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THE EFFECT OF HYPERVITAMINOSIS A ON THE INTERDENTAL EPITHELIUM OF RAT[1]

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ABSTRACT

The investigation was performed to show the effect of hypervitaminosis A on the keratinization of interdental epithelium. Materials obtained from 30 female *Sprague-Dawley* rats were fed with vitamin A test diet containing different amounts of vitamin A for 32 to 68 days. They were studied macroscopically and microscopically. Examinations revealed loss of appetite, hyperirritability and loss of hair in hypervitaminotic groups. Large doses of vitamin A stimulated metaplastic proliferation of the interdental epithelium which was found nonkeratinized. The administration of vitamin A resulted in redifferentiation of the interdental epithelium into structures resembling dental lamina and enamel organ from which the interdental reduced enamel epithelium derived. The deviation from optimal supply of vitamin A can alter the characteristics of epithelial tissues: Large dose of vitamin A can bring about a progressive alteration in form.

For many years, investigators have been trying to find out the primary cause of periodontal breakdown in order to treat this stubborn disease. Among numerous etiological factors, both general or local, the structural vulnerability of the interdental epithelium was noted by Becks (1), Skillen (2), Cohen (3-5), Kohl (6), and Stahl (7). They found that this vestigial epithelium lined the interdental tissues in the morphology of a "col", and it was not able to produce keratin or to heal. According to their findings, they postulated that the interdental epithelium might be the primary location of initial periodontal breakdown. The author studied the structure in problem on the viewpoints of its morphology (8), its histochemical reactions (9), and its developmental origin (10) in 1964, 1965 and 1966 respectively, and proved that the interdental epithelium, derived from the reduced enamel epithelium, was vulnerable because its nonkeratinization and low repair ability which play important role in the initiation of periodontal disease.

In order to further check the plausibility of the forementioned hypothesis, the author observed the interdental epithelium of rats under deficient state of vitamin A which was concerned primarily with the processes of differentiation and keratinization of epithelial cells. The interdental epithelium was found still not keratinized, but with morderate degree of squamous metaplasia and slight parakeratosis, however, the keratinized layer of the oral epithelium of the hypoviminotic rats became thicker than that of the control group (11).

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In spite of the failure in inducing keratinization of the interdental epithelium by means of avitaminosis A, the changes of the structure in problem under the condition of hypervitaminosis A are still worthwhile to be observed to complete this series of studies.

MATERIALS AND METHODS

The experimental animals of choice were *Spraque-Dawley* rats of femal sex. Sixty weaning rats of 40-50 grams of body weight were used. At that time their first molars had appeared but their second and third molars were still unerupted in the oral cavity (12). This was the ideal dental pattern for the study of interdental epithelium. They were fed on normal diet for three days. Their body weights were taken at the end of this period. The rats gaining too much or too little weight as compared with the average gain was discarded in order to avoid the irregular intake of vitamin A. Thirty rats were selected for study after the above screening. They were grouped and fed with synthetic vitamin A test diet (13) in which different amounts of vitamin A (with one tenth of vitamin D_2, preparation of Roch) were added: Group I, the control group, was fed on diet containing 100 I. U. of vitamin A per day; Group II, the group of mild hypervitaminosis A, with 500 I. U. of vitamin A in diet per day; Group III, the marked hypervitaminosis A group, on diet with 5000 I. U. of vitamin A per day.

The body weight of the rats was taken on the day starting to feed on different diets. The rats were then examined every four days to observe the symptoms and signs developed. The animals were sacrificed after different periods of feeding, from 32 to 68 days, by means of over doses of ether inhalation.

After sacrifice, the both jaws of each rat were dissected immediately, and the pertinent tissues cut into small blocks. The minimal amount of tissus desired for study consisted of at least two teeth, in situ, in each block. Totally 120 blocks were obtained from the sacrificed animals. All the blocks were immediately fixed in 10% formalin for five days, and then decalcified in 5% nitric acid for another five days. The interdental tissues of each block were cut into bucco-lingual serial sections of six microns. The sections were stained by hematoxylin-eosin stain and modified Mallory's connective tissue stain (14).

The stained sections were examined microscopically. After repeated screening, 30 of them were kept for interpretation and photomicrography.

RESULTS

The body weight of all the rats follow normal body weight curves without apparent differences between groups although the appetite of the hypervitaminotic rats is mildly impaired. Hyperirı itability presents in the hypervitaminotic rats after one month of feeding.

Macroscopically, the coats of the hypervitaminotic rats become rough. Moderate degree of loss of hair is noted in group II and group III. Even the control group reveals loss of hair but in less severity. Neither the eyes nor the bones of all the groups show abnormal changes.

