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EFFECT OF VITAMIN A DEFICIENCY ON THE STRUCTURE OF RAT INCISOR

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ABSTRACT

This investigation was performed to show the effect of avitaminosis A on the structure of rat incisor. Specimens were obtained from thirty weaning female *Sprague-Dawley* rats which were divided into three groups. They were fed with vitamin A test diet containing different amounts of vitamin A for 32 to 68 days. The right lower incisor of each animal was cut in paraffin sections and stained by hematoxylin and eosin for microscopic study. Macroscopic examination was performed to ascertain the deficiency of vitamin A. Microscopic examination revealed that the mandibular incisor of vitamin A deficient rats produced distinctive defects in this study. There are moderate degeneration of ameloblasts and increase of the thickness of dentin on the labial side and excessive increase of thickness of predentin on the labial and lingual side. However, the width of dentin on the lingual side are not obviously changed. Another consequence of the defective formation of dentin is plication and buckling of the wall of dentin with cell inclusions. The odontoblasts on the labial side are in palisade-like arrangement and high columnar in shape, While the odontoblasts on the lingual side become shortened and in irregular arrangemnt or eventually absent in some places. Both lingual and labial dentin are poorly calcified with numerous interglobular spaces. The possibility of the invading of the proliferating ameloblasts into the pulp chamber to promote the formation of osteodentin in the pulp was discussed. However, the osteodentin thus formed is not evident in this study.

As a large catagory of oral diseases, the developmental anomalies of teeth have been studies by numerous investigators. Many factors were found in close relation with the abnormal development of teeth. A variety of experimental animals were used for such studies, among them, the rat incisor was thought ideal and most frequently used for this purpose due probably to its continuous growth and the founctional similarty of its odontogenic cells (1). The rat incisor develops from an elliptical structure at the apical end of the tooth known as odontogenic epithelium which proliferates throughout the life of the animal. This odontogenic epithelium has three founctions similar to those of the enamel organ in human teeth: it determines the shape of the dentino-enamel and dentino-cemental junctions; it has an organizing influence on the nearby mesen-

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chyme causing the cells to differentiate and promote the formation of dentine; the cells of the labial third of the odontogenic epithelium become enamel organ to produce enamel (2).

Vitamin A is known as an essential factor for the differential growth and the maintenance of normal epithelial structures. Therefore, the deficiency of vitamin A would lead to disturbances of the differentiation of the formative cells and, in turn, the entire process of tooth formation. There would be various degrees of changes in either odontogenic epithelium or the dentine and/or pulp tissues in vitamin A deficient rats.

MATERIALS AND METHODS

The animals used in this experiment were sixty weaning female *Sprague-Dawley* rats weighing 40 to 50 grams. In order to avoid the irregular intake of vitamin A, a preliminary selection of the animals was performed before the study. The rats were fed on normal diet for three days, and their body weights were taken at the begining and the end of this period. Thus, thirty rats, which maintained their body weight at an average level, were selected for study. Other rats with their body weight gaining too much or too little were discarded.

These thirty rats were evenly divided into three groups by random. They were fed with vitamin A test diet (3) containing different amount of vitamin A. Group A was fed on a vitamin A free diet; Group B was fed on a hypovitamin A diet with daily intake of 1 I. U. of vitamin A. Group C was a control group with daily intake of 100 I. U. of vitamin A. These rats were examined every four days throughout the experiment. The changes of body weight, eye, hair, teeth and oral mucosa of the rats were recorded on specially designed cards. All the animals were sacrificed after thirty to sixty-eight days of observation by means of overdoses of ether inhalation. Their right mandibular incisors were immediately dissected, these specimens were fixed in 10% formalin for five days and decalcified in 5% nitric acid for another five days and then embedded in praffin and cut into longitudinal serial sections of 7 microns. The sections were stained with hematoxylin and eosin. All the stained sections were examined under microscope, and some of them were photomicrographed.

