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# EFFECT OF OOPHORECTOMY ON THE RAT INCISOR[1]

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## ABSTRACT

Thirty matured Long-Evans female rats were divided into three groups. Oophorectomy on group A and group B were performed. Group B received an injection of 0.5 micrograms of estradiol dipropionate daily from the ninth day after operation. Sham operation were performed on group C rats for control. Vaginal smears were examined daily before and after operation. From the sixteenth day after operation the rats were sacrificed successively at intervals of two weeks. Left lower incisor of each rat was dissected and sectioned by paraffin method for microscopic examination. Atrophy of ameloblasts, hypoplasia of enamel matrix, numerous interglobular dentin on the labial side, osteodentin in the pulp, slightly increased thickness of dentin, folding of enamel matrix and lingual dentin were found in oophorectomized rats. The main effect of oophorectomy on rat incisor might be on the metabolism and acceleration of growth at the basal end of the incisor. The balance of pituitary growth hormone and gonadal hormone action seemed to be disturbed after operation, therefore some tissues of rat inciosr grew and developed abnormally.

The oral cavity contains a number of different structures which may be influenced characteristically by local and systemic diseases. The gingiva and oral mucosa reflect metabolic disturbances of the present (blood dyscraseas, nutritional deficiencies); on the other hand, enamel and dentin give permanent and chronologic record of systemic influences of the past (enamel hypoplasia). Disturbances in maturation of the teeth may offer evidence of thyroid deficiency, while overgrowth of the mandible and maxillae may be a sign of acromegaly. Oral mucosa has also been suspected of presenting evidence of certain endocrinopathies in particularly those associated with the gonad. It must be pointed out that, in human subjects, the abnormalities of oral structures are usually caused by the dysfunction of more than one endocrine organ (7). In some experimental studies, however, we are able to obtain relatively pure cases of underfunction or overfunction of the endocrine organ. In this investigation the effect of oophorectomy on the rat incisor is studied in order to elucidate the relationship between the development of tooth and the dysfunction of ovarian gland.

## MATERIALS AND METHODS

This study is based on the histological

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examination of rat incisor. Sixty weaning female Long-Evans rats were used for this study. Their body weight were around 40-50 grams. They were fed on ordinary diet (chicken feed, Taiwan Sugar Co., Taipei) to the age of 80 days in order to insure sexual maturity (3). Vaginal smears using lavage method and Wright stain for the examination of the changes of the keratinized cells during the estrous cycles of these rats were performed in this period. Then, thirty rats, with the least variation of their estrous cycles, were selected as the experimental animals, and they were evenly divided into three groups by random as A, B and C.

An operation of removal of ovaries of rats of group A and graup B were performed on the day of grouping. A small portion of the upper horn of the uterus was removed to insure that all the ovarian tissue was removed completely. Sham operation for rats of group C, the control group, were performed on the same day. From the ninth day after operation on, 0.5 micrograms of estradiol dipropionate (CIBA Pharmaceutical Company, Summit, N. J., U. S. A.) in 0.05ml olive oil was given to each rat of group B subcutaneously daily to maintain persistent estrus (3), and vaginal smears of each experimental animal was studied daily to determine the percentage of keratinized cells until the sacrifice of the rats.

From the sixteenth day following oophorectomy, two or three rats of each group were sacrificed successively at the intervals of two weeks by means of overdose of ether inhalation. The last sacrifice was on the sixtieth day after operation. The left lower jaws with the incisor *in situ* were dissected immediately after sacrifice. All the blocks were immediately fixed in 10% formalin for 5 days. Then decalcified in 5% nitric acid. Serial longitudinal paraffin sections of 6 microns was cut and stained by hematoxylin and eosin. All the sections were examined under a microscope.

## RESULTS

The percentage of keratinized cells in the vaginal smears of the three groups of rats revealed no difference before operation. It yeilded significant information after oophorectomy. The number of keratinized cells in group A was greatly reduced down to a level almost reached the zero point throughout the experiment (Fig. 1). In group B the percentage of keratinized cells was as low as group A after operation, but it soon rised and maintained at the level between group A and group C after the injection of estradiol on the ninth day after opertion (Fig. 1). The percentage of keratinized cells of group C remained in regular changes of normal estrous cycles throughout the experiment.

In microscopic examination of the sections, the structure of dentin, enamel, ameloblasts, odontogenic epithelium and pulp tissue in group C were found as usual (Fig. 2).

