone was deposited on the surfaces mesial to the roots, whereas the bone distal to the roots was dominated by resorption lines and discontinuities of the lamellar systems (Plate 4, Fig. A). In the deficient rats, the mesial bone exhibited apposition, as in the control animals; but also, the distal bone was characterized by apposition indicated by apposition lines and intact lamellae (Plate 4, Fig. B). The periodontal membranes of the deficient rats appeared to be narrower than that of the controls. This observation could be made only when strictly comparable sections of a deficient animal and its pair-fed control were available. These instances were too few to permit any conclusions from actual measurements.

The tissues examined histologically included portions of the minor sublingual salivary glands. The gland tissue in the deficient rats displayed a reduced number of acini, atrophy, but no keratinization, of the duct epithelium, fibrosis, and varying degrees of atrophy of the secreting cells (Plate 4, Figs. C and D). Marked lymphocytic infiltration, especially of serous glands, was also noted.

DISCUSSION

Gingival changes

The interpretation of changes observed in the interdental papillae of rats is encumbered with the lack of standardization of the influence of the outer environment. Coarse diets may give rise to foreign body impaction with resulting pathologic changes of considerable extent (*Frandsen* 1962). Purified diets of powdery consistency cannot exert mechanical traumata on the interdental papillae, but rats raised on such diets may exhibit

pathologic changes caused by hair impaction. The technique of amalgam impaction was employed in the present study in order to effect a severe and somewhat standardized trauma of the interdental papilla. However, even though it was attempted to insert particles of the same size with the same pressure, it was inevitable that variations did occur. Further, it was noted at autopsy that the retention time of the amalgam varied greatly.

An attempt to overcome these inaccuracies was made by comparing control and deficient animals with approximately the same degrees of interdental traumata. The soft tissue changes (Plate 1, Figs. A and B) indicate trends rather than specific alterations. The illustrations, which are representative of the material, show a tendency towards increased epithelial proliferation and pocket formation as a response to foreign body impaction. Whereas this epithelial hyperplasia is in accordance with that found in other studies on rats (*Glickman & Stoller* 1948, *King* 1940, *Mellanby* 1941, *Mellanby & King* 1934, *Miglani* 1959), dogs (*King* 1935, *Mellanby* 1930), and monkeys (*Tomlinson* 1939), the hyperkeratinization of the interdental papillae and the gingiva described by most of these authors was not noted in the present material. As a possible explanation, it is worth mentioning that the gingiva of the rat normally is markedly keratinized. A distinction between normal and increased keratinization may therefore be a matter of a rather subtle quantitative judgement. The undisturbed interdental papillae of control and deficient rats were covered by reduced enamel epithelium which was not keratinized. Damage to the interdental papilla resulted in ingrowth of oral epithelium which replaced the reduced enamel epithelium. Thus it was observed that, subsequent to trauma, the interdental tissues were covered by keratinized oral epithelium in both control and deficient rats.

The foreign body impaction which complicated the interpretation of the interdental tissue changes was practically never seen at the gingival margins mesial to the first or distal to the third molars. Therefore, these regions offered possibilities for exact comparisons of control and deficient animals. Soft and hard deposits in the gingival sulcus of these areas were frequent findings in control as well as deficient animals. While this could elicit inflammatory changes of the sulcular tissues, the outer

gingival surface normally remained unaffected. The changes illustrated in Plate 2 were located to the outer gingival surface. They were considered specific vitamin A deficiency changes in that they were distinct pathologic alterations frequently encountered in the deficient animals, and never were observed in the control animals. For a long time it has been maintained that "the specific tissue change due to deprivation of fat-soluble vitamin A is replacement of various epithelia by stratified squamous keratinizing epithelium" (*Wolbach & Howe* 1925). The present study and a foregoing one (*Baume & Frandsen* 1953) offer evidence that even epithelium, which is normally stratified, squamous and keratinizing may undergo changes in this deficiency.

