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EFFECTS OF OOPHORECTOMY ON THE INTERDENTAL EPITHELIUM OF RAT[1]

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ABSTRACT

Effect of oophorectomy on the interdental epithelium was studied on rats. Materials were obtained from 30 sexually matured female *Long-Evans* rats. One-half microgram of estradiol was given to the ten out of twenty oophorectomized rats daily. The remaining rats received sham operation. Vaginal smears of each rat were examined everyday to obtain the curves of keratinized cells as a criterion for analysis. The animals were sacrificed successively in 60 days, and their interdental tissues cut into bucco-lingual sections for microscopical examination. The interdental tissue between two contiguous teeth was found always in col shape and nonkeratinized. Various degrees of metaplastic proliferation, with some of them similar to dental lamina, were disclosed in the interdental epithelium of the oophorectomized rats. No apparent macroscopical changes of oral tissues were found. The actions of estrogen and the relationship between ovary and oral tissues were discussed.

In as early as 1930 Skillen (15) studied on interdental papillae and indicated that the interdental epithelium is not only subject to infection but also affords little or no protection to the underlying connective tissue since there is no keratinized oral epithelium to back up it. Cohen (1, 2, 3) and Shyu (9, 11) observed the morphological pattern of the interdental epithelium and its developmetal origin. They found that the interdental structure between two contiguous teeth is "col" shaped and the newly formed col is covered by reduced enamel epithelium, a degenerated or at least atrophic vestigial tissue without the two foremost characteristics of the protective integument of gingiva, namely, the ability of producing keratin and the capacity to heal. Some other investigators (6, 16) and the author's (10) studies had confirmed the morphological features of the interdental papillae and agreed that this region might be the primary site of the initial periodontal breakdown. All the above studies indicated that the nonkeratinization and low repair ability play important role in the initiation of periodontal disease.

Keratinization of epithelium may be influenced by many factors, among which vitamin A and estrogen appear to be the most important ones. The role of vitamin A in keratinization of the interdental epithelium has been studied in this laboratory. It has been shown that, in rats, avitaminosis A did not induce keratinization of the interdental epithelium (12), and hypervitaminosis A resulted in some metaplastic proliferation and

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the redifferentiation of the interdental epithelium into structures resembling dental lamina and enamel organ (14). These results indicated that vitamin A may be not related to the pathogenesis of periodontal disease.

In regards to estrogen, Schultz-Haudt and From (8) indicated that estrogen might influence the keratinization of both the stratified squamous epithelium and nonkeratinized epithelia. The effect of estrogen on the interdental papilla, however, has never been studied. In this experiment, the author attempted to observe the effect of estrogen insufficiency on the keratinization of interdental epithelium by means of the removal of the ovaries of experimental animals in order to further check the plausibility of the hypothesis which relates the interdental epithelium to the initiation of periodontal disease.

MATERIALS AND METHODS

The experimental animals of choice were sixty weaning female *Long-Evans* rats of 40-50*g* body weight. They were fed on laboratory diet until 80 days of age to insure sexual maturity (5). Then, three estrous cycles were followed with vaginal smears using lavage method and Wright stain. Thirty rats with little variation according to the record of these three estrous cycles were thus obtained. They were evenly grouped into three groups by random.

On the first day after grouping, the rats of groups A and B were operated to remove their ovaries. A small portion of the upper horn of the uterus was removed simultaneously to insure complete removal of all the ovarian tissue. Sham operations were performed on group C rats as control. From the ninth day after grouping on, 0.5 micrograms of estradiol dipropionate (CIBA) in 0.05 ml olive oil was given to each of group B rat subcutaneously daily to maintain persistant estrus (5), and vaginal smears of every experimental animal were studied daily to determine the percentage of keratinized cells until sacrifice.

From the sixteenth day following oophorectomy, the rats of each group were sacrificed successively two or three at a time at the intervals of two weeks by means of excessive dose of ether inhalation. The last sacrifice was on the 60th day after operations.

Both jaws of the rats were dissected immediately after sacrifice. The pertinent tissues were cut into small blocks. The minimal amount of tissues desired for study consisted of at least two teeth, *in situ*, in each block. All the blocks were immediately fixed in 10% formalin for five days. After decalcified in 5% nitric acid solution, processed and embedded in paraffin wax, the interdental tissues of every block were cut into bucco-lingual serial sections of 6 microns, and stained with hematoxylin-eosin and modified Mallory's connective tissue stains (13).

The sections from above steps togather with the vaginal smears made during pre- and postoperative stages were studied microscopically and analysed.

