1966

The Generation Cycle of Epithelial Cells in the Molar Interdental Papillae of Ovariectomized Mice

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**Recommended Citation**


THE GENERATION CYCLE OF EPITHELIAL CELLS IN THE MOLAR
INTERDENTAL PAPILAE OF OVARIECTOMIZED MICE

by

Francis Lawrence Bachnik

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science

June
1966
I dedicate this thesis to my wife Peggy,
whose encouragement and understanding
made this work possible.
ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Dr. Anthony W. Gargiulo for his personal interest and guidance during the preparation of this thesis.

The author wishes to thank Dr. Patrick D. Toto for his unlimited patience and constant advice.

The author is also indebted to Dr. John J. O'Malley for his helpful suggestions and continuous assistance.

The author is grateful to Dr. Vincent J. Sawinski for his help with the mathematical analysis of the data.

The author wishes to thank Mrs. Gerry Duyvejonck for the final preparation of this thesis.
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INTRODUCTION

This investigation was prompted by clinical and histological observations which indicated that alterations in ovarian hormone levels, particularly estrogen, produced significant changes in epithelial tissues. Increased estrogen levels have been reported to increase the number of mitoses and reduce the mitotic time (Bullough and Van Oerdt, 1950). Conversely, regressive histologic changes have been reported in women with diminished estrogen levels after menopause (Richman and Abarbanel, 1943). The effect of ovariectomy on the kinetics of mouse oral epithelium has not been investigated.

The purpose of this study was to observe the effects of ovariectomy on the synthesis of deoxyribonucleic acid (DNA) and the migration of epithelial cells of the mouse molar interdental papillae. This tissue was examined autoradiographically using tritiated thymidine.

The lack of ovarian hormones will significantly prolong the generation cycle of gingival epithelial cells and decrease the number of epithelial cells synthesizing DNA. The migration time of epithelial cells in the molar interdental papillae is prolonged while the overall pattern of movement of these cells remains the same.
REVIEW OF LITERATURE

Histology of the Mouse Molar Interdental Papilla:

The mouse molar interdental papilla has been an important site for recent investigations concerning the biology of the gingival sulcus. The epithelium lining this gingival sulcus is stratified squamous nonkeratinized epithelium which is characteristic of human as well as rodent sulcular epithelium (Orban, 1963). The apical portion of the sulcular epithelium is bound to the tooth surface by neutral and acid mucopolysaccharides. These mucopolysaccharides cement the epithelial cells not only to the tooth, but to each other, and to the underlying connective tissue (Toto and Sicher, 1964: Toto and Grandel, 1965).

Pattern of Mitosis in the Normal Mouse:

Beagrie and Skougaard (1962) studied the life cycle of gingival epithelial cells in the molar region of the mouse autoradiographically. At the one hour sacrifice interval, labeled cells were seen not only in the basal cell layer, but in the cells of the epithelial attachment approximating the surface of the tooth at the cemento-enamel junction. The percentage of labeled cells increased during the first 24 hours and then declined. Cell migration was found to be horizontal in the apical portion of the epithelial attachment and more oblique in the remainder. They concluded that epithelial cells in the attached epithelium were completely renewed every 24 hours, and that labeled cells required between 3 to 5 days to reach the coronal tip of the interdental papilla.
Greulich (1962) investigated the epithelial attachment collar of the mouse molar with autoradiographs. Soon after the administration of tritiated thymidine, he found labeled cells in the stratum germinativum. Labeled cells were seen adjoining the enamel space close to the cemento-enamel junction. At later intervals, more labeled cells were observed coronally; these were presumed to be daughter cells migrating toward the gingival surface.

Joglekar (1964) studied the epithelium lining the molar interdental papillae in the normal mouse with autoradiographs at regular intervals beginning at one-half hour through five days. At one-half hour, labeled cells were visible not only in the basal cell layer, but in the prickle cell layer of cells of the epithelial attachment, directly approximating the enamel surface of the tooth. She reported a doubling in the percentage of labeled cells 10 hours after the injection of tritiated thymidine. The percentage of labeled cells maintained a plateau from 10 to 22 hours and then declined steadily to a low level at 5 days. The labeled cells migrated from the basal cell layer and the prickle cell layer to the surface cell layer. The labeled cells were desquamated into the gingival sulcus or from the tip of the interdental papilla.

