1968

Cell Kinetics of the Oral Epithelium of Adrenalectomized and of Hypophysectomized Mice

Elliott Howard Dickler
Loyola University Chicago

CELL KINETICS OF THE ORAL EPITHELIUM OF ADRENALECTOMIZED AND OF HYPOPHYSECTOMIZED MICE

by

Elliott Howard Dickler

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

1968

I dedicate this thesis to my wife Phyllis, whose constant understanding and encouragement made this work possible.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Anthony W. Gargiulo for his guidance and personal interest in this work.

The author wishes to express his sincere appreciation and gratitude to Dr. Patrick D. Toto whose infinite patience and constant advice and encouragement made this thesis possible.

LIFE

Elliott Howard Dickler was born in Baltimore, Maryland, October 16, 1931.

He was educated in the Baltimore Public Schools where he was graduated from Forest Park High School in 1949. The next four years he attended the University of Maryland and received his Bachelor of Science degree in 1953. He then entered the Baltimore College of Dental Surgery, University of Maryland. In June 1957 he was graduated with the degree of Doctor of Dental Surgery.

In September 1966, he enrolled in the Oral Biology Graduate Program of Loyola University School of Dentistry in Chicago, Illinois.

TABLE OF CONTENTS

INTRODUCTION

Recent investigations have shown that a significant change occurs in the mitotic rate and cell turnover of epidermis when animals are subjected to deprivations of hormones from the pituitary gland and from the adrenal glands (Bachnik, 1966; Bullough, 1949; Bullough and Laurence, 1959 and 1961; Chaudhry and Halberg, 1956 and 1961). Studies have also been made upon the rate of cell turnover of oral epithelium of interdental papilla in the normal mouse (Joglekar, 1964) and the ovariectomized mouse (Bachnik, 1966).

The effects of adrenalectomy and of hypophysectomy upon the cell kinetics and desoxyribose nucleic acid doubling time of the oral epithelium of the mouse have not been investigated. This study was limited to an investigation of these hormone deficiencies upon the oral squamous epithelium of the interdental papilla and the attached epithelium.

Using tritiated thymidine to label the cells preparing for mitosis, cell counts were used to determine doubling time of oral epithelial cells of the interdental papilla between the mandibular first and second molar of the mouse. The lack of hormones in adrenalectomy and hypophysectomy may significantly change the rate of DNA synthesis, hence change the rate of cell renewal and cell migration.

REVIEW OF LITERATURE

Control of Mitosis

The mitotic activity is subjected to controls from various sources each acting as a balancing force toward the other. These controls have been widely investigated as to their exact nature and mode of action. Ziskind, Daniel, and Blackberg (1940) using hypophysectomized and castrated rhesus monkeys reported degenerative changes such as loss of keratin, poorly preserved intercellular bridges, and superficial epidermal layers missing. They found no significant changes in vessels, inflamatory response or mitosis to account for them, and the evidences of tissue degeneration were more marked in the hypophysectomized group as a whole than in the female castrate group.

Bullough (1949 a), using male mice, found that an increase in blood sugar via subcutaneous starch injections caused an increased mitotic rate in the epithelium of the esophagus and salivary glands. He also states that the mitotic rate is decreased by lowering the blood sugar level with insulin. He explains this apparent contradiction by concluding that the critical factor in the control of mitotic cycles is the concentration of sugar in the tissues themselves, because the diurnal changes in blood sugar are the reverse of those for mitotic activity, i.e., that blood sugar per se is not the crucial factor which determines the form of the diurnal cycles. The actual control would appear to be exercised by some finer mechanism which is only crudely affected by unnaturally great changes in blood sugar concentration.

He hypothesizes that the mitoses (needing sugar to energize the process) takes place most actively when sugar is deposited from the blood into the tissues and a clear understanding of the factors controlling such deposition is necessary to explain the variations in mitotic rates. Again Bullough (1949 b) states that it appears that sugar is the vital factor involved, and that the sugar content of tissues is used up quickly during high mitotic activity. In 1952 (a) Bullough stated that the factors which effect mitosis can be roughly divided into hormonal and nutritional but at least some of their action is by interference in carbohydrate metabolism. ". . .with direct mitogenic hormones excepted, one of the most remarkable conclusions to be drawn from these observations is the relative unimportance of any factor other than that of carbohydrate supply and utilisation."

Later in 1952 (b), Bullough also found that mitotic activity is depressed by stress situations. He found greatly increased adrenal gland size in such stressed animals. The presumption is that the increase in its size is accompanied by an excessive secretion of adrenalin from the medulla and glucocorticoids from the cortex, and furthermore that the mitotic depression was the result of these hormones. The medullary hypertrophy was unexpected since it does not hypertrophy after unilateral adrenalectomy and since it was thought to play an active part only during the first few minutes of stress (Selye, 1950). Adrenalin action through the anterior pituitary production of ACTH, induces secretion of glucocorticoid hormones from the adrenals. Thus, according to Bullough, the antimitotic activity of adrenalin may be due to cortisone-like substances.

To further describe the system of interdependence and interrelationships of the various mitotic controls, Best (1952) quoting Koster (1930), and Griffiths (1941), states that hypophysectomy creates atrophy of the acinar tissue of the pancreas thus increasing the ratio of islet tissue to acinar tissue in hypophysectomized rats even though the islet tissue has not increased in amount.

The mitotic control of the pituitary growth hormone is really exerted by action on the pancreas, as shown by Young (1953) and Bullough (1954). Their works accept the dependence of epidermal mitotic activity in mice on the rate of glucose entry into the cells and its transformation into energy. Bullough (1954) further states that insulin increases the number of mitoses while growth hormone acts as an inhibitor.* When introduced together

*Mitogenic Effect of Insulin in Vitro
Substrate——0.02M Glucose

		0.5u	5u	50u	250u
Mitosis	Alone	Insul.	Insul.	Insul.	Insul.
Recorded by	9.1	14.3	17.7	18.7	17.0
Colchicine	± .52	± 1.19	± .96	± .8	± .89

Bullough hypothesises the influence of insulin to be wholly due to speeding the reaction: Glucose ⟶ Glucose -6- phosphate on the cell wall, they counteract each other. The existence of separate fractions in pituitary hormone, one of growth hormone and one of diabetogenic nature as suggested by Raben and Westermayer (1952), is not corroborated by Young (1953). The purified hormone described by Cotes, Ried, and Young (1949) has both growth and diabetogenic factors.

The direct action of the pituitary hormone upon the pancreas is best described in the work of Richardson and Young (1939), Krichesky (1936), and Marks and Young (1939). Their evidence indicates that pituitary growth hormone causes stimulation to islet tissue of pancreas. Marks and Young reported huge increases in the amount of insulin in the pancreas of experimental animals after two weeks of daily treatment with crude anterior pituitary extract. This increase was in the order of 130%-250%; however, no immediate report of its effect on mitosis was given.

Bullough (1952 a) theorises that there are two main mitosis-controlling forces, the sum of the effects of the external hormonal controls being only one of these forces. This is the more superficial, easily analysed and is responsible for the diurnal (hour-to-hour and day-to-day) cycles. The second mitosis-control force is apparently inherent in the species of tissue, which he calls "chalone," because their primary function is to slow growth.

The mitotic rates of various tissues at any given moment seem to be determined by two main groups of factors. The first group contains a number of hormones with direct relationships on cells preparing for mitosis. The second group evidently includes a large number of tissue-specific substances the nature of which is largely unknown, but which are possibly proteins (Bullough, 1962). He states further that there appears to be only one of these substances produced by each tissue and that it reacts on that same tissue to control the mitotic rate. Hormones which possess a specific power to inhibit mitoses are glucocorticoids and adrenalin, and the mitotic rate

may rise greatly after either hypophysectomy or adrenalectomy. However, both of these hormones inhibit mitosis more powerfully in some tissues, such as growing hair bulbs. Therefore, it is clear that their action is more tissue-specific than mitosis-specific (Bullough, 1962). Furthermore, the evidence shows that while mitotic rate of tissues may be influenced by hormones, it is not controlled by them. The ultimate control of mitotic rate evidently resides within the tissues themselves, and in appropriate circumstances the cells of most tissues are capable of indefinite growth and mitosis; each tissue-specific control mechanism must be anti-mitotic. Since the primary function of these anti-mitotic control substances is to slow growth, the name Chalone is more appropriate than Hormone (Bullough, 1962).