Healing

The decreased capacity for healing may be connected with a deficient inflammatory response (Plate 1, Figs. C and D), and it focusses the attention on the effect of vitamin A on the resistance to infection. It has been assumed that vitamin A has a specific effect as an anti-infective vitamin (*Boynton & Bradford* 1931, *Green & Mellanby* 1928). However, this specific effect has been questioned (*Clausen* 1938), and the fact that vitamin A deficient animals often die from some kind of infection has been explained by general debility or by the decreased resistance of lining membranes secondary to gland atrophy. Lately, a study of vitamin A deficient germ-free rats (*Beaver* 1961) presented evidence that vitamin A is not a specific anti-infective agent. In these rats, death supervened independently of the germ-free state, and survival time was not increased. The deficient rats of the present study were severely debilitated, and this is offered as an explanation, although not quite satisfactory, for the failing healing of the interdental tissues. The absence of sequestration or resorption of necrotic bone may be a contributory factor.

Bone changes

The bone changes, which are also considered specific, appear to be the consequences of suppressed osteoclast function. The effects of this suppression as regards the healing socket after tooth extraction and the process of endochondral ossification

were described earlier (*Frandsen & Becks* 1962). The findings of that study are consistent with the behavior of the alveolar bone in the present study. Thus the resorption of the alveolar crest seen in the control animals in connection with inflammatory changes of the interdental papilla was absent in the deficient animals (Plate 1, Figs. B and C); the marrow cavities of the deficient animals became partly obliterated by bone trabeculae which were unchecked by resorption (Plate 3); and finally, the alveolar bone surfaces, which normally exhibited resorption coincident with the distal movement of the rat molars, were not resorbed, but became the seat of bone apposition (Plate 4, Figs. A and B).

The alveolar bone of the upper incisors, which was not examined in the present study, was investigated in vitamin A deficient rats by *Schour et al.* (1941) and *Irving* (1949, 1956). They found an increased thickness of the fundic alveolar bone with multiple tongue-shaped bone spicules projecting toward the tooth. *Irving* (1949) further described widening of the maxillary labial bone and apposition on surfaces of the alveolar bone where normally resorption takes place. He also noted a suppression of osteoclastic activity, and maintained that this was a matter of the osteoclasts being overshadowed by excessive activity of the osteoblasts. However, this explanation was questioned by *Frandsen & Becks* (1962), who demonstrated retarded bone formation in the healing tooth socket in vitamin A-deficient rats.

Changes of the incisor alveolar bone similar to those mentioned above were reported by *Irving, Pindborg, Fitzhugh, Weinmann, & Schour* (1952) in rats fed sodium sulfite, which produced symptoms of vitamin A and E deficiencies. Thus, despite conflicting explanations it appears that reasonable consistency exists regarding the changes of the molar periodontal tissues reported in the present study and those of the incisor periodontal tissues described previously.

The objection may be raised, that the impaction of amalgam between the first and second molars could exert orthodontic forces and thus influence the bone picture. This objection is not considered valid because the amalgam was not retained for any considerable length of time, and secondly, it could not exert a

continuous force. Further, the control animals displayed no evidence of orthodontic forces, and the bone pattern ascribed to vitamin A deficiency could also be observed around the second molars.

Although it was not a topic for the present study, it is felt that observations of the root-bone relationships in vitamin A deficiency combined with measurement of tooth movements may furnish information for further evaluation of the principles underlying physiologic tooth movements.

Salivary gland changes

With regard to the salivary glands, little has been added to the excellent account of these and other tissue changes in vitamin A deficiency given by *Wolbach & Howe* (1925). The present findings (Plate 4, Figs. C and D) were consistent with earlier descriptions, with the modification that atrophy of the duct epithelium was more conspicuous than hyperkeratinization. The lack of hyperkeratinization of the gingiva and the salivary ducts observed in the present study, and the fact that *Beaver* (1961) found no cases of hyperkeratinization of skin, hair follicles or sebaceous ducts in vitamin A deficient germ-free rats, indicate a need for further clarification of the postulated specificity of the epithelial hyperkeratinization in vitamin A deficiency.