The labeled basal cells and prickle cells give rise to daughter cells which migrate to the surface cell layer to desquamate. The labeled cells in the epithelial attachment migrate up along the tooth surface to exfoliate into the gingival sulcus. The migrating cells then are desquamated along the entire surface of the sulcular epithelium and also at the tip of the interdental papilla (Toto, 1966).
Ovarian Hormones:

Four distinct groups of ovarian hormones have been recognized: estrogens, progestins, androgens, and relaxin.

Estrogen is the name given to a group of steroids which includes estradiol-17 B, estrone, and estriol. Allen and Doisy first demonstrated this hormone in 1923 and called it the "ovarian follicular hormone" or "primary ovarian hormone" (T.R. Forbes, 1960). The source of estrogen in the ovary is probably the theca interna of the follicle (Dempsey and Bassett, 1943), and it has also been reported in the corpus luteum by Tepperman (1965), Forbes (1960), and Grollman (1941). Also, estrogens are produced by the adrenal cortex, placenta, and Leydig cells of the testis (Williams, 1963). Several synthetic estrogens, commonly used in research, are diethylstilbestrol and a-estradiol benzoate.

Progestins, the best known of which is progesterone, are steroids, secreted by the corpus luteum of the ovary, the adrenal cortex, testis, and placenta. Progestins in the adrenal cortex and the testis are thought to be precursors in the secretory products of these glands (Williams, 1963).

Androgens are produced in small amounts in the ovaries, apparently as intermediary compounds in the synthesis of estrogen. The androgens of the ovaries are of little physiological significance (Williams, 1963).

Relaxin is a non-steroid compound found in the ovaries and thought to facilitate delivery of the newborn in some species (Williams, 1963). Little is known about its relationship with epithelial tissue.

The secretion of ovarian hormones is supervised through an elaborate
feed-back system via the hypothalamus. The hypothalamus regulates the anterior pituitary secretion of gonadotrophic hormones, which control the level of ovarian hormones. (R.H. Williams, 1963).

The fate of ovarian hormones is not completely understood. Specifically, estrogen and progestins are known to be degraded in the liver. Some probably are consumed while eliciting a response in the end organs upon which they act. Others, either are transformed into inactive compounds or are excreted in the urine. (T.R. Forbes, 1960).

It must be emphasized that the above data concerns ovarian hormones obtained from primates, and it would be unjustifiable completely to correlate them with those of the mouse.

The Effect of the Ovarian Cycle Upon Epidermis of the Mouse:

The female mouse is considered to be sexually mature at 30 to 40 days of age when the first ovulation occurs (Ben-or, 1963). The estrus cycle recurs at an interval of 4 to 6 days unless interrupted by pregnancy. There is a cyclic variation in the thickness of vaginal epithelium in the mouse, similar to that in humans, corresponding to the changes in estrogen level during the estrus cycle (Grollman, 1941).

H.F. Bullough (1943) discovered a relationship between the thickness of skin and the normal ovarian cycle, due to a variation in the mitotic activity of the epidermis.

In the epidermis of the female mouse, the number of mitotic figures varies with the stage of the estrus cycle. An increase in the level of estrogen not only increases the number of mitotic figures, but also reduces
the mitotic time. During periods of low blood-tissue levels of estrogen, the epidermal cell division in the female mouse takes two hours. At maximum levels, this time was reduced to 3/4 hour (W.S. Bullough, 1950).

The Effects of Injections of Estrogen on the Epidermis of the Mouse:

W.S. Bullough and Van Oerdt (1950) reported that estrogenic hormones administered to castrated three or four month old male mice caused an increase in the epidermal mitosis rate and a reduction in the mitotic time from 2½ to 1 hour.

H.F. Bullough (1947) likewise noted that the mitotic activity and the thickness of epidermis are normally increased by estrogenic hormones, but a prolonged excess of such substances could produce the opposite effect. Also, Hooker and Pfeiffer (1943) found that injections of estradiol benzoate over a long period of time resulted in a decreased epidermal thickness, due to inhibited mitotic activity.

The Effect of Injections of A-estradiol Benzoate on Oral Epithelium of the Mouse:

Several investigators have studied the effects of estrogen derivatives on mouse gingival tissues. Nutlay, et al. (1953) observed that injections or subcutaneous pellets of a-estradiol benzoate produced an apical proliferation of epithelium along the molar root surfaces. The gingival epithelium and interdental papillae of the molar region also increased in size. The earliest of these changes was found to occur three weeks after the initiation of this experiment. Eventually, deep interdental and interradicular pockets were
found in the molar region.

