DNA Metabolism of the Basal Cell Layer of the Epithelium of the Tongue in the Mouse

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DNA METABOLISM OF THE BASAL CELL LAYER
OF THE EPITHELIUM OF THE TONGUE
IN THE MOUSE

by
William A. Schoenheider, Jr.

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
1960
TO MY WIFE

DEON
LIFE

William Albert Schoenheider, Jr. was born in Chicago, Illinois on October 27, 1931. His primary education was received at Marquette Grammar School (Chicago), while his secondary education was obtained at Lindblom High School (Chicago).

From 1949 to 1952 he attended Purdue University, West Lafayette, Indiana, in the pre-dental curriculum. In September, 1952, he started his dental education at Loyola Dental School, Chicago College of Dental Surgery, and received his D.D.S. degree in June of 1956.

Upon graduation, two years were spent with the United States Navy serving as a Lieutenant in the Dental Corps.

In July, 1953, he began a two year graduate program at Loyola University leading to a Master of Science Degree in Oral Anatomy.
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CHAPTER I

INTRODUCTION

This study was undertaken to examine the synthesis of deoxyribonucleic acid (DNA) of the basal cell layer of the epithelium of the tongue in the mouse by the autoradiograph technique. The cells were labeled with radioactive thymidine.

The study covered a period of fifty hours, commencing with the injection of the isotope.
CHAPTER II

REVIEW OF THE LITERATURE

A. Histology of the Tongue in the Mouse.

Snell (1941), as editor for the Jackson Laboratory Manual on the Laboratory Mouse, gave a gross microscopic description of the tongue.

Kutuzov and Sicher (1953), macroscopically and microscopically described the dorsum of the mouse tongue.

Medak (Ph.D. dissertation 1959) gives a detailed account of the oral epithelium of the mouse, except that of the tongue.

**Microscopic Description**

Dense connective tissue forms the thin lamina propria that forms the core of the fungiform and vallate papillae. The lamina propria is tightly connected with the musculature of the tongue.

**Mucosa of the Dorsum of the Tongue**

The dorsum of the tongue can be divided macroscopically into three areas by the type of papillae present:

1.) Anterior zone of simple conical papillae
2.) Intermolar eminence with giant papillae
3.) Posterior zone of filiform papillae

The stratified squamous epithelium of these three areas, as well as all
other oral epithelium (cheek, palate), is keratinized.

Anterior Area

The dorsum and the lateral borders of this zone are covered with simple conical papillae separated by narrow interpapillary fields. The spines of the papillae are curved posteriorly. Fungiform papillae are distributed over the entire area; in the region of the median sulcus they are arranged in a sagittal row.

Intermolar Eminence

This area is characterized by the giant papillae.

Kutuzov and Sicher state:

The dermal core of the papillae is a truncated cone strongly compressed anteroposteriorly and crescent shaped in a horizontal section. At its crest the compressed truncated cone carries two slender secondary dermal papillae. In the epithelium of the giant papilla, the central prickle cells on the concave surface of the dermal core form a common epithelial sheath for both of the secondary papillae with a resultant single keratinized buttress.

Special staining showed the area of the intermolar eminence to be the same as the anterior area, but a higher degree of hornification was present.

Posterior Area

This region is characterized by regularly arranged filiform papillae. Each papilla terminates in two or three spines; the papillae are separated by relatively wide interpapillary fields.

In horizontal sections the dermal core of the filiform papillae resembles that of the giant papillae, but is much smaller in size, and the secondary papillae are taller and more slender than those of the giant papillae.
Kutuzov and Sichel also state:

The epithelium of the filiform papilla undergoes the same process of differentiation as that of the giant papilla. However, the central prickle cells of the posterior surface of the papilla, which differentiates independently for each of the secondary papillae, are not as flat as the analogous cells of the giant papilla of the mouse. Their nuclei are not pycnotic and the cytoplasm of the cells is eosinophilic. The resultant spine is long and thin with a strong posterior curvature.