The observation of salivary gland changes brings up the question whether xerostomia was a factor in producing periodontal alterations. No qualitative or quantitative saliva analyses were performed, but gross observations during the experiment and at autopsy indicated no striking dryness of the rats' mouths. Further, histologic examinations of the glands did not show complete destruction or severe reduction in the number of acini. Thus, while some disturbances in salivary secretion must be presumed to have occurred, it is not likely that they were of major importance.

The fact that no severe destruction of the periodontal tissues occurred in these animals, which suffered from a degree of deficiency barely compatible with life, may lend support to the concept that vitamin A deficiency is not a major causative factor as regards human periodontal disease.

SUMMARY

Forty-five male rats were divided into three groups: Eighteen rats received the vitamin A-free diet from the age of 14 days, 18 rats served as pair-fed controls, and nine rats were given the control diet *ad libitum*. At the age of 60 ± 2 days amalgam was inserted into the interdental area between the right first and second lower molars. Groups of deficient and control animals were killed at ages ranging from 60 to 119 days. The right mandibles were disarticulated, and central sagittal sections of the molar areas were prepared and stained with hematoxylin and eosin.

Gross deficiency symptoms were manifested after approximately 30 days on the diet, whereafter suboptimal maintenance doses of vitamin A were required to ensure survival throughout the experiment. Histologic examination of the periodontal tissues revealed changes, specific and nonspecific, of the gingiva as well as the alveolar bone. The specific changes comprised focal lesions of the gingiva characterized by epithelial atrophy and liquefaction, and hypercellularity of the lamina propria. Other specific changes were observed in the alveolar bone, and suppression of bone resorption was considered to be the responsible factor. Salivary gland changes comprised atrophy of the duct epithelium, reduced number and atrophy of acini, fibrosis, and sialoadenitis.

The non-specific alterations were tendencies towards an increased proliferation of the interdental papilla epithelium, a deficient inflammatory response, and reduced capacity for healing subsequent to trauma of the interdental tissues.

RÉSUMÉ

ALTÉRATIONS DES TISSUS PARODONTAUX DE JEUNES RATS PRIVÉS DE VITAMINE A

Quarante-cinq rats mâles ont été répartis en trois groupes: Dix-huit rats on reçu, depuis l'âge de 14 jours, un régime privé de vitamine A, 18 rats, nourris par paires avec les premiers, ont été utilisés à titre de contrôle, et neuf rats ont reçu le régime de contrôle ad libitum. A l'âge de 60 ± 2 jours, de l'amalgame a été inséré dans la région interdentaire de la première et de la

seconde molaires inférieures droites. Des groupes d'animaux de carence et d'animaux de contrôle ont été tués à des âges allant de 60 à 119 jours. Les mandibules droites ont été désarticulées, et des coupes sagittales des régions molaires ont été preparées et colorées à l'hématoxyline-éosine.

Après avoir reçu le régime pendant 30 jours environ les rats ont présenté des symptômes macroscopiques de carence, et il a fallu ensuite administrer des doses suboptimales de vitamine A pour assurer la survivance des animaux pendant le reste de l'expérience. L'examen histologique des tissus parodontaux a montré des altérations spécifiques et non-spécifiques de la gencive et de l'os alvéolaire. Les altérations spécifiques comportaient des lésions focales de la gencive caractérisées par l'atrophie et la liquéfaction de l'épithelium, et par l'hypercellularité de la lamina propria. D'autres altérations spécifiques ont été observées dans l'os alvéolaire, où on a consideré comme élément résponsable la suppression de la résorption osseuse. Les altérations des glandes salivaires comportaient l'atrophie de l'épithélium du canal excréteur, l'atrophie et la réduction du nombre des acini, la fibrose, et l'inflammation des glandes salivaires.