Stahl and his associates (1950) injected a-estradiol benzoate into male and female mice and examined the molar region histologically. They found no changes in the oral epithelium of the epithelial attachment. However, the rests of Malassez in the periodontal ligament space appeared to increase in number and size similarly in male and female mice.

The Effect of Progesterone in the Mouse:

Investigations concerning the influence of progesterone on epithelial tissues of the mouse have been very limited. Forbes (1960) reported finding endometrial stromal cells with large rounded vesicular nuclei in the uteri of mice during proestrus. The appearance of such cells has been attributed specifically to the action of progesterone. Another investigator, Grollman (1941), described the rodent corpus lutea as being poorly developed and probably non-functional. The corpus lutea of the primates is the primary ovarian source of progesterone, so it is not clear as to where progesterone is produced in the mouse ovary. Furthermore, there is no information as to the effect of progesterone on oral epithelial tissues in the mouse.

Ovariectomy in Experimental Animals:

The effects of ovariectomy on oral epithelial tissues in the mouse has not been investigated. However, Ziskin and his coworkers (1933) examined the oral mucosa of thirty ovariectomized young adult rats, and reported the epithelial changes as insignificant.

Forbes (1960) observed that ovariectomy caused an atrophy in uteri, mammary glands and vaginal mucosa in sexually mature animals. He found that
Estrogens administered to ovariectomized primates in adequate dosages for ten days would produce endometrial hypertrophy. Ziskin and others (1936) reported that the gingival papillae in ovariectomized monkeys developed the clinical appearance of necrotizing ulcerative gingivitis. Following estrogen injections, there were increases in the number of mitoses in the epithelium and in keratin formation, and a greater ability of the gingiva to resist infection, resulting in a restoration of a normal state of health in this tissue.

**Estrogen in Humans:**

The vast majority of literature concerning ovarian hormones has been devoted to estrogen. Estrogenic hormones are regarded as growth stimulators, their effect being, in general, anabolic and not limited to the tissues of the reproductive organs (Calkutt, 1942). During embryonic growth, estrogen is responsible for full development of the uterus and vagina. Before puberty, a low level of estrogen is maintained to safeguard against precocious sexual maturity. After puberty, estrogen output is increased and the primary and secondary sexual characteristics develop (Tepperman, 1965).

**Epidermal Response to Estrogen in Women:**

There seems to be a gradation of epithelial tissue sensitivity to estrogen depending upon the location of this tissue in the body. Vaginal mucosa is very sensitive to estrogen (Wiener, 1956), and subsequently, reflects the changes in estrogen level throughout the menstrual cycle. A high level of estrogen produces a thick, keratinized vaginal epithelium, while a low level of estrogen has the opposite effect (Piroshaw and Glickman, 1957).
Estrogen is known to stimulate proliferation in the endometrium and myometrium and is necessary for the development of the alveolar ducts in the breast. It has also been found to increase the water content and thickness of the skin (Williams, 1963).

The Effects of Increased Estrogen on Human Oral Epithelium:

Ziskin and his associates (1933) reported that in women with high estrogen levels, as a result of pregnancy, menstruation, or puberty, the gingiva showed a marked hyperplasia of the stratum germinativum with an elongation of the rete pegs. The etiology of these changes was discussed in a later article by Ziskin and Nesse (1946). They felt that the immediate cause of these changes appeared to be a diminished utilization of estrogen or a modification of the estrogen metabolism in the gingival epithelium.

Orban and Maier (1949) reported similar histopathologic findings with significant increase in mitotic activity in the gingiva of pregnant women, but they considered the primary etiological factor to be of local irritative origin modified by the pregnancy.

The Effects of Decreased Estrogen on Human Oral Epithelium:

Conversely, certain regressive histologic changes occur in the oral mucosa of women with lowered estrogen levels, resulting from either the cessation of menstruation or the removal of both ovaries. There is an atrophy of the epithelium, primarily in the germinal cell layers. The prickle cell layer is affected most noticeably. Treatment with injections of estradiol or synthetic estrogen restored a normal appearance to the stratum germinativum and
increased the thickness of the epithelium, although hyperkeratosis was never observed (Richmond and Abarbanel, 1943). In a group of women receiving estrogenic (Progynon B) and gonadotropic (Follutein) hormones for several gynecologic disorders, Ziskin (1937) observed a hyperkeratinization of the gingiva and a hyperplasia of the prickle cell layer of the epithelium.