Microscopically, the interdental gingiva, as a whole, between two contiguous teeth is col in shape. The interdental epithelium of the hypervitaminotic rats reveals metaplastic proliferation and parakeratosis of various degrees (Fig. 2-6) in comparing with a normal interdental col (Fig. 1). Of great interest is the metaplastic proliferation of the reduced enamel epithelium covering the interdental col of a group III rat (Fig. 7). The proliferating epithelium forms structures closely resembling dental lamina (Fig. 7, L) and enamel organ (Fig. 7, E) both in their general appearance and their cellular structures.

The dental lamina consists of three or five layers of cuboid cells surrounded by proliferating connective tissue cells. The enamel organ is in the shape of typical early bell stage. Its layers of inner enamel epithelium, outer enamel epithelium and stellate reticulum can be clearly identified. The superficial side of the enamel organ is still in partial union with the perforated dental lamina. The whole enamel organ is surrounded by proliferating connective tissue cells. This makes the entire structure a complete tooth germ.

The interdental epithelium of all the groups reveals no keratinization (Fig. 1 to Fig. 7) but some mild degree of parakeratosis (Fig. 4, 6, 7). The connective tissue underlying the interdental epithelium of the control group rat (Fig. 1) is clear. However, the interdental connective tissues of hypervitaminotic rats is infiltrated in various degrees in propotional to the proliferation of its covering epithelium (Fig. 2 to Fig. 7).

DISSCUSION

The general appearance of the interdental gingiva between two contiguous teeth is always col in shape and nonkeratinized both in this study or in previous investigations on human being (7, 15, 16) or on different species of experimtnal animels (8-11), under natural (7-10, 15, 16) or vitamin A deficient (11) conditions.

Little is known of the function of vitamin A in biologic system. Vitamin A is an unsaturated alcohol which may function as an oxidation-reduction catalyst. The needs of body depends on weight, not on metabolic activity. This implies that it is chiefly necessary for cellular structure rather than fundtion (17). Seward and coworkers suggested that vitamin A is required in mitochondrial membranes at an optimal concentration. Deviations from optimum may induce functional changes in enzymes associated with oxidation phosphorylation (18).

In addition to its important role in visual processes and growth, vitamin A is also necessary for the maintenance and differentiation of epithelial tissues. Its deprivation results in keratinizing metaplasia of various epithelia (11, 17, 19-21). However, the enamel epithelium of the interdental col does not show any keratinization but moderate degree of squmous metaplasia in vitamin A deficiency (11).

Weiss and James exposed cells to vitamin A in tissue culture. They found that the skin of the chick embryo was no longer capable of producing keratin but instead changed to produce a cuboid or columnar epithelium (22). The classical work of Fell and Mellanby went a step farther, producing a mucous-secreting epithelium from stratified squamous epithelium after exposure to vitamin A in tissue culture (22). Vitamin A is therefore known to have an antikeratinization effect and to be concerned primarily with the processes of keratinization and differentiation of epithelial cells, probably through its oxidation-reduction catalyst action (17) or its effect on oxidation phosphorylation (18). Lawrence and Bern found that vitamin A greatly stimulated cell division and increased the mitotic activity of epithelia (24). Parnell and Sherman also showed that the administration of physiologic amounts of vitamin A caused significant increase in the mitotic index of epithelia, whereas high toxic doses of vitamin A greatly diminish mitotic activity in A deficient and normal rats (25).

Vitamin A certainly plays a part in the nutrition of a tissue. Epithelial tissue is capable of change under different environmental states. When vitamin A is deviated from its optimal supply, it can alter the characteristics of epithelial tissues. Large dose of vitamin A can bring about a progressive alteration in form; whereas

in vitamin A deficiency, regressive alteration in form takes place (25). The proliferation and redifferentiation of the reduced enamel epithelium of the interdental col into the structures resembling dental lamina and enamel organ in this study is a positive evidence for the above hypothesis. Optimal supply of vitamin A is therefore necessary to retain the morphological status of certain epithelial tissues.

Rats fed on high vitamin A diet (30,000 to 40,000 I. U. to rats weighing 100 grams daily) would cause bone resorption, bone fracture and skeletal malformation; coats becomes rough; cachexia; catarrhal conjunctivitis, hemorrhagic rhinitis and diarrhea; disturbance of growth or even death (26, 27). In human being, the toxic effects of vitamin A can only be resulted by intake of 300-500 grams of polar-bear liver which may contain 10,000 times of minimal requirement of vitamin A. There may be loss of appetite, hyperirritability, appearance of sensitive lumps in extremities, cortical thickening of bone, loss of scalp hair, jaundice, severe headache, polyarthralgia, vomiting and diarrhea (20, 26).

The rats of the hypervitaminotic group in this study were fed on diet containing only 5,000 I. U. of vitamin A to each rat daily. There were only moderate loss of appetite, hyperirritability, and mild degree of hair changes. Nothing else of toxic syndrome of hypervitaminosis A could be found.

