1959

Mitotic Activity of Human Oral Epithelium When Exposed to Hydrogen Peroxide

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The professional man has no right to be other than a continuous student.

Black

Education without ideas is not only useless, it is above all things harmful.

Whitehead

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by

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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 in Oral Anatomy

February

1959

LIFE

Anthony W. Gargiulo, the last of three siblings, was born May 21, 1930 in Chicago, Illinois.

He was graduated from the Richard T. Crane High School Chicago, Illinois in February 1947. From 1947 to 1949 he attended the University of Illinois. In 1949 he continued his undergraduate studies at Roosevelt University and received the Bachelor of Science Degree in January of 1951. From 1951 to 1952 he was employed as a research associate with the Research Division of the American Can Company.

In September 1952 he began studies at Loyola University School of Dentistry and received the Doctor of Dental Surgery degree in June 1956. His graduate studies began in the Department of Oral Anatomy of Loyola University in June 1956. At the same time he was awarded a research fellowship in the Department of Periodontics in the School of Dentistry.

In September 1957 he was appointed a research fellow by the National Institutes of Health, United States Public Health Service.

ACKNOWLEDGEMENT

To Doctor Frank M. Wentz, under whose suggestion this problem was undertaken, I wish to gratefully acknowledge his constant advice, supervision and untiring assistance. He has enhanced my desire for basic research to an even greater magnitude. The author wishes to sincerely thank Doctor Harry Sicher for his constant guidance and constructive criticism. It has been a real privilege to work under his tutorage.

In addition, I extend my gratitude to Doctor Balint Orban for his many suggestions. I also wish to express my indebtedness to the specimen donors. To Doctor John O'Malley my thanks for his aid with the photomicrographs.

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CHAPTER I

INTRODUCTION

According to Marwah's findings (1956), the mitotic frequency in human gingia is 50% higher in older age groups (50 to 78 years of age) than in younger age groups (25 to 35 years of age). This served to support Bullough's findings (1949) of considerably higher mitotic activity in the mouse epidermis of middle aged animals as contrasted with that of young animals. These findings contradict previously accepted concepts of aging. It is generally accepted that bodily processes slow down and the regenerative capacity of the tissues diminish with age. Marwah and Weinmann state that one might relate carcinogenesis in older age groups to the possibility of a loss of the regulative mechanisms governing mitotic frequency and normal cellular proliferation.

Orban (1942), when investigating the effects of a 30% hydrogen peroxide solution on chronically inflamed human gingiva, incidentally reported an apparent simultaneous increase in the mitotic activity of the epithelium. This additional finding established the purpose for this study.

The present study was to investigate the morphologic changes of the normal gingival epithelium, in two age groups, when influenced by this chemical stimulant, thus establishing more quantitatively valid data.

The findings will be significant in establishing norms of mitotic index, mitosis patterns, cell density and their possible alterations when

under the direct influence of a cellular stimulant, especially those of the older age individuals.

CHAPTER II

REVIEW OF THE LITERATURE

The early investigations of mitotic activity were concerned with describing and labeling the various processes and stages involved (Tyson, 1870, Flemming, 1882). Flemming's (1884, 1885) was the first quantitative study of mitotic activity in an attempt to correlate this activity with cell loss and replacement.

Minot (1908) added further impetus to the quantitative analysis of mitotic activity by studying the epidermis of rabbit embryos at varying age ranges. He found different mitotic indices in various tissues, thus establishing evidence of differential rates of growth. Minot described the mitotic index (MI) as "the number of cells to be found at any given moment in the active process of division out of a total of 1000 cells." This index has been a very useful tool for many years since.

A. Variations in Mitotic Activity

(See Tables I,II.)

Regional differences: Thuringer (1924, 1928) established the earliest quantitative counts of cell division. He counted one mitosis for 2,414 cells on the scalp, for 378,325 cells on the leg, and 268,275 on the ear.

In 1938 Cooper and Schiff reported a mitotic index of 4.6 in eight day old human prepuce.

Again in 1939 Cooper reported a mitotic index of 3.8 in newborn human prepuce. Broders and Dublin (1939) reported a mitotic index of 6.4 in newborn prepuce.

Sutton (1938), reported a renewal time of 7-11 days for the stratum corneum in normal human skin.

In 1953 Pinkus charted these findings using Minot's mitotic index. In Pinkus' review it is seen that Thuringer's mitotic index of adult human epidermis ranges from 1.6 to 0.1 A summary of these values is found in Table II as charted by Pinkus.

Mitotic rhythmicity: It is generally recognised that a periodic mitotic rhythm exists in the regeneration of plants and animals. Rhythmic cycles were first established in plants by Killicott (1904). Fortuyn-van Leyden (1916) was the first to report this phenomenon in animals.

Many investigators have shown the existence of a daily rhythm in both animals and humans; however, as yet, there is no agreement in the timing of peak mitotic activity.

It has recently been indicated that the mitotic rhythm varies from organ to organ in the same animal (Blumenfeld, 1942).

Henry (1952) found a maximal activity of 7.2 and minimal of 3.8 mitoses per 1000 cells in rabbit oral mucosa, three and one-half months old, at 1 p.m. and 10 p.m. respectively.

Halburg, et al. (1952) reported a night low and morning high in mitoses in the retromolar epithelium and periodontal membrane in five month old black

male rats. No day and night difference was detected in the interdental papilla.

The above discrepancies encountered may be due to the differences of species, methodology, laboratory conditions, or biometric analysis. The values for some of the investigators are summarised in Table III.

Distribution of mitosis: The site of regenerative activity in the epithelium is the stratum germinativum.

Thuringer (1924), and Henry (1952), and Marwah (1956) have gathered data in regards to the distribution of mitosis in the different strata. Thuringer found 12 per cent of mitoses in the basal layer and 88 per cent in the spinous layer of cat epidermis. Henry found 60 per cent in the basal layer and 40 per cent in the spinous layer of rabbit buccal mucosa. Marwah reported 23 per cent of mitoses in the basal layer and 77 per cent in the inner spinous layer of human attached gingiva. These values are summarised in Table IV.

Some earlier investigators, prompted by the supposedly insufficient rate of epidermal mitoses, attributed regeneration to the underlying connective tissue (Burckhardt, 1859; Hamburger, 1880; Bostroem, 1928 and Levander, 1950) and the transformation of lymphocytes into epithelial cells (Andrew and Andrew, 1949).

TABLE I

Mitotic Index-Animals

Author and Year	Animal	Region	Age	Mitotic Index
Carleton (1934)	Mice	Abdominal Skin	1-7 days	2-23 (Av. 9.8)
Blumenfeld (1939)	Mice	Abdominal Skin	28 days	0.8
Thuringer (1939)	Cat	Foot Pad	Adults	2.37
Cooper and Franklin (1940)	Mice	Ear Skin	50 days	0-3.93 (Av.1.4)
Cooper and Reller (1942)	Mice	Ear Skin	2½ months	0.8-1.5
Cowdry and Thompson (1944)	Mice	Foot Pad	10 days	Basal 25.2 Spinous 27.0
Glucksmann (1945)	Mice	Interscapular Skin	2 months	2.0
Knowlton and Hempelmann (1949)	Mice	Ear Skin	42-56 days	1.69
Andrew and Andrew (1949)	Rat	Abdominal Skin	300 days	0.58
Knowlton and Widner (1950)	Mice	Ear Skin	42-56 days	0.75
Henry (1951)	Rabbit	Oral Mucosa	100 days	5.1