In group A and group B, the authors found quite a number of changes in the structure of the teeth. Part of ameloblasts became atrophied, hypoplastic enamel matrix with numerous round spaces like a network was found in many incisors (Fig. 3). In some specimens ameloblasts and enamel matrix became folded (Fig. 4). The folding enamel matrix also presented hypoplasia with numerous round spaces which appeared in figure 3 and figure 4. The main feature of foldings exhibited by the enamel matrix and the ameloblasts was the repeated occurance of folds perpendicular to the dentin surface. After a projection in the pulp direction, the enamel bended again to its previous level.

After a small distance another bend occured. It repeated to assume an accordion-like appearance (Fig. 4). Foldings were also found on lingual dentin at the basal end of the incisor (Fig. 5). A calcified tissue mass, which was surrounded by a great number of odontoblasts, was found in the pulp near the base (Fig. 5). The labial dentin presented a lot of interglobular dentin (Fig. 3, 4). The labial or lingual dentin in some specimens increased in thickness (Fig. 3, 4, 5). Lack of periodontal ligament was noted in oophorectomized rats (Fig. 3, 4).

## DISCUSSION

Under normal condition, the estrous cycles of rat depends upon a periodical activity of the ovaries. It is divided into five periods and extends over four days (3). The separation of these five stages depends on the examination of vaginal smears. According to the records in this study the percentage of keratinized cells from group C reveals no difference from the normal estrous condition, while in group A only very few or even no keratinized cells can be detected after the removal of ovaries. Apparently it is due to the lack of estrogenic hormone to induce the estrous cycles. The changing of keratinized cells in group B follow the same style of change as group A after operation, but from the ninth day after operation the number of keratinized cells increases due to the injection of 0.5 micrograms of estradiol dipropionate which induces estrous cycles again. The above phenomena are in harmony with the principle of estrous cycle of rats. (3).

The influence of the lack of female sexual hormone on the rat incisor is demonstrated in this study with the evidence of atrophy of ameloblasts, hypoplasia of enamel matrix and numerous interglobular dentin. It has been well-established that female sex hormone may influence the metabolism of fat, protein, carbohydrate, creatine and calcium, as well as basal metabolism, growth, etc. (5, 9).

As a result of declining ovarian activity, manifested clinically by the cessation of menstruation (menopause), atrophic changes may occur in the oral mucosa similar to those developing in the vulvovaginal tract. This atrophic process apparently stems from a defeciency of the ovarian steroids, estradiol and its derivatives (6). Therefore, in this study, the oophorectomy should exert the same effect on the rat incisor as on the oral mucosa, but in some rats the incisors remain intact. It was suggested that the steroid may have an extragonadal source such as suprarenal cortex (6).

The pituitary growth hormone acts to accelerate the rate of somatic growth (7, 8). On the other hand, the gonadal and gonadotropic hormones, by initiating maturation, serve to limit the time of active growth and inhibits the action of the pituitary growth hormone. In this experiment, the ovaries of the experimental animals are removed. The very low blood concentration of estrogens stimulates the secretion of gonadotropic hormones in pituitary gland. However, the follicle-stimulating hormone is no longer effective to restore the normal estrogen concentration in blood because there is no more ovary at all in these rats. Therefore, a condition of deprivation of estrogen is resulted in spite of the high concentration of gonadotropic hormone in blood. The mechanism of action differs in pituitary growth hormone and gonadal hormones. The former acts upon the rate, while the latter regulate the duration of growth. A balance of the two effects is essential to normal growth and development (1, 2, 7, 8). Since the sexual hormones bring an end

to the period of active growth and initiate maturation, a deficiency in the secretion of the gonadal hormones before puberty postpones the maturation, and prolong the growth period (8). Foldings of enamel epithelium, enamel matrix and lingual dentin are disclosed in the sections of the experimental animals (Fig. 3, 4). It is believed that the balance of pituitary growth hormone and gonadal hormones is disturbed after removal of ovaries. Therefore the growth and development of the incisor becomes abnormal. When the limitation of the time of active growth is withdrawn, the acceleration of the growth rate of the incisor at the basal end is faster than normal, therefore folds result. The eruption rate of incisor may be not in proportion to the growth rate. This disproportion is responsible for the foldings (9). Degeneration of periodontal ligament is usually found in young rat incisor after oophorectomy (4). In this study the lack of periodontal ligament may be due to a degenerative change. The presence of an increased pressure on periodontal ligament of normal quantity or norml pressure on lesser quantity of periodontal ligament will both result in folding of the tissue (9). Apparently the presence of folding in this experiment belongs to the latter.