The diurnal cycles are the result of fluctuations in the supply of essential nutrients (carbohydrate in the form of glucose). When carbohydrates are available, mitotic rate rises steeply. Conversely, when carbohydrates are eliminated and fail to reach the tissues, the mitotic activity ceases. Therefore, high mitotic activity is typical of well-fed resting animals and low mitotic activity is typical of starved animals at hard work (Bullough, 1955). Cori (1950) states that certain hormones, adrenalin and insulin, can affect rate-limiting reactions in carbohydrate metabolism. In the case of mammalian epidermis, the suggestion has already been made that mitotic rate may vary directly with the degree of activity of the enzyme glucokinase, and further this enzyme is susceptible to hormone influence. Cori (1950) in an address to the first International Congress of Biochemistry, indicated one hormone, insulin, which stimulates the glucokinase

system and two hormones, pituitary growth hormone and adrenal glucocorticoid hormone which inhibit it. Cori confirms that growth hormone antagonises insulin by inhibiting the glucokinase reaction. Bullough tested this in vitro in 1954 using glucose substrate, and found growth hormone to be a mitotic inhibitor "although it is evident that relatively large doses are required to produce any great effect" (results on page four). He states (that) the fact that pituitary growth hormone prevents development of mitotic activity is so surprising that further consideration was necessary. The suggestion by Rabin and Westermeyer (1952), that the hormone extracts were not pure, but contaminated by a second hormone closely similar chemically, was disproved by Young (1953). Bullough (1955) continues, "the alternative theory must be examined, that growth hormone is indeed an inhibitor of carbohydrate metabolism and therefore of mitotic activity of such tissues as epidermis. . . . it has been suggested that in normal circumstances a high rate of growth hormone secretion is countered by a high rate of insulin secretion, and that insulin is in fact the real growth hormone." Therefore, a strong case can be made in favor of growth promotion by insulin secreted in response to growth hormone stimulation. As stated another way, Young (1953), quoting his 1940 work reports, ". . . growth promoting action of anterior lobe extracts may be contingent upon the ability of the pancreas to secrete sufficient insulin to induce nitrogen retention and promote carbohydrate oxidation." Young concludes that in youth the ability to secrete insulin is responsible for growth, but a depressed or lost ability of the pancreas to elaborate insulin, as in an adult, results

in diabetes. Best (1952) showed this to be true by injecting insulin into hypophysectomized young rats, and they grow to adult size. Bullough (1955) further states that adrenalin plus adrenochrome, both act in glucose metabolism in one or more of many biochemical reactions. Moreover, "the value of adrenalin secretion in an emergency must, therefore, remain in some doubt. The glucose released from the liver in the blood cannot be readily taken up and utilized and evidently the stimulus to the muscles is mainly if not wholly concerned with glycolysis."

The action of ACTH and glucocorticoid hormones is one of moderate stimulation, and cortisone one of depression of mitosis (not at the glucokinase level), Swann (1958). Bullough (1955) concludes that hormones which stimulate epidermal mitosis, do so through stimulation of the glucokinase system. Examples are insulin, estrogens, weak estrogenic steroids, and possibly a contaminent of ACTH.

Hormones which inhibit epidermal mitosis do so in more complex fashion interfering in a number of points, one of which may be the glucokinase system. It appears to be a natural function of all cells to grow, and divide, and the fact that most tissues are limited in their capacity to do these things may be taken as indicating that this natural function is normally kept in check. Researching further into control systems for mitotic activity, Bullough (1959) states that mitotic activity in unwounded epidermis is normally suppressed by some inhibitor produced within the skin. He claims that it is probable that it is the function of cells, unless prevented, to replicate, grow, and divide. Therefore, no extraneous or

exogenous stimuli need be required, and substances for inhibition not stimulation may be the essential factors in any differentiating tissue. Therefore, a stimulus to repair or regneration may in fact be a lack of inhibition. Also a stimulus to overgrowth may be from the action of a hormone which could temporarily suppress the specific inhibitor. Chaudhry, Halberg, and Bittner (1956) found inhibition of mitosis by epinephrine in pinnal epidermis of mice, confirming the related studies by Bullough.

Stafne (1950) contradicting some of the cited works, concludes that a deficiency in the secretion of the growth hormone of the pituitary gland results in a decrease in the growth of all tissues soft and skeletal.

Bronstein, Gray, and Parrott (1952) using an acid acetone extract of human plasma, has shown it to be active in partially maintaining the weight and histological structure of the adrenals of hypophysectomized rats and claim that ACTH may be demonstrable in human plasma.

Chaudhry and Halberg (1961) emphasized the important role of the mammalian adrenal cortex in the co-ordination of physiologic function along the 24 hour scale and in addition showed the distinctive effects of gland removal. Adrenalectomy lowers the amplitude of the animals (hamsters), temperature rhythm, and obliterated its pinnal mitotic rhythm.

Bullough and Laurence (1961) reported that the suppression of epidermal mitotic activity during starvation is due primarily to the increasing concentration of a mitotic inhibitor. They suggest that this inhibitor is adrenalin secreted in excess during the stress of starvation. They state that adrenalectomy is followed by a considerable rise in the epidermal

mitotic rate and by the elimination of the diurnal mitotic rhythm. They further suggest that this rhythm is normally based on the hour-by-hour variations in the rate of adrenalin secretion. The awake mouse is active, with a raised adrenalin level giving a low mitotic rate. Conversely, the sleeping mouse is inactive with a low adrenalin level giving a high mitotic rate.

Their experiments also showed the effect of adrenalin in vitro and in vivo by washing out the adrenalin from the tissues, which allowed mitoses to continue. On five adrenalectomised mice a considerable increase occurred in the epidermal mitotic rate. Adrenalin injections on these animals reversed the trend, and lowered the mitotic rate.

ACTH in physiological concentrations does not exert an antimitotic action (Bullough, 1955). The same is true of such mineralocorticoids as desoxycorticosterone (Bullough, 1952 b). Cortisone, on the other hand, can powerfully depress the epidermal mitotic rate in high quantities (Bullough, 1952 b). It is appropriate at this point to note that stress can occur to experimental animals from noise and/or handling in cages and in transferring them to other cages.

To elaborate further on tissue inhibitors, Bullough (1964) states that under the circumstances of wound healing, the epidermal mitoses become insensitive to adrenalin. He suggests that this may be due to a fall in the concentration of the mitosis-inhibitor of the tissues, which must be present in order for adrenalin to act in the way generally reported. He declares that "all available evidence is in agreement with a theory that neither the

epidermis-specific mitotic inhibitor nor adrenalin is able to function alone, and that in normal epidermis they act together to limit and so to control the epidermal mitotic rate."

Studies on Oral Epithelium

Toto and Ojha (1962) studied the generation cycle of oral epithelium of the tongue in 36 mice with tritiated thymidine. They determined ten hours to be the DNA synthesis time and this figure (for DNA synthesis) is corroborated by Joglekar (1964), using the interdental papilla tissue of mice. Toto and Ojha reported the percentage of labeled mitotic figures as follows:

1/4 hour - 0%	9 hour - 89%
1/2 hour - 11%	10 hour - 98%
1 hour - 39%	12 hour - 15%
2 hour - 44%	15 hour - 0%
4 hour - 56%	24 hour - 0%
6 hour - 66%	48 hour - 0%

Dhawan and Toto (1965), using tongue, and palate epithelium of mice, also reported the highest labeled percentage on the palate at ten hours. This value was given as 95% of the cells labeled. The ten hour figure was also highest for the dorsum of the tongue (94.5%) and the ventral surface of the tongue (98%)

Joglekar (1964), using tritiated thymidine reported on the oral epithelium (interdental papilla), of normal mice. She showed a labeled cell

doubling time for DNA synthesis occurs from one half hour to ten hours. This confirms the results reported by Toto and Ojha (1962), and by Dhawan and Toto (1965). Joglekar showed that more labeling occurred in the basal cell layer than in the prickle cell layer during the first 18 hours after injection of the isotope. This does not concur with the findings of Krajewski, Gargiulo, and Staffelino (1964), who reported on human female oral epithelium. Their findings show 78% of the cells of mitosis in the deep prickle layer and only 22% in the basal layer. Joglekar showed that after 22 hours the greater number of labeled cells occurred in the prickle cell layer, but this was due to cell migration.

Hormonal Influences on Oral Epithelium

Shklar (1963) reported decreased density of the periodontal membrane, decreased osteoblastic activity, and atrophy of gingival epithelium occurring after bilateral adrenalectomy of albino rats. Labelle and Schaffer (1966) reporting on the effects of 2.5 mg. cortisone acetate daily and induced local factors (wire ligature irritation), confirm that the appearance of the periodontium was that of decreased cellular activity exhibited by a decrease in number and size of fibroblasts and osteoblasts. When cortisone is administered in the presence of irritants, the response to that irritant is lessened.

Bachnik (1966) studied the effects of ovarectomy upon the synthesis of DNA, and the migration patterns of oral epithelial cells of the mouse interdental papilla. He found, by means of labeling with tritiated thymidine,

that ovariectomy delayed DNA synthesis and thereby delayed the doubling time of labeled epithelial cells.

Histology of the Mouse Molar Interdental Papilla

Greulich (1961) using thymidine autoradiograms revealed that DNA synthetic activity and presumably, therefore, mitotic activity in the region of the gingival margin and attached epithelial cuff is considerably greater than that of the adjacent oral mucosal epithelium. These findings support the concept that the tissues of the gingival margin and attached epithelial cuff are modified to perform supportive and protective functions specifically associated with the teeth and should be considered to be functionally separate from the oral epithelium as a whole. In 1962 Greulich further states that since no evidence of keratinization has been noted and since a continuous cell migration occurs, a firm attachment to the enamel seems unlikely. On the other hand, it is stated in Orban's Periodontics (from Stern, 1963) "the cells of the epithelial attachment appear to attach to the tooth by a system of hemidesmosomes. The system resembles the mode of attachment of the basal cells to the basement membrane." Toto (1964) states that the nature of the epithelial relationship to tooth is one of close adherence maintained by a mucopolhysaccharide cement.

Autoradiography

Gross, Bogoroch, Nadler, and Leblond (1951) showed autoradiography to be a method for labeling, tracing and detecting radioisotopes based on their ability to affect the silver bromide crystals of photographic emulsion.

Firket and Vesley (1956) showed thymidine to be a specific precursor of DNA.

Leblond, Messier, and Kaprirva (1959) showed that if tritiated thymidine is administered during DNA synthesis, the nuclei of these cells will be labeled before mitosis takes place. The labeled DNA is shared equally by the nuclei of the daughter cells. Messier and Leblond (1960) reported that in renewing cell populations, large numbers of labeled nuclei appear after administration of thymidine-H^3. Their number then rapidly decreases and disappears as the cells redivide and are finally lost. Hughes, et al 1958 reported from their work that the time interval that tritiated thymidine is available for cell uptake is one hour. Moreover, after one hour, 95% of the label is either utilized in DNA replication or excreted. Skougaard (1964) states that the mode of administration is an important factor in determining the cell renewal time. He claims that an increase occurs in the mean grain count corresponding to the time the tracer was available in the blood plasma. Intravenous injection gave a fast uptake while the intraperitoneal and intramuscular routes of administration were considerably lower.

