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Renewal of Cell Population in Palate and Tongue Epithelium of Mice

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RENEWAL OF CELL POPULATION IN PALATE AND TONGUE EPITHELIUM OF MICE

by

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LIFE

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CHAPTER I

INTRODUCTION

There is a dynamism of many cell populations. Normally, the number of cells entering is equal to the number of cells leaving. Whatever the reasons may be, cells are continuously in a state of flux, being lost from the organism and an equal number added from the generative compartment of the tissues. The average length of time a cell remains in a given compartment is equal to the ratio of the size of the compartment to the total outflow of the cells. The generation cycle of cells has been studied in the past by various workers employing the mitotic index method of calculation, the stage duration index method of calculation and a direct graphic method. This study is a part of a general study of the effect of ageing on the oral epithelium. In particular this study employs the graphic method to study the generation cycle in the oral epithelium of sixty day old mice.

CHAPTER II

REVIEW OF LITERATURE

Histology of the Oral Mucosa in Mice - Medak (1959) adequately described the composition of the oral mucosa in mice as having a lamina propria consisting of connective tissue cells and fibers, a basement membrane with collagenous and reticular fibers and a stratified squamous epithelium.

The basal layer of the epithelium has a single row of columnar cells. They are perpendicular to the basement membrane. This plane varies in the lateral part of the palate and the cheek. The cytoplasmic nucleic acid contents are higher in these cells than the cells forming the rest of the epithelium. These nucleic acid contents decrease peripheralward. The basal cells stain very well, but this stainability is lost with the decrease of the nucleic acid contents. The cells of the basal layer are mainly responsible for the mitotic activity and, thus, the production of new cells to replace those cells which are lost at the surface of the epithelium.

The stratum spinosum has large polygonal cells with star-shaped outlines and intercellular connections. They undergo division and contribute from 2% - 35% of the cells to the general cell population. The greater the number of cell layers, the greater is

their contribution. Keratohyaline granules are found in the cells of the stratum spinosum in later stages of cellular differentiation. Glycogen, alkaline phosphatase and sudan stainable lipids are absent in the spinous cellular layers. Tonofibrils, cell membrane and intercellular bridges are more prominent adjacent to the compact keratin areas than to the loose keratin areas.

The stratum granulosum has no residual cytoplasm and the unstained granules are of uniform size only adjacent to the compact keratin areas. There is phosphamidase activity in the keratohyaline granules.

Medak (1959) furthermore distinguished between loose keratin and compact keratin. Loose keratin is present in the cheek and the floor of the oral mucosa of the mouse. It is chemically unstable and is altered by salivary action. Compact keratin is found in the lateral and central parts of the palate. It is stable and resistant to salivary action. The thickness of the keratinous layer increases with the thickness of the cellular layer. The thick keratin wears off more readily and is, therefore, less economical. The oral mucosa resembles epidermal epithelium but contains no sudan stainable lipids in keratin.

Leblond (1956) described the morphological changes in columnar cells of the basal layer. As the cells move outwards, they change from columnar to polyhedral and finally to flattened, granule-containing cells, which then transform abruptly into a homogeneous, glassy layer. This, in turn, changes into soft keratin

layers. The surface releases thin squames with ragged edges, which are the remnants of individual dead cells.

Schoenheider (1960) reviewed the composition of deoxyribonucleic acid (D.N.A.). It contains deoxypentose carbohydrate, the essential component of the chromatin of cell nuclei, which is strictly confined to the nucleus. On complete hydrolysis, it yields pyrimidine, purine bases, a ribose sugar and a phosphoric acid component. The pyrimidines are cytosine and thymine, while purines are adenine and guanines.

Regeneration of Cells - Ris (1955) stated that amitosis consists of a mere nuclear fragmentation, a process which generally precludes further proliferation. Leblond (1956) reviewed in detail the process of the renewal of cell population. He reported that mitosis is the only means of cell reproduction and the number of mitoses occuring in a tissue represents the number of new cells formed in this tissue. Cell production is balanced by cell loss. An adult animal, theoretically, cellularly is in a steady state: as many cells leave as are produced. This steady state varies with the time (diurnal) in 24 hours and in females with estrous cycle. Hoffman (1953) observed that mitosis occurs at random in most cell populations. Leblond (1956) further stated that the period of cell life following mitosis may last as long as the life of the organism or may sooner or later be followed by another mitosis. The period between the two mitoses is called an inter-

phase. Prior to a series of visible changes which a cell undergoes, it doubles its D. N. A. contents. This is the duplication stage. Toto (1962) conducted an investigation of the generation cycle of the epithelium of the tongue in mice in which he concluded that the duplication stage lasts for 10 hours. After this, D. N. A. synthesis there is a rest period of 20 minutes (called G_2).

Leblond (1956) described four distinct phases of mitosis. Stevens (1953) stated that prophase is the first visible sign of cell division. The chromatin is more basophilic and the size of the cell increases while the visibility of threads, spherical shape of the cell and the presence of nuclear membrane mark this stage. During metaphase the nuclear membrane disappears and the chromosomes are clumped to form an equatorial plate in the center of the cell. At this phase there is no divergence of chromosomes from the equator. Leblond (1956) stated that in the next phase, anaphase, the chromosomes separate and begin to diverge. The divergence proceeds until there is a division of the cell body. This is telophase and is marked by the appearance of the nuclear membrane in the two daughter cells. Messier (1960) in a radioautographic study of cell proliferation and migration in rats and mice found it difficult to delimit prophases and telophases.

Pinkus (1954) stated that the basal cells divide and renew the population. Bostroem (1928) and Levander (1950) pointed out that, contrary to some belief, the cells do not come from the

underlying connective tissue. Andrew (1949) stated that no transformed lymphocytes contributed to the renewal of cell population. Leblond (1956) stated that the presence of mitoses can be recognized in the area of rapid nenewal. In slowly renewing areas there are fewer mitoses; therefore, it is difficult to locate them. Storey (1949) found that the mitosis at a spinous level may not always lead to complete separation of the daughter cells, since binucleated cells are common in the upper half but not in the lower half of the spinous layer.