The Effects of Progesterone in Women:

Progesterone is concerned with the differentiation rather than the proliferation of epithelial tissues as it induces maturation of glandular tissues, such as alveoli of the breast, and stimulates secretion, while in other actions it is directly antagonistic to estrogen. Progesterone is known to have a catabolic effect on protein, and it is believed to block endometrial growth (Williams, 1963).

It is not known whether changes in progesterone levels directly influence epithelial tissues in other parts of the body.

Autoradiography:

Proliferative cells obtain precursors which are synthesized into deoxyribonucleic acid (DNA). Radioactive thymidine, one synthetic precursor, is available in the tritiated form (thymidine-\(H_3\)) and is a specific component of DNA (Lajtha, Phil, and Oliver, 1958).

If tritiated thymidine is administered during DNA synthesis, the nuclei of these cells will be labeled before mitosis takes place. Such nuclei will share their radioactive material during the actual mitosis and pass on equal amounts to their daughter cells (Leblond, 1959).
Tritiated thymidine overcomes most difficulties of other DNA precursors. Tritiated thymidine is only utilized for DNA synthesis. Small dosages can be used to reduce the danger of radiation damage and allow observation of cells for a period of months. Beta rays are emitted from this isotope with suitable low energy to permit intra-cellular resolution. The beta rays probably expose most photographic grains within 1 micron of the source (Leblond, Messier, and Korpriwa, 1959).

Belanger and Leblond began using the procedure of painting the tissue sections with photographic emulsion. Nuclear Track Emulsion $B^1$ (NTB), a liquid emulsion, is the most sensitive to beta particles for its grain size (Boyd and Williams, 1948).

MATERIALS AND METHODS

Twenty-three CD1 strain 1 ovariectomized white mice, 45-50 days old, with an average weight of 30 grams, were selected for this experiment. Each mouse was injected intraperitoneally with tritiated thymidine 2 , having a specific activity of 1.9 curies per millimole, at a dose rate of 1 microcurie per gram of body weight, three days following ovariectomy. The mice were injected within a period of one hour to minimize the effect of diurnal variation. One animal was sacrificed one-half hour after injection and at subsequent intervals of 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 36, 40, 44, 48 hours; 3 days, 4 days, and 5 days following the injection.

The mandibles with the attached soft tissues were fixed in a 10% solution of formalin for one week. Three days after immersion the formalin was changed. Following fixation the specimens were demineralized in a solution of sodium citrate and formic acid (Table D). This solution was changed every day for three days, at which time demineralization was complete as determined by roentgenographs. The tissues were dehydrated in ascending alcohol solutions; 75%, 95%, and absolute alcohol; three changes each for one-half hour.

The specimens were then embedded in paraffin 3 . The embedding was done in Vacuo, at a temperature of 56.5°C under a pressure of 15 pounds per square inch for a period of 15 minutes.

Paracentral mesio-distal serial sections, 6 microns thick, of the molar region were cut on a rotary microtome and each sixth section was selected for

1. The Charles River Mouse Farms, Inc., North Wilmington, Massachusetts.
preparation of autoradiographs.

The mounted sections were then deparaffinized by immersion for ten minutes in each of three changes of xylol followed by descending solutions of alcohols; five minutes in absolute, and three minutes in 95% and 75%, and finally washed in distilled water for five minutes.

Autoradiography Technique:

The slides were dipped in liquid photographic emulsion\(^1\) for three seconds and then air dried for ten minutes and placed in a black, light-proof exposure box, section side up. Lithium chloride was placed in the box to maintain a low humidity, and black masking tape was used to light-seal the box. The slides were exposed for two weeks at a temperature of 4 degrees C. During this exposure time the box was maintained in a position which kept the sections upright.

Developing:

The slides were rinsed in distilled water for thirty seconds and placed in acid fixer reducing solution for ten minutes. They were then washed in running tap water for thirty minutes and allowed to dry in a stream of air. When the slides were dry, they were dipped in water for thirty seconds and the excess emulsion was trimmed from the slides. The sections were then stained with a nuclear fast red, indigo carmine sequence\(^2\) (Table D). Selected sections were also stained with hematoxylin and eosin without the autoradio-

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1. Eastman Kodak Company, Nuclear Track Emulsion Type NTB\(_2\).
2. Micheline Montreuil-Langlois, Dept. of Comparative Anatomy and Histology, Faculty of Science, Univ. of Paris, Rue Victor Cousin, Paris, France.
graphic technique.