MATERIALS AND METHODS

Twenty-five Caesarean Derived #1[1] adrenalectomized white mice and twenty-five C.D. #1 hypophysectomized white mice 45-50 days of age with average weight of 30 grams were utilized for these studies. Each animal received tritiated thymidine via intraperitoneal injection. The specific activity of the isotope was 1.9 curies per millimole, and the dose rate was one microcurie per gram of body weight three days following the adrenalectomy or hypophysectomy. The injections were carried out within a one hour time span in order to minimize the effects of the diurnal rhythm of mitosis. The first animals for each study were sacrificed one half hour after the injections and thereafter at the following time intervals; 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 22, 24, 28, 32, 36, 40, 44, 48 hours; three, four, and five days.

The mandibles and soft tissue coverings were disected out and fixed in a ten percent formalin solution for one week. The formalin bath was changed after three days. Following fixation the specimens were demineralized in a solution of sodium citrate and formic acid as follows:

Solution A - 50 grams of sodium citrate and
250 ml of distilled water.

Solution B - 125 mg of 90% formic acid and
125 ml of distilled water.

Solutions A and B were mixed in equal parts.

---

[1]The Charles River Mouse Farms, Inc., North Wilmington, Massachusetts.

Specimens were treated in this solution until demineralization was complete as determined radiographically. The demineralization solution was changed every day for three days. Specimens were then dehydrated in alcohol solutions in sequence from 75% to 95% and finally to absolute alcohol. The tissues were then embedded in paraffin (Tissue Mat-Fisher Company). This was carried out in a Vacuo at a temperature of 56.5° C at 15 pounds per square inch for 15 minutes.

Sectioning was performed on a rotary microtome at a thickness of six microns. These were cut on a mesiodistal axis through the molar area and each sixth section was utilized for autoradiographs. The sections were then mounted and deparaffinized by immersion for ten minutes in each of three changes of Xylol, followed by three descending concentrations of alcohol, five minutes in absolute alcohol, and three minutes each in 95% and 75% alcohol. Finally the sections were washed in distilled water for five minutes.

Under darkroom conditions, the slides were dipped into liquid photographic emulsion (Nuclear Track Emulsion Type NTB$_3$-Eastman Kodak Company) for three seconds, air-dried for ten minutes and placed into a black, light-tight exposure box, section side up. Lithium Chloride was placed into the box to maintain low humidity, and black masking tape was used to light-seal the box. The slides were allowed to expose the emulsion for a period of two weeks at 4° C. During this time the box was positioned to keep the sections upright.

Developing

The slides were placed in a rack and developed for five minutes at 60° F (18° C Kodak D 196 developer). The slides were then washed in distilled water for thirty seconds and placed into acid fixer reducing solution for ten minutes. The slides were washed in running tap water for 30 minutes and allowed to dry in a stream of air. When the slides were dry, they were dipped into water for 30 seconds and the excess emulsion was trimmed from the slides. The sections were stained with a nuclear fast red, indigo carmine sequence.[1] Also, a representative section from each time period was stained with hematoxylin and eosin without an autoradiograph being made. For each time period, six slides were prepared by autoradiographic technique with each slide containing three to six sections. The sections were mesiodistal through the interdental papillae of the molar area.

The counts were performed on the papilla between the first and second molar. At least one section on each slide prepared was examined and recorded, making a minimum of six labeled cell counts per time interval.

The hematoxylin and eosin slides were used in a random sampling to find the average total epithelial cell population per papilla. The epithelial cell population per papilla was determined. The determination of the labeled cell populations was next carried out. Each of the slides for every sacrifice period was examined in both the adrenalectomized mice

---

[1] Micheline Mortreull-Langlois, Department of Comparative Anatomy and Histology, Faculty of Science, Univ. of Paris, Rue Victor Cousin, Paris, France.

series and the hypophysectomized mice series. For more detailed localization of the labeled cells, the papillae were arbitrarily divided into three zones using the thickness (depth) of the epithelium as a measure. Zone I began at the apical end of the attached epithelium, and continued coronally until the epithelium was three cells wide (deep). Zone II began at this point and continued coronally to a point corresponding to the end of the connective tissue (lamina propria). Zone III consisted of all epithelium coronal to the connective tissue (See Figure 1). Generally, Zone II varied from four to six cells deep and Zone III six and more cells deep. Zone III terminates at the tip of the papilla.

To further clarify the location of the labeled cells we recorded their occurrence in one of three layers of epithelium; the basal layer cells are on the basement membrane, the prickle layer cells above the basal layer, and surface cells on the surface ready for desquamation.

A statistical analysis of the occurrence of labeled cells in the adrenalectomized mouse and the hypophysectomized mouse was prepared and compared to the results obtained for the normal mouse (Joglekar, 1964) and for the ovarectomized mouse (Bachnik, 1966).

FINDINGS

The interdental papilla of the mouse is basically triangular in outline, with slightly concave sides to accommodate the curvature of the enamel surface of the molar tooth with which it is in close contact. Microscopically, it consists of a dense fibrous connective tissue core covered by stratified squamous epithelium, resting on a basement membrane. There is a basal layer, and prickle cell layer but no granular-cell layer or stratum cornium in the sulcus epithelium. The surface layer contains polygonal prickle cells containing granular cytoplasm, and pyknotic nuclei. The prickle cell layer contains polyhedral cells, and varies in thickness, increasing from the end of the epithelial attachment to the tip of the interdental papilla.

Adrenalectomized Mice

The average epithelial cell population for each section of the interdental papilla of an adrenalectomized mouse was determined by counting the epithelial cells of 31 papillae. The cell populations ranged from a low of 103 cells to a high of 313 cells. The mean epithelial cell population per section of interdental papilla for adrenalectomized mice was 173 cells.

At one half hour 12.5 labeled cells (7.2%) were seen and at one hour this value fell to 9.0 (5.2%). Then there was a gradual rise in the labeled cell population to 18 cells. The doubling time of the initial labeled cell population occurred at 14 hours. (Tables A and B).

Basal cell layer: The greatest number of labeled cells occurred in the basal layer for the first 24 hours (Table A). The average labeled population for one half hour was 8.9 cells. At one hour there were 5.7 labeled cells, and thereafter this value increased to a peak of eight cells at the 24 hour period. Thereafter, the number of labeled cells per section dropped sharply to 4.6 cells at the 44 hour period, 1.8 cells for the three day period, 2.1 cells for the four day period and 0.6 cells for the five day period.

Prickle cell layer: The labeled cell count of the prickle cell layer began with an average of two cells at the one half hour period for all zones. The cell count gradually increased through the early sacrifice hours to reach a peak of 21.3 cells at 20 hours. The number decreased steadily to 9.3 at 44 hours, thereafter to 5.5 cells at the three day interval, 5.1 cells for the four day period, and 4.6 cells at five days.

Surface cell layer: At one half hour the surface layer showed an average of 1.8 labeled cells. The figure steadily increased to 8.5 cells at the 44 hour period. Later periods showed a decline in the number of surface labeled cells ending with a count of 5.6 at the five day period.

Zone distribution: In the early hours, the count of the labeled cell population was concentrated in Zone I, beginning with 6.5 cells at the one half hour period. Zone II showed five cells, and Zone III showed one cell for the one half hour period. Subsequently, the zones showed increases up to peak points followed by declining counts as follows: Zone I reached a peak of nine labeled cells at 20 hours and declined to 0.8 at five days.

Zone II reached a peak of 21.1 labeled cells at 40 hours, and declined to 4.6 cells at five days. Zone III peaked at four days with an average count of 7.3 labeled cells ending at 5.2 cells for five days (Table B).

Percentage of labeled cells: The percentage of labeled cells at one half hour was 7.2%. The labeled cell numbers gradually rose to a maximum of 19.4% at 20 hours and then gradually declined through the remaining time periods, ending with 6.1% at five days.

The percentage of labeled cells doubled from 5.2% at one half hour to 10.5% at 18 hours. The curve of the logarithm values of the percentages (Figure 2) of labeled cells shows a straight line to the 26 hour period. The rate of increase diminished slightly from 30 hours to 40 hours, and then declined gradually to the five day period (Table E).

Hypophysectomized Mice

The average cell population for hypophysectomized mice was determined by cell counts on sections of 15 interdental papillae. The figures determined for these sections ranged from a low of 123 cells per papilla section to a high count of 250 cells. The mean was 190 epithelial cells per section of papilla.

At one half hour, the sections showed a labeled cell population of 9 cells. This was followed by a gradual rise in the labeled cell frequency to 18. This occurred at 16 hours.

Basal cell layer: The basal layer contained the greatest number of labeled cells in the early hours, and continued so up to 16 hours. At one half hour the average count was 6.6 labeled cells per papilla. The average

increased to nine cells at the 16 hour period. This was maintained up to 20 hours. The counts then declined to eight labeled cells at 24 hours, six at 48 hours, three at three days, 2.3 at four days, and finally two labeled cells at the five day sacrifice period.

Prickle cell layer: The prickle cell layer showed an average number of labeled cells of less than one cell at one half hour. This value increased to 14.3 at 28 hours. The increases continued less sharply to the 44 hour period which showed an average value of 16.5 labeled cells. Then the value declined to 8.6 at three days, seven at four days, and ended at five days with an average count of 4.5 labeled cells per section.