Henry (1952) investigated the oral mucosa of rabbits and stated that the mitotic activity showed a slight tendency to cluster in localized areas. He distinguished a distinct layer of cells forming the inner layer of the stratum spinosum. In the renewal of the cell population, this layer contributed 40.2% and the basal layer 59.8% of the total cells.

Toto (1962), while investigating the generation cycle of the epithelium of the tongue in mice, found that the duration of mitosis was 40 minutes. Medak (1959) concluded that the mitosis required one hour in the oral mucosa of mice. In case of rabbits' oral epithelium it was found to require 64 minutes by Henry (1952). Leblond (1956) suggested that there are variations in the estimation of the duration of mitosis probably due at least in part to the observational difficulties.

Mitotic Index, as described by Leblond (1956), is the number of cells undergoing mitosis at any one time, over the total number

of cells present. The nuclei are counted since in most cases one cell has only one nucleus. Mitotic index is used to measure the renewal time. He pointed out that this varies in different animals. Medak (1959) discovered that the M. I. in the center of the palate of mice is 1.65 and in the lateral part of the palate it is 1.74, in the floor of the mouth, 2.02, and in the oral mucosa of the cheek it is 2.43. In case of buccal mucosa of the normal rabbits, mitotic index is found to be 5.1 by Henry (1952), which in the colchicine biopsies is 25.7 and there are diurnal variations from 3.8 - 7.2.

Messier (1960) stated that M. I. is not altogether satisfactory to assess the rate of cell proliferation because the mitotic figures are not recognized in the small and the elongated cells. Also the counts of mitoses in section are proportional not only to the number of cells entering mitosis, but also to the time taken by these cells to complete visible mitosis.

Toto (1962) estimated that the generation cycle of the tongue epithelium of mice is 100 hours. Bertalanffy (1960) investigated the whole digestive tract of Albino rat and found that the number of cells entering mitosis varied from 7% in the lip to 79% in the intestinal mucosa. The significance of such variations may be seen, for example, in the tongue and he suggested this could be due to mechanical factors, since mitotic rate is higher on the dorsum than the ventral surface of the tongue. He further speculated that it could be due to some enzymes or other inherent

factors. Leshar (1961) found that the generation cycle of the epithelium of young and middle-aged mice is 11.5 hours. The rate of regeneration of the oral mucosa in rabbits is 0.48% per hour and for the entire epithelium is 208 hours as investigated by Henry (1952). This period is identical with the average life span or the intermitotic time of the cells. It occupies a position between skin and the intestinal mucosa. Bertalanffy (1954) assumed that most organs and tissues of the male rat grow in proportion to the body weight and the number of cells increases in a parallel manner.

There are various methods for the determination of the rate of regeneration of cells preferred by different workers for different reasons. Some types of cells can be labeled with radioactive isotopes, vital dyes, etc., and then traced through their life cycle; this was pointed out by Leblond (1956). Hevesy (1948) stated that the phosphorus present in D. N. A. is incorporated during synthesis of this substance at the duplication stage of mitosis. Therefore, the amount of D. N. A. formed in a tissue may be estimated from the uptake of radiophosphorus into the D. N. A. fraction. He suggested that phosphorus-containing precursors could be used for the purpose of cell labeling. Lajtha (1954) attributed the use of p^{32}, C^{14} adenine and C^{14} formate for the same purpose. Tritiated thymidine was used by Toto (1962), Messier (1960) and many other workers. Shoenheider (1960), in his study of D. N. A. metabolism of the epithelium of the tongue

in mice, reviewed the method of autoradiography, for detecting radioisotopes based on their ability to affect the silver bromide crystals of photographic emulsion. He assumed that the chemical behavior of the labeled substance is identical with that of its stable counterpart. It is like a "tracer". Toto (1962) stated that there is no minimal threshold for tritiated thymidine at which injury to the chromosomes does not occur. Quastler (1959) discovered that within 5 minutes following the injection of tritiated thymidine into mice, the amount of labeling in the intestinal epithelium is half and the epithelium gets saturated in 10 - 20 minutes. He suggested that this method of investigation is not physiological, because of the growth factor in young animals, the stress of injection and the injury to the chromosomes caused by the tritiated thymidine. Messier (1960) used thymidine H^3 for his studies of the cell proliferation and migration in male rats and mice. The frequency of labeled cells is proportional to the duration of the duplication stage. A close relationship exists between the labeled thymidine uptake and mitosis in the tissues. He further stated that the radioactive index is a rough index of the rate of cell division, since it is influenced by the rate of incorporation of the label into the intermediate substances leading to D. N. A. synthesis and by the duration of the period of D. N. A. synthesis. The statistics relating the generation cycle were derived by Leshar (1961) from the percentage of mitotic figures labeled as the function of time.

Colchicine, a plant alkaloid, has a property of blocking mitotic divisions at metaphase and was used by Henry (1952) in the oral mucosa of rabbits. The effect of colchicine on the accumulation of mitosis is evident since it is 25.5% in the normal specimens as reported by him. There is an increase of mitosis in the colchicinated specimens of 20.4 cells / 1000 during 6 hours or 3.4 cells / 1000 entering mitosis every hour--a difference of 6.34% per hour. In the colchicinated specimens, metaphase is a higher fraction of the total number of mitoses. The prophase, percentage is lowered significantly in the colchicinated specimens. The corresponding percentage increase in the frequency of the cells in mitosis is found to be due to almost wholly the increase in the metaphases which constitute 0.23% of all the cells in normal material. After 3 hours interval, the increase in metaphase is 1.47% while after 6 hours interval it is found to be 0.23%. In the colchicinated treated material, anaphases and the telophases are constant while prophases decreased from 0.17 to 0.12% in the 3 - 6 hour specimen.

Lesher (1961) suggested the use of x-ray irradiations for the study of the regeneration of cells. It stops early prophases in the material treated in this manner.