RESULTS

The percentage of keratinized epithelial cells in the vaginal smears of all the three groups of rats follow similar curves (Fig. 1) before operations. After oophorectomy, the curve of group A drops down abruptly and maintains at a very low level near zero throughout the experiment. The curve of group C rats changes as regularly as the normal curve before operation. In group B the percentage of keratinized cells in vaginal smears is as low as group A after oophorectomy until the estradiol is

given. The curve then rises and varies irregularly between the curves of groups A and C. It varies around 50% of keratinized cells. Its estrus and diestrus are not apparent, and the intervals between stages of the estrous cycles are no longer regular. The curve B keeps in such variation throughout the entire experiment (Fig. 1).

There is no apparent difference between either the macroscopic appearence of oral tissues of the three groups of rats, or among their body weight curves.

In microscopic examination of the interdental tissues, the interdental gingivae between two contiguous teeth, as a whole, is in col shape (Fig. 2-7). The reduced enamel epithelium covering the cols of all the rats is not keratinized (Fig. 2-7). The interdental epithelium of group C rats is thin with its underlying connective tissue free from inflammatory infiltration (Fig. 2). Various degrees of squamous metaplasia and parakeratosis of the interdental epithelium can be noticed togather with chronic inflammatory infiltration in its connective tissue in group B (Fig. 3-5) and group A (Fig. 6, 7). The keratinized layer of the oral epithelium extends from the gingiva up to the tips of the interdental papillae of the rats of every group, then disappears at the junction of the oral and enamel epithelia (Fig. 2-7). The metaplastic proliferation of the interdental epithelium of some of the oophorectomized rats reveals resemblance of its structure to dental lamina (Fig. 5-7).

DISCUSSION

It is quite uniformly found in the previous investigations (2-11) and in this study that the morphology of the interdental tissues between two contiguous teeth is always col in shape and nonkeratinized regardless the different sources of materials and the various conditions of experimental environment.

Ovary is the main organ producing estrogenic hormones. Estrogens have many actions other than their influence on sexual organs and secondary sexual characteristics, such as protein synthesis, lipid peroxidation, collagenogenesis, calcium metabolism and keratinization. But the precise role played by estrogen is not very clear.

In the lower animals, the estrogenic hormones induce estrus, a series of changes in the female reproductive system accociated with ovulation. The changes may be most sensitively detected by the histological appearance of the vaginal smear as the author has done in this study. The estrous cycle of rat, as described by Long and Evans, extends over four days, and is devided into five periods. In most cases, it may extend through the fifth day (5). This is also quite true in this study according to the regular changes of the curve of keratinized cells in vaginal smears of group C rats (Fig. 1, curve C).

It appears to be clearly demonstrated that the phenomenon of estrous cycle is depended upon a periodical activity in the ovaries. When the ovaries are completely removed, the cycle ceases. Under this condition, there are only very few or even no keratinized cells in all the vaginal smears because of the lacking of estrogen (Fig. 1, curve A).

According to Griffin and Farris (5), 0.5-1.0 microgram of estrone given to spayed young rat subcutaneously once per day will result at least 50% cornification in vaginal smear. This is in harmony with the result of this experiment (Fig. 1, curve B). However, Davidson *et al.* (4) indicate full cornification following administration of 0.2 microgram of estrogen per day to spayed rat.

The metaplastic proliferation and its resemblance to the architecture of dental lamina (Fig. 3-7) are quite similar to the effect of hypervitaminosis A on the interdental epithelium of rat (14); that is to say, bringing about progressive alterations in its form. This resemblance implies that there might be some common mode of action exerted on the epithelial tissues by these two conditions, and optimal supply of vitamin A as well as estrogen are therefore necessary to retain the morphological status of certain epithelial tissues.

In human being, evidence has been presented that certain gonadal disturbaces may be reflected in the oral mucosa and periodontal tissues. The most familiar examples of diminished estrogen are pregnancy and climaterium. A number of oral manifestations can be noticed in these cases, such as: gingivitis, gingival bleeding, pregnancy tumor, pale, dry and atrophic oral mucosa, etc., Langley and Cheraskin have described in their book that, in animal experiment, female sex hormones apparently cause very definite alterations in the periodontal tissues (7). However, the author does not find such changes in this study. It is presumable that the above mentioned clinical findings in pregnancy and climaterium are resulted from some local irritating agents superimposed upon a systemic background which is still to be clarified. Much work is needed to clarify the effects of female sex hormones on the oral and periodontal tissues.