Ten anteroposterior paracentral sections of interdental papillae from the molar region were selected for each sacrifice period. Papillae from between the first and second, and second and third molar were used. Care was taken to avoid obliquely sectioned or distorted specimens.

To find the average number of epithelial cells in a papilla, the total cells in two papillae from each time period were counted and this total (8790 cells) was divided by the total number of papillae (46) counted. An average papilla was found to have 191 cells. The average number of epithelial cells in one-half of a papilla was then considered to be 96 cells. This number was used to calculate the percentage of labeled cells in each one-half papilla for each time period.

To facilitate description, the papillae were hypothetically divided into mesial and distal halves. The labeled cells were counted in the more typical half-papilla. Labeled cells which overlapped the dividing line of a papilla were counted and included in the tables. The average number of labeled epithelial cells present in a half-papilla was calculated from the ten sections in each sacrifice period.

To localize the labeled cells in a half-papilla, the epithelium was subdivided into three zones depending upon the thickness of the epithelium. (Figure 3). Zone I began at the apical end of the attached epithelium and extended coronally until the epithelium was three cell layers wide. Zone II consisted of that epithelium which varied from four to five cell layers in thickness. Zone III was six to twelve cell layers thick and began at the
coronal end of Zone II and terminated at the tip of the papilla. Zone I and Zone II consisted of potentially germinative cells (Greulich, R.C., 1962; Joglekar, 1964.). For descriptive purposes we distinguished two cell types in these zones; the basal cells, which are found along the basement membrane, and the more superficial surface cells, or prickle cells, which in these two zones closely approximated the enamel of the tooth. Zone III was divided into basal cell, prickle cell, and surface cell layers.

A statistical analysis of the percentage of labeled cells in the ovariectomized mouse as compared to the normal mouse (Joglekar, 1964), between one-half hour and 5 days, was determined by several methods:

1. The Pearson product moment correlation (r).

2. A linear regression analysis for the regression coefficient (b).

3. The t-test.
FINDINGS

An anteroposterior paracentral section through a mouse interdental papilla in the molar region is triangular in shape and consists of a dense, fibrous connective tissue core covered by nonkeratinized stratified squamous epithelium. (Figure 2).

Cells of the basal layer appeared to be polyhedral in shape, lying on a distinct basement membrane. The prickle cell layer varies in thickness and contains polyhedral cells. In the surface layer the cells have a flattened appearance. The epithelium of the gingival sulcus is not keratinized and attaches apically to the surface of the tooth to form the attached epithelium. The precise surface outline of a papilla depends largely upon the contour of the approximating teeth with the surface of the epithelium following the curvature of the enamel surface.

Labeled epithelial cells were observed in all the interdental papillae taken at the stated intervals from one-half hour to five days (120 hours), following the administration of tritiated thymidine.

DISTRIBUTION OF LABELED CELLS IN EPITHELIAL CELL LAYERS (TABLE A)

**Basal Cell Layer:**

For the first 20 hours most labeled cells were in the basal cell layer.

There was an average of 3.4 labeled cells in the basal cell layer of all three zones at one-half hour. This number gradually increased to a maximum of 6.4 labeled basal cells per section at 14 hours. A near maximum of labeled cells in the basal cell layer continued to the 20 hour period, and then
decreased gradually to 3.0 labeled cells in the basal layer at 24 hours, 2.3 labeled cells at 36 hours, and 1.2 labeled cells at 48 hours. After 48 hours less than one labeled basal cell was seen in each half-papilla studied.

**Prickle Cell Layer:**

An average of 1.3 labeled cells per section was present in the total prickle cell layer of zones I, II and III combined at one-half hour. Labeled prickle cells were observed approximating the enamel surface of the tooth at this time in zones I and II. The number of labeled cells doubled to 3.0 at 14 hours, reaching a maximum number of 4.8 cells at 16 hours.

From 22 to 40 hours the most numerous labeled cells were in the prickle cell layer. There was an average of 3.4 labeled prickle cells for the next 24 hours, followed by a gradual decrease to 2.6 at 48 hours. The number of labeled prickle cells averaged .9 for the next three days.