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LEGEND OF FIGURES

Fig. 1. An interdental col of a rat on normal diet. (× 100)

Fig. 2. Interdental col of a rat of group II. The covering epithelium shows only very little proliferation. (× 100)

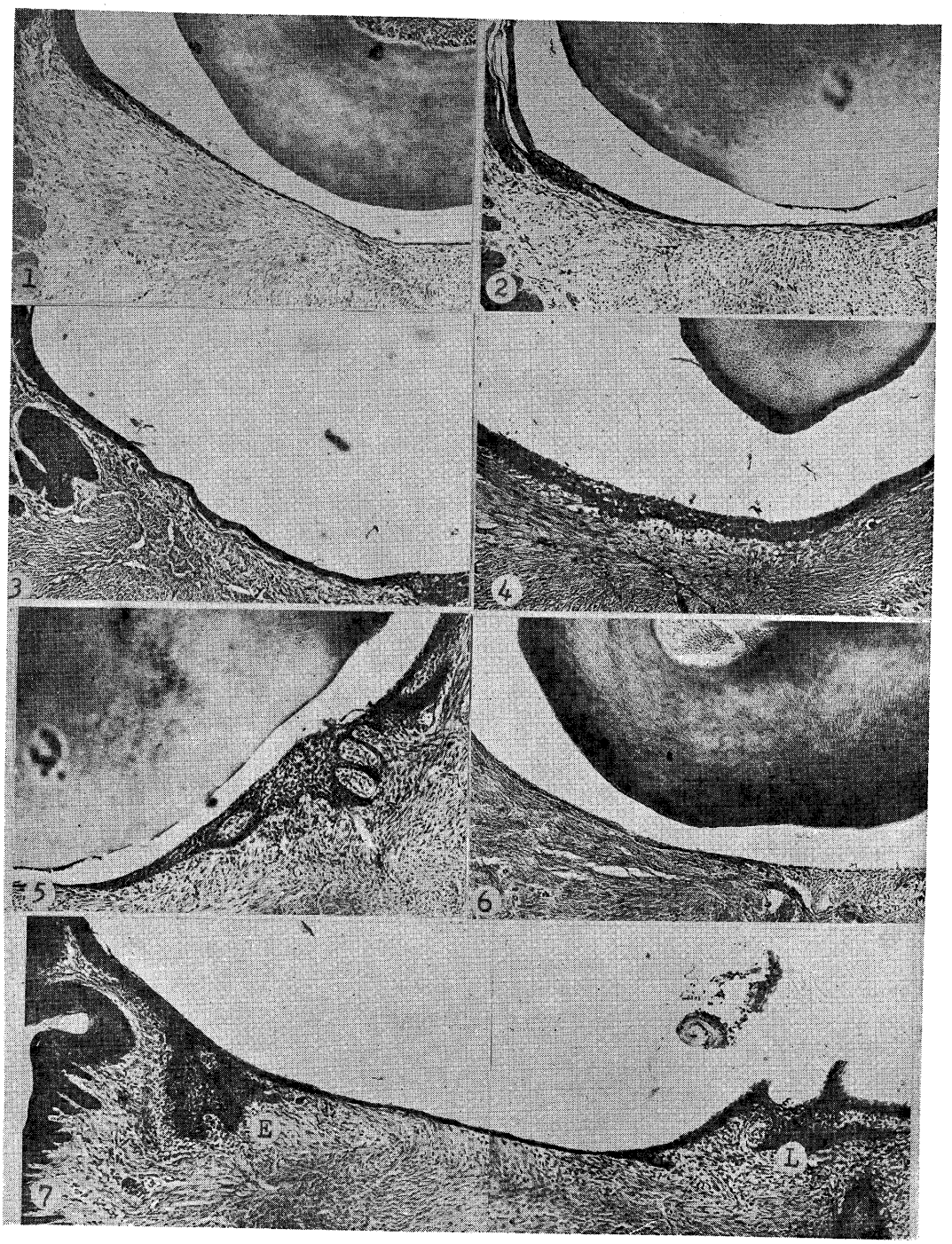
Fig. 3. Mild hyperplasia of the interdental epithelium of a group II rat. (× 100)

Fig. 4. Metaplastic proliferation and parakeratosis of the interdental epithelium of an other group II rat. (× 100)

Fig. 5. Moderate metaplastic hyperplasia of the covering epithelium at the center of an ihterdental col of a group III rat. (× 100)

Fig. 6. Moderate metaplasia of the interdental epithelium (Rt. lower corner) with a little parakeratosis. (Mallory's stain, × 100)

Fig. 7. The metaplastic proliferation of the interdental epithelium of a group III rat. The proliferating epithelium forms the structures resembling dental lamina (L) and enamel organ (E) (× 100)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