Lesher (1961) pointed out that the regeneration time computed by the stage duration index method formula -

$$\frac{\text{NUMBER OF CELLS IN MITOSIS}}{\text{NUMBER OF CELLS IN INTERPHASE}} = \frac{\text{DURATION OF MITOSIS}}{\text{DURATION OF INTERPHASE}}$$

is unreliable proportional to the degree to which these estimates of the mitotic duration are unreliable. In the study of intestinal epithelium of mice, he used the graphic method for estimating the generation time of cells. Leshar calculated the generation cycle of cells by adding the D. N. A. synthesis period (S), a rest period (G_2), the duration of mitosis (M) and the interphase (G_1), which is the period between the end of one mitosis and the start of the next generation cycle. He further stated that in the course of 2 hours the curve rose rapidly from 0 - 100% and remained there for 5 hours. The curve descends at 11 hours, after which another cycle begins. He suggested that the graphic method is superior to the stage duration index method because it requires the precise knowledge of the size of proliferative pool and of the duration of a particular stage of the generation cycle which are difficult to find. In this method, same conclusions are reached whether 50% ascending limb or the mid-plateau points are used. The stage duration index method has the advantage of applying without observing the complete generation cycle..

There are various factors influencing the renewal time of the cells, which are recorded by different workers. Bullough (1948) reported that there are diurnal variations and he associated higher mitotic activity with sleep and lower mitotic activity with those of wakefulness. Experimentally, the proliferation could be either increased by inducing sleep with an anaesthestic or decreased by having animal run in a revolving box to keep him awake.

He further stated that the glycogen level of many tissues is higher in resting than in active animals and suggested that the mitotic activity is largely dependent on the amount of glycogen present in the cells. Bertalanffy (1960) noticed that the mitotic activity is higher in the afternoon in the stratified epithelium than that of the columnar epithelium. Storey (1949) described seasonal variations in the mitotic activity of epidermis. He recorded the turnover time of epithelium during summer (9.8 days), in the autumn (16.3 days), and in the winter (18.8 days). Diller (1946) pointed out that starvation lowers mitotic activity in the rat intestine. Many workers in the past have discovered that endocrinological influences are important to the rate of mitotic activity. Eartly (1951) showed that thyroxin in small doses depresses the mitotic activity. Bullough (1943) found that ester cycle in female increases the mitotic rate; Eartly (1951) discovered that testosterone in male enhances the rate of cell division. Leblond (1955) stated that growth hormone accelerated the mitosis. Diller (1946) attributed increase of mitosis to nutrition. Storey (1950) also found a rise of mitotic activity at a temperature of 25° - 30° C. in rats epidermis. Leblond (1956) is of the opinion that in an integrated animal there is probably no effect because of the internal temperature regulation mechanism. Berfurth (1891) and Peters (1955) suggested that irritation causes cell proliferation, since it is noticed that at the edges of a wound the mitotic activity tends to fill up the gap. Thringer

(1939) reported that irritative agents which first produce desquamation of cornified cells from the epidermis, may on repetition lead to a thickening of the cornified layers - Henry (1952) reported that colchicine depresses mitosis in the oral mucosa of rabbits. Leshar pointed out that there are interanimal variations in the generation cycle of the cells. He also stated that the increase of the generation time may be due to "wearing out" with age or the hormonal, humoral factors (a part of the generative integrated system). Quastler (1959) concluded that whatever mechanism is responsible in old age, the reproduction of epithelial cells in the intestinal crypt of mice is changed, the generation time and the heterogenicity of the cell population is increased.

Causes of Renewal Phenomena - Leblond (1956) reviewed the process of renewal and attributed to the environmental influences like air, food, and urine, etc., as potential irritating agents. He stated that it is self evident from the fact that the non-renewing epithelia are in contact with blood or the extracellular fluids. The current theory assumed by Grant (1944-45) isthat mitotic activity is a regeneration in response to irritation damage. Nicholas (1955) calls it a physiological regeneration. Vulpe (1954) found that irritation due to urine causes mitosis in the transitional epithelium of the bladder. Leblond (1956) pointed out that the smaller animals have abundant mitosis since they are subjected to

unknown irritational agents like parasites, urine, etc. The mitoses in the bladder are due to repair processes. Peters (1955) and Berfurth (1891) suggested that the cells damaged by the irritants are believed to release "EVOCATORS" of mitosis inducing their neighbors to divide, as it takes place in wound healing. Leblond (1948) demonstrated that most renewal systems, though responsive to environmental influences, are capable of renewing in their absence, e.g., hemopoetic tissues and the seminiferous tubules. Accordingly, the ability to proliferate is an inherent property of the cells of the renewal systems.

The departure of a cell from a renewal system or from a given compartment is due to emigration, death or a combination of these processes. As seen in spermatozoa, red blood corpuscles which senescence or the differentiating epidermal cells, as attributed by Leblond (1956). He further stated that the movements of healthy cells takes place because of overcrowding as it happens in the respiratory epithelium of trachea or they are squeezed out by the muscular contraction of the intestines. The cells are pushed toward the lumen as a result of pressure arising from the accumulation of newly formed cells in the basal region.

Bierman (1955) suggested that cells may be eliminated by hormonal factors as is seen in case of lymphocytes by the adrenal cortical hormone. He further described the destruction of granulocytes by tissue action.

Leblond (1952) stated the interaction of various influences of cell production, migration and losses takes place in testes, by synchronous evolution of a generation of cells present in the epithelium. The process is similar in the bone marrow after the withdrawal of blood from the body of an organism; the mitotic activity is increased in the hemopoietic tissues. It corresponds to the compensatory hypertrophy as seen after the extirpation of a kidney or a lung. Therefore, under physiological conditions, the continuous loss of red blood cells lead to a continuous compensatory mitotic activity of the erythrocytic series.

Mitotic index of several cells per thousand occurs regularly in the epidermis of very young animals. Leblond (1956) furthermore, described that in the older animals, such a high number of mitosis occurs only in the oral mucosa and in the intestinal mucosa. Beyond infancy, the epidermal epithelium has a lower order of mitotic activity. The highest epidermal index is reached in extreme youth because of functional hyperplasia.

Leblond (1956) stated that in the region of high functional demands the mitotic index is high. The mitotic activity in the plantar epidermis approximates that of the oral mucosa. In the intestinal mucosa very high percentage of cell population is in mitosis. The mitotic activity of the oral mucosa is intermediate between that of the epidermal epithelium and the intestinal mucosa.

Furthermore, Leblond (1956) described that some renewal systems are complex (as seen above) while others are the basic pro-

perties of the cells, for example reproduction and ameboidism. These are the established property of the organism.