Surface cell layer: At one half hour the surface layer contained an average of 1.6 labeled cells. The number of these cells increased to 13 at the 44 hour period. Thereafter, the number diminished rapidly, showing a 5.5 labeled cells at 48 hours; 7.6 labeled cells were present at three days, and 4.5 at four days, and 5.6 labeled cells at five days.

Zone distribution: Zone I contained the great majority of labeled cells beginning with the one half hour period. At this point the average labeled cell population for Zone I was eight, Zone II was 1.3, and Zone III was zero. Each zone gradually increased to a peak and then declined.

Zone I had the highest average count at 16 hours with a figure of 9.5 labeled cells. The decline was abrupt to six labeled cells at 18 hours, and held near this figure to 48 hours. The decline was then to 3.3 cells at three days; 1.5 at four days; and one at five days. The Zone II average rose from 1.3 cells at the one half hour period to 19.3 cells at

44 hours. The three day count was eight labeled cells; the four day count was 5.6 cells, and the five day count was four labeled cells. The Zone III labeled cell population remained less than one for up to eight hours. It remained low (two, three, or four), up to 40 hours. At the 44 hour point the figure rose sharply to ten cells labeled. The decline thereafter was more gradual as follows: six labeled cells at 48 hours, eight cells at three days, seven cells at four days, and eight cells at five days.

Percentage of labeled cells: The percentage of labeled cells per section of papilla doubled from 4.8% at one half hour to 9.5% at ten hours and 9.7% at 14 hours. This value increased regularly to 13.2% at 28 hours. The decline thereafter was gradual, showing 9.7% at 36 hours, 11.6% at 48 hours, 10.3% at three days, 7.4% at four days, and 6.7% at five days.

From the data recorded in Table F, one notices a distinct similarity between the labeled cell counts of the adrenalectomized mice and the hypophysectomized mice.

Statistical analysis of the values obtained in this study consisted of determinations of the means, standard deviations, and t-test probabilities (Table G).

DISCUSSION

When the adrenal glands or the hypophysis are removed from mice, the frequency of labeled squamous cells of the oral epithelium is generally depressed. The smaller number of labeled cells is seen within the first few hours after tritiated thymidine administration. However, in the later hours of the cell cycle, the labeled frequency rises above that of the normal animals. This indicates that the cell renewal time is slowed to the extent that the labeled cells remain in the tissue sections for longer periods of time than the normal animals reported by Joglekar (1964).

There seems to be a general slowdown in total cell proliferation and cell migration (Table E). This slower cell migration and cell proliferation indicated by the delay in DNA doubling time, is probably due to the hormonal imbalance created in the experimental groups. It is suggested that the labeled cells, which are fewer in number than reported for normal animals, remain in the tissue longer than normal. The final reduction in the labeled cells is caused by the surface exfoliation of such cells after they migrated from the basal layer through the prickle cell layer and lost at the surface.

In the adrenalectomized mice the cell layer distribution (Table A) shows that most of the labeled cells remain in the basal layer for the first 16 to 22 hours. The prickle cell layer showed the greatest number of labeled cells from 18 hours to the 40 hour time period. After that these cells reached the surface and were lost. Therefore, all or most of the mitosis occurs in the basal and lower prickle cell layers where the highest

labeling frequencies occur within the doubling times of the labeled population.

The greatest labeled cell frequency for the adrenalectomized mice occurs in Zone I for the first six hours. Zone II shows the greatest number of labeled cells from ten hours to approximately three days. Therefore, most of the mitotic acitivity is concentrated in Zones I and II. The four and five day zone distribution shows the highest labeled count in Zone III. These results show that the orderly procession of cells from layer to layer and from zone to zone is not altered by adrenalectomy except that the rate of movement is reduced as compared to normal (Joglekar, 1964), and ovariectomized (Bachnik, 1966) mice, and the number of mitoses per unit time also is reduced. In addition, the total number of cells per papilla section is also reduced. In addition, the total number of cells per papilla section is also reduced in comparison to the normal counts as reported by Joglekar.

In the hypophysectomized animals a similar pattern is evident, although the time sequences are different. The cells first are labeled with tritiated thymidine in the basal cell layer and account for the larger number of labeled cells seen here early in the study (Table C). The labeled cells undergo mitotic division while they remain in this layer within the period of doubling time. Therefore, in this group also the vegetative activity of these cells occurs in the basal and lower prickle cell layers. Then the majority of the labeled cells is seen in the higher prickle cell layer up to four days. At five days, the surface layer contains the greatest value.

The migration through the zones follows the pattern of the adrenalectomized mice (Table D). The Zone I count is the highest from one half hour to 12 hours, and the Zone II count is highest from 14 hours to 48 hours. Zone III has the greatest number of labeled cells in the three, four, and five day counts. Again, one sees the same orderly procession of labeled cells, from layer to layer and from zone to zone, as is observed in the adrenalectomized group.

The variations from normal in the labeled cell counts are the result of the upset to the delicate endocrine balance, caused by hormone deprivation resulting from the removal of these glands. This same pattern of disturbance was reported by Bachnik (1966) on ovariectomized mice. However, in spite of the upset to the endocrine balance caused by these gland removals, one is struck by the fact that the basic functions of the cells examined in this study continued to operate, no matter the altered rate. As stated by Bullough (1959), "it is the function of cells unless prevented, to replicate their component molecules, to grow, and to divide."

The magnitude of the fluctuations from the normal compared to our groups and the ovariectomized group is worthy of note. There is seen in the graph (Figure 2) the logarithm of the percent of labeled cell counts. The straight line feature of the early hours of the study indicates that in the experimental animals, growth - as represented by cell proliferation - is a geometric expression albeit at a slower pace than reported for normal animals (Joglekar, 1964). These curves are very close, indeed they cross at one point and then continue together throughout the experimental period.

There is, according to the t-test of probability, very little chance that these values are accidently similar ($p > .001$). The difference in the values for the times of doubling of the labeled cell populations are also different. It is interesting to note the closeness of these values to those obtained by Bachnik (1966) for doubling time of initial labeled cell population, cell layer distribution, and zone distribution, and compare all these data to the normal values reported by Joglekar (1964). Although the absolute values reported for the ovariectomized group (Bachnik, 1966) are different from ours, the pattern of the change, and the sequence of the shifts in labeled cell populations are very similar. Therefore, considering all together - adrenalectomy, hypophysectomy, and ovariectomy (Bachnik, 1966), and comparing these data to the results of the normal mice (Joglekar, 1964) - one sees that the nature of the endocrine system disturbance created is not any more important than the fact itself that a disturbance exists, when the DNA doubling time of oral epithelial cells is used as a measure of such disturbance. Nevertheless, such disturbances produce significant depressions in the cell renewal cycle.

These data, however, do not agree with those of Bullough and Laurence (1961), and Chaudhry and Halberg (1961). In their experiments, on pinnal epidermis of mice using starvation as a stress factor, increased mitotic inhibitor (presumably adrenalin) is secreted. They also showed adrenalectomy to produce a considerable increase in the epidermal mitotic rate and destruction of the diurnal mitotic rhythm of epidermis.

Their in-vitro studies also showed adrenalin to inhibit the mitosis, and followed with the washing out of the adrenalin, whereupon the mitosis resumes. It must also be remembered that in 1959 Bullough and Laurence propounded the theory of tissue-produced inhibitors (named Chalones in 1964) which are necessary to act in conjunction with adrenalin to produce the mitotic inhibition. In 1952, Bullough showed that stress produced by overcrowding caused epidermal mitotic rates in mice to fall 60%. He concluded that this result was due to increased adrenal output due to adrenal hypertrophy. At this time the explanation for this apparent contradiction between our results for adrenalectomy and those of Bullough and Laurence (1961), and Chaudhry and Halberg (1961) escapes us.

There are significant differences in the means of labeled cells between 28 hours and 44 hours in adrenalectomized and hypophysectomized animals which showed a divergence from each other which then narrowed again to a very close range of differences after 44 hours ($p < .001$). The rate of labeling is faster (or slower) in one series versus the other.

The general pattern of labeling frequency is decreased from normal as reported by Joglekar (1964) throughout the first 24 hours. After this time the percentage of labeled cells in the experimental groups (ovariectomy included) is higher than that reported as normal (Table E) due primarily to the depressed rate of cell proliferation. The labeled cell count in the experimental group exceeds normal after 24 hours of the study because the entire pattern (doubling time and cell migration) of the experimental group is slowed.

Therefore, after 24 hours, a greater percentage of labeled cells has

become lost from the normal animals, and the labeled population is still present in the sections of the experimental group (Table E). This slower movement and slower doubling time is shown by the increased time lapse necessary to complete the doubling of the initial labeled population. Therefore, the experimental labeled cell population remains insitu longer and becomes, in effect, an older cell population than normal. This delay in cell movement explains the higher numbers of labeled cells seen in the experimental group after 24 hours.

If ten hours is to be taken as the DNA doubling time for normal animals in this type of study (Toto and Ojha, 1962; Dhawan and Toto, 1965; Joglekar, 1964), then our results for adrenalectomy at 14 hours, and hypophysectomy at 16 hours as interpreted from the graph (Figure 2), do indeed represent a changed (slowed) pattern. However, our doubling times as interpreted from the graph are not appreciably different from each other, and that of ovariectomized mice (Bachnik, 1966) being 14 hours.

SUMMARY

A histological and cell kinetic study was undertaken on the oral epithelium of the interdental papilla of adrenalectomized and of hypophysectomized C.D. #1 white mice 45-50 days of age weighing approximately 30 grams. Tritiated thymidine (specific activity 1.9 curies per millimole) was injected intraperitoneally at a rate of one microcurie per gram of body weight. The animals were sacrificed at one half hour, one hour, and two hours, and regular two hour intervals up to 24 hours. In addition, sacrifices were made at four hour intervals up to 48 hours and at the end of three, four, and five days.