Mucosa of the Ventral Surface of the Tongue

No literature of the ventral surface of the tongue of the mouse was available for review.

The mucosa of the ventral surface of the tongue is smooth and relatively thin. The epithelium is keratinized.

B. Autoradiography

Autoradiography is a method for detecting radioisotopes, based on their ability to affect the silver bromide crystals of photographic emulsions. Such crystals act as micro-detectors of radiation and are, therefore, useful in visualizing locations of radioelements. Practically, an autoradiograph is obtained by placing a tissue section in contact with photographic emulsion, allowing sufficient time for exposure, and then developing as in ordinary photography. The resultant autoradiogram consists of accumulations of black silver granules overlying those areas in the tissue section which contain the radioactive material, and, by comparing the photographic image with the tissue section, such areas of activity can be fairly accurately assigned to definite microscopical structures.

As with other methods involving the use of radioisotopes, it is assumed that the chemical behavior of a labeled substance is identical with that of
its stable counterpart. This will also be true of the biological behavior provided (1) the amount of radioactivity administered be small enough not to have a significant radiochemical effect, and (2) the amount or weight of material injected be sufficiently small not to produce a significant increase in the amount of the substance in the circulation. The labeled substance will then truly act as a "tracer" of normal metabolism.

Tauxe (1959) describes in detail the etymology of autoradiography. Many terms have been applied since the first autoradiographic technique in 1896, among them autoradiography, radioautography, autophotography, and histoautoradiography. The results of the technique have, accordingly, been called autoradiography, autoradiogram, radioautogram, autophotograph, histoautoradiograph, and curieograph.

For uniformity throughout this paper, the term autoradiography will be used to describe the technique and autoradiogram to designate the result of the technique.

Becquerel (1896) after Roentgen's discovery of x-rays wrapped a photographic plate in black paper, placed a crystal on top, and exposed the combination to sunlight. After developing the plate a faint outline of the crystal was observed, the first autoradiogram. Although he discovered the facts of radioactivity, he failed to understand them.

London (1904) was the first to use autoradiography for locating radioactivity in minerals. He exposed a frog to radium emanation, and after its death, placed it on a photographic plate, resulting in an autoradiogram.

Bouchard et al. (1904) were using the microscopic autoradiographic
method to study the distribution of inhaled radium emanation in tissue sections of a guinea pig.

Kotzareff (1922) showed a concentration of radium in the cortex of the kidney of the guinea pig. He was also the first to inject radium into a tumor, and produce an autoradiogram.

Lacassagne (1924) and his co-workers studied the distribution of polonium and other elements in histological specimens, while Lamholt (1930) apposed a tissue section to a plate and later removed them for staining. Utilizing Lamholt's technique, the same section making the autoradiogram could be studied histologically.

Leblond (1930), working independently of Lamholt, started mounting sections on glass plate for apposition with the photographic plate to study the distribution of polonium.

Graven et al. (1938) produced the first autoradiogram of induced radioactivity when they showed that neutron irradiated iridium blackened a photographic plate.

Bulliard and his associates (1938) were the first to produce a biological autoradiogram with induced radioactivity when they demonstrated phosphorus -32 in the adrenal gland.

Belanger and Leblond (1946) developed a technique for locating radioactive elements in tissue by painting histological sections with liquid emulsion. Pelc (1947) described a technique utilizing stripping film emulsion.

Nadler and Leblond (1951) concluded experimentally that the most
satisfactory autoradiogram is obtained when the thickness of the emulsion and tissue, plus the space between the two, is reduced to a minimum.

Leblond and associates (1959) investigated tritiated thymidine as a tool for studying the renewal of cell populations. The mouse tongue was utilized, but only results for eight hours and six months were reported.

The latest work, done by Cronkite, Bond, Fliedner and Pubini (1959) utilized autoradiography in the study of DNA synthesis, and cell turnover in hematopoietic tissues.