TABLE II

Mitotic Index
Human Epidermis

Author and Year	Region	Age	Mitotic Index
Thuringer (1924)	Scalp	Adult	10 mitosis per sq. mm.
Thuringer (1928)	Prepuce	3 years	0.74
	Scalp	Adult	0.41
	Ear	Adult	0.0037
	Leg	Adult	0.0026
Cooper (1939)	Prepuce	Newborn	1.4-6.8 (Av. 3.8)
Cooper (1939)	Prepuce	6-11 days	4.1
Broders and Dublin (1939)	Prepuce	Newborn	7.4
Andrew and Andrew (1949)	Anticubital Region	Adult	0.13
Katsberg (1952)	Abdominal Skin	0-20 years	basal 0.23 spinous 0.26
		21-40 years	basal 0.48 spinous 0.34
		41-60 years	basal 0.73 spinous 0.37
		61-80 years	basal 0.76 spinous 0.36
Pinkus (1952)	Forearm, flexor surface	46 years	1.59

TABLE III

Mitotic Rhythmicity

Author and Year	Source of Material	Organ	Time of Mitotic Cycles	
			Maximal Activity	Minimal Activity
Fertuyn-vanLeyden (1916)	Young kittens	Thymus Spleen Lymph Node	10:30 P.M.- 2:00 A.M.	10:30 A.M.
Ortis-Picon (1933)	Mice	Skin	12:00 A.M.	12:00 P.M.
Carleton (1934)	Mice	Hair Follicle	8:00 P.M.	12:00 A.M.
Blumenfeld (1939)	Rat	Skin	8:00 A.M.	early evening
Bullough (1947)	Mice	Skin	6:00 A.M. and 2:00 P.M.	10:00 A.M. and 8:00 P.M.
Henry (1952)	Rabbits	Oral buccal mucosa	1:00 P.M.	10:00 P.M.

TABLE IV

Distribution of Mitosis

Author and Year	Source of Material	Region	Per Cent Distribution of Mitosis	
			Basal Layer	Spinous Layer
Thuringer (1924)	Adult Man	Scalp	12%	88%
Thuringer (1939)	Cat	Foot Pad	44.5%	55.5%
Cowdry and Thompson (1944)	Mouse	Foot Pad	27.5%	72.5%
Thuringer and Cooper (1950)	Adult Man	Abdomen	~50%	~50%
Pinkus (1952)	Adult Man	Forearm	61%	39%
Henry (1952)	Rabbits	Oral buccal mucosa	60%	40%
Marwah (1956)	Adult Man	Attached gingival epithelium	25 yrs. 22% 50 yrs. 24%	25 yrs. 78% 50 yrs. 76%

B. Mitotic Activity In Oral Epithelium

The first mitotic index of human oral mucous membrane was reported by Marwah (1956). He reported the age difference in the mitotic index of the human attached gingival epithelium. The ages ranged from 25 to 35 years and from 50 to 78 years. The older group was found to have 50% more mitotic activity than the younger age group; thus it is seen that gingival epithelium behaves like the epidermis of man and mice. Marwah reported an average mitotic index of 0.98 in the young group and 1.56 in the older group.

Mitotic indices have been determined in the buccal mucosa of adult rabbits by Henry (1951), and in several areas of the adult rat gingiva by Halberg (1954), Muhlemann (1954, 1955) and Hirt (1955). Henry's main objective was to establish the pattern of mitotic rhythmicity in the oral mucosa of rabbits. These values are summarized in Table V.

TABLE V

Mitotic Index - Male Oral Mucosa

Author and Year	Source of Material	Region	Age	Mitotic Index
Henry (1951)	Rabbits	Buccal mucosa	3.5 months	5.1
Halberg (1954)	Rats	Retromolar epithelium	5 months	11.0
Muhlemann (1954)	Rats	Retromolar epithelium	5 months	4.4
Muhlemann (1955)	Rats	Interdental epithelium and Epithelial attachment	5 months	39.81
Marwah (1956)	Human	Attached gingival epithelium	25-35 yrs. 50-78 yrs.	0.98 1.56

C. Factors Influencing Mitotic Activity

Among the factors which will effect the daily variation in mitotic activity are:

1) physical stimulation; Carleton (1933) found the continuous exposure of animals to light caused an alteration in the rhythmical periodicity of mitosis.

2) hormonal: Bullough (1946) presented evidence that the estrene levels in the female mouse affected the renewal of epidermal cells.

3) temperature; Bullough (1949) concluded that mitotic activity is high during lowered temperatures and low during higher temperatures.

4) blood sugar levels; Bullough (1949) observed the existence of a direct relationship between mitotic activity and blood sugar levels.

5) glucose oxidation; Bullough (1951) concluded that mitotic activity could be increased by stimulating the glucose oxidation. In order for this action to be effective it had to occur just prior to prophase.

6) stress; Bullough (1952) demonstrated stress as an influencing factor in mitotic activity. The glucocorticoid hormones play a role in the anti-mitotic mechanisms while testosterone was seen to induce mitotic activity.

7) age; Marwah (1956) reported a marked increase in mitoses in older human gingival epithelium. On the other hand, Kiljuner (1956) reported the mitotic index in young rat epidermis to be higher than in older ones.

D. Action Of Oxygen On Epithelial Tissues

Rothmann (1954) believes there is evidence that the main steps in energy metabolism in the skin proceed as in other tissues, namely, by glycolysis of carbohydrates; oxidation of the products of glycolysis, fats, and amino acids via the Krebs citric acid cycle; and, finally a transfer of electrons through the cytochrome system to molecular oxygen.

The cellular portion of epithelium, exclusive of the inert stratum corneum, is more active in the utilization of oxygen than other tissues.

Orban (1942) using a repeated application of 30% hydrogen peroxide, which in turn produced monomeric oxygen, was able to produce significant changes on human gingival epithelium. The basal cell layer was found to form from four to six layers of cells with a remarkable increase in mitoses. He attributed an epithelial proliferation and an intensified hornification to the great increase in mitotic activity. In addition a marked decrease in the inflammatory cell infiltration occurred.

Barron et al. (1948) reported the oxygen uptake for fetal skin to be greater than that for human adult skin. There is also a marked decrease in glycolysis of the adult skin.

In 1949 Bullough reported the relationship between the epidermal mitotic activity and the blood sugar level of the mouse. He found an increase in epidermal glycogen content during sleep, when the mitotic rate increases. With the onset of sleep, glucose is deposited in the tissue in the form of glycogen. He considers the glucose, or glycogen, to be the critical substance

effecting the mitotic activity in the epidermis of the adult mouse and human.

Glickman (1950) discouraged the assumption that the artificial introduction of oxygen would appreciably hasten the normal cellular processes in the course of gingival healing.

Bullough (1951) investigated the relationship of mitotic activity and oxygen tension. The number of mitosis can be increased by stimulating the rate of energy production from glucose oxidation. This has its effect immediately prior to the prophase (antephase) which appears to be a critical time in the histo-physiology of mitosis.

Bullough (1952) reported mitotic activity as being related to some stage of carbohydrate metabolism. Thus, carbohydrate supply and utilization (oxidation) are the relative important factors. Tissues having a daily mitotic cycle (e.g. epithelium) are at the lower end of a priority scale in glucose supply and utilisation. Thus, these tissues are normally "half-starved" in spite of being constantly profused with the glucose of the blood stream.

Holman (1957) feels that agents which inactivate catalase or produce hydrogen peroxide should have a detrimental effect on tumors.

CHAPTER III

MATERIAL AND METHODS

The area selected for a study of the mitotic activity of normal oral epithelium, prior to and after treatment with a 30% hydrogen peroxide solution, was the interdental gingival epithelium. Plates I, II, III, IV. This portion of the masticatory oral mucosa was selected for its ease of biopsy and because other workers, Marwah et al (1956), had previously established a mitotic index for this tissue. The investigation of normal interdental gingiva also permitted an independent evaluation of the mitotic index in different age groups.