The mandibles were removed, fixed and embedded in paraffin by following the usual standard procedures. Sections were cut at a thickness of six microns on a mesiodistal axis through the molar interdental papilla. Each sixth section was used for autoradiography. The sections were stained with a nuclear fast red, indigo carmine sequence.

Six specimens prepared in this manner from each sacrifice animal were selected for examination. The average number of labeled cells compared to non-labeled cells as well as the location of the labeled cells in the papilla was recorded. The position of the labeled cell in the papilla was recorded by dividing the epithelial covering into three zones based on the thickness of the epithelium. The average number of labeled cells and the percentage of labeled cells was calculated for each sacrifice period. These values were compared to those determined for the normal mouse (Joglekar, 1964), and those determined for the ovariectomized mouse (Bachnik, 1966).

Statistical analyses for the adrenalectomized and for the hypophysectomized mice consisted of means, standard deviations, and t-test determinations.

CONCLUSIONS

The initial sections at one half hour showed that the first labeled cells appeared in all layers and all zones in both series of animals. The only exception to this pattern is the complete lack of labeled cells in Zone III of the hypophysectomized group. The greatest majority of these labeled cells occurred in the Zone I areas and in the basal layers of the epithelium.

After the time lapse of 14 hours in the adrenalectomized group and 16 hours in the hypophysectomized group (the time elapsed for doubling of labeled cell populations) the greatest numbers of labeled cells are still present in the basal and prickle cell layers. This means that the cells which undergo mitotic division in the oral mucosa of mice are located in the basal and lower prickle cell layers.

The labeled cells migrated obliquely in a coronal direction and towards the tooth surface, where they were lost into the sulcus.

The rates of DNA synthesis and cell mitosis are very similar in both groups. Both adrenalectomy and hypophysectomy show these rates to be about equal as the labeling frequencies are essentially the same for both experimental groups.

The doubling time for labeled oral epithelium of the interdental papilla in adrenalectomized mice is 14 hours, and that doubling time in hypophysectomized mice is 16 hours.

These data suggest to us that the inter-relationships between the adrenal gland and hypophysis (and their hormones) is so close that the

removal of one gland creates a disturbance in the other gland, and furthermore, that the end result patterns are similar.

BIBLIOGRAPHY

Bachnik, Francis L. THE GENERATION CYCLE OF EPITHELIAL CELLS IN THE MOLAR INTERDENTAL PAPILLAE OF OVARIECTOMIZED MICE. Thesis, Submitted for M.S., Loyola University, 1966.

Best, Charles H. INSULIN. Diabetes, Vol. 1, No. 4, 1952.

Bornstein, J., Gray, C. H., and Parrott, D. M. ADRENOCORTICOTROPHIC-LIKE ACTIVITY IN PLASMA. J. Endocr., 8:40, 1952.

Bornstein, J., Reid, E., and Young, F. R. THE HYPERGLYCAEMIC ACTION OF BLOOD FROM ANIMALS TREATED WITH GROWTH HORMONE. Nature, 168:903, November, 1951.

Bourne, Geoffrey H. AUTORADIOGRAPHY. Biological Reviews of the Cambridge Philosophical Society, 27:108, 1952.

Bullough, W. S. AGE AND MITOTIC ACTIVITY IN THE MALE MOUSE, MUS MUSCULUS L. J. Exp. Biol., 26:261, 1949.

Bullough, W. S. THE RELATION BETWEEN THE EPIDERMAL MITOTIC ACTIVITY AND THE BLOOD-SUGAR LEVEL IN THE ADULT MALE MOUSE, MUS MUSCULUS L. J. Exp. Biol., 26:83, 1949.

Bullough, W. S. THE ENERGY RELATIONS OF MITOTIC ACTIVITY. Biological Reviews of the Cambridge Philosophical Society, 27:133, 1952.

Bullough, W. S. STRESS AND EPIDERMAL MITOTIC ACTIVITY: THE EFFECTS OF THE ADRENAL HORMONES. J. Endocr., 8:265, 1952.

Bullough, W. S. A STUDY OF THE HORMONAL RELATIONS OF EPIDERMAL MITOTIC ACTIVITY IN VITRO II INSULIN AND PITUITARY GROWTH HORMONE. Exp. Cell Res., Vol. 7, 1954.

Bullough, William S. HORMONES AND MITOTIC ACTIVITY. Vitamins and Hormones, 13:261, 1955.

Bullough, William S. THE CONTROL OF MITOTIC ACTIVITY IN ADULT MAMMALIAN TISSUES. Biological Reviews of the Cambridge Philosophical Society, 37:307, 1962.

Bullough, William S. GROWTH REGULATION BY TISSUE-SPECIFIC FACTORS, OR CHALONES. Cellular Control of Mechanisms and Cancer. Edited by P. Emmelot and O. Muhlbock. New York: Elsevier Publishing Co., 1964.

Bullough, William S., and Johnson, M. A SIMPLE TECHNIQUE FOR MAINTAINING MAMMALIAN EPIDERMAL MITOSIS IN VITRO. Exp. Cell Res., Vol. 2, 1951.

Bullough, William S., and Laurence, E. B. THE CONTROL OF EPIDERMAL MITOTIC ACTIVITY IN THE MOUSE. Proc. Roy. Soc. Lond. Ser., 151:517, 1959.

Bullough, William S., and Laurence, E. B. MITOTIC CONTROL BY INTERNAL SECRETION: THE ROLE OF THE CHALONE-ADRENALIN COMPLEX. Exp. Cell Res., 33:176, 1964.

Chaudhry, A. P., Halberg, F., Bittner, J. J. EPINEPHRINE AND MITOTIC ACTIVITY IN PINNAL EPIDERMIS OF THE MOUSE. J. Appl. Physiol., 9:265, 1956.

Chaudhry, A. P., and Halberg, F. ADRENALECTOMY EFFECT ON TEMPERATURE AND EPIDERMAL MITOTIC RHYTHMS IN HAMSTERS. J. Dent. Res. (Abstract), Vol. 40, No. 4, July-August, 1961.

Cohen, B. STUDIES OF THE INTERDENTAL EPITHELIAL INTEGUMENT. PROCEEDINGS OF THE SEVENTH ANNUAL MEETING OF THE I.A.D.R., April, 1959. J. Dent. Res., 38:1219.

Cori. ADDRESS TO FIRST INTERNATIONAL CONGRESS OF BIOCHEMISTRY. Cited by W. S. Bullough, 1955.

Cotes, P. M., Reid, E., and Young, F. R. DIABETOGENIC ACTION OF PURE ANTERIOR PITUITARY GROWTH HORMONE. Nature, 164:209, August, 1949.

Firket, H., and Vesly, W. G. AUTORADIOGRAPHIC VISUALIZATION OF DNA IN TISSUE CULTURE WITH TRITIUM LABELED THYMIDINE. Nature, 181:274, 1957.

Greulich, R. C. CELL PROLIFERATION AND MIGRATION IN THE EPITHELIAL ATTACHMENT COLLAR OF THE MOUSE MOLAR. I.A.D.R., March, 1962.

Greulich, R. C. EPITHELIAL DNA AND RNA SYNTHETIC ACTIVITIES OF THE GINGIVAL MARGIN. J. Dent. Res. (Abstract), Vol. 40, No. 4, 1961.

Gross, J., Bogoroch, R., and Nadler, N. J., and Leblond, C. P. THE THEORY AND METHODS OF RADIOAUTOGRAPHIC LOCALIZATION OF RADIOELEMENTS IN TISSUE. Amer. J. Roentgen., 65:420, 1951.

Henry, J. L., Meyer, J., Weinmann, J. P., and Schour, I. PATTERN OF MITOTIC ACTIVITY IN ORAL EPITHELIUM OF RABBITS. A.M.A. Arch. Path., 54:281, September, 1952.

Hughes, W. L., Bond, V. P., Bucher, G., Gronkit, E. P., Painter, R. B., Queastler, H., and Sherman, F. G. CELLULAR PROLIFERATION IN THE MOUSE AS REVEALED BY AUTORADIOGRAPHY WITH TRITIATED THYMIDINE. Proc. Nat. Acad. Sci., 44:476, 1958.

Joglekar, A. A. CYTOLOGICAL STUDY OF THE MIGRATION OF THE CELLS OF THE INTERDENTAL PAPILLAE IN THE MOUSE. Thesis, Submitted for M.S., Loyola University, 1960.

Krajewski, J. J., Gargiulo, A. W., and Staffelino, H. MITOTIC ACTIVITY IN THE ORAL EPITHELIUM OF THE HUMAN FEMALE. Periodontics, 2:267, 1964.

Krichesky, Boris. PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 34:126, 1936.

Labelle, R., and Schaffer, E. THE EFFECTS OF CORTISONE AND INDUCED LOCAL FACTORS ON THE PERIODONTIUM OF THE ALBINO RAT. J. Periodont. Vol. 37, No. 6, November-December, 1966.

Leblond, C. P., Messier, B., and Kapriwa, B. THYMIDINE-H^3 AS A TOOL FOR INVESTIGATION OF THE RENEWAL OF CELL POPULATIONS. Lab. Invest., 8:296, 1959.

Lettre, Hans. SOME INVESTIGATIONS ON CELL BEHAVIOR UNDER VARIOUS CONDITIONS: A REVIEW. Cancer Res., 12:847, December, 1952.

Levin, Harry L. SOME DENTAL ASPECTS OF ENDOCRINE DISEASES. Oral Surg., Oral Med., and Oral Path., 19:466, 1965.

Marks, H. P., and Young, F. C. THE "PANCREOTROPIC" ACTION OF ANTERIOR PITUITARY EXTRACTS. Chemistry and Industry (Abstract), Vol. 58, No. 27, July, 1939.