C. Structure of Deoxyribonucleic acid (DNA)

When a cell is about to divide, its DNA content doubles. As a result, a normal complement of DNA will be passed on to each daughter cell. If a radioactive DNA precursor is administered, then radioactivity appears in those nuclei which undergo DNA synthesis. These cells may be detected by autoradiography. Furthermore, if animals are sacrificed at various time intervals after the administration of the DNA precursor, it is possible not only to detect the dividing cell soon after injection, but also to trace some of the newly formed cells.

In 1871 Miescher discovered nucleic acids as constituents of animal cell nuclei, and their presence in plants was demonstrated some eighteen years later by Altman (1899).

The views on the general structure of the nucleic acids, which are found in most textbooks, represent a considerable over-simplification. But many of the essential points -- particularly with regard to the nature of the sub-units, the nucleotides, and their constituents are recognized.
Two types of nucleic acids were, and are still, recognized: that in which the carbohydrate is pentose and that in which it is deoxypentose. The acid prepared from yeast (ribonucleic - RNA) was regarded as characteristic of the first type, and the latter type deoxyribonucleic acid (DNA) was the acid obtained from the thymus gland. Our discussion will be concerned only with the second acid, DNA.

DNA has been known for a long time as an essential component of the chromatin of the cell nuclei. Cytochemists, using the specific Feulgen reaction, and biochemists, analyzing isolated nuclei and cytoplasmic elements, have shown that DNA is strictly confined to the nucleus and that all nuclei contain DNA.

Complete hydrolysis of DNA yield pyrimidine and purine bases, a sugar component and phosphoric acid. Partial hydrolysis yields compounds known as nucleosides and nucleotides. Each of these component parts will be discussed in turn.

**Pyrimidine bases**

The pyrimidine bases are derivatives of the parent compound - pyrimidine and the two derivatives found in DNA are cytosine and thymine.

**Purine bases**

Both types of nucleic acids contain the same purine base, adenine and guanine; they are derivations of the parent compound, purine.

**Nucleosides**

A purine or a pyrimidine base may be condensed with a pentose or a deoxypentose sugar to form a nucleoside, namely deoxyadenosine, deoxya-
denosine, deoxyguanosine, and thymidine.

**Nucleotides**

The nucleotides are phosphoric esters of the nucleosides.

**D. Feulgen Technique**

The Feulgen technique was introduced by Feulgen and Rossenbeck in 1924 for the demonstration of thymonucleic acid in tissues.

Stowell (1946) states that with proper precautions the Feulgen technique for thymonucleic acid is one of the most specific histochemical reactions. Wyckoff and Eberling (1932) observed that ultraviolet studies agree with the Feulgen reaction in the localization of the thymonucleic acid within the cells. Ultraviolet photography may be used for quantitative estimations of minute amounts of nucleic acids in tissue according to Caspersson (1936), and properly performed Feulgen reaction is practically specific for DNA, as has been demonstrated by many workers. DNA removal through the use of purified pancreatic deoxyribonuclease will render a tissue Feulgen-negative, proving the validity of the Feulgen technique.

Feulgen reaction brings a mild hydrolysis of its nucleoproteins. Hydrolysis releases some of the aldehyde groups of the sugar present in the nucleic acid molecule, and one can then test for aldehyde by means of fuchsin-sulfurous acid. This is an excellent histologic test for the nucleoprotein.

The Feulgen method is an adaptation of the Schiff reaction for aldehydes to histologic technique. The reagent used is a leuco-fuchsin, made as follows:
Dissolve one gram basic fuchsin in 200 cc. boiling distilled water; cool to 50\textdegree{} C.; filter and add 10 cc. N hydrochloric acid and two grams potassium metabisulfite ($K_2S_2O_5$); allow to bleach for 24 hours; then add 0.5 grams neutral activated charcoal; shake for one minute and filter rapidly through coarse filter paper.

The solution should be light yellow. If not, take a different sample of fuchsin and try again. The same lot of fuchsin generally gives consistent results, and if this technique is to be employed frequently, a sample found satisfactory for this method should be set aside for it.