Only human adult males were utilized since the hormonal influence of the menstrual cycle, in females, could add a variable influencing the rate of mitosis.

The biopsy contributors were 30 healthy students and faculty members of the dental school. Fifteen individuals, ranging in age from 22 to 27 years, comprised the younger age group; fifteen, ranging in age from 48 to 63 years comprised the older age group.

A biopsy was taken from each individual, before the experimental treatment, in order to establish the norms of mitotic activity. Each donor received applications of a neutralized 30% hydrogen peroxide solution (Superoxol-Merck) three times per week for a period of 30 days. The agent was applied directly to the same interdental papillae by use of a small cotton pellet held in a cotton pliers. This was allowed to remain for 1 minute and then irrigated well

with plain tap water. This particular time interval was previously ascertained through pilot studies. Previous pilot studies of normal human gingiva were performed in order to determine: the best biopsy technique; the fixative agent; the best stain technique; the effect of single and multiple application of the 30% hydrogen peroxide solution; the optimal dosage of the solution; and the best time for biopsy of the experimental specimens. The 30 day interval was selected because of its apparent optimal mitotic activity.

Biopsy technique and specimen preparation: All the biopsies were taken between 11:30 A.M. and 12:30 P.M. in order to minimize any variation in mitotic counts which may be due to a time factor.

The which was exposed to the 30% hydrogen peroxide was biopsied 24 hours after the last application. These specimens will later be referred to as the "oxygenated experimental group" in contrast to the "normal control" group.

In preparation for biospy, the alveolar mucosa was infiltrated with a local anesthetic agent. The interdental papillae between the lower premolars and molars were excised (Plate V). The tissue was washed in room temperature tap water and immediately fixed in Zenker-Formalin solution for 8 hours. The specimens were then washed in cold tap water for 24 hours. The tissue was embedded in paraffin, sectioned at 6 microns and stained with hematoxylin and eosin. The sections were cut at right angles to the surface of the epithelium.

Method of analysis: One section of each specimen was selected at random. Tracings of microscopic projections from each specimen, magnified 100 times, were made. The exact magnification was determined each time by

projecting a stage micrometer to verify the measurement. The boundry between the free gingiva and attached interdental gingiva was demarcated. The attached interdental gingiva, selected for study, was free of inflammatory cells (Plate VI). The attached interdental gingiva was examined microscopically (high dry 500-X) for the presence of epithelial cells in the active process of mitosis. The mitotic figures were located on the magnified tracings and their phase, location and plane of division recorded in chart form.

Measurement of cell populations: Utilising a surface planimeter, calibrated in square centimeters, the total surface area of epithelium was measured. A small area of epithelium was designated on the tracing as the sample area. This sample area was measured and the total number of cells within its boundaries were counted with the aid of a hand tally mechanical counter. From this, the total number of epithelial cells in the entire attached gingiva of the specimen was computed.

Mitotic index: The mitotic index, expressed as the number of counted mitotic figures per 1000 cells, was computed as a simple proportion utilising the estimated total cell population and the total mitotic count.

In addition each specimen was examined for the degree of hornification, suprapapillary thickness of epithelium, and height of the basal cells and the presence or absence of a granular cell layer. The number of cells in the layers of the stratum spinosum was also recorded. All measurements were taken by using a disc micrometer.

Phases of mitosis: Identification of the stages of mitosis was based on established standards (De Robertis, Nowinski, and Saez 1949).

1. Prophase was identified by an increase in the size of the cell, an increased visibility of chromatin threads, its tendency to assume a spherical form and an increased basophila of the nucleus (Plate VII).
2. Metaphase was recognised by the absence of a nuclear membrane and the arrangement of chromosomes in an equatorial plane (Plate VIII).
3. Anaphase was characterised by a divergence of chromosomes from the equatorial plate in the undivided cell body (Plate IX).
4. Telophase was recognised by the constriction or division of the cell body, into two daughter cells (Plate X).

CHAPTER IV

FINDINGS

A. Mitotic Index

Control biopsy specimens. The mitotic index (MI) represents the number of observed mitoses per thousand cells in the total epithelium. The average mitotic index for the young age group (25 years) was 0.79, and the average for the old age group (56 years) was 1.69. Thus, the average mitotic index was approximately twice as high in the group of old individuals.

The difference between the two age groups in the distribution of mitotic indices are further demonstrated in Plate XV. All the mitotic indices are divided into three large divisions. Those ranging from 0 to 0.5 were found in 6 of the 15 young age specimens and 1 of the 15 old age specimens. Indices ranging from 0.5 to 1.5 were found in 7 of the young age and 7 of the old age groups respectively. An index above 1.5 occurred in 2 young age and 7 of the old age group. From this it is seen that the most striking contrast lies in the higher incidence of a mitotic index of from 0 to 0.5 in the young group, as opposed to the greater frequency of a mitotic index above 1.5 in the older age group.

Experimental biopsy specimens. After treatment with 30% hydrogen peroxide the fifteen young individuals average mitotic index increased to 6.50. In the old age group the index increased to 8.76. The mitotic index in the young age group had increased from 0.79 to 6.50, approximately eight times.

In the old age group the mitotic index increased from 1.69 to 8.76, or about five times. See Plate XVI.

The mitotic index of the young age group ranged from 1.4 to 22.3 and the old group from 4.6 to 15.7. Once again the mitotic indices are divided into three large divisions. Those ranging from 0 to 5.0 were found in 8 of the 15 young age specimens and 3 of the 15 old age specimens. Indices ranging from 5.0 to 10.0 were found in 4 of the young age specimens and 7 of the 15 old age specimens. An index above 10.0 was found in 3 of the young age and 5 of the old age group. See Plate XVII.

B. Cell Density and Its Relation to Mitotic Index

Control biopsy specimens. In Plate XVIII, the cell densities are plotted against age and the mitotic index. The number of cells per $(100\ u)^2$, averaged 62 for the young age group and 70 for the older age group. There is no direct correlation between cell density and mitotic index. See Table VI.

Experimental biopsy specimens. The number of cells per $(100\ u)^2$ averaged 58 for the young age group and 65 for the old group. The overall cell density in the experimental specimens is seen to decrease.

TABLE VI

Cell Density and Mitotic Index

Group		Average Cell Density (100 u)	Average Mitotic Index	
			Above Average Cell Density	Below Average Cell Density
Control Group	Young Age	62	0.71	0.48
	Old Age	70	1.70	1.69
Experimental Group	Young Age	58	4.24	9.89
	Old Age	65	7.82	10.15

C. Differential Counts of Phases in Mitosis

Control biopsy specimens. The frequency distribution of the various phases of mitosis in the control specimens is tabulated in Tables VII and VIII.

The respective equivalent proportion of cells in prophase, metaphase, anaphase and telaphase is as follows:

Young age 2:3:2:4

Old age 4:-2:2:3

This can be compared with the experimental group below.

Experimental biopsy specimens. The distribution of the various phases of mitosis in the experimental specimens is tabulated in Tables VII and VIII.

The respective equivalent proportion of cells in prophase, metaphase, anaphase and telaphase are as follows:

Young age 10:-6:2.5:1

Old age 11:6:1:2

This can be compared with the control group above.