Leblond (1956) pointed out that because of frequent occurance of the renewal process in the most exposed areas of the organism they are subjected to mechanical, toxic or infectious agents. The ordinary repair mechanism cannot efficiently maintain tissue integrity when a constant supply of the young cells anticipate a damage and prevent occurance of a weakened area or a gap. Such a renewal system in the course of evolution has become an established and hereditary characteristic due to selection advantage to the species. Leblond (1956) concluded that there are many ways by which a balance is achieved between the cell production, a cell loss and the speed of the renewal processes.

CHAPTER III

MATERIALS AND METHODS

Fifty-four female mice of C_{57} strain; white, 60 days old are injected interaperitoneally with tritiated thymidine (specific gravity 1.9 Curies per milli-mole) at a dose rate of one micro Curie per gram of animal weight.

The average weight of the animal is 35 grams. They are killed under ether anesthesia at one quarter, one half hour, one hour, two hours and, thereafter, at two hour intervals up to one hundred and two hours.

The tongues are removed from the mouth and cut midsaggitally, and a five millimeter square of the palate mucosa is stripped from the bone. The tissues are fixed in neutral formaline for twenty-four hours. Later the tissues are washed in running water, dehydrated with ascending series of alcohol, cleared with xylene and embedded in paraffin. Sections of 3 micron thickness are made. Such sections are treated with xylene, a descending series of alcohol and water. Autoradiograms are prepared using liquid emulsion Kodak N T B_3. The sections are exposed for one week. The autoradiograms subsequently are stained with hematoxylin and eosin.

The times at which the first labeled prophase and telophase

are seen, are recorded. The average number of epithelial cells per oil immersion field in the palate, dorsal and ventral surfaces of the tongue is calculated, for two hour and one hundred hour intervals following the injection of tritiated thymidine. The number of labeled and non-labeled cells in their metaphase and anaphase of mitosis is counted. The number of mitotic cells per thousand is calculated, which is the mitotic index. Two hundred mitotic figures for each interval of time are observed for each of the specimens under present investigations.

The percentage labeled mitotic figures for each interval is calculated. Curves are plotted using the percentage of labeled mitotic figures against each interval of time. The D. N. A. synthesis is estimated by extrapolation of the coordinates on the curve at 50 percent labeling of the mitotic figures.

CHAPTER IV

FINDINGS

It is observed that two or three adjacent basal cells start dividing approximately at the same time and approximately at similar sites on various papillae (at crest, base or in between) of the epithelium in a particular slide specimen. The interval or the distance between these sites of mitotic activity looks uniformly equal. The distribution of mitotic figures with uniform and regular interval in between them gives an impression to the observer, that the mitotic phenomenon occurs in a wave-like fashion.

The average numbers of epithelial cells per oil immersion field at two hours following the injection of tritiated thymidine were two hundred and thirty, one hundred and ninety, one hundred and sixty for the dorsal surface of the tongue, palate and the ventral surface of the tongue, respectively, (Table 1). There is a significant decrease in the cell population, per oil immersion field as the time of sacrifice of the animal increases following the injection of tritiated thymidine. For example, the number of cells per oil immersion field at one hundred hours following the injection of tritiated thymidine is one hundred and ninety, one hundred and thirty, and one hundred and ten for the dorsal surface

of the tongue, palate and the ventral surface of the tongue, respectively, (Table II).

The mitotic index, two hours following the injection of tritiated thymidine is 2.9, 2.5, 2.2 for the dorsal surface of tongue, palate and the ventral surface of the tongue, respectively, (Table I). The mitotic index is considerably higher at one hundred hours following the injection of tritiated thymidine. It is 4.4, 4.1, 3.5 for the dorsal surface of the tongue, palate and the ventral surface of the tongue, respectively, (Table II).

At any given time, there are always some cells in preparation for mitosis; within the first hour of injection tritiated thymidine is either incorporated in the nuclei of some cells which are synthesizing D. N. A. or it is excreted. The labeled cells are randomly distributed in the basal and the prickle cell layers. The first labeled prophase among these cells is observed at thirty minutes and the first labeled telophase at one hour following the injection with tritiated thymidine.

The peak of the labeled mitotic figures on the ventral surface of the tongue is observed at ten hours following the injection of tritiated thymidine. This is two hours longer than that seen either in the palate or the dorsum of the tongue,(Table V, VII, VII).

There is a sharp rise and sharp decline in the percentage distribution of the labeled mitotic figures in the epithelia of the tongue and the palate. There never was a one hundred percent

labeling in any sample. There was, however, a small plateau which extended from two to two and one half hours in the palate and the dorsal surface of the tongue (Figures 1 and 2).

After sixteen to eighteen hours following the injection of tritiated thymidine, no labeled mitotic figures are seen either in the epithelia of the tongue or the palate, (Tables V, VI, VII).

The extrapolated D. N. A. synthesis time for the dorsal surface of tongue and the palate is eight hours while in the ventral surface of the tongue the D. N. A. synthesis time is eight and one half hours.

On the dorsal surface of the tongue at ninety-eight hours following the injection of tritiated thymidine, an unusually large number of cells at different sites are seen to be arrested in the prophase stage of mitosis. The keratin layer seems to be looser as compared to the previous specimens.

One hundred and two hours following the injection of tritiated thymidine, on the ventral surface of the tongue, abnormal looking labeled mitotic figures are seen. The chromatin material seems to be fragmentated and the nuclei are hyperchromatic. The keratinous layer seems to be thinner than the other specimens.

The size of the granules and the size of the granular layer increases in the three areas of the oral epithelium as the time of sacrifice of the animal increases following the injection of the tritiated thymidine.

CHAPTER V

DISCUSSION

The reproduction of the stratified squamous epithelium is mainly the function of the basal layer of cells. This view is supported by Medak (1959) who stated that in the oral epithelium of mice, the basal cells contributed 63% - 98% in the renewal of the cell population. Henry (1952) in his studies on the oral epithelium of rabbits described that the basal cells contributed 59.8% in the renewal of the cell population.

The mitotic figures are found in peculiarly similar locations in the basal cell layer. At different intervals of time, the locations of the mitotic figures appear to change in the basal layer.