The staining technic is as follows:

1. Bring paraffin sections through xylene and alcohol to water.
2. Rinse one minute in N hydrochloric acid at 60\textdegree{} C. for 4 minutes or at 50\textdegree{} C. for 20 minutes.
3. Rinse one minute in cold N hydrochloric acid.
4. Immerse 2 hours in leuco-fuchsin solution.
5. Drain and give 3 successive 10 minute treatments in closed Coplin jars in acid sulfite solution. This compound is composed of 6 cc. 10\% potassium metabisulfite, 6 cc. N hydrochloric acid, and 120 cc. distilled water. Place 44 cc. of this in each jar. Wash 5 minutes in tap water.
6. Rinse in distilled water. Counterstain in 0.5\% alcoholic fast green.
7. Dehydrate in alcohol.
8. Clear in xylene and mount.
E. Tritiated Thymidine

Individual nucleosides derive their names from the purine and pyrimidine bases they contain: the purine nucleosides adding the suffix "osine", the pyrimidine nucleosides the suffix "idine". In DNA hydrolyzates, deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine are found.

Cronkite, Bond and Fliedner and Rubini (1959) state that:

"Although thymidine is apparently not a normal precursor of DNA, it can enter the synthetic chain and label DNA at the time of DNA doubling prior to mitosis. Thus, if the label is sufficiently intense and permanent, one can, by autoradiography, follow cells from the time of DNA synthesis to ultimate death."

Hughes, Bond, Beecher, Cronkite, Painter, Quastler, and Sherman (1958) state that:

In order to distinguish all renewal from the renewal of cellular constituents, the label must be incorporated into a fixed component of the cell which is not lost during the cell's lifetime; and present knowledge suggests that a label incorporated into DNA should be most useful for this purpose. The specific advantage of labeled tritium lies in the very high resolution which can be obtained because of the very weak energy and consequently short range of its B radiation. The maximum range in tissue of a B ray from tritium is only 6 u and half of the B's will travel less than 1 u. Consequently the activated silver grains of an autoradiogram should largely lie within 1 u of their source. Tritiated thymidine is apparently rapidly absorbed by all cells when injected into mice. The concentration of tritium hydroxide reaches a maximum within 1 hour following intraperitoneal injection. Therefore, the precursor pool of DNA synthesis must simultaneously become exhausted suggesting that all labeling of nuclei occurs during the first hour after injection. This also means a similar rate of exhaustion of thymidine from DNA synthesizing cells as from tissue as a whole.

Tritiated thymidine of a specific activity of approximately 300 curies
mole was obtained from Schwarz laboratories. It was labeled on the pyrimidine portion of the molecule (presumably by the exchange with that hydrogen bound to carbon in the pyrimidine ring) and it was free of labeled impurities as judged by recrystallization with carrier thymidine. It is diluted with isotonic sodium chloride to a convenient concentration for injection. The very short range of B-rays from tritium limits the average number of silver grains activated per disintegration to approximately 1 with tritium dissolved in the emulsion. The time required for the appearance of labeled mitotic figures following injection measures the interval between completion of DNA synthesis and the beginning of mitosis.

Lajtha, Phil, and Oliver (1959) confirm thymidine as a specific component of DNA, important because it labels only DNA. The range of beta particles has a maximum of 8 u and an average of 1.5 u. Tritium has a half life of 12.26 years and a disintegration rate of $0.016 \text{ day}^{-1}$.

Leblond, Messler, and Kapriwa (1959) state that it is known that mitosis is preceded by a synthesis of DNA. If a radioactive DNA precursor is administered at that time, the nucleus becomes radioactive and may be recognized by autoradiography, even before mitosis takes place. Such nuclei retain the label during the actual mitosis and pass it on to the daughter cells. A new DNA precursor, thymidine labeled with tritium, makes it possible to overcome the difficulties encountered with the other DNA precursors. The danger of radiation damage seems to be reduced with tritium, and it is possible to use doses which allow cells to be traced over periods of months in the body without apparent sign of damage to these or other cells. Further-
more, the sections need not be subjected to chemical extractions since DNA is the only labeled substance present in significant amounts in the sections following thymidine H³ administration, and DNAse treatment eliminates all autoradiographic reactions.