Mc Hugh, W. D., and Zander, H. A. STUDIES ON THE MIGRATION OF CELLS IN GINGIVAL CUFF EPITHELIUM. J. Dent. Res., Vol. 43, No. 5, September-October, 1964.

Messier, B., and Leblond, C. P. CELL PROLIFERATION AND MIGRATION AS REVEALED BY RADIOAUTOGRAPHY AFTER INJECTION OF THYMIDINE-H^3 INTO MALE RATS AND MICE. Amer. J. Anat., 106:247, May, 1960.

Raben, M. S., and Westermeyer, V. W. DIFFERENTIATION OF GROWTH HORMONE FROM THE PITUITARY FACTOR WHICH PRODUCES DIABETES. Proc. Soc. Exper. Biol. Med., 80:83, 1952.

Richardson, K. C., and Young, F. G. THE "PANCREOTROPIC" ACTION OF ANTERIOR PITUITARY EXTRACTS. Jour. Physiol., 91:352-364, 1937.

Shklar, Gerald. THE EFFECT OF BILATERAL ADRENALECTOMY ON THE PERIODONTIUM AND ALVEOLAR BONE OF THE ALBINO RAT. I.A.D.R., 41:63, March, 1963.

Skougaard, M. INFLUENCE OF THE ROUTE OF ADMINISTRATION OF H^3-THYMIDINE ON THE DETERMINATION OF CELL RENEWAL IN GINGIVAL EPITHELIUM OF THE MARMOSET. J. D. Res., 43:762, September-October, 1964.

Stafne, E. C. DENTAL ROENTGENOGRAPHIC ASPECTS OF SYSTEMIC DISEASE. J.A.D.A. 40:265, 1950.

Stern, I. B. ELECTRON MICROSCOPIC OBSERVATIONS OF THE DENTOGINGIVAL ATTACHMENTS IN RAT INCISORS. Cited by Grant, Stern, and Everett. Orban's Periodontics. St. Louis: The C. V. Mosby Co., 1963.

Swann, M. M. THE CONTROL OF CELL DIVISION: A REVIEW. Cancer Res., 18:1118, November, 1958.

Toto, Patrick D., and Grandel, Eugene R. ACID MUCOPOHYSACCHARIDES IN THE ORAL EPITHELIUM. Periodontics, Vol. 4, No. 3, 1966.

Toto, Patrick D., and Ojha, Gokul. GENERATION CYCLE OF ORAL EPITHELIUM IN MICE. J. D. Res., Vol. 41, No. 2, 1962.

Toto, Patrick D., and Sicher, Harry. THE EPITHELIAL ATTACHMENT. Periodontics, 2:154, 1964.

Trott, J. R., and Gorenstein, S. L. MITOTIC RATES IN THE ORAL AND GINGIVAL EPITHELIUM OF THE RAT. Arch. Oral Biol., 8:425, 1963.

Young, F. G. GROWTH HORMONE AND EXPERIMENTAL DIABETES. J. J. Clin. Endocr., 11:531, 1964.

Young, F. G. THE GROWTH HORMONE AND DIABETES RECENT PROGRESS IN HORMONE RESEARCH. J. Clin. Endocr., 8:471, 1953.

Zander, Helmut A. A METHOD FOR STUDYING "THE EPITHELIAL ATTACHMENT." J. Dent. Res., 35:308, 1956.

Ziskind, D., and Blackberg, S. N. THE EFFECT OF CASTRATION AND HYPOPHYSECTOMY ON THE GINGIVAL AND ORAL MUCOUS MEMBRANES OF RHESUS MONKEYS. J. Dent. Res., 19:381, 1940.

TABLE A

Adrenalectomized Mice

The Average Distribution of Labeled Cells According to Cell Layer at Various Time Intervals Per Molar Interdental Papilla Section. Mean Average Determined from Six Sections in Each Time Period.

Time	Basal Cell Layer	Prickle Cell Layer	Surface Cell Layer	Average Total Number	*Average Total Number Normal	**Average Total Number Ovariectomy
½ hr	8.9	2.0	1.8	12.5	11	4.8
1 hr	5.7	2.3	1.0	9.0	10	4.4
2 hr	4.1	3.3	2.3	9.8	15	2.8
4 hr	4.6	6.0	2.8	13.5	19	6.2
6 hr	5.6	3.9	3.1	12.5	16	6.2
8 hr	0.1	0.0	0.3	0.5	17	5.2
10 hr	6.1	5.3	4.5	16.0	27	7.8
12 hr	7.1	5.0	2.6	14.8	7	5.8
14 hr	3.8	6.5	4.0	14.3	25	10.6
16 hr	5.6	10.1	3.8	18.0	—	10.1
18 hr	5.8	8.1	4.3	18.3	26	11.0
20 hr	7.0	21.3	5.5	33.6	17	10.7
22 hr	6.0	7.6	4.8	20.1	26	7.0
24 hr	8.0	6.6	6.6	21.2	16	6.7
28 hr	3.0	8.8	4.8	16.6	18 (26 hr)	6.5
32 hr	1.3	6.0	4.1	11.5	17	6.9
36 hr	3.4	6.8	3.6	13.8	9	6.5
40 hr	4.3	13.3	6.0	23.6	10 (42 hr)	7.8
44 hr	4.6	9.3	8.5	22.5	10	6.1
3 day	1.8	5.5	1.3	9.3	7	4.2
4 day	2.1	5.1	5.6	13.0	14	2.0
5 day	0.6	4.6	5.6	10.8	3	2.8

* Joglekar (1964)
** Bachnik (1966) - ½ papilla
*** 8 hr - poor slides, unreliable reading

TABLE 3

Adrenalectomized Mice

Average Distribution of Labeled Cells According to Zones at Various Time Intervals per Molar Interdental Papilla Section. Mean Averages Determined from Six Sections in Each Time Period.

Time	Zone I	Zone II	Zone III	Average Total I, II, III	Zones I, II, III Average *Total Normal	Zones I, II, III Average **Total Ovariectomy
½ hr	6.5	5.0	1.0	12.5	11	4.8
1 hr	4.3	4.0	0.7	9.0	10	4.4
2 hr	5.1	3.8	1.3	9.8	15	2.8
4 hr	4.6	4.5	4.3	13.5	19	6.2
6 hr	6.5	5.0	0.5	12.5	16	6.2
8 hr***	0	0.1	0.3	0.5	17	5.2
10 hr	7.5	8.0	2.1	16.0	27	7.8
12 hr	5.1	8.5	1.1	14.8	7	5.8
14 hr	3.8	9.5	1.0	14.3	25	10.6
16 hr	3.8	10.5	2.0	18.0	--	10.1
18 hr	6.1	10.1	2.0	18.3	26	11.0
20 hr	9.0	21.1	5.1	33.6	17	10.7
22 hr	8.3	10.5	1.3	20.1	26	7.0
24 hr	8.0	11.8	1.0	21.2	16	6.7
28 hr	4.1	10.6	2.0	16.6	18 (26 hr)	6.5
32 hr	4.1	6.3	1.0	11.5	17	6.9
36 hr	4.4	4.6	4.8	13.8	9	6.5
40 hr	5.0	13.5	5.1	23.6	10 (42 hr)	7.8
44 hr	7.3	8.5	6.6	22.5	10	6.1
3 days	1.5	6.8	1.0	9.3	7	4.2
4 days	4.6	1.8	7.3	13.0	14	2.0
5 days	0.8	4.6	5.2	10.8	3	2.8

* Joglekar (1964)
** Bachnik (1966) - ½ papilla
*** 8 hr - poor slides; unreliable reading

TABLE C

Hypophysectomized Mice

Average Distribution of Labeled Cells According to Cell Layer at Various Time Intervals per Molar Papilla Section. Mean Averages Determined from Six Sections in Each Time Period.

Time	Basal Cell Layer	Prickle Cell Layer	Surface Cell Layer	Average Total Number	*Normal Total No.	**Ovariectomy Total No.
½ hr	6.6	0.8	1.6	9.1	11.0	4.8
1 hr	6.1	1.5	1.1	9.0	10.0	4.4
2 hr	7.0	1.6	3.1	11.6	15.0	2.8
4 hr	4.6	4.0	2.3	11.0	19.0	6.2
6 hr	3.5	2.1	1.5	7.1	16.0	6.2
8 hr***	----	----	----	----	----	----
10 hr	9.3	4.6	4.0	18.0	27.0	7.8
12 hr	4.0	4.2	4.0	12.2	7.0	5.8****
14 hr	3.6	11.6	3.3	18.6	25.0	10.6
16 hr	9.0	5.5	7.0	21.3	----	10.1
18 hr	7.5	5.1	5.5	18.0	18.0	11.0
20 hr	9.0	7.4	3.2	19.4	17.0	10.7
22 hr	6.3	6.1	6.0	18.5	26.0	7.0
24 hr	8.0	9.0	6.5	23.5	16.0	6.7
28 hr	6.0	14.3	4.5	25.0	----	6.5
32 hr	4.6	6.4	8.6	19.6	17.0	6.9
36 hr	4.4	8.3	5.5	18.5	9.0	6.5
40 hr	3.0	5.3	5.3	13.5	10.0	7.8
44 hr	7.0	16.5	13.0	36.3	10.0	6.1
48 hr	6.0	10.8	5.5	22.0	9.0	6.4
3 days	3.0	8.6	7.6	19.5	7.0	4.2
4 days	2.3	7.0	4.5	14.0	14.0	2.0
5 days	2.0	4.5	5.6	13.0	3.0	2.8

* Joglekar (1964)

** Bachnik (1966) - ½ papilla

*** 8 hour - no sections

**** 12 hour - poor sections

TABLE D

Hypophysectomized Mice

Average Distribution of Labeled Cells According to Zones at Various Time Intervals per Molar Papilla Section. Mean Averages Determined from Six Sections in Each Time Period.