TABLE VII

Per Cent of Mitotic Phases

Group		Total No. of Mitosis	Prophase	Metaphase	Anaphase	Telophase
Control Group	1. Young Age	50	18%	28%	18%	36%
	2. Old Age	150	37%	16%	18%	29%
Experimental Group	3. Young Age	439	40%	25%	11%	23%
	4. Old Age	456	56%	28%	5%	11%

A total comparative proportion of the above per centages would be as follows:

1. 2 : 3 : 2 : 4
2. 4 : -2 : 2 : 3
3. 10 : -6 : 2 : 1
4. 11 : 6 : 1 : 2

TABLE VIII

Phases of Mitosis

Group		Total No. of Mitosis	Prophase	Metaphase E	Metaphase L	Anaphase E	Anaphase L	Telophase E	Telophase L
Control Group	Young Age	50	9	9	5	5	4	6	12
	Old Age	150	55	21	3	18	10	17	26
Experimental Group	Young Age	439	174	64	45	33	16	22	85
	Old Age	456	256	114	14	12	11	7	42

E - Early stage of the given phase

L - Late stage of the given phase

D. Location of Cells in Mitosis and Their Respective Plane of Division

Control biopsy specimens. Mitosis occurred either in the basal cell layer or in the deeper layers of the stratum spinosum. Almost one-half of the total number of mitotic figures in either age group were in the basal cell layers and the other half in the stratum spinosum.

An attempt was made to determine the plane of division of mitosis in relation to the basement membrane. Since it is difficult to ascertain the plane of division of cells in prophase and early metaphase, they were eliminated from this tabulation.

Sixty-five per cent of the two recorded phases were perpendicular to the basement membrane. The remaining mitosis, totaling thirty-five per cent, were dividing in a parallel plane. There was no significant difference between the two age groups. These figures are a combined sample for the cells undergoing division in both the basal and prickle cell layers. See Table IX.

Since some investigators believe that the basal cell layer is autonomous and reproduces only itself, particular attention was given to the plane of mitotic division in the basal cell layer. Therefore, in addition to the aforementioned tabulation, the planes of division were "cross-matched" with the respective cell layer in which they were located. Table X.

Experimental biopsy specimens. There was no significant difference in the two age groups. Fifty-five per cent of the total number of mitotic figures observed in both groups were in the basal cell layer and forty-five

per cent in the prickle cell layer. Sixty-three per cent were perpendicular, and thirty-seven per cent were parallel to the basement membrane.

Despite the great increase in mitotic index, the ration of the planes of division is almost the same in the control and experimental groups. See Tables IX and X.

TABLE IX

Location and Planes of Mitosis

Group		Total No. of Mitosis	Location of Mitosis		Plane of Mitosis	
			Basal Cell Layer	Prickle Cell Layer	Perpendicular	Parallel
Control Group	Young Age	50	48%	52%	61%	39%
	Old Age	150	51%	49%	68%	32%
	Average Both Groups	200	49.5%	50.5%	65%	35%
Experimental Group	Young Age	439	54%	46%	56%	44%
	Old Age	456	55%	45%	69%	31%
	Average Both Groups	895	54.5%	45.5%	62.5%	37.5%

TABLE X

Plane of Mitosis and Respective Layer Location

Group		Cell Layer	Plane of Mitosis to Basement Membrane	
			% Perpendicular	% Parallel
Control Group	Young Age	Basal Cell Layer	29.6%	18.5%
		Prickle Cell Layer	29.6%	22.2%
	Old Age	Basal Cell Layer	33.9%	14.1%
		Prickle Cell Layer	29.5%	22.4%
Experimental Group	Young Age	Basal Cell Layer	34.6%	22.4%
		Prickle Cell Layer	21.1%	21.8%
	Old Age	Basal Cell Layer	44.6%	22.5%
		Prickle Cell Layer	22.2%	9.6%

E. Degree of Keratinisation, Suprapapillary Epithelial Thickness, Height of Basal Layer and Incidence of Granular Layer.

Control biopsy specimens. Of thirty cases, a cornified layer was present in twenty-nine. In different areas of the same specimens the cornified seam ranged in thickness from 3.2 to 17.0 microns. There was some apparent variance in thickness of the keratin layer between the young and old age groups. The thickness of the keratin layer in the young age group ranged from 3.2 to 16.0 microns, with an average of 8.16. In contrast to this the old age group ranged from 6.0 to 17.0 microns and averaged 14.10. There was no apparent correlation between the degree of cornified layer and the mitotic index. Table XI and XII.

The suprapapillary epithelial width measurements were recorded. This measurement was difficult because of the uncertainty in the tissue specimen definitely being cut at an absolute right angle to the surface. In order to lessen the errors in this measurement, specimens with a tangential section were eliminated. Three readings were taken for each specimen and these were averaged. The measurements were taken from the tip of the papillae to the base of the keratin layer. The number of cell layers in the young age group ranged from 11 to 16 with an average of 12.9 rows. In the old age group these values ranged from 10 to 15 cell layers with an average of 13.1. The thickness of the young age group ranged from 70.4 u. to 125.6 u. with an average of 106.0 u. The old age group ranged from 76.8 u. to 160.0 u. and averaged 126.7 u. Table XI.

A final measurement was made of the height in a single row of basal cells.

There was no significant difference in the young and old age group. In the young age group the height of the cells varied from 4.0 u. to 9.6 u., and averaged 6.38 u. In the old age group the range was from 6.4 to 9.6 and averaged 6.81 microns. Table XI.

From the total number of thirty specimens, fifteen showed cells containing keratohyaline. If a granular layer was present its thickness varied from 1 to 3 cell rows. on an average those cases without the granular layer showed a higher mitotic index. In this respect both the young and old aged groups behaved the same. The young age group without a granular layer had an average mitotic index of 1.29 and that with a granular layer, a mitotic index of 0.41. In the old age group, those without a granular layer had a mitotic index of 2.14 and with a granular layer a mitotic index of 0.88.

Experimental biopsy specimens. A hornified layer was observed in 29 of the 30 specimens examined. The degree of cornification varied, but appeared to be somewhat more compact than in the normal specimens. The thickness of the keratin layer in the young age group ranged from 3.0 to 19.2 microns with an average of 11.9. The old age group ranged from 3.2 to 25.6 microns and averaged 14.4. Table XI and XII.

The suprapapillary epithelial width was ascertained in the same manner as before. The number of cell layers in the young age group ranged from 9 to 17 with an average of 14.3 rows. In the old age group these values ranged from 10 to 21 with an average of 13.1 cell rows. The thickness of the young age group ranged from 80.0 u. to 144.0 u. and averaged 124.4 u. The old age

group ranged from 76.8 u. to 144.0 u. and averaged 121.5 u. Table XI.

The height of the basal cell layer once again showed no difference between young and old age groups. However, there is a marked contrast between the basal cells of the control and experimental groups. In the experimental specimens the basal cell layer is more "columnar" in appearance, well demarcated and the pedicles more prominent. In the young age group the height of the cells ranged from 6.0 to 12.8 microns and averaged 10.1. The old age group ranged from 6.4 to 15.0 and averaged 10.1 microns. Table XI.

The granular cell layer was also ascertained from this group of specimens. When the granular layer was present it was seen to be much clearer and easier to identify in comparison to the normal specimens and its thickness varied from 1 to 4 cell layers. Once again it was seen that the specimens without a granular layer had a higher mitotic index. The young group without a granular layer had an average mitotic index of 8.0 and those with a granular layer a mitotic index of 3.6. In the old age group, those without the granular layer, a mitotic index of 8.67.