Finally, due to the low beta-ray energy of tritium, the photographic grains produced by tritium containing structures are found within 2 or 3 u, most of them within 1 u from the source as measured within NTB-3 emulsion. It may be concluded thymidine H³ is an adequate tool for the autoradiographic detection of newly formed DNA, and, therefore, may be used to locate the sites of cell formation.
CHAPTER III

MATERIALS AND METHODS

Materials

Twelve 200 day old mice, a strain from C57 Black and Albino, weighing an average of 37 grams, and being fed a diet of Wayne Lab Blox for mouse or rat, were injected intraperitoneally with 50 uc. of tritiated thymidine. They were sacrificed at intervals of 15, 30, 45, and 60 minutes, 2, 4, 12, 24, 44, 46, 48, and 50 hours following the injection.

The tongues were dissected out, cut mid sagittally, fixed in formalin, dehydrated, and embedded in paraffin.

Serial longitudinal sections, cut at three microns, were prepared. Each tenth section was selected and stained by the Feulgen method. Additional sections from each time period were stained by H and E.

The sections stained by Feulgen were kept in distilled water immediately following the staining procedure. This permitted the selection of the best slides to be used for the autoradiographs. Five slides from each of the sacrifice periods were selected for autoradiography.

Methods

Autoradiograph Strip Film Technique

The technique used for autoradiography was modified from that described by Fitzgerald (1953).
1. Under dark room conditions, a Wratten #1, Red Safelite 10 watt bulb is used. A humidity of 70% and below is recommended.

2. Kodak, AR-10 fine grain emulsion is placed on a cutting jig, and cut into twelve squares with a scalpel.

3. Using a scalpel, a corner of the square is picked up, stripped from the glass plate, and floated, emulsion side down, in a glass dish containing distilled water 4 inches deep.

4. The histologic slide (section side up) is inserted into water. The slide is brought up under the floating emulsion and engaged.

5. The slide is then air dried for 10 minutes.

6. Ten slides are placed in a black, light proof exposure box, section sides up. Lithium chloride is placed in the box for maintaining a low humidity, and black masking tape is used to seal the box.

7. The box is exposed for 30 days at low humidity and temperature. During the exposure time the box must be maintained in a position which keeps the sections upright.

Developing

1. The slides are placed in a staining rack and developed for 5 minutes at 60°F. (130°C. in Kodak D196 developer.)

2. The slide is rinsed in distilled water for 30 seconds.

3. Place in acid fixer for 10 minutes.

4. Wash in running tap water for 30 minutes.

5. Cover the slides and staining dish with Kleenex (to prevent dust from settling on slides) and allow to dry in a stream of air.
6. When the slides are dry, they are dipped in water for 30 seconds and the excess emulsion is trimmed from the slide.

7. The slides are then dipped in 95% alcohol, xylene I, and xylene II.

8. Cover slips are finally mounted with Canada Balsam.

The region anterior to the glossopiglottic fold was utilized for recording the findings on the dorsal surface; while the area on the anterior region, where the simple conical papillae end, was utilized for the ventral surface.

1. Percentile of labeled nuclei: Under high dry magnification two separate 100 basal cell fields were counted. For each time interval three to five slides were utilized. The number of labeled nuclei observed in each 100 cells was then recorded, and from this, the mean and standard deviation were determined. The ventral and dorsal surfaces were recorded separately.

2. Cell population: Utilizing an H and E stain, an area of 100 basal cells in length was determined. All epithelial cells within the 100 basal cell length were counted. An average of six histologic slides was calculated. Both ventral and dorsal surfaces were recorded.

3. Mitotic index (M.I.): All mitotic figures in the entire epithelial layer were counted. One slide from each time interval was utilized. The total number of basal cells were then counted. By transposing the cell population per 100 basal cells, the total number of epithelial cells was calculated.