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LITERATURE CITED

1. COHEN, B. 1959. Morphological factors in the pathogenesis of periodontal disease. *Brit. Dent. J.*, **107**: 31-39.
2. COHEN, B. 1959. Pathology of the interdental tissues. *Dent. Practit. and Dent. Rec.*, **9**: 167-173.
3. COHEN, B. 1960. Comparative studies in periodontal disease. *Proc. Roy. Soc. of Med.*, **53**: 275-280.
4. DAVIDSON, J. M., E. R. SMITH, C. H. RODGERS, and G. J. BLOCH. 1968. Relative thresholds of behavior and Somatic respones to estrogen. *Physiol. Behav.* 3: 227-229.
5. GRIFFIN, J. Q., and E. J. FARRIS, 1942. *The Rat in Laboratory Investigation*. Lippincott, Philadelphia. pp. 3, 52, 103, 319.
6. KOHL, J. T., and H. A. Zander, 1961. Morphology of interdental tissues. *Oral Surg. Oral Med. and Oral Path.*, **14**: 287-295.
7. LANGLEY, L. L., and E. CHERASKIN. 1956. *The Physiological Foundation of Dental Practice*. Mosby, St. Louis. pp. 504-515.
8. SCHULTZ-HAUDT S. D., and S. FROM. 1961. Dynamics of periodontal tissues, I. the epithelium. *Odontologisk Tidskrift*, **69**: 431-460.
9. SHYU, K. W. 1964. Morphological studies on interdental epithelium in the etiology of periodontal disease. *Chinese Med. J.*, **11**: 137-152.
10. SHYU, K. W. 1965. Histochemical studies. on interdental epithelium in the etiology of periodontal disease. *Bull. Inst. Zool., Academia Sinica.*, 4: 1-9.
11. SHYU, K. W. 1966. Development weakness of the interdental epithelium. *Bull. Inst. Zool., Academia Sinica.*, 5: 23-28.
12. SHYU, K. W. 1967. Effect of avitaminosis A on the keratinization of the interdental epithelium of rat. *Chinese Med. J.*, **14**: 375-384.
13. SHYU, K. W. 1967. A modified Mallory's anilin blue collagen stain for keratin. *Chinese Med. J.* **14**: 276-278.
14. SHYU, K. W. 1968. Effect of hypervitaminosis A on the interdental epithelium of rat. *Bull. Inst. Zoll., Academia Sinica*, **7**: 49-54.
15. SKILLEN, W. G. 1930. Normal characteristics of the gingiva and their relation to pathology. *J. Amer. Dent. Asso.*, **17**: 1088-1110.
16. STAHL, S. S. 1963. Morphology and healing patterns of human interdental gingiva. *J. Amer Dent. Asso.*, **67**: 48-53.

LEGEND OF FIGURES

Fig. 1. Curves Showing the percentage of keratinized cells in vaginal smears made during pre- and postoperative stages. (op.: oophorectomy or sham operation)

Fig. 2. An interdental col of a rat of control group. (×100)

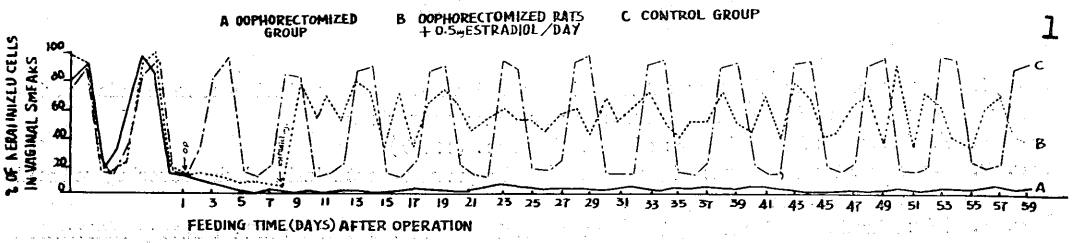
Fig. 3. Interdental col of a rat of group B. Mild squamous metaplasia of the covering reduced enamel opithelium is seen. (×100)