TABLE XI

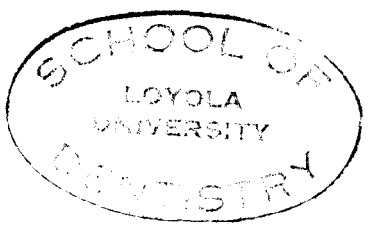
Measurements of
Keratin Layer, Suprapapillary Epithelium and Basal Cell Layer

Group		Keratin Layer Thickness (microns)		Suprapapillary Epithelial Thickness				Basal Cell Layer Height (microns)	
		Range	Average	Range Cell Layers	Average Cell Layers	Range (u.)	Average (u.)	Range (u.)	Average (u.)
Control Group	Young Age	0-16	8.16	11-16	12.9	70-125	106	4-9	6.38
	Old Age	6-17	14.10	10-15	13.1	76-160	111	6-9	6.81
Experimental Group	Young Age	3-19	11.90	9-17	14.3	80-144	124	6-12	10.10
	Old Age	0-25	14.40	10-21	13.1	76-144	121	6-15	10.10

TABLE XII

Keratin Layer and Mitotic Index

Group		Average Keratin Layer Thickness (microns)	Average Mitotic Index	
			Above Average Keratin Layer	Below Average Keratin Layer
Control Group	Young Age	8.16	0.58	0.86
	Old Age	14.10	1.85	1.51
Experimental Group	Young Age	11.90	4.69	11.70
	Old Age	14.40	9.11	8.35



CHAPTER V

DISCUSSION

A. Possible Mechanism of Hydrogen Peroxide Action

The induced increase of the mitotic index, following prolonged exposure to 30% hydrogen peroxide, appeared in both extremes of age. In an attempt to explain the greatly increased mitotic index, there are several points which we must take into consideration.

The first viewpoint which one might obviously consider is that the increased mitotic index is a true expression of a proportional increase in mitotic activity. However, the increased mitotic index need not represent an associated increased mitotic activity, but possibly a retardation or arrest of mitosis similar to that produced by colchicine. In addition to the two aforementioned viewpoints, the increased mitotic index may be due to a relative increase in mitoses and associated with a more prolonged mitotic period in one of the phases.

From the viewpoints which have thus far been expressed, the latter appears to be the more likely phenomenon. This statement is based on two observations which may possibly be the key to the mechanisms involved. The first observation is based upon a clinical finding of the tissue specimen before biopsy. When this tissue was first exposed to the 30% hydrogen peroxide there was extreme "blanching" and for a prolonged period. Following repeated exposure, and at the end of the 30 day period, the tissue did not blanch easily nor as intensely as the earlier applications. In addition to

this clinical phenomenon, we observe that the mitotic index does not show a dramatic increase until a lag period of three weeks after the initial hydrogen peroxide application.

The possible mechanisms to explain the above mentioned phenomenon may lie in alterations of the normal tissue metabolic processes. If two hydrogen atoms combine with an oxygen molecule, in normal tissue respiration, the product is not water but hydrogen peroxide (Gerard, 1949). The hydrogen peroxide is a strong oxidizing agent and in any quantity is very poisonous to cells. In accord with this, there are two enzymes (oxidases) which are responsible for the breakdown of the possible toxic accumulation of hydrogen peroxide in animal tissue. These are catalase and peroxidase.

Tissue catalases function appears to be in the splitting of hydrogen peroxide, which might otherwise accumulate during biological oxidation and be toxic to living cells. Catalase will aid in the breakdown of hydrogen peroxide to yield gaseous oxygen (Kleiner, 1951):

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2 \quad \text{(molecular oxygen)}$$

Peroxidase, if present, will act upon hydrogen peroxide and organic peroxides to yield an active oxygen which in turn oxidizes the substrate. These are usually phenols.

$$H_2O_2 \xrightarrow{\text{peroxidase}} H_2O + O \quad \text{(atomic oxygen)}$$

$$M + O \quad MO \quad \text{(metabolite)}$$

It is further hypothesized that cells in the area of prolonged hydrogen peroxide exposure have lessened or exhausted their ability to produce sufficient

catalase, thus the clinically observed phenomenon of less "blanching" in the later days of hydrogen peroxide exposure. This may be due to a diminished breakdown of hydrogen peroxide, thus some unknown quantity of hydrogen peroxide would be able to enter the tissue and produce a possible poisoning of the cells.

An increased mitotic activity, to compensate for the increased loss of the poisoned cells, may well be the explanation for a true proportional increase in mitotic index. On the other hand a "colchicine like" action of the hydrogen peroxide may have occurred with only a relative increase in mitoses and a prolonged period in one of the phases.

The latter viewpoint gains credence when we review the work on the action of hydrogen peroxide in controlling tumorous growths. This phenomenon of retarding cell growth with the use of peroxide has been used experimentally in the treatment of adenocarcinoma (Holman, 1957). Numerous animals and some humans have already been treated with marked results.

Holman feels that agents which inactivate catalase or produce hydrogen peroxide should have a detrimental effect on tumors. In essence this same thing is thought to occur in the use of radiation therapy. Ionizing radiations have long been known to destroy malignant cells, and this may be explained as being due to the production in the presence of oxygen in fluid systems in vivo, of hydrogen peroxide, the free powerful oxidising radicals HO_2, HO and atomic oxygen as well as the destruction of the enzyme catalase. It is a known fact that tumor cells are low in catalase, and thus the ability of the

peroxides to enter the tissue and retard their uncontrolled growth (Greenstein, 1941).

In various experiments (Holcroft 1952, Makino 1953, Holman 1957) hydrogen peroxide was administered by intraperitoneal injection, intravenous routes, or simply placing small quantities of hydrogen peroxide in drinking water in order to control tumorous growths. Holman feels that the only effective way to destroy the malignant cells which are already deficient in catalase and sensitive to over-oxidation is to keep up a continued administration of an active oxidizing agent. Hydrogen peroxide is the oxidising agent of choice, it is an excellent ionising solvent, and is obviously of great importance in most living cells.

Loiselaur (1953) has pursued a series of experiments in which he demonstrates that the effects of irradiation result from primary radioactivation of the molecular oxygen found in solution in the irradiated tissues.

Horgan et al. (1957) showed two types of reactions with various organic peroxides. On a basis of total peroxide content, autoxidized linoleic acid was most toxic by intraperitoneal injection than any of the simple peroxides. They found evidence consistent with the view that radiation toxicity is due to initiation of chain autoxidation of essential fatty acids producing lethal doses of peroxides.

Anderson (1957) showed that cells are more sensitive to irradiation during mitosis. The most sensitive stage of mitosis appears to be prophase or late preditotic phase. If cells are irradiated at this time they show a

delay or inhibition of mitosis.

B. Mitotic Index

In the normal specimens the mitotic index of the young age group was approximately doubled in the old age group. The increase was from 0.79 to 1.69. Marwah et al. (1956) state that this finding would seem to contradict the common concept of ageing, according to which bodily processes slow down and the regenerative capacity of the tissues diminishes with age.

When normal tissue of a similar age range was subjected to 30% hydrogen peroxide for a period of 30 days, the mitotic index of both age groups was increased. The young age group had an eightfold increase in its mitotic index in going from 0.79 to 6.50. The old age group had a fivefold increase in going from 1.69 to 8.80. From these figures we can see that the old age group did not show the same proportional response as the young group.

If our hypothesis, as stated in the initial discussion, is true in regard to a "colchicine like" action of hydrogen peroxide then the above findings would seem to contradict Marwah's previous statement. Since the increase in the mitotic index was in proportion greater in the young than in the old, this would seem to indicate a greater number of cells entering mitosis in the young when both are under the same circumstances, or a less damaging effect of hydrogen peroxide in the old.

From this "colchicine like" effect we can also see that the use of mitotic index as a measuring device is only a relative mechanism. Therefore, we feel that mitotic index as a tool is only as important as the set of cir-

cumstances under which it is utilized and evaluated.