RESULTS

The body weight of the three groups revealed no apparent difference within ten days. After that, there was a gradual divergence of the body weight curves (Fig. 1), which became exaggerated after one month of feeding. The control group rats gained the most, while the rats of avitaminotic group began to lose their weight after the second month of feeding (Fig. 1).

The appetite of the avitaminotic rats was mildly impaired and hair began to lose after the tenth day, and ultimately marked alopesia found on most of them. The same manifestations were also found on group B rats in less severity, after about three weeks of feeding. Only two rats of the control group revealed loss of hair.

Different degrees of eye conditions were found in the different groups. The eyes of group C rats were intact. However, in group A and B, various conditions were disclosed, such as watery, sensative to light, slight swelling, congestive or hemorrhagic rings, conjunctivitis or the combinations of two or more of these signs. Neither bloody nor purulent exudate nor opacity of cornea was found in all the groups. The lips of the rats of groups A and B became more grayish than that of the control group. The mentioned signs appeared earlier and more severe in group A than in group B.

In microscopic examination of the sections, following findings were disclosed: In group C, the odontogenic epithelium can be clearly identified at the basal end of the mandibular incisor (Fig. 2). The part of the epithelial organ covering the convex surface of the incisor extended for the entire length of the tooth, while the part corresponding to the concave surface of the tooth consists only of inner and outer enamel epithelia; it extended for a short distance from the root apex and was comparable to Hertwig's epithelial root sheath in human tooth germ (Fig. 2). The enamel matrix was stained basophilic. Its labial surface was smooth (Fig. 3). The average thickness of enamel matrix at the middle third of the tooth measured around 140 microns, while the average height of ameloblasts was 45 microns (Fig. 3, arrow). The dentin was stained pink and measured 135 microns in width at the middle third of the tooth on labial side but a little thinner on lingual side. The predentin was light eosinophilic. Its average width was 10 microns. The pulp surface of predentin was found smooth (Fig. 3). The pulp consisted predominantly of evenly distributed fibroblasts. Blood vessels and nerves presented as usual. The odontoblasts were tall columnar cells of 40 microns in height. Both odontoblasts and ameloblasts arranged in palisade pattern (Fig. 1). Separations between ameloblasts and enamel matrix, and between enamel matrix and dentin might be artifacts caused by shrinkage (Fig. 3). Cementum layer was very thin and stained deep blue

In group A and B, there was mild degree of atrophy of ameloblasts, and their height was reduced. The enamel matrix layer was well-formed with occasional microhypoplastic defects. The average width of the labial dentin was thicker than that of the control group (Fig. 4). The predentin layer, at the middle third of the tooth, was much thicker than that in group C (Fig. 4). The pulpal surface of the dentin and predentin on lingual side showed plication and buckling. Many cell inclusions might be found in the dentin and predentin (Fig. 5, 6). However, the pulpal surface of dentin and predentin on labial side was found smooth (Fig. 4). The odontoblasts on the labial side arranged regularly in a palisade pattern (Fig. 4). But those on lingual side were shorter and disorientated or even discontinued in arrangement (Fig. 5, 6). In comparison to the control group, the odontoblasts in vitamin A deficient rats were shorter. Both labial and lingual dentin were poorly calcified with many interglobular spaces (Fig. 4, 5, 6).

The pulp tissues, except odontoblasts, and the odontogenic epithelium at basal end of the apex were intact.

DISCUSSION

According to the macroscopic and microscopic manifestations of the rats in this study, the animals were found under vitamin A deficient conditions after certain period of feeding, when young animals previously fed a diet containing vitamin A are deprived of this vitamin. They continue to grow for a period dependent upon the amount of vitamin A which they have stored in their livers and kidenys (3) and the amount of experimental diet eaten (4). In this study, about ten days were found to be the duration during which the stored vitamin A maintains the similar growth rate of all the three groups. After that the body weight curves diverged gradually (Fig. 1). While the stored vitamin A was being used up, this divergence became apparent after one month's feeding. The real loss of body weight was found only in avitaminotic group after two months of feeding.