Les altérations non-spécifiques comprenaient des tendences à une augmentation de la prolifération de l'épithélium de la papille interdentaire, une réaction inflammatoire diminuée, et une diminution de la cicatrisation après contusion des tissus interdentaires.

ZUSAMMENFASSUNG

DIE PARODONTALEN GEWEBSVERÄNDERUNGEN BEI JUNGEN RATTEN BEI MANGEL AN VITAMIN A

45 männliche Ratten wurden in 3 Gruppen eingeteilt. 18 von diesen wurden — 14 Tage alt — mit Vitamin A-freier Nahrung gefüttert. Weitere 18 Ratten stellten paarweise gefütterte Kontrolltiere dar, während 9 Ratten mit der Kontrollnahrung in beliebiger Menge gefüttert wurden. Als die Tiere 60 ± 2 Tage alt waren, wurde Amalgam in den Interdentalraum zwischen erstem und zweitem Molar des Unterkiefers gepresst. Die Gruppe der hypovitaminotischen Ratten und die der Kontrolltiere wurden sodann im Altersintervall zwischen 60 und 119 Tagen getötet.

Das rechte Mandibel wurde exartikuliert, wonach man zentrale, sagittale Schnitte von den Molarregionen herstellte. Diese Schnitte wurden mit Hämatoxylin und Eosin gefärbt.

Die hypovitaminotischen Ratten wiesen makroskopische Mangelsymptome auf, nachdem sie ungefähr 30 Tage lang die Versuchskost erhalten hatten. Nach diesem Zeitpunkt war es erforderlich, suboptimale Erhaltungsdosen von Vitamin A zu verabfolgen, um diese Ratten bis zum Abschluss des Versuchs am Leben erhalten zu können. Die histologische Untersuchung der parodontalen Gewebe wies sowohl spezifische als unspezifische Veränderungen an der Gingiva und in dem die Alveole umgebenden Knochengewebe auf. Die spezifischen Veränderungen bestanden in örtlichen Gingivaläsionen, die durch Atrophie und Verflüssigung des Epithels und durch eine Zellvermehrung in der lamina propria charakterisiert waren. Andere spezifische Veränderungen fand man im alveolaren Knochengewebe. Diese hat man als eine Folge gehemmter Knochenresorption aufgefasst. In den Speicheldrüsen wurde eine Atrophie des Epithels der Ausführungsgänge, eine solche der Acini und eine herabgesetzte Anzahl derselben, sowie Fibrose und Entzündung der Drüsen beobachtet.

Die unspezifischen Veränderungen bestanden in einer Neigung zu vermehrter Proliferation des Epithels der Interdentalpapillen, einer mangelhaften Entzündungsreaktion und einem herabgesetzten Heilungsvermögen nach Beschädigungen des interdentalen Gewebes.

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PLATES

Plate 1.

Interdental tissues of lower molars. Hematoxylin-eosin stain. Original magnification × 64.

A. Area between second and third molars from a 75 day old control rat. Notice epithelial and connective tissue reaction to hair impaction.

B. Same area from a 109 day old deficient rat. Hair impaction is associated with pronounced epithelial proliferation and a diminished inflammatory response of the connective tissue. Pocket formation has occurred at the second molar.

C. Area between first and second molars from a 69 day old control rat five days after insertion of amalgam. There is moderate epithelial proliferation and round cell infiltration. Osteoclasts are resorbing the crest of the interdental septum.

D. Same area from a 70 day old deficient rat five days after insertion of amalgam. Notice pronounced epithelial proliferation and pocket formation, necrosis of the crest, and little evidence of bone resorption.