The mitotic index was determined by using the mitotic figure count and the total epithelial cell count. An average mitotic index was calculated from all the time intervals.
4. Grain count: With oil immersion microscopy, 25 labeled nuclei for each time interval (12, 24, 48 hours) were observed. The number of black silver grains over each nuclei was recorded and an average for each time interval was calculated.
CHAPTER IV

FINDINGS

At the fifteen minute time interval radioactive nuclei were observed in the basal cell layer of the epithelium. The nuclei appeared in groups of from three to seven in a random distribution (Figure 3).

The synthesis of the injected tritiated thymidine reached a maximum of 7.75% of the basal cells on the dorsal surface at one hour. On the ventral surface, 6.37% of the basal cells had synthesized the thymidine within one hour (Table I).

Within eleven hours after synthesis the number of labeled cells on the dorsal surface had slightly more than doubled from 7.75% to 17.10%. From 17.10%, the number of labeled nuclei decreased to less than 1% at fifty hours.

On the ventral surface, the number of labeled nuclei increased from 6.37% to 14.50% in eleven hours. By fifty hours the maximum percentage of 14.50 decreased to less than 1%.

Between the four and twelve hour intervals there was a definite decrease in the number of grains over the labeled nuclei (Figures 5 and 6).

The labeled nuclei were first observed in the prickle cell layer at the twelve hour period (Figure 4). At fifty hours, only rarely was a labeled nuclei observed in the basal layer (Figure 7).
The cell population of an area 100 basal cells in length was calculated.

An average of 320 epithelial cells for the dorsal and 244 epithelial cells for the ventral surface was determined (Table II).

The mitotic index (M.I.) average for all the time periods was 1.50 and 1.77 for the dorsal and ventral surfaces respectively (Table III).

The grain count at the time intervals of 12, 24, and 48 hours varied from 38 to 44 on the dorsal surface, and 31 to 37 on the ventral surface of the tongue (Tables IV, V, VI).
CHAPTER V

DISCUSSION

The observation of random grouping of the labeled nuclei at the early time intervals indicates that the formation of new epithelial cells takes place in a random distribution. This is indicative of an asynchronous pattern of DNA metabolism. If it were possible to follow the cells upward and determine if a basal layer grouping of three nuclei resulted in a group of six nuclei in the superficial layers, this concept could be proved. Originally this project was to follow the newly formed basal epithelial cells upward until they were exfoliated, but due to keratinization of the epithelium studied, this was impossible. It is a basic histologic concept that during keratinization the nuclei are lost; the radioactive isotope, therefore, would be lost, and the resulting autoradiogram would be blank.

It is believed by Cronkite and Lesher that the effective availability time of tritiated thymidine is about sixty minutes during which time it is either degraded or incorporated into DNA. At forty-five and sixty minutes the percent of labeled cells on the dorsal surface was fairly constant (7.75%). It may be concluded that at one hour approximately 8% of the basal cells have synthesized DNA. In the ventral surface approximately 6.5% of the basal cells have synthesized DNA.

Between sixty minutes and twelve hours there was an increase of slightly
over one hundred percent in the number of labeled cells. During the four and twelve hour interval there was a decrease in the number of grains over each labeled nuclei. The decrease in the grains is caused by the diluting of the isotope by mitosis. Prior to mitosis, a labeled nuclei, for example, contains one hundred grains; after mitosis each daughter cell will contain fifty grains. The increase of labeled cells, therefore, must be the consequence of mitosis.

A grain count between one and up to twelve hours was impossible because the silver grains appeared as one homogenous mass.

Leblond did a limited amount of work relative to DNA metabolism of the mouse tongue. His results for the malpighian cells of the ventral surface of the tongue was 1.6% labeled cells after eight hours. The amount of tritiated thymidine injected was not disclosed. The weight of his animals was only 13 to 14 grams compared to an average of 37 grams in this experiment. Since this study dealt only with the basal layer, it is difficult to correlate his results with those discussed here.