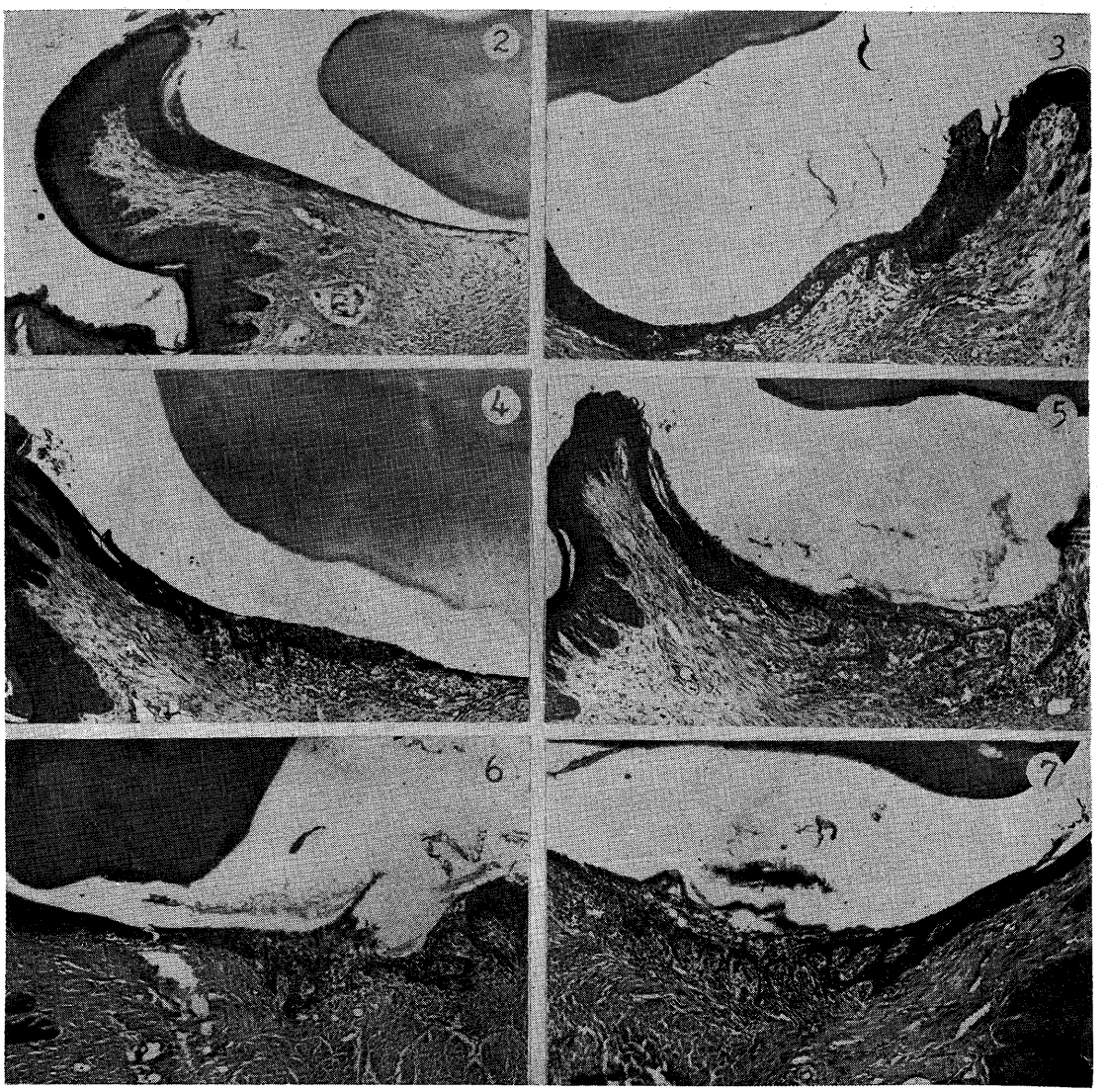
Fig. 4. Metaplastic proliferation of the interdental col and the chronic inflammatory infiltration of its connective tissue of a group B rat. (×100)

Fig. 5. An interdental col of a group B rat showing moderate metaplastic proliferation of the covering enamel epithelium with structures resembling dental lamina, and inflammatory infiltration in connective tissue. The keratinized layer of the oral epithelium extending from gingiva disappears at the junction of the oral and enamel epithelia. (×100)

Fig. 6. Moderate degree of squamous metaplasia of the interdental epithelium of a group A rat. (×100)

Fig. 7. The metaplastic proliferation and parakeratosis of the interdental epithelium of the col of a group A rat. Note the presence of structures resembling dental lamina. (×100)


A OOPHORECTOMIZED GROUP
B OOPHORECTOMIZED RATS + 0.5㎎ESTRADIOL/DAY
C CONTROL GROUP
1
% OF NEBAINUCLU CELLS IN VAGINAL SMEARS
100
80
60
40
20
0
C
B
A
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
FEEDING TIME(DAYS) AFTER OPERATION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