C. Cell Density

The density of the cell population of the old age group as compared with the young was slightly greater in both the control and experimental specimens. In comparing the control and experimental specimens, the overall cell density in the latter group was seen to decline. The experimental group's cell density decrease could be attributed to a larger size of the cells and/or to a greater intercellular space. Due to the irregular shape of the prickle cells even more estimates of size would be a rather formidable task. The basal cells were seen to increase in size in the experimental group. Therefore, this could account for the overall decreased cell density and possibly the apparent decreased reproductive capacity of the experimental cells.

The above statements could once again support the hypothesis that there is a potential poisoning of cells occurring. The decreased number of cells and the increased size of the cells are more evidence to reaffirm the initial premise of a colchicine like action occurring from hydrogen peroxide.

D. Significance of Differential Counts of Phases in Mitosis

The incidence of the various phases of mitoses were somewhat altered with the differences in age and specimens. The most obvious alteration was in the greater incidence of prophase in both the young and old age experimental specimens.

This finding could add further impetus to the colchicine like action of

hydrogen peroxide. The prolonged exposure to hydrogen peroxide could be retarding or "holding" the cells undergoing mitosis in prophase.

E. Location of Mitosis and Plane of Division

Mitosis occurs mainly in the basal layer and the one immediately overlaying the basal layer, or the suprabasal area. Very few mitosis were located in the higher strata of the rete ridges.

The "waviness" of the junction of the lamina propria and epithelium renders it difficult to be sure if a given nucleus is in the basal or suprabasal layer. If a rete ridge is cut tangentially, cells which actually are in the basal layer may appear to be in much higher strata of the rete ridge. This error can only be avoided by comparing serial sections. Becker (1952) in support of his claim that all Langerhans cells are in the basal layer, using serial section, has limited the source of mitosis to this layer. One other aid is the difference in staining of the cytoplasm of basal cells which is more eosinophilic than the darker and definitely basophilic prickle cells.

Approximately fifty per cent of mitosis was in the basal layer. This is similar to the results of Pinkus (1952) and Rothman (1954) which were obtained with human skin. The oxygenated specimens had a slightly higher number of mitosis in the basal layer. However, the results were reasonably the same for the two variables. This result did vary a great deal with Marwah (1956). He had reported a mitosis location of 23 per cent in the basal layer and 47 per cent in the prickle layer of human attached gingiva.

In ascertaining the plane of cell division, only cells in anaphase and telophase were utilized. Many times it is difficult to determine the plane of cell division in prophase and metaphase, therefore they were eliminated. Approximately less than two-thirds of the cells were found to be dividing at a plane perpendicular to the basement membrane. Due to the many cells found in a relationship to the basal layer, it can definitely be said that the basal layer is one of the "contributing" factors to the mitosis in the suprabasal layer. It seems obvious that many of the daughter cells must have ascended into the suprabasal layer and may or may not have undergone further multiplication. This may give further light to Cowdry's (1944) concept of vegative and differentiating intermitotics.

The vast similarity of control and experimental groups planes of division, may once again indicate a potential "holding" mechanism being placed on the cells by the hydrogen peroxide.

F. Significance of Epithelium Size Changes and Granular Layer Differences

There was no apparent correlation between the degree of hornification and mitotic index. The most significant observation was in the greater thickness of the hornified seam with an increase in age. This was seen to once again increase in the oxygenated specimens. The hornified seams in the experimental specimen was somewhat more complete, uniform, and denser than that found under normal circumstances.

In one comparable case, the hornified seam was seen to double in the experimental specimens as compared to its counterpart in the control specimens.

The basal cell layer appeared to be rather constant in both the young and old age groups. The evident contrast existed between the control and experimental specimens. The size of the basal layer cells, on an average, increased by about 50 per cent in height. Thus we can say that there was a definite size increase of the basal cells in relationship to the use of hydrogen peroxide.

Upon calculating the mitotic index for tissue specimens with or without a granular layer, it was found to vary in each instance. In the absence of a granular cell layer the average mitotic index is usually seen to be greater than if a granular layer is present. However, too much emphasis could be placed on the relationship of increased mitosis and the absence of a granular layer. This conclusion has already been established by some authors, but only where the random sampling of specimens was used. By using random sampling of specimens it seems possible that the tissue as a whole may have a granular layer which may or may not be present in the sample at hand. Therefore, it seems to be an error to place a great deal of importance on the direct association of mitotic activity and granular layer.

The only real significant observation in the granular layer was in the oxygenated specimens. When the granular layer was present, the keratohyaline granules were much clearer, as were the granular layer cells themselves. In addition the granular cell layer appeared to be thicker.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to study the mitotic activity of normal human oral epithelium, and in addition the possible alteration in mitosis patterns when exposed to hydrogen peroxide.

The following were the major observations:

1. The epithelium of human gingiva was studied and mitotic indices established. In order to establish a control the normal gingiva of two age ranges was studied, a young age group (Av. 25 years) and an older age group (Av. 56 years). The mitotic index in the control specimens was 0.79 for the young age and 1.69 for the old age group.

2. The mitotic index was greatly altered upon exposing the two age groups to 30% hydrogen peroxide for 30 days. The mitotic index in the experimental young age group increased eight fold, from 0.79 to 6.50. The old age group increased by five fold, from 1.69 to 8.80.

3. The overall cell density of the experimental group was decreased when compared with that of the control group. However, in both groups the old age had a greater density than the young age specimens.

4. After single exposures of 30% hydrogen peroxide for a 30 day period, an apparent prolongation of the mitotic period occurred. This seemed to be more specific in prophase, thus the apparent increase in mitotic index was not necessarily a true reflection of the mitotic activity. Therefore, the increased mitotic index is only relative to the set of circumstances under which it is utilised and evaluated.

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VIII ILLUSTRATIONS

Plate I

Figure 1. Diagramatic illustration of the anatomic areas of the oral mucous membrane.

Figure 2. Area of the dentegingival junction.