The reduction in the cell populations observed in the tongue and palate could be attributed to the injury to the nucleus due to radiation over a period of one hundred hours. It is noted that there are labeled prophases seen at ninety-eight hours, but subsequently many cells show nuclear fragmentations with failure of metaphase to appear. Also, there is an increase in the mitotic index with time. To account for the decrease in the cell population in the face of an increased mitotic index, it is suggested that the cells become arrested at the metaphase and anaphase

stages of mitosis, thereby increasing the ratio of the mitotic figures in the cell population. Also, in addition, the nuclear degeneration could account for the reduction in the cell population.

Another observation which may be related to reduced cell population is the decreased number of prickle cells, and an increased number of granular cells and thickness of the keratin layers. It is suggested that as the number of cells in the population is reduced, the cells remain in the epithelium for a longer period of time. This permits them to mature, accounting for the increased granular and keratin layers. Moreover, this mechanism is the only one by which the epithelium could function to protect the underlying connective tissue, i.e., flattening out and forming keratin. Along this line of reasoning, the ageing epithelium ultimately would lose its keratin and in the absence of a regenerating population this could result in ulceration. Such are the observations made by Chase and Toto (1961) in a study of the effect of roentgen radiation of the normal human oral epithelium. They noted an increase in the number of keratinized cells but a decrease in probable generating cells.

Before a visible phase of mitosis, the cell duplicates its D. N. A. It is during this stage that the injected tritiated thymidine enters the D. N. A. molecule in place of the naturally occurring thymine; thus the cell is radioactively labeled, permitting one to follow it subsequently, by autoradiography. All

those cells which take up tritiated thymidine at a particular period are in D. N. A. synthesis phase (S) but some may be at the beginning, some at the middle, others still, at the end of the phase.

The first labeled prophase is observed at thirty minutes, but not at fifteen minutes following the injection of the tritiated thymidine. It is assumed that the rest period following the D. N. A. synthesis, which is called G_2 by Lesher (1961), is between fifteen and thirty minutes, i.e., approximately twenty minutes. The first labeled telophase is observed at one hour period following the injection of the tritiated thymidine. Therefore, by subtracting the rest period, G_2, from one hour, the period of approximately forty minutes is estimated to be required by mitosis. The G_2 period of twenty minutes and the forty minutes required by the cell division are also estimated by Toto (1962) in the investigation of the oral epithelium of mice.

The increase in percentage of labeled cells with increase of time of sacrifice of the animal is due to the division of the labeled cell population.

There are many cells which are in D. N. A. synthesis at the time of injection but for various reasons they did not take up thymidine or other cells which did take up thymidine or other cells which did take up thymidine but the very small quantity is undetectable. Thus it is suggested that one cannot find a one hundred percent labeled cell at any period and in any sample of

the specimen.

Lesher (1961) while working with tritiated thymidine on the intestinal mucosa of mice concluded that the broad plateau on the curve shows that the many labeled cells are slowed or stopped mitosis because of toxic effect of tritiated thymidine. Therefore, it is suggested, in the present studies, that a plateau of two hours and two one half hours in the palate and the dorsal surface of the tongue are probably due to the similar reasons.

D. N. A. synthesis time for the dorsal surface of the tongue and the palate is eight hours while for the ventral surface it is eight and one half hours. The peak of the labeled mitotic figures reached two hours later in the ventral than the dorsal surface of the tongue and the palate. These two observations could be explained that the rate of loss of cells is greater on the dorsal surface of the tongue and the palate than the ventral surface of the tongue in mice. This suggests that the generation cycle is expected to be of longer duration in the ventral surface than the dorsal surface of the tongue and the palate.

Lesher (1961) stated that the generation cycle consists of the D. N. A. synthesis (S), a rest period (G_2), the duration of mitosis (M) and that period till the next mitosis begins (G_1). In the present studies, the calculations based on such a method show that the new generation cycle for the palate and the dorsal surface of the tongue begins at ninety-six hours period and for the ventral surface of the tongue at ninety-eight hours following the

injection of the tritiated thymidine. The D. N. A. synthesis time for the palate and the dorsal surface of the tongue is eight hours while for the ventral surface of the tongue it is eight and one half hours. Rest period (G_2) is twenty minutes and the duration of mitosis is forty minutes for the three areas under investigation. Therefore, it is concluded that the generation cycle for the palate and the dorsal surface of the tongue is eighty-seven hours while for the ventral surface of the tongue it lasts for eighty-eight and one half hours. This observation is very close to the generation cycle of eighty-nine hours estimation by Toto (1962) in an adult mouse, by the stage duration index method. Since he used the adult animal and the present studies are based on young mice, it is expected that the generation cycle be shorter in this case. Such a difference may be explained on the basis of the normal deviations and due to the difference of methods used in the two investigations. The beginning of the second generation cycle is not observed by Toto (1962) but it is only estimated. Hence, there is a chance of error in his estimations. Moreover, the beginning of the second generation cycle with the injection of the tritiated thymidine is not regarded as physiological and the possibility of growth factor in the present studies may give some erroneous results.

Medak (1959) estimated that the generation cycle in the center of the palate lasts for twenty-five days and in the lateral palate it lasts for twenty-four days. Such a great difference in

the generation cycle as compared to this investigation may be accounted for by the greater reliability of the graphic method as compared to the mitotic index method used by him. The graphic method has the distinct advantage of directly observing the actual length of time of the generation cycle. The mitotic index method depends only upon calculations. Messier (1961) pointed out that the mitotic index method gives erroneous results since one does not observe the actual generation cycle of the cells.