A number of organs might be changed both structurally and functionally in vitamin A deficiency. By the time the body reserves of vitamin A had been depleted and growth ceases, the animal usually

developed xerophthalmia, impaired appetite, loss of hair, xerostomia and various other mainfestations (4-8). In this experiment, most of these menifestations were found in the vitamin A deficient rats in various degrees.

Vitamin A is necessary for the maintenance and differentiation of epithelial tissues (2, 6, 8, 9). The specific or primary consequence of its deprivation is a keratinizing metaplasia of epithelial cells throughout the body (4, 10, 11, 12). Being one of the epithelial structures, the odontogenic epithelium would be affected in the differential growth of its formative cells by vitamin A deprivation. Therefore, this vitamin is essential to the entire process of tooth growth. Such influence of vitamin A has been demonstrated in this study in the evidence of shortening of the ameloblasts. Since the rat incisors are continuously growing at a rapid rate throughout the life, when vitamin A is deficient, defective formation of tooth structures may develop. The developmental anomalies, such as plications or buckling of the dentin wall, cell inclusions in the dentin and predentin, slight thickness of labial dentin, thinness of lingual dentin and marked thickness of predentin are present in this study. These findings are in harmony with Jenkins' conclusions (2). The odontoblasts degenerate at irregular rates during the period of vitamin A deficiency, so isolated groups of cells cease to form dentin while their neighbors remain active. The inactive cells become surrounded by dentin to form cell inclusions, and dentin wall may be folded or plicated or lined by pulp connective tissue cells. There is no satifactory explanation of the differences in response of labial and lingual odontoblasts to vitamin A deficiency. It is remarkable that the former showed increased dentin production at a stage of the vitamin A deficiency at which other odontoblasts have completely disappeared and the body as a whole is losing weight. On the labial side, which is covered by enamel, the odontoblasts become more active and the dentin becomes abnormally thick although it is poorly calcified with many interglobular spaces. It has been suggested (2) that in the rodent incisor, the enamel or enamel organ may exert some restraining influence over the odontoblasts throughout its life. This influence would be removed if the enamel organ degenerates as in vitamin A deficiency. However, this hypothesis has not yet been widely accepted. Investigators agrees that the ameloblasts can exert organizing influence on the mesenchymal cells to stimulate the latter to differentiate into odontoblasts. Jenkins assumes that in regions where the dentin is very thin or absent, cells from the odontogenic epithelium enter the pulp and at their vicinity irregular masses of osteodentin are formed (2, 13-16). Apparently, under the condition of vitamin A deficiency, the odontogenic epithelim fails to undergo normal histodifferentiation and morphodiferentiation. The result is an increased rate of cell proliferation (1, 8). This would be a reasonable explanation of the invasion of the epithelial cells into the pulpal tissue. Where they produce their organizing influence on the pulpal cells to promote the latter to differentiate into odontoblasts and osteodentin is formed. However, the osteodentin thus formed in the pulp tissue is not evident in this study. Jenkins (2) also indicated that the degenerated ameloblasts may be converted into a stratified epithelium, and become hornified. Hunt and Paynter (17) described that both the oral and enamel epithelia have the potential to form a stratified squamous keratinizing epithelium. The authors can not find any positive evidence in this study to support their postulations.

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LEGEND

Fig. 1. Body weight curves of the experimental animals.