**Surface Cell Layer of Zone III:**

At 4 hours .6 labeled cells were seen in the surface layer of zone III. The number of labeled cells had doubled to 1.2 by the twelfth hour and averaged 1.1 until 40 hours, when the number increased to 2.7. From 44 hours to 5 days, the most numerous labeled cells were in the surface cell layer. The highest number of labeled cells (3.2) was seen at 44 hours. The number declined to 2.6 at 48 hours and 2.0 after 3 days. At the 5 day sacrifice period their number was reduced to 1.8.

**Distribution of Labeled Cells in Zones I, II, and III (Table E):**

From one-half hour to 8 hours, more labeled cells were observed in zone
I than in either zone II or III. The highest number of labeled cells in zone I (3.5) was observed at 18 hours, and this number declined until labeled cells in this zone became quite rare after 40 hours.

The number of labeled cells increased in zone II from one-half hour to 14 hours, with a maximum number of labeled cells in the zone (3.3) occurring at 16 hours. This number decreased until after 44 hours there was fewer than one labeled cell per section in zone II.

From 10 hours to 5 days, the majority of labeled cells was observed in zone III. A maximum number of labeled cells (7.3) was recorded at 14 hours. The number of labeled cells in zone III remained high through 48 hours, (5.2) and dropped to 2.2 after 5 days.

**Percentage of Labeled Cells (Table C):**

The percentage of labeled cells at one-half hour was about 5.0. This number increased to 11.0 at 14 hours. The maximum percentage of labeled cells occurred at 18 hours (11.5). Between 20 and 22 hours the number declined rapidly. The percentage of labeled cells decreased very slowly from 22 to 48 hours. At 24 hours the percentage of labeled cells was 7.0; at 36 hours 6.8; at 48 hours 6.7. At 3 days the number was down to the one-half hour level again (4.4). After 5 days the percentage of labeled cells was 2.9.

The t-test showed a significant difference (p < 0.05) between the percentage of labelled cells in the normal mouse (Joglekar) and the ovarioctomized mouse from one-half hour to 5 days. (Figure 1). This indicates that the probability of a significant difference existing between these groups of animals is 95%. The correlation coefficient and the regression analysis substantiated this finding.
DISCUSSION

The relation between ovarian hormones and oral epithelial tissues is not completely understood. It is difficult to determine the exact role of individual ovarian hormones because of the complex interrelationship which exists between the compounds themselves, and the endocrine system as a whole.

Many investigators have introduced ovarian hormones into experimental animals and studied their effects upon epithelial tissues. Conversely, we used the ovariectomy in the mouse as a tool to study the effects of a loss of ovarian hormones on the oral mucosa. Specifically, we were interested in changes which might take place in the generation cycle of an epithelial cell in the mandibular intermolar papillae.

The percentage distribution of labeled epithelial cells in the interdental papillae of the ovariectomized mouse followed a pattern similar to that found in the normal mouse as reported by Joglekar (1964). (Table C, Figure 1). However, there were significantly fewer labeled epithelial cells in the ovariectomized mouse for the first 36 hours. This lower percentage of labeled cells could be at least partially explained by the effects of the elimination of ovarigenic estrogens in the ovariectomized animal.

A loss of ovarian estrogen in the ovariectomized mouse possibly could increase the generation time of the epithelial cells, thus accounting for the delay in doubling of the labeled cell population. There are substantial general and specific effects of estrogens upon epidermis of skin and oral mucosa respectively in converse agreement with the increase in generation time as a consequence of ovariectomy.
H.F. Bullough (1943, 1947) found that estrone increased both the number of mitoses and their speed of completion in the epidermis of the female mouse. W.S. Bullough and Van Oerdt (1950) found that estrogenic hormones increased the mitotic rate and reduced the duration of epidermal mitosis from 2½ hours to a new elapsed time of 1 hour in adult female and castrated male mice.

Nutlay, et al. (1953) found that α-estradiol benzoate caused an increased downgrowth of the attached epithelium along the root surface and furcation areas of the molar region in the adult mouse. Also, the gingival epithelium between the first and second molars proliferated extensively. These investigations concluded that an increased estrogen level produced a stimulatory effect on epithelial tissues, decreasing the generation time and increasing the number of mitosis.

These studies were limited to only α-estradiol and it is believed that other ovarian hormones exert an influence on epithelial tissues (Williams, 1963). However, our findings indicated that a decreased level of estrogen could increase the generation time of epithelial cells and decrease the number of mitoses as a consequence of fewer cells entering DNA synthesis.