CHAPTER VI

SUMMARY

Fifty-four female mice, sixty days old white C_{57} strain, were injected with tritiated thymidine intraperitoneally. They were sacrificed at quarter hour, half hour, one hour, two hours, and, thereafter, every two hour period up to one hundred and two hours following the injection. The mitotic activity of the epithelium of the palate, and both surfaces of the tongue was studied. Two hundred metaphases and anaphases were counted in each period observed. The number of cells per oil immersion field at two hour intervals following the injection of the tritiated thymidine were two hundred and thirty, one hundred and ninety, one hundred and sixty, and the mitotic index was 2.9, 2.5, and 1.9 for the dorsal surface of the tongue, the palate and the ventral surface of the tongue, respectively. The number of cells per oil immersion field at one hundred hours following the injection of the tritiated thymidine was one hundred and ninety, one hundred and thirty, one hundred and ten, and the mitotic index 4.4, 4.1, 3.5, for the dorsal surface of the tongue, the palate, the ventral surface of the tongue. Three curves are plotted with the percentage of mitoses as the function of time. Rest period (G_2) is twenty minutes and the duration of mitosis forty minutes, for the three areas of in-

vestigation. The synthesis time is eight hours for the palate and the dorsal surface of the tongue, eight and one-half hours for the ventral surface of the tongue. The generation cycle for the palate and the dorsal surface of the tongue is ninety-six hours and for the ventral surface of the tongue, ninety-eight hours. The radiations injure the epithelial cells and change the morphology of the epithelial layers.

CHAPTER VII

CONCLUSIONS

Rest period (G_2) is twenty minutes and the mitotic duration (M) is forty minutes in the palate, the dorsal and the ventral surfaces of the tongue in sixty day old mice.

The synthesis time is longer in the ventral surface of the tongue than the palate and the dorsal surfaces of the tongue.

The generation time for the palate and the dorsal surface of the tongue is ninety-six hours, the ventral surface of the tongue, ninety-eight hours.

The radiations injure the epithelial cells and affect the morphology of the layers of the oral epithelium in mice.

There is a greater amount of keratin accumulation following the injection of the tritiated thymidine.

The mitotic index rises with the start of the second generation cycle. This may be due to mitotic arrest.

Graphic method is reliable for the estimation of the generation cycle of the oral epithelium in mice.

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TABLE I

MITOTIC INDICES OF TONGUE (DORSAL AND VENTRAL SURFACES) AND PALATE OF THE MICE, TWO HOURS FOLLOWING THE INJECTION OF TRITIATED THYMIDINE.

EPITHELIUM	NUMBER OF CELLS PER OIL IMMERSION FIELD	NUMBER OF FIELDS OBSERVED	NUMBER OF METAPHASES COUNTED	M. I.*
TONGUE - DORSAL SURFACE	230	35	24	2.9
PALATE	190	23	11	2.5
TONGUE - VENTRAL SURFACE	160	17	5	1.9

TABLE II

MITOTIC INDICES OF TONGUE (DORSAL AND VENTRAL SURFACES) AND PALATE OF THE MICE, ONE HUNDRED HOURS FOLLOWING THE INJECTION OF TRITIATED THYMIDINE.

EPITHELIUM	NUMBER OF CELLS PER OIL IMMERSION FIELD	NUMBER OF FIELDS OBSERVED	NUMBER OF METAPHASES COUNTED	M. I.*
TONGUE - DORSAL SURFACE	190	20	31	4.4
PALATE	130	18	12	4.1
TONGUE - VENTRAL SURFACE	110	20	8	3.5

* M. I. = MITOTIC INDEX

TABLE III

PERCENTAGE DIFFERENCE IN THE MITOTIC INDEX IN TONGUE (DORSAL AND VENTRAL SURFACES) AND THE PALATE TWO HOURS FOLLOWING TRITIATED THYMIDINE INJECTION.

PERCENTAGE DIFFERENCE IN M. I.	TONGUE DORSAL SURFACE	PALATE	TONGUE VENTRAL SURFACE
TONGUE - DORSAL SURFACE	0	17	34
PALATE	17	0	17
TONGUE - VENTRAL SURFACE	34	17	0

TABLE IV

PERCENTAGE DIFFERENCE IN THE MITOTIC INDEX IN TONGUE (DORSAL AND VENTRAL SURFACES) AND THE PALATE ONE HUNDRED HOURS FOLLOWING TRITIATED THYMIDINE INJECTION.

PERCENTAGE DIFFERENCE IN M.I.	TONGUE DORSAL SURFACE	PALATE	TONGUE VENTRAL SURFACE
TONGUE - DORSAL SURFACE	0	7	20
PALATE	7	0	14
TONGUE - VENTRAL SURFACE	20	14	0

TABLE V

EPITHELIUM - DORSAL SURFACE OF THE TONGUE IN MICE.

THE NUMBER OF MITOTIC FIGURES COUNTED IN EACH INTERVAL OF TIME IS TWO HUNDRED.

HOURS (FOLLOWING THE INJECTION OF TRITIATED THYMIDINE)	MEAN LABELED MITOTIC FIGURES	% AGE LABELED MITOTIC FIGURES
¼	0	0
½	0	0
1	6	3
2	44	22
4	96	48
6	145	72.5
8	184	92
10	189	94.5
12	108	54
14	24	12
16	4	2
18	0	0
20	0	0
96	8	4
98	21	10.5
100	28	14
102	37	18.5

TABLE VI

EPITHELIUM OF THE PALATE IN MICE.

THE NUMBER OF MITOTIC FIGURES COUNTED IN EACH INTERVAL OF TIME IS TWO HUNDRED.

HOURS (FOLLOWING THE INJECTION OF TRITIATED THYMIDINE)	MEAN LABELED MITOTIC FIGURES	%AGE LABELED MITOTIC FIGURES
¼	0	0
½	0	0
1	7	3.5
2	38	19
4	89	44.5
6	138	69
8	188	94
10	190	95
12	122	61
14	56	28
16	3	1.5
18	2	1
20	0	0
96	9	4.5
98	18	9
100	27	13.5
102	30	15

TABLE VII

EPITHELIUM - VENTRAL SURFACE OF THE TONGUE IN MICE.

THE NUMBER OF MITOTIC FIGURES COUNTED IN EACH INTERVAL OF TIME IS TWO HUNDRED.

HOURS (FOLLOWING THE INJECTION OF TRITIATED THYMIDINE)	MEAN LABELED MITOTIC FIGURES	%AGE LABELED MITOTIC FIGURES
¼	0	0
½	0	0
1	2	1
2	8	4
4	37	18.5
6	98	49
8	162	81
10	196	98
12	155	77.5
14	102	51
16	22	11
18	4	2
20	0	0
96	0	0
98	4	2
100	21	10.5
102	62	31

FIGURE I.