At twelve hours the labeled cells made their first appearance in the superficial layers. This is assumed to be due to the mitotic activity of non-labeled cells which forced the labeled cells upward.

Fifty hours after injection, the decrease in the number of labeled cells in the basal layer was due to one of two things:

1. Mitotic activity, by dilution, eliminating the isotope after the first mitotic wave.

2. Moving upward of the cells into the superficial layers.
A grain count of 12-24 hours varied only slightly from 33 to 64 grains nuclei in the dorsal and 31 to 37 grains nuclei in the ventral, so that the diluting factor may be ruled out. The decrease may then be due to the moving upward of the labeled nuclei.

It then may be stated that 8% of the basal cells synthesize DNA within one hour after injection. Eleven hours after synthesis, the percentage of labeled cells increased slightly more than one hundred percent. The labeled cells go through an interphase, and then decrease to less than one percent forty-nine hours after synthesis. A similar observation was noted on the ventral surface, only the number of basal cells synthesizing DNA one hour after injection was 6.5%.

The epithelium on the ventral surface is much thinner than the dorsal surface. The thicker dorsal surface, and the variability of the papillary projections would account for the increased number of the epithelial cell population between the dorsal and ventral surfaces.

One facet of the study which presented a problem was the difficulty in observing a labeled mitotic figure. Due to the large number of grains over the nuclei, the location of a labeled mitotic figure in the early time intervals was all but impossible. A further study is recommended, with a decreased exposure time, in order that such a figure may be readily observed.

Medak, in his thesis, determined the mitotic index (M.I.) of the palate and buccal mucosa of the mouse, but no investigations were performed relative to the tongue. The M.I. of the structures he studied was between 1.65 and 2.43. In this study the average M.I. of the tongue was 1.50 for the dorsal
and 1.77 for the ventral, which would fall well within Medak's M.I. range.

If it would have been possible to determine the percentage of labeled mitotic figures between one and twelve hours, a steady rise of labeled mitotic figures would appear inversely proportional to the M.I. That is, as the number of labeled mitotic figures increased in each time interval, the M.I. should decrease proportionately. According to Table III there was a slight decrease in the M.I. between one and twelve hours, but not significantly enough to correlate with an anticipated increase of possible labeled mitotic figures. Of all the phases of this study the M.I. appears to be the weakest point.

The literature offers much about the radiation effect of the injected tritium, but little has been written concerning the problem. Experimentation with doses up to 2.0 uc gm. of tritiated thymidine produced none of the classic effects of radiation. It is possible that tritium may not be able to produce gross chromosomal aberrations as seen after external irradiation. On the other hand Cronkite stated: "Even though we have seen no obvious cytologic effects of radiation in mammals, we feel strongly that the material should be administered only to human beings with short life expectancy and with no likelihood of procreating."
CHAPTER VI

SUMMARY

Twelve two hundred day old mice, a strain from C57 Black and Albino were injected with 50 uc of radioactive thymidine. They were sacrificed at twelve intervals between fifteen minutes and fifty hours.

The tongue was sectioned mid-sagittally, cut longitudinally at three microns and the sections stained by the Feulgen method for DNA.

The nuclei which had synthesized the radioactive thymidine gave off radiation.

The mounted stained sections were used to expose small squares of Kodak stripping emulsion for thirty days. The slides were developed and covered, using Canada Balsam.

The number of labeled nuclei located in the basal layer were counted for each time interval.

At fifteen minutes, labeled nuclei were observed in the basal epithelium layer of the dorsal surface in a random distribution. By one hour, 7.75% of the basal cells had synthesized DNA. Through mitosis the synthesized cells had more than doubled in number within eleven hours. At fifty hours the number of labeled cells had decreased to less than 1%. The same observation held true for the ventral surface, only in a smaller percentage.

At twelve hours labeled nuclei were observed to have moved upward into
the superficial layers.

The cell population of the dorsal and ventral surfaces was 320 epithelial cells and 244 epithelial cells respectively. An area of 100 basal cells in length was utilized.