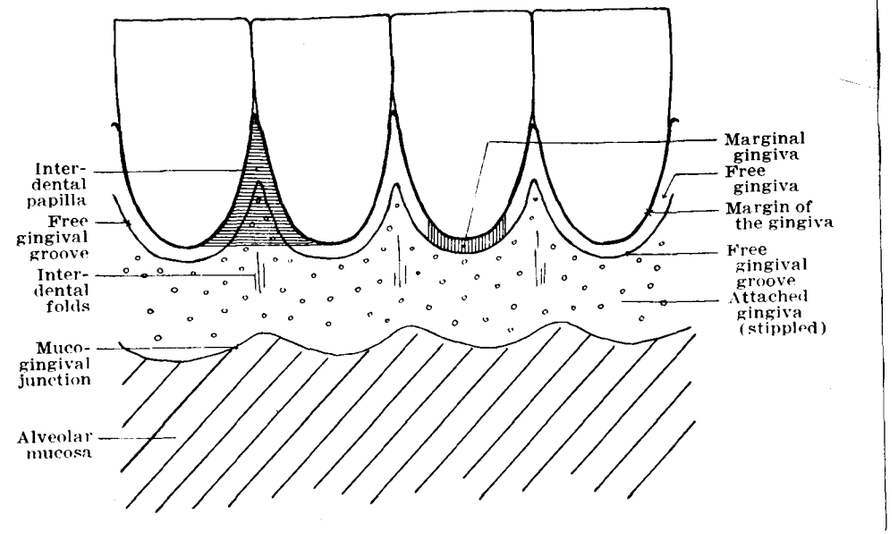

Figure 1

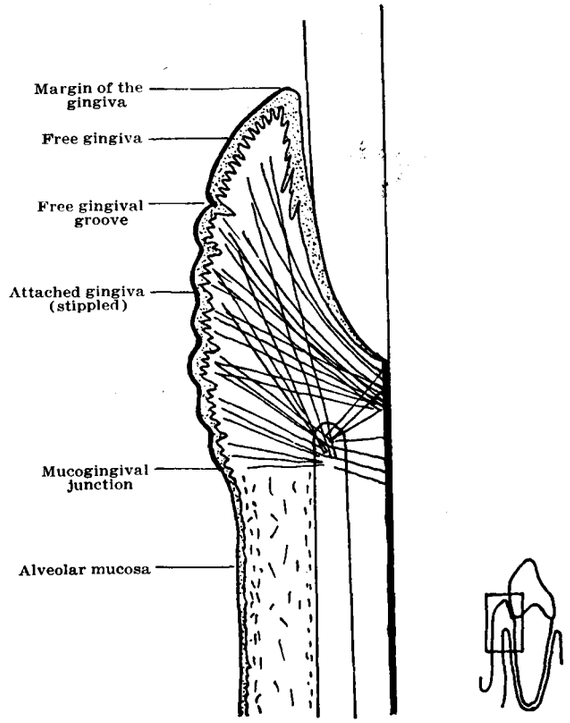

Figure 2

Plate II

Figure 1. Diagramatic illustration of the gingival oral mucous membrane in the region of the dento-gingival junction(gingiva).

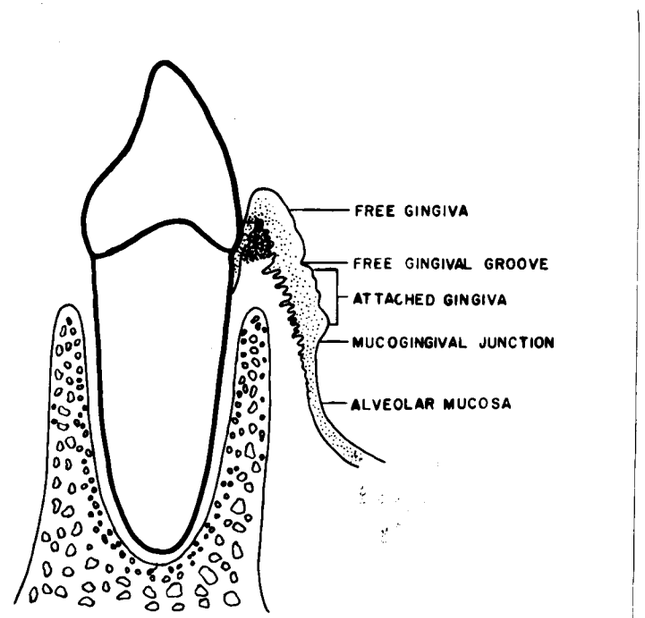

Figure 1

Plate III

Figure 1. Diagramatic histologic illustration of oral epithelium.

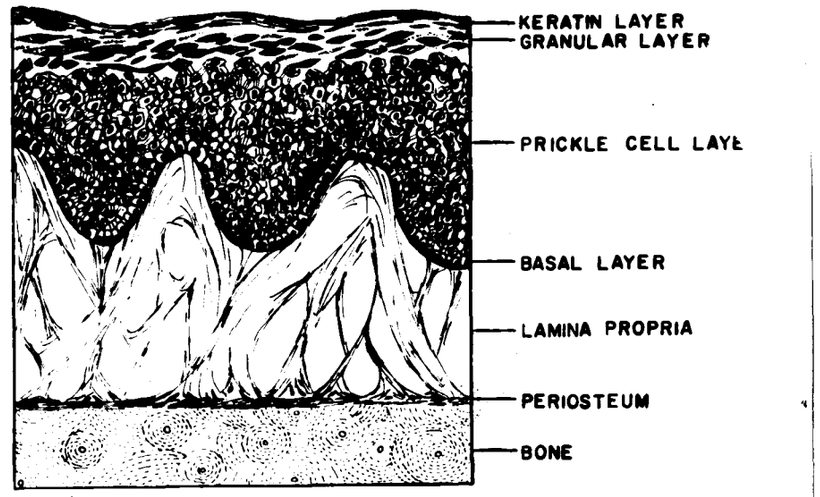

Figure 1

Plate IV

Figure 1. Photomicrograph of human attached gingival epithelium. X 325

Abbreviations:

A. - Keratin layer
B. - Granular cell layer
C. - Spinous cell layer
D. - Basal cell layer
E. - Underlying connective tissue of lamina propia.

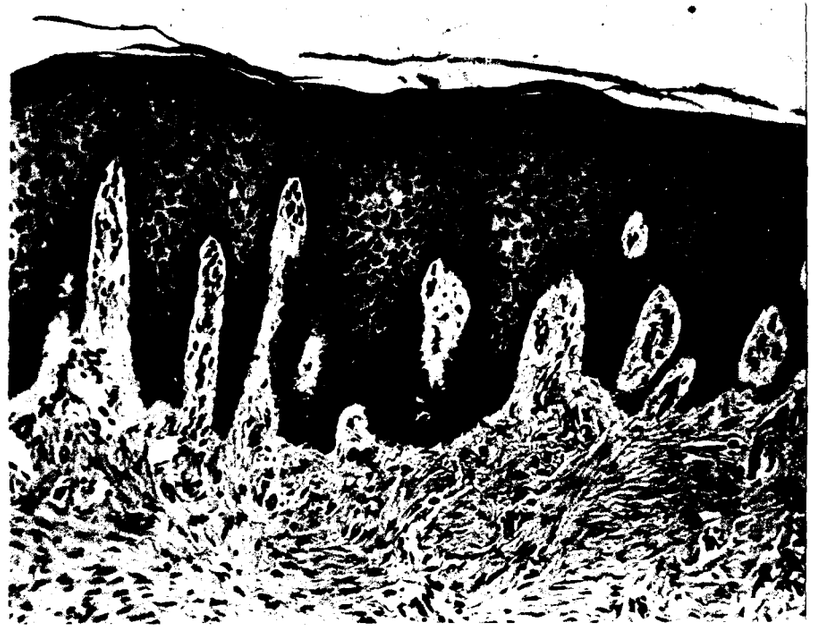

Figure 1

Plate V

Figure 1. Clinical illustration of the biopsy technique.

Figure 1

Plate VI

Figure 1. Photomicrograph of gingival biopsy specimen. X 25

Abbreviations:

A. - Free gingiva
B. - Attached gingiva
C. - Inflammatory cells in free gingiva
D. - Normal connective tissue of attached gingiva without inflammatory cells.

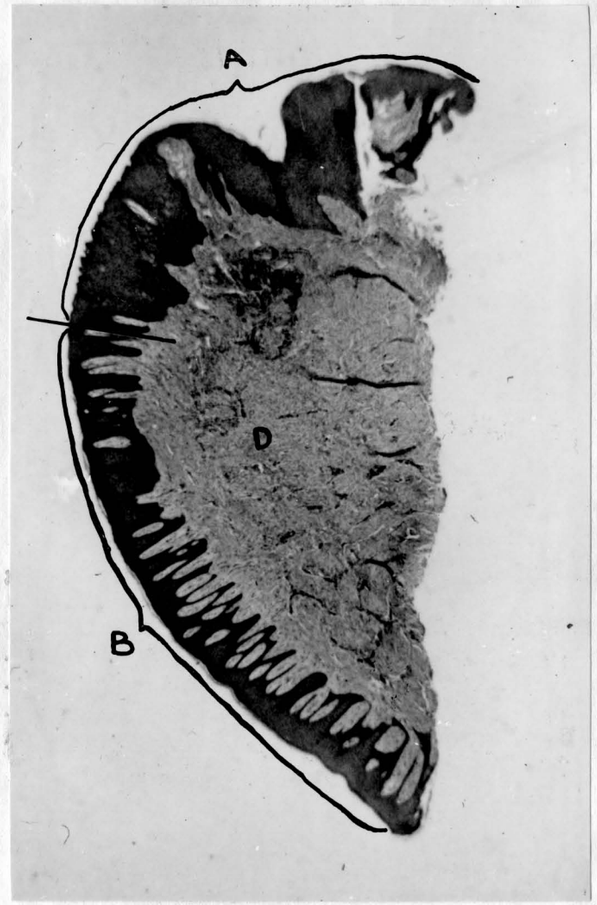

Figure 1

Plate VII

Figure 1. Photomicrograph of oral epitelium. x 500
Note cell in prophase at A.