PERCENTAGE DISTRIBUTION OF THE LABELED MITOTIC FIGURES IN THE EPITHELIUM OF THE DORSAL SURFACE OF THE TONGUE IN SIXTY DAY OLD MICE.

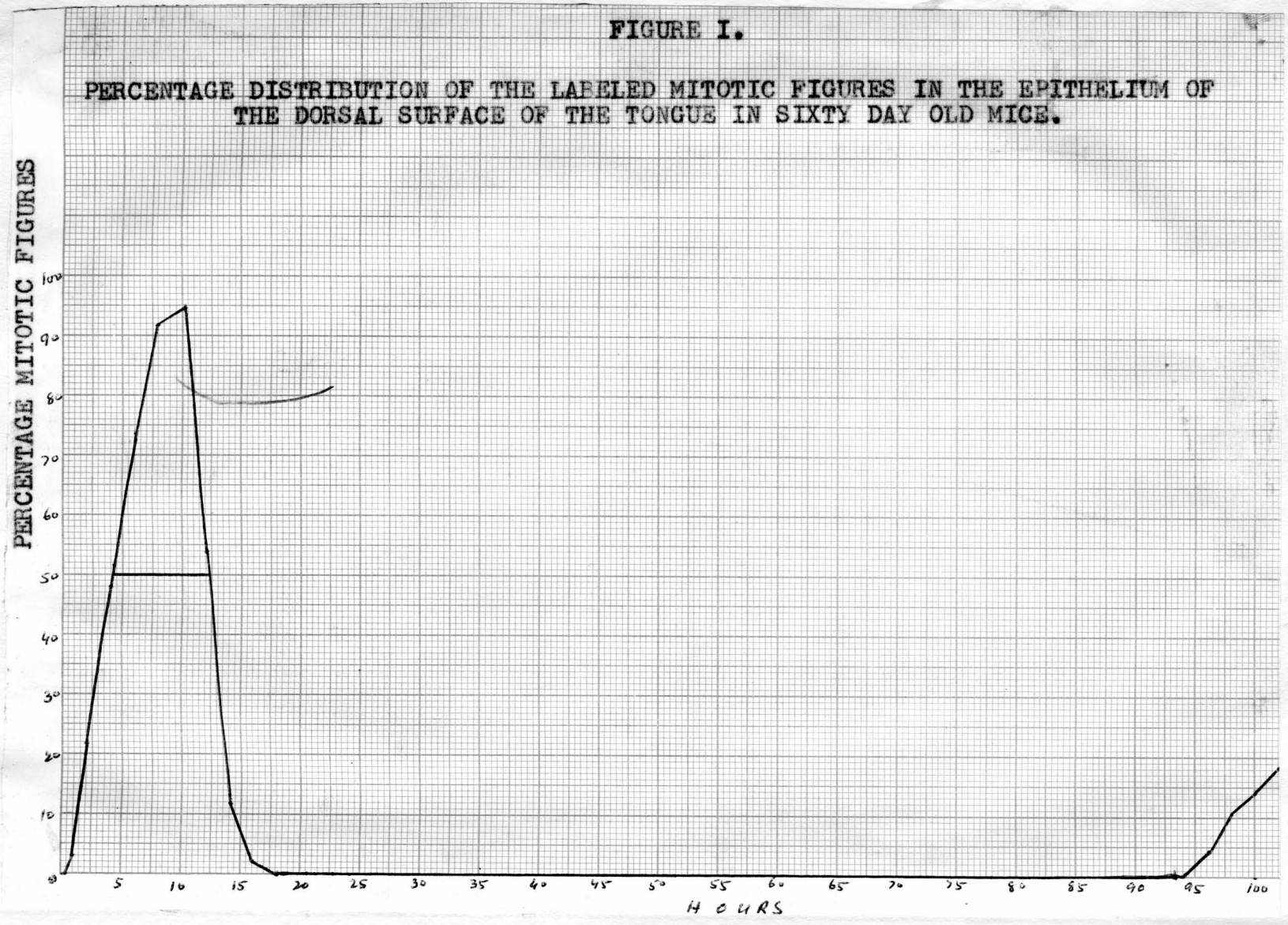


FIGURE II.

PERCENTAGE DISTRIBUTION OF THE LABELED MITOTIC FIGURES IN THE EPITHELIUM OF THE PALATE IN SIXTY DAY OLD MICE.