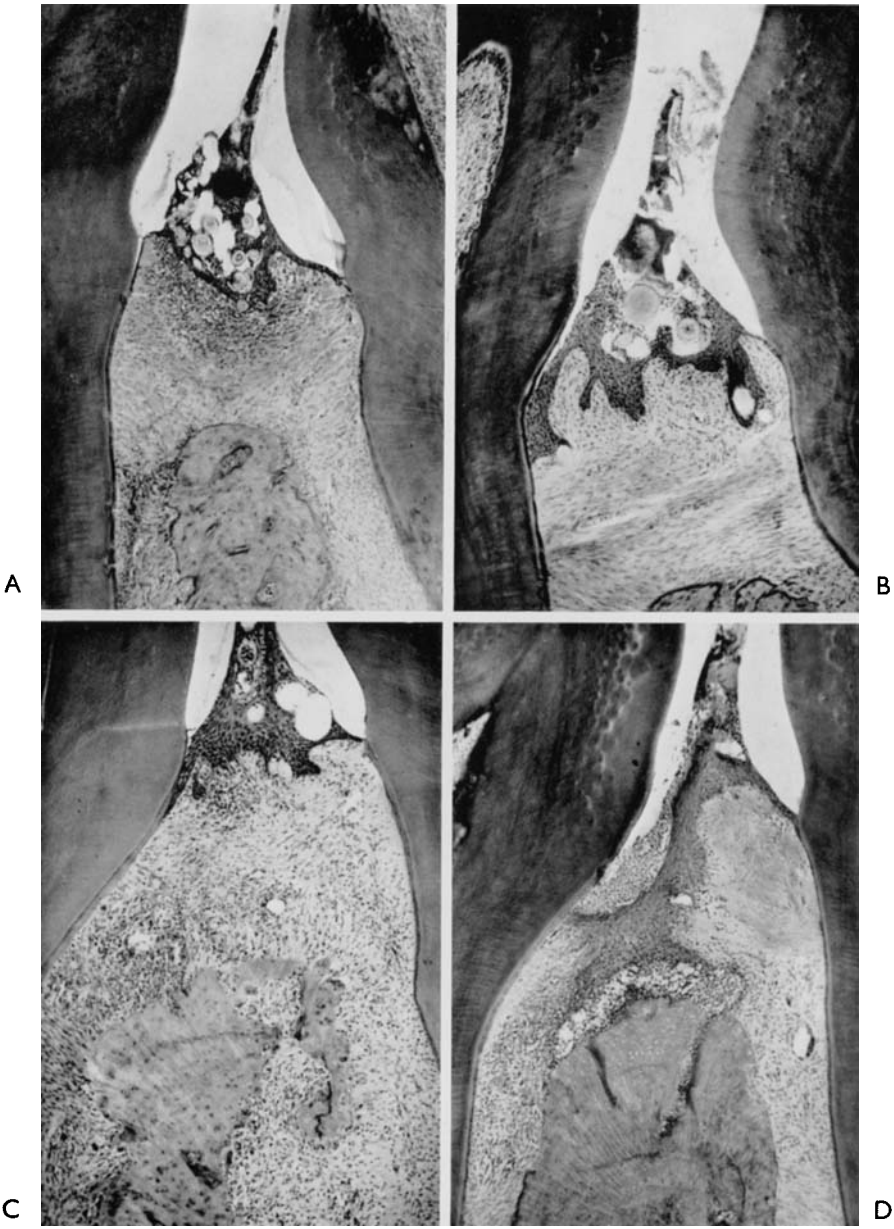
A
B
C
D

Plate 2.

Gingiva mesial to the right lower molar. Hematoxylin-eosin stain.

A. 69 day old control rat. A mild inflammatory reaction to the presence of deposits on the enamel may be seen. The gingival crest and outer surface is covered by a thick squamous keratinizing epithelium. Original magnification × 64.

B. 76 day old deficient rat. Notice proliferation of the sulcular epithelium and foci of atrophy of the outer epithelium. Original magnification × 64.