The time required for labeled epithelial cells to double is approximately 40% greater in the ovariectomized mouse as compared to the normal mouse. (Table C). The percentage of labeled cells in the ovariectomized mouse was found to increase by 100% within 14 hours after the injection of tritiated thymidine. Joglekar found a similar increase in the percentage of labeled cells in the normal mouse after just 10 hours. Evidently, the duration of mitosis is extended in the ovariectomized mouse. This again could be due to a decreased estrogen level, which according to Bullough and Bullough is direct-
ly related to the duration of mitosis. There is no reference in the literature concerning the specific effects of other ovarian hormones on mitotic time.

The labeled epithelial cells of the interdental papillae in the ovariec-
tomized mouse were found to move more slowly and exfoliate less rapidly than the epithelial cells in the normal mouse. This slow movement may result from a reduced rate of cell renewals as indicated by the increased time for labeled cells to double in number. In effect, the cell population remains in situ longer and, therefore, is an older population. This delay in cell movement explains the higher numbers of labeled cells seen from 36 to 48 hours in the ovariec-
tomized mouse. (Figure 1). In the normal mouse, the cells have moved and exfoliated while in the ovariec-
tomized animals, the labeled cells have undergone mitotic division and have remained longer than normal in situ.

From one-half hour to 5 days, the labeled epithelial cells moved in a cor-
ronal direction in the interdental papilla. At the same time they migrated from the basal cell layer, through the prickle cell layer and into the surface cell layer of the epithelium. The number of labeled cells in the specimens declined as the epithelial cells were exfoliated from the surface layer into the gingival sulcus. (Tables, A, B).

For the first 20 hours, most labeled cells were basal cells, indicating that the majority of desoxyribonucleic acid synthesis was occurring in this layer. At one-half hour a few labeled cells were also seen in the prickle cell layer immediately adjoining the enamel space in zones I and II. These cells in the stratum germinativum, that is in the basal and lower prickle cell layers, remained in their same positions as vegetative intermitotic cells or
became differentiating intermitotic cells if they moved into more superficial layers.

In zone I differentiating intermitotic cells moved horizontally toward the surface of the enamel or coronally into the prickle cell layer of zone II. In zone II these labeled cells moved horizontally toward the surface of the enamel or coronally into the prickle cell layer of zone III. Labeled cells in zones I and II, upon reaching the enamel surface, moved coronally along it and were shed into the gingival sulcus.

From 22 to 40 hours the most numerous labeled cells were in the prickle cell layer, primarily in zone III. This was attributed to the mitotic division of labeled prickle cells in zone III and the migration of differentiating intermitotic cells into this area from zones I, II, and III.

The differentiating intermitotic cells from the prickle cell layer gradually migrated into the surface cell layer of zone III, reaching a peak at 44 hours. Thereafter, the number of labeled surface cells diminished, presumably as these cells were exfoliated into the gingival sulcus or from the tip of the papilla. The few remaining labeled epithelial cells seen at 5 days were found in the coronal part of the papilla.

The path of migration and fate of these epithelial cells in the normal mouse has been described by several investigators. Our findings were in general agreement with Joglekar (1964), Beagrie and Skougaard (1962), Greulich (1962).

The depression of new cell formation and migration in the sulcular epithelium associated with ovariectomy in the female mouse cannot be directly
compared to changes in the oral mucosa of women having correspondingly lower levels of ovarian hormones in the bloodstream. Unfortunately, there is little information relating oral epithelial activity in ovariectomized states.

However, after menopause, a decreased level of estrogen frequently is accompanied by degenerative changes in the oral mucosa. There is an atrophy of the germinal cell layers in the epithelium, particularly in the prickle cell layer (Richman and Abarbanel, 1943). Treating these patients with estrogen compounds reversed these changes. In contrast, women with high estrogen levels, as a result of pregnancy, menstruation, or puberty, often exhibit marked hyperplasia of the stratum germinativum of the oral mucosa (Ziskin, 1933).

Obviously, oral epithelial tissues are sensitive to fluctuations in estrogen levels. However, any changes seen in oral tissues are a reflection of the interrelation between all of the ovarian hormones and the entire endocrine system.
SUMMARY

A histologic study of the epithelium of the molar interdental papillae of the ovariectomized mouse was undertaken. Mandibular specimens were obtained from twenty-three white female mice, 45-50 days old, weighing an average of 30 grams. Three days after ovariectomy, each mouse was injected intraperitoneally with one microcurie of tritiated thymidine for each gram of body weight. An animal was sacrificed at an interval of one-half hour, 1 hour, 2 hours, and at intervals of every 2 hours up to 24 hours, and at intervals of every 4 hours up to 48 hours; 3, 4, and 5 days.