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## LEGEND OF FIGURES

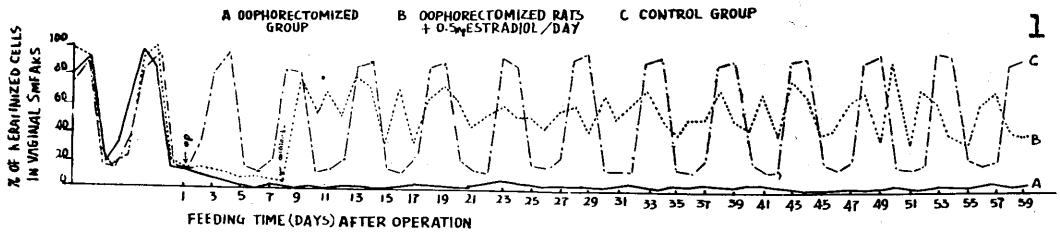
Fig. 1. The curves showing the percentage of keratinized cells in vaginal smears made during pre-and postoperative stages. (Op.: oophorectomy or sham operation).

Fig. 2. Normal structure of the incisor of control rat. (× 160). A., ameloblasts; E., enamel matrix; O., odontoblasts; D., dentin; P., pulp.

Fig. 3. Labial portion of rat incisor after oophorectomy, showing enamel hypoplasia, atrophy of ameloblasts and numerous interglobular dentin. (×160)

Fig. 4. Labial portion of rat incisor after oophorectomy, showing folding enamel epithelium, enamel matrix and hypoplasia of enamel matrix. (× 160)

Fig. 5. Lingual portion of the dentin at the basal end of rat incisor after oophorectomy, showing folding of dentin and formation of osteodentin in the pulp. (× 160)


A OOPHORECTOMIZED GROUP
B OOPHORECTOMIZED RATS + 0.5γ ESTRADIOL/DAY
C CONTROL GROUP
1
% OF KERATINIZED CELLS IN VAGINAL SMEARS
100
80
60
40
20
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C
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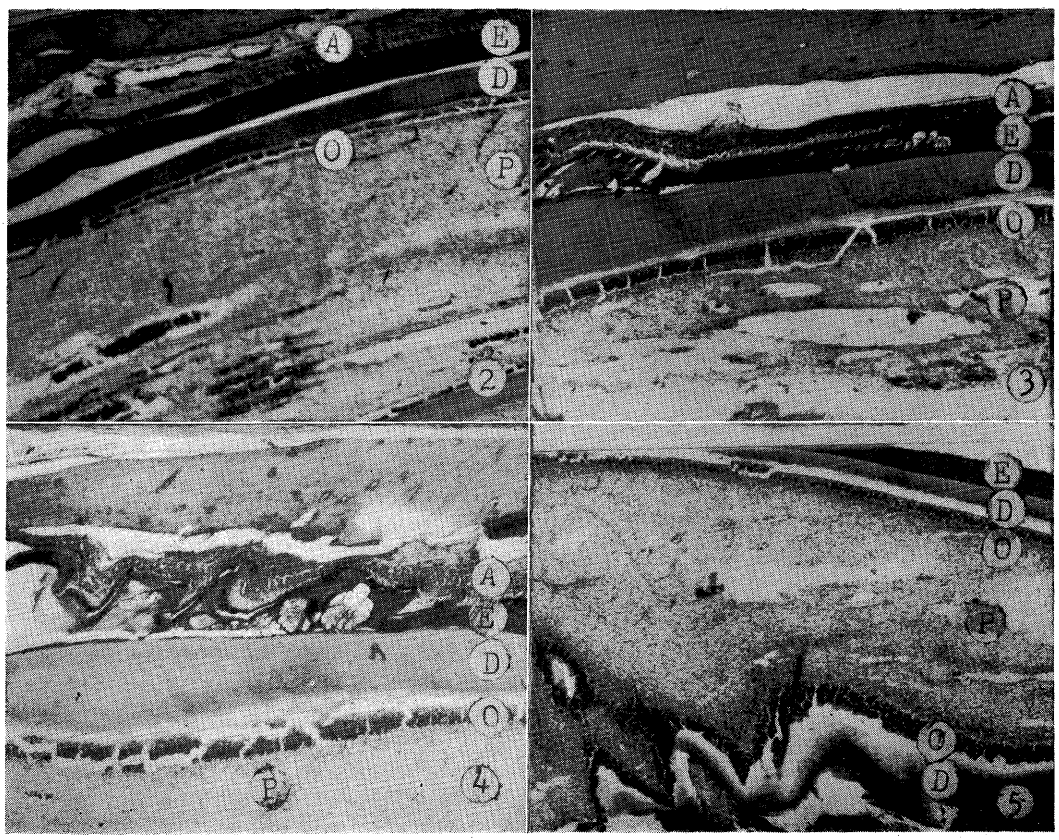
FEEDING TIME (DAYS) AFTER OPERATION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