Time	Zone I	Zone II	Zone III	Total Cells Zones I, II, III	*Normal Average Total Number	**Ovariectomy Total Number
½ hr	8.0	1.3	0.0	9.0	11.0	4.8
1 hr	3.6	4.3	0.8	9.0	10.0	4.4
2 hr	7.0	4.3	0.3	11.6	15.0	2.8
4 hr	4.0	6.3	1.0	11.0	19.0	6.2
6 hr	2.5	4.3	0.3	7.0	16.0	6.2
8 hr	----	----	----	----	17.0	5.2
10 hr	8.0	7.6	2.0	18.0	27.0	7.8
12 hr	5.4	5.0	1.0	12.2	7.0	5.8
14 hr	3.6	12.0	3.0	18.6	25.0	10.6
16 hr	9.5	10.5	1.3	21.3	----	10.1
18 hr	6.0	9.0	2.5	18.0	26.0	11.0
20 hr	5.4	11.0	3.0	19.4	17.0	10.7
22 hr	5.6	9.0	4.0	18.5	26.0	7.0
24 hr	5.6	15.0	3.0	23.5	16.0	6.7
28 hr	5.3	15.3	4.1	25.0	18.0 (26 hr)	6.5
32 hr	5.6	9.8	4.0	19.6	17.0	6.9
36 hr	5.0	9.3	4.0	18.5	9.0	6.5
40 hr	4.0	6.6	3.0	13.5	10.0 (42 hr)	7.8
44 hr	6.8	19.3	10.0	36.3	10.0	6.1
48 hr	6.0	10.6	6.0	22.0	9.0	6.4
3 days	3.3	8.0	8.0	19.5	7.0	4.2
4 days	1.5	5.6	7.0	14.0	14.0	2.0
5 days	1.0	4.0	8.0	13.0	3.0	2.8

* Joglekar (1964)
** Bachnik (1966) - ½ papilla

TABLE E

Adrenalectomized Mice and Hypophysectomized Mice

Percentage of Labeled Cells in Epithelium of Interdental Papilla Sections at Various Time Intervals.

Time	Adrenalectomized	Hypophysectomized	**Ovariectomized	*Normal
½ hr	7.2%	4.8%	5.0%	6.0%
1 hr	5.2%	4.7%	4.6%	6.0%
2 hr	5.6%	6.3%	2.9%	9.0%
4 hr	7.8%	5.8%	6.5%	10.5%
6 hr	7.2%	3.7%	6.5%	9.0%
8 hr	0.3%	-----	5.4%	10.0%
10 hr	9.2%	9.5%	8.1%	15.0%
12 hr	8.5%	6.4%	6.0%	4.0%
14 hr	8.2%	9.7%	11.0%	14.5%
16 hr	10.4%	11.2%	10.5%	-----
18 hr	10.5%	9.4%	11.5%	15.0%
20 hr	19.4%	10.2%	11.1%	10.0%
22 hr	11.6%	9.7%	7.3%	15.0%
24 hr	12.2%	12.1%	7.0%	9.0%
26 hr	-----	-----	-----	10.4%
28 hr	9.3%	13.2%	6.8%	-----
32 hr	6.5%	10.2%	7.2%	10.0%
36 hr	7.9%	9.7%	6.8%	5.0%
40 hr	13.6%	7.1%	8.1%	-----
44 hr	13.0%	19.1%	6.4%	6.0%
48 hr	-----	11.6%	6.7%	5.0%
3 days	5.3%	10.3%	4.4%	4.0%
4 days	7.5%	7.4%	2.1%	8.0%
5 days	6.1%	6.7%	2.9%	1.7%

* Joglekar (1964)
** Bachnik (1966) - ½ papilla

TABLE F

Adrenalectomized Mice and Hypophysectomized Mice

Comparison of Average Total Number of Labeled Cells per Section at Various Time Intervals.

Time	Adrenalectomized	Hypophysectomized	**Ovariectomized	*Normal
½ hr	12.5	9.1	4.8	11.0
1 hr	9.0	9.0	4.4	10.0
2 hr	9.8	11.6	2.8	15.0
4 hr	13.5	11.0	6.2	19.0
6 hr	12.5	7.1	6.2	16.0
8 hr	0.5	----	5.2	17.0
10 hr	16.0	18.0	7.8	27.0
12 hr	14.8	12.2	5.8	7.0
14 hr	14.3	18.6	10.6	25.0
16 hr	18.0	21.3	10.1	----
18 hr	18.3	18.0	11.0	18.0
20 hr	33.6	19.4	10.7	17.0
22 hr	20.1	18.5	7.0	26.0
24 hr	21.2	23.5	6.7	16.0
26 hr	----	----	----	18.0
28 hr	16.6	25.0	6.5	----
32 hr	11.5	19.6	6.9	17.0
36 hr	13.8	18.5	6.5	9.0
40 hr	23.6	13.5	7.8	10.0
44 hr	22.5	36.3	6.1	10.0
48 hr	----	22.0	6.4	9.0
3 days	9.3	19.5	4.2	7.0
4 days	13.0	14.0	2.0	14.0
5 days	10.8	13.0	2.8	3.0

* Joglekar (1964)
** Bachnik (1966) - ½ papilla

TABLE G

Comparison of Mean, Standard Deviation, and T-Test for Adrenalectomized and Hypophysectomized Mice.

Time	Adrenalectomized Mice Mean	Adrenalectomized Mice Standard Deviation	Hypophysectomized Mice Mean	Hypophysectomized Mice Standard Deviation	T-Test	Probability
½ hr	12.5	3.2	9.1	2.9	4.8	p > .001
1 hr	9.0	3.68	9.0	4.9	0.0	p > .001
2 hr	9.8	3.55	11.6	5.3	2.2	p > .001
4 hr	13.5	3.8	11.0	4.6	3.0	p > .001
6 hr	12.5	6.44	7.1	2.85	7.0	p < .001
10 hr	16.0	9.34	18.0	5.73	1.0	p > .001
12 hr	14.8	6.11	12.2	4.55	2.8	p > .001
14 hr	14.3	8.5	18.6	7.06	4.0	p > .001
16 hr	18.0	7.55	21.3	8.47	3.0	p > .001
18 hr	18.3	1.9	18.0	4.22	0.4	p > .001
20 hr	33.6	13.38	19.4	9.9	10.0	p < .001
22 hr	20.1	2.78	18.5	5.87	2.0	p > .001
24 hr	21.2	2.9	23.5	7.44	2.5	p > .001
28 hr	16.6	2.18	25.0	7.28	9.8	p > .001
32 hr	11.5	2.84	19.6	3.94	11.1	p < .001
36 hr	13.8	4.6	18.5	4.03	18.5	p < .001
40 hr	23.6	5.03	13.5	7.5	10.0	p < .001
44 hr	22.5	10.17	36.3	8.89	10.6	p < .001
3 days	9.3	2.25	19.5	8.75	4.08	p > .001
4 days	13.0	1.88	14.0	4.12	1.4	p > .001
5 days	10.8	1.9	13.0	5.05	2.7	p > .001

TABLE H

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. Illustration of the three zones of epithelium in interdental papilla of the mouse selected for counting convenience.

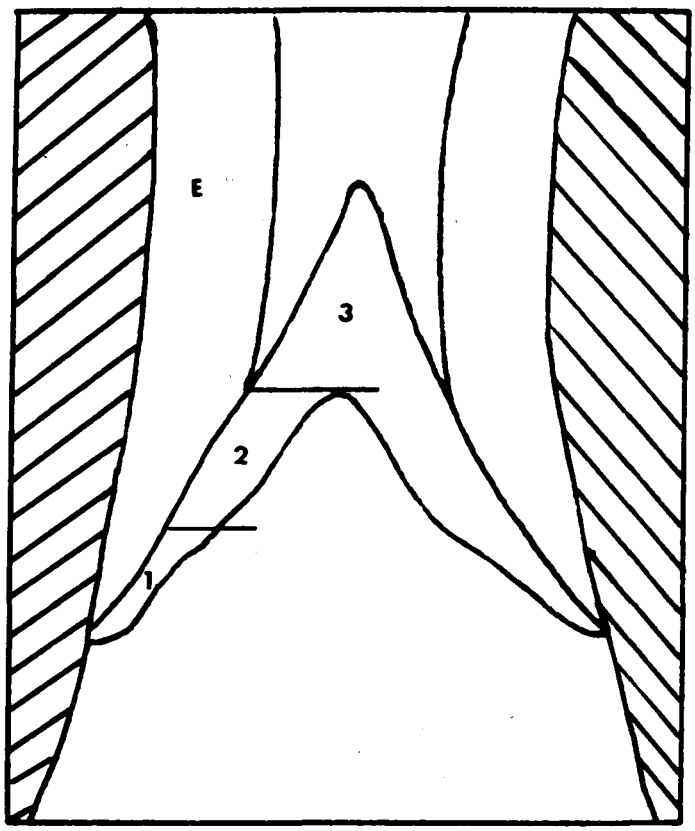

E
3
2
1

Figure 2. A graph representing the logarithm of the percent values of labeled cells in the oral epithelium of interdental papilla of adrenalectomized and of hypophysectomized mice. The perpendicular lines represent the time elapsed for the doubling of each initial labeled cell population.