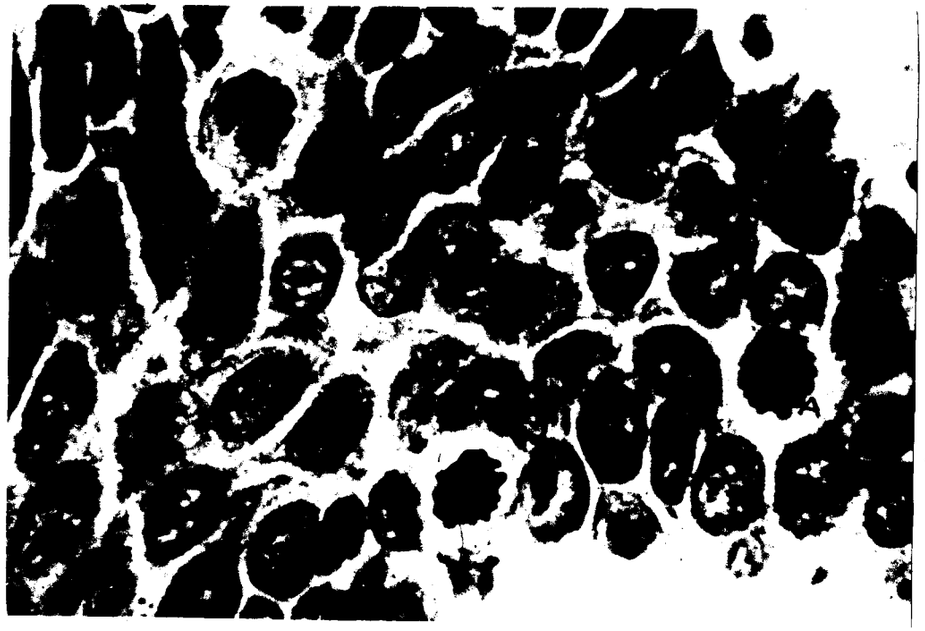

Figure 1

Plate VIII

Figure 1. Photomicrograph of oral epithelium. X 500
Note cell in metaphase.

Figure 1

Plate IX

Figure 1. Photomicrograph of oral epithelium. X 500
Note cells in anaphase.

Figure 1

Plate I

Figure 1. Photomicregraph of oral epithelium. X 500
Note cells in telophase. The intermediate body of Fleming is prominent.

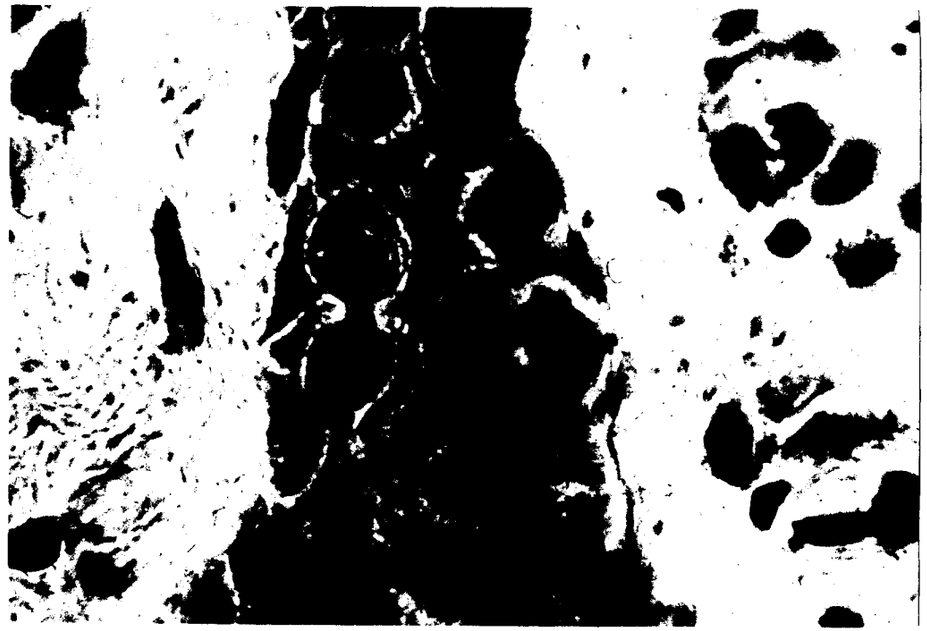

Figure 1

Plate XI

Figure 1. Photomicrograph of oral epithelium. X 250
Note the attached gingiva immediately after the application of 30% hydrogen peroxide. The oxygen bubbles are found almost entirely in the epithelium.

Abbreviations:
A. - Epithelium
B. - Underlying connective tissue
C. - Oxygen bubbles

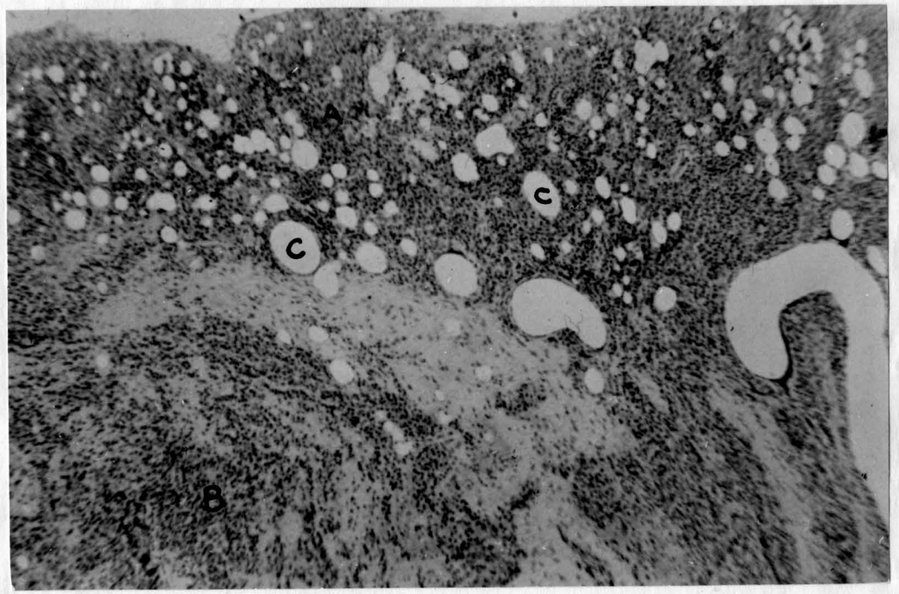

Figure 1

Plate XII

Figure 1. Photomicrograph of oral epithelium. X 250
Note attached gingiva 15 minutes after the application of 30% hydrogen peroxide.
The distension of the connective tissue by the oxygen is visible as "bubbles."

Abbreviation:
A. - Oxygen bubbles in connective tissue.