C. Higher magnification of the upper enclosed area from Fig. B. Liquefaction of the stratum germinativum, and hypercellularity and slight lymphocytic infiltration of the lamina propria are noted. Original magnification × 260.

D. Higher magnification of the lower enclosed area from Fig. B. Notice atrophy of the epithelium and hypercellularity of the lamina propria. Original magnification × 260.

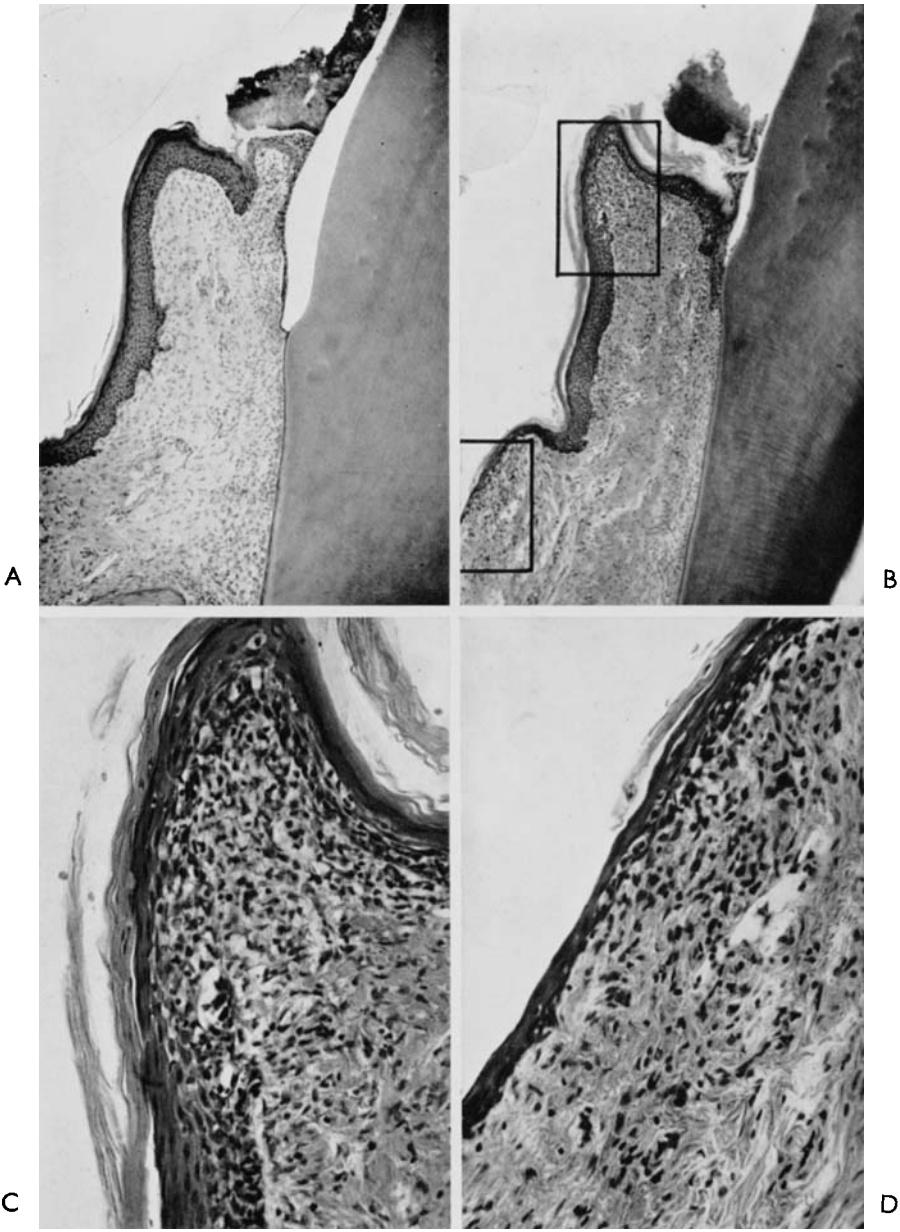
A
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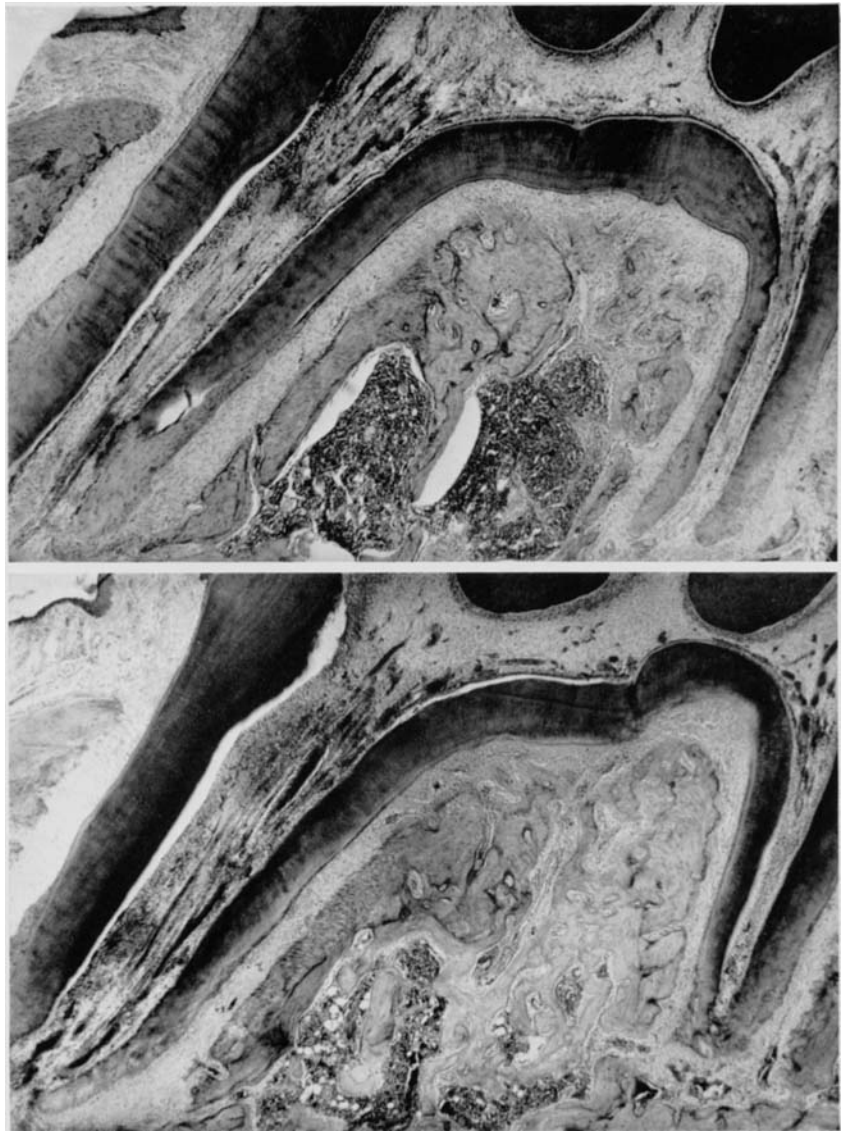
Plate 3.