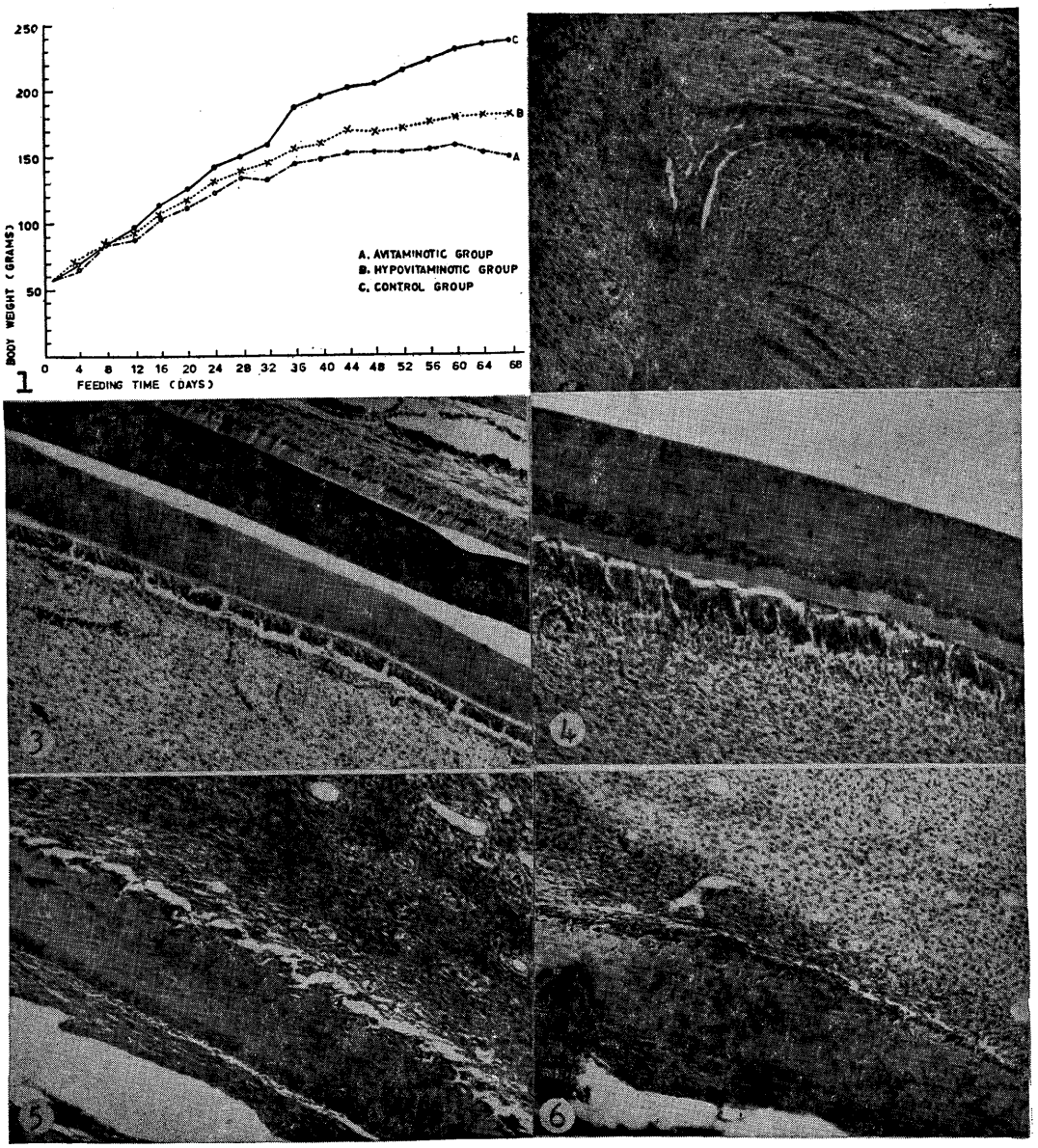
Fig. 2. Normal odontogenic epithelium at the basal end of apex of incisor (× 100.)

Fig. 3. Structure of a normal rat incisor (× 100.)

Fig. 4. Vitamin A deficiency. Note the thickness of predentin and presence of interglobular spaces of dentin (× 100.)

Fig. 5. Vitamin A deficiency. Note the lack of odontoblasts, plication and buckling of the pulp wall, and the presence of many cell inclusions and interglobular spaces (× 100.)

Fig. 6. Vitamin A deficiency. Note the odontoblasts in irregular arrangement, plication and buckling of the interglobular spaces (× 100.)


250
200
150
100
50
BODY WEIGHT (GRAMS)
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68
FEEDING TIME (DAYS)
C
B
A
A. AVITAMINOTIC GROUP
B. HYPOVITAMINOTIC GROUP
C. CONTROL GROUP
1
3
4
5
6