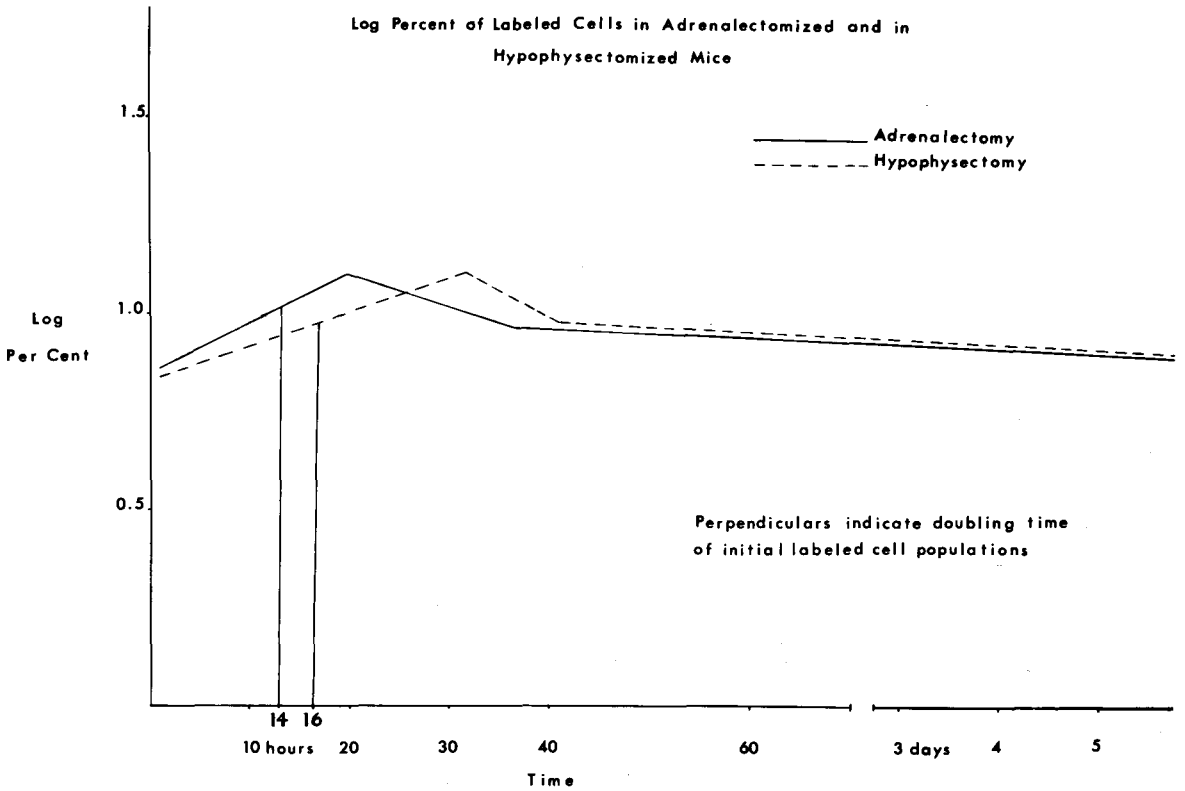

Log Percent of Labeled Cells in Adrenalectomized and in Hypophysectomized Mice
Adrenalectomy
Hypophysectomy
Log
Per Cent
1.5
1.0
0.5
Perpendiculars indicate doubling time of initial labeled cell populations
14
16
10 hours
20
30
40
60
3 days
4
5
Time

Figure 3. One hour specimen adrenalectomized mouse interdental papilla autoradiogram stained with nuclear fast red and indigo carmine. Original magnification X25.

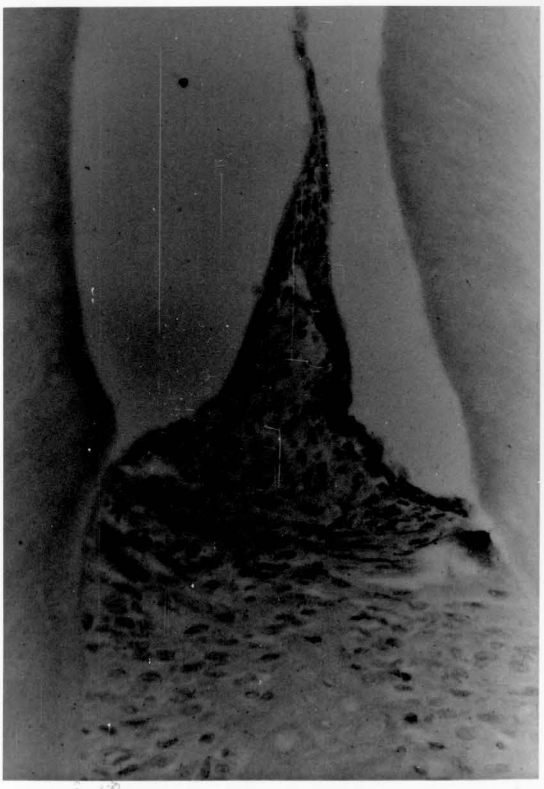


Figure 4. Fourteen hour specimen adrenlectomized mouse interdental papilla autoradiogram stained with nuclear fast red and indigo carmine. Original magnification X25.

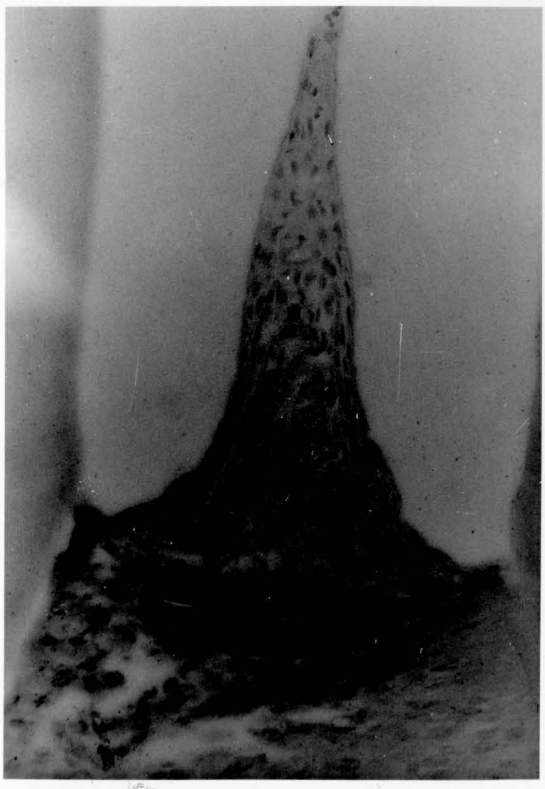


Figure 5. One hour specimen hypophysectomized mouse interdental papilla autoradiogram stained with nuclear fast red and indigo carmine. Original magnification X25.

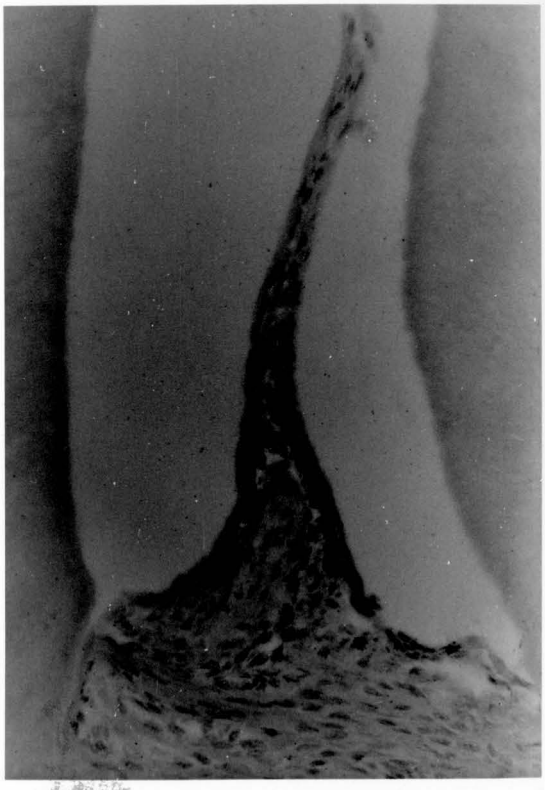
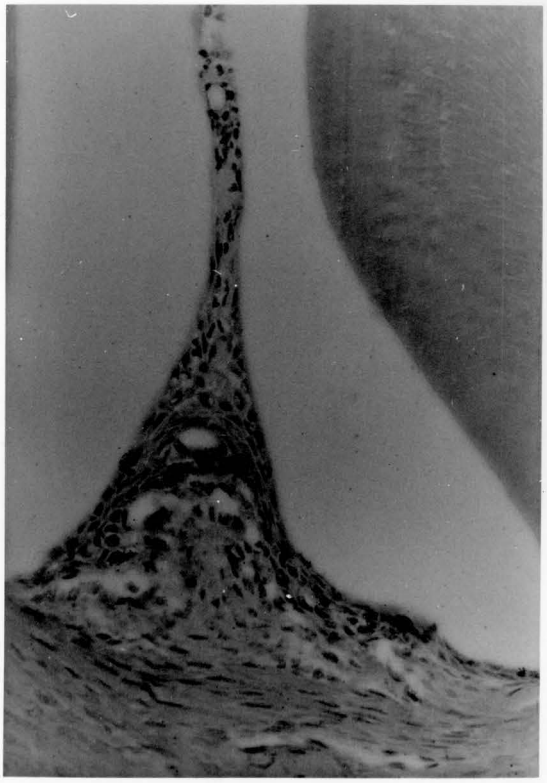


Figure 6. Sixteen hour specimen hypophysectomized mouse interdental papilla autoradiogram stained with nuclear fast red and indigo carmine. Original magnification X25.



APPROVAL SHEET

The thesis submitted by Dr. Elliott Howard Dickler 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.

May 10, 1968
Date

Patrick D. Tate
Signature of Advisor