The mandibular molar region was excised, fixed in 10% formalin, demineralized, dehydrated, and embedded in paraffin. Paracentral mesio-distal serial sections were made at a thickness of 6 microns and each sixth section was deparaffinized and dipped into NTB3 emulsion for autoradiography. The sections were then stained with a nuclear fast red, indigo carmine sequence.

Ten specimens from each sacrifice interval were selected and the positions of the labeled cells were recorded on diagrams. The epithelium of the molar interdental papilla was divided into three zones based upon the thickness of the epithelium. The average number of labeled cells and the percentage of labeled cells were calculated for each sacrifice interval. A comparison was made between the percentage of labeled cells in the normal mouse (Joglekar) and the ovariectomized mouse, and statistical analysis showed a significant difference of $p < .05$ for the two groups.
CONCLUSIONS

There is a decrease in the frequency distribution of oral epithelial cells of the molar papillae in DNA synthesis in the ovariectomized mouse.

The time for generation of epithelial cells in the papillae is 1/4 hours, 40% longer than normal.

Epithelial cells are lost more slowly due to a reduction in the number of reproducing cells and the increased generation time. This results in an older average cell population.

Epithelial cells in zones I and II migrate obliquely toward the tooth surface and coronally to the gingival sulcus where they are exfoliated. In zone III, epithelial cells move from the basal and prickle cell layers to the surface cell layer and are lost along the entire sulcular surface or from the tip of the papilla.
BIBLIOGRAPHY


Medak, H. HISTORY OF ORAL EPITHELIUM IN THE ADULT MOUSE. Thesis Submitted for Ph.D., Univ. of Ill., Chicago, 1959.


Toto, P.D. PERSONAL DISCUSSION. May 9, 1966.


The average distribution of labeled cells in different cell layers of epithelium at different time intervals in a molar half-papilla. This average was derived from ten sections in each sacrifice period.

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<tr>
<th>TIME</th>
<th>BASAL CELL LAYER</th>
<th>PRICKLE CELL LAYER</th>
<th>SURFACE CELL LAYER</th>
<th>AVERAGE TOTAL NUMBER</th>
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## Table B

### Number and Distribution of Labeled Cells in the Half-Papilla of Ten Sections According to Zones

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<th>Zone II</th>
<th>Zone III</th>
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<td>5 days</td>
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<td>1.7%</td>
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</table>

*Joglekar, 1964.*
TABLE D

Histologic Formulas and Procedures

Demineralization Solution:

Solution A consisted of fifty grams of sodium citrate and 250 cc of distilled water. Solution B consisted of 125 cc of 90% formic acid and 125 cc of distilled water. Solutions A and B were mixed together in equal parts. Specimens were allowed to remain in this solution until demineralization was complete as determined by roentgenographs.

Nuclear Fast Red, Indigo Carmine Sequence:

The slides were placed for one to five minutes in a nonaqueous solution of 5% aluminum sulfate, containing 0.1 nuclear fast red (Kernechtrot Chroma-Grubler Stains), and then washed in running water for two minutes. Next they were immersed in a saturated solution of picric acid to which 0.25% of indigo carmine had been added for thirty seconds. Then they were quickly dehydrated by three changes of absolute alcohol, cleared in two changes of xylene, and mounted in Canada balsam.
Figure 1. A graph comparing the percentage of labeled cells in the ovariectomized (ovex) and in the normal mouse from one-half hour to 5 days.
% of labeled cells in the normal & the ovariectomized mouse

% of Per Cent

HOURS

TIME

DAYS

NORMAL

OVEX
Figure 2. A paracentral mesio-distal section through the mouse molar interdental papilla.

H & E stain. (X 300)
Figure 3. An illustration of the three zones of epithelium in the molar interdental papilla of the mouse.
Figure 4. Labeled cells in zone 1 at one-half hour.
Nuclear fast red, indigo carmine stain.
(X 1000)
Figure 5. Labeled cells in the basal cell layer of all three zones at 6 hours. Nuclear fast red, indigo carmine stain. (X 450)
APPROVAL SHEET

The thesis submitted by Dr. Francis Lawrence Bachnik has been read and approved by three members of the Graduate School Faculty.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

5-21-64
Date

Signature of Advisor