Alveolar bone of lower first molars. Hematoxylin-eosin stain. Original magnification × 26.

A. 67 day old control rat. Large marrow cavities of the interradicular bone are seen.

B. 73 day old deficient rat. Marrow cavities are occupied by bone trabeculae.



A

B

Plate 4.

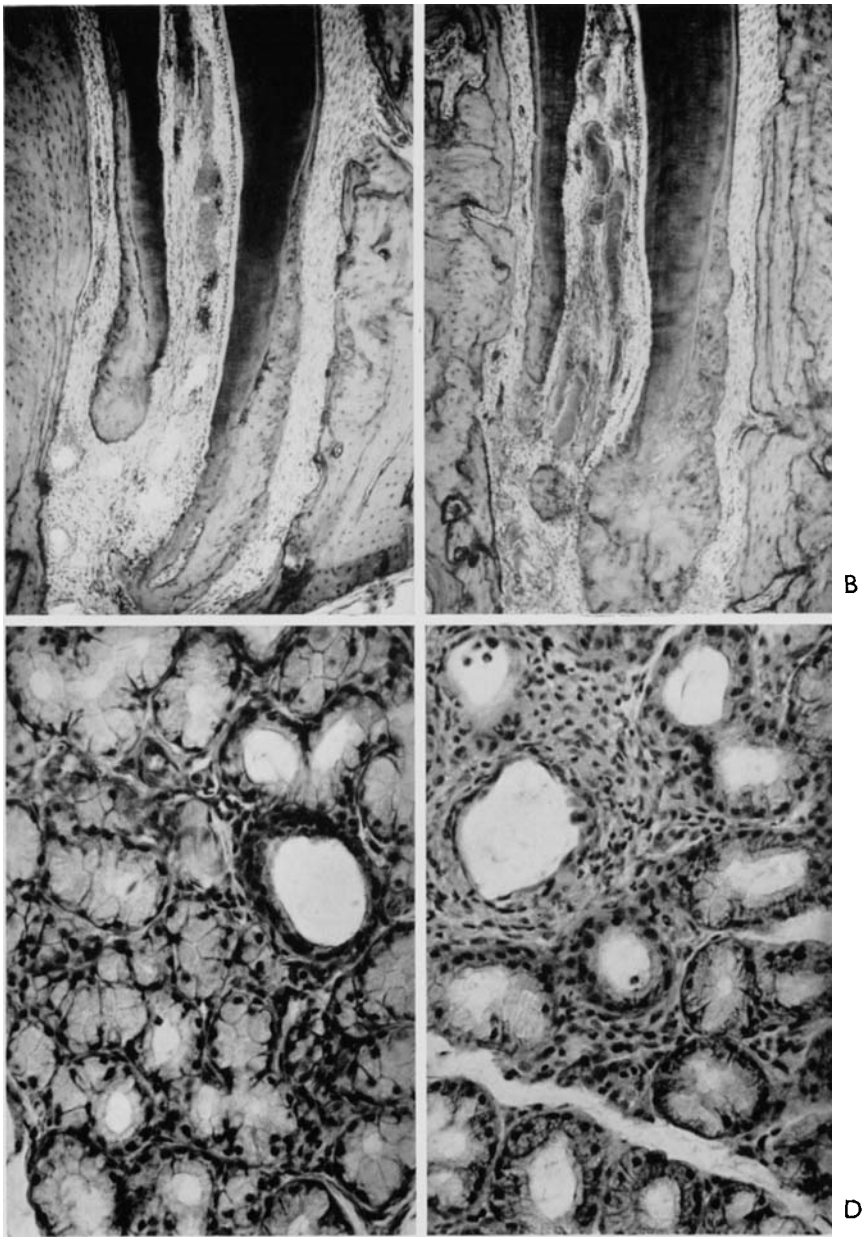
Distal roots of lower first molars, and mucous glands. Hematoxylin-eosin stain.

A. 109 day old control rat. The alveolar bone mesial to the root is characterized by intact lamellar systems, while that distal to the root exhibits discontinuities of the lamellae. Original magnification × 64.

B. 109 day old deficient rat. The surfaces of the alveolar bone display evidence of bone apposition on the mesial as well as the distal side of the root. Original magnification × 64.

C. Mucous acini and duct from a 69 day old control rat. Original magnification × 260.

D. Mucous acini and duct from a 109 day old deficient rat. Notice atrophy of the duct epithelium, fibrosis, and atrophy and reduction in number of acini. Original magnification × 260.


A
B
C
D