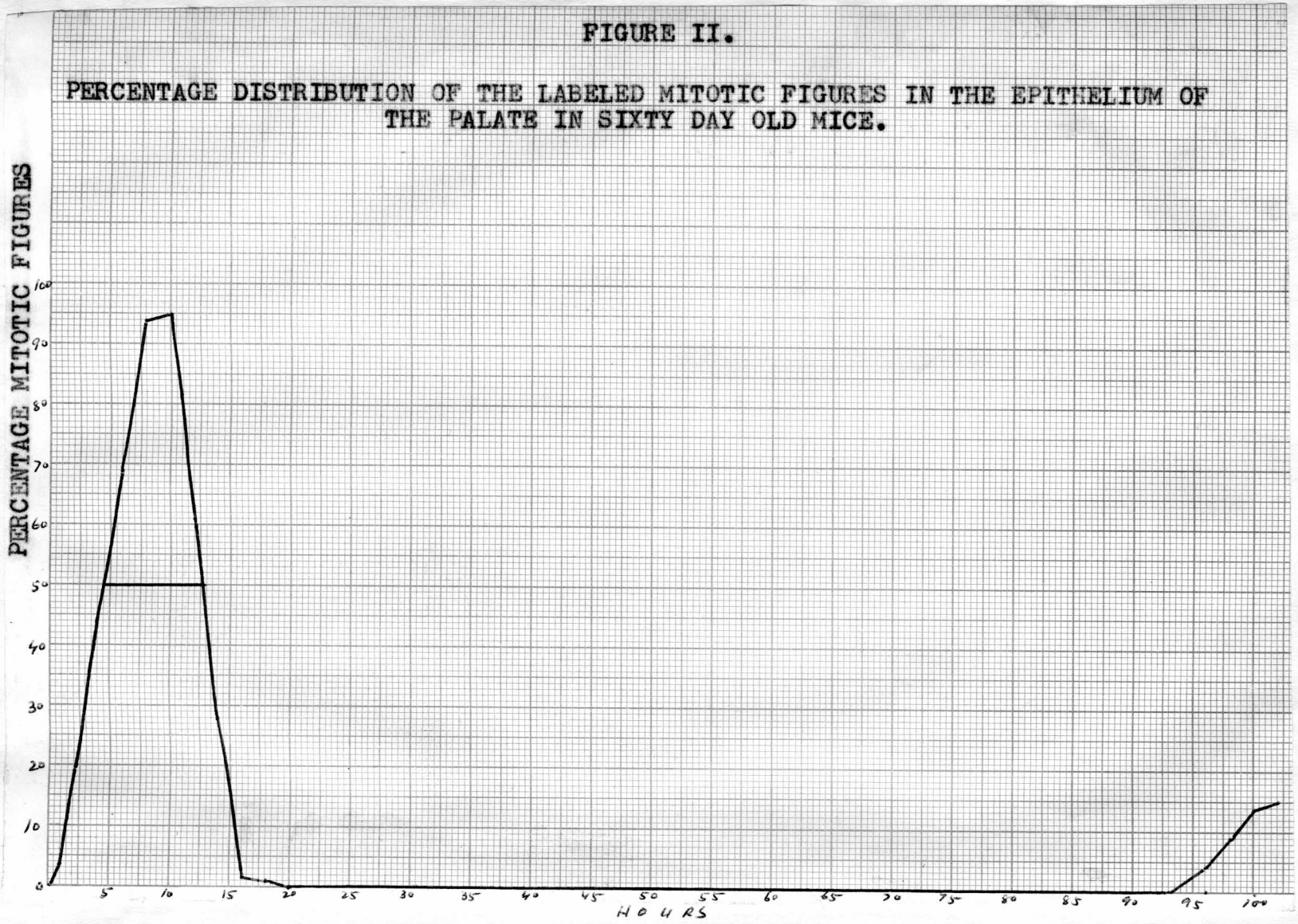
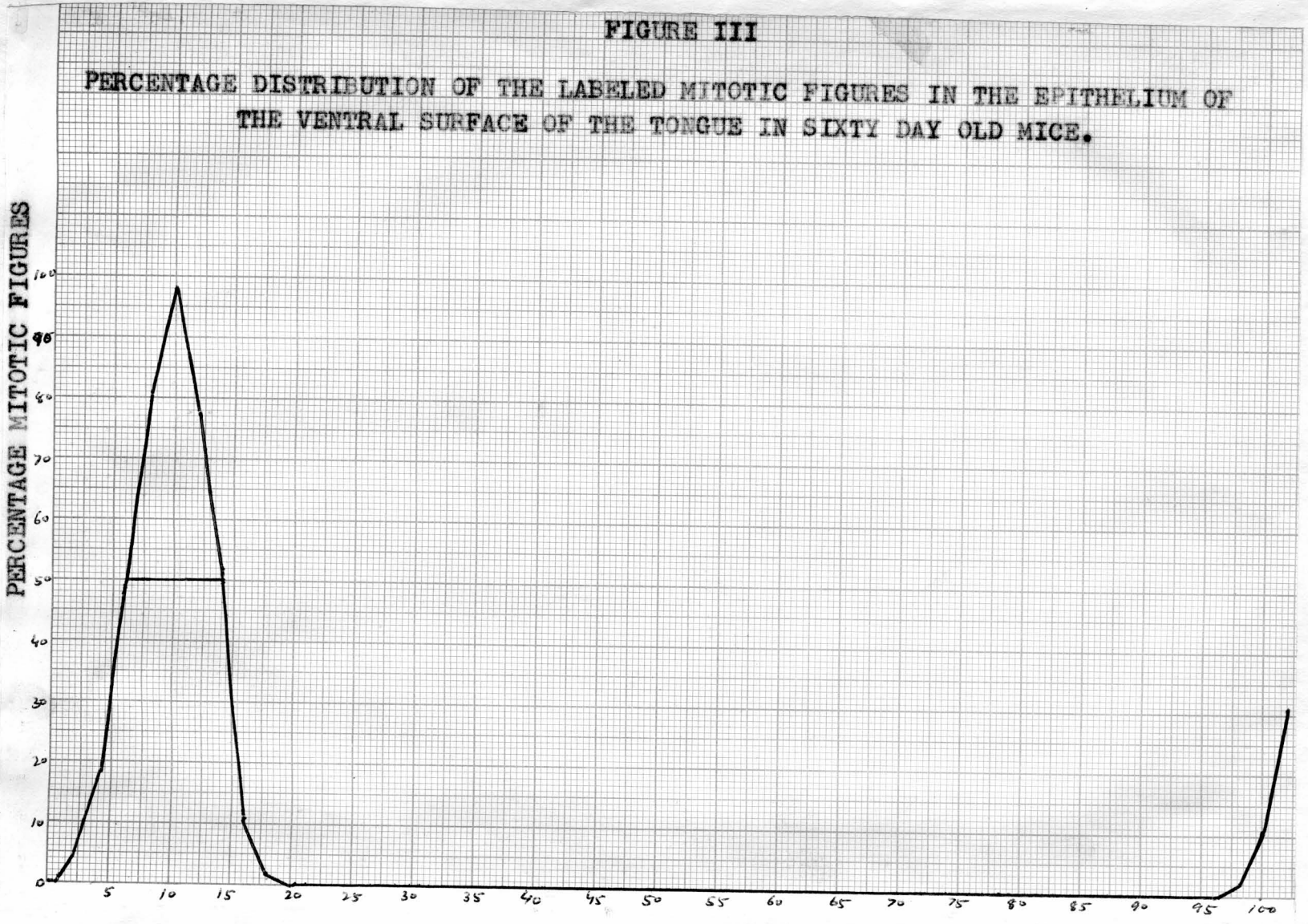


FIGURE III

PERCENTAGE DISTRIBUTION OF THE LABELED MITOTIC FIGURES IN THE EPITHELIUM OF THE VENTRAL SURFACE OF THE TONGUE IN SIXTY DAY OLD MICE.



APPROVAL SHEET

The thesis submitted by Ajit Singh Dhawan 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.

May 26 1964
Date

Gotwick D Sab
Signature of Advisor