The mitotic index averaged 1.50 for the dorsal, and 1.77 for the ventral surface.

Between four and twelve hours there was a definite decrease in the number of silver granules over the labeled nuclei.

A count of the number of silver granules over each nuclei was made for the time periods 12, 24, and 48 hours. The count fluctuated only slightly in the 36 hours.

A count of the silver granules at the 4 hour period was impossible due to the heavy concentration of grains.
CHAPTER VII

CONCLUSIONS

1. Eight percent of the basal cells on the dorsum of the tongue synthesize DNA within 1 hour, while on the ventral surface 6.5% of the basal cells synthesize DNA within 1 hour.

2. The basal cell population which has undergone DNA synthesis will double in number in 11 hours.

3. The cells which are synthesizing DNA appear in a random distribution of from 3 to 7 cells per group. This indicates an asynchronous pattern of DNA metabolism.

4. The basal cells which synthesize DNA disappear from the basal layer in slightly more than 50 hours. The decreased number of labeled cells in the basal layer at 50 hours can be attributed to replacement by non-labeled cells produced by mitosis.

5. The cell density of the dorsal surface is higher than the ventral surface of the tongue.

6. The mitotic index of the dorsal surface is less than the mitotic index of the ventral surface. This mitotic activity is characteristic of the range of variability of oral epithelium.
BIBLIOGRAPHY


Figure 1. Normal epithelium on the dorsal surface of the tongue. (H & E stain X400)
APPENDIX

A. Photomicrographs

PLATE I

Figure 1
Figure 2. Normal epithelium on the ventral surface of the tongue.
(H & E stain X400)
PLATE III

Figure 3. Random distribution of labeled nuclei. Fifteen minute interval (Feulgen stain X100)
PLATE IV

Figure 4. Labeled nuclei in lower prickle cell layer.
Twelve hour interval
(Feulgen stain X400)
Figure 5. Heavy concentrations of grains. Four hour interval (Feulgen stain X400)
PLATE VI

Figure 6. Light concentration of grains
Twelve hour interval
(Feulgen stain X400)
PLATE VII

Figure 7. Labeled nuclei in lower prickle cell layer, with absence of labeled nuclei in basal cell layer. Fifty hour interval (Feulgen stain X400)
Figure 7
PLATE VIII

Figure 8. Mitotic figure
(Feulgen stain X400)
Figure 9. Labeled nucleus
Four hour interval
(Feulgen stain)
PLATE X

Distribution of labeled nuclei from 15 minutes to 50 hours.
B. Graphs

PLATE X

![Graph showing percent of labeled cells over time.](image)

**GRAPH 1**

- **Percent of Labeled Cells**
- **Time in Hours**
- **Dorsal**
- **Ventral**

Figure 10
PLATE XI

Distribution of labeled nuclei from 15 minutes to 4 hours.
Figure 11

Graph 2

Time in Hours

Percent of Labeled Cells

Dorsal: solid line
Ventral: dashed line
TABLE I

PERCENTILE OF LABELED NUCLEI IN BASAL CELL LAYER

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<th>Ventral</th>
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<td>1.12</td>
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Mean 6.91
Standard Deviation 5.14

TABLE II

CELL POPULATION

(One Hundred Basal Cells)

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Total 1921  Total 1465
Average 320 Average 244
### TABLE III

**MITOTIC INDEX**

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**Average** 1.50

**Average** 1.77

Average for both surfaces 1.63
### TABLE IV

**GRAIN COUNT**

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Average: 44.04

Average: 37.60


**TABLE V**

**GRAIN COUNT**

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Average 38.00

Average 31.88
TABLE VI

GRAIN COUNT

Forty-Eight Hours

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</table>

Average 10.72

Average 34.28
APPROVAL SHEET

The thesis submitted by William A. Schoenheider, Jr. has been read and approved by three members of the faculty of the Graduate School.

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.

Date May 23, 1960

Joseph G. Kostrubala, M.D., D.D.S.
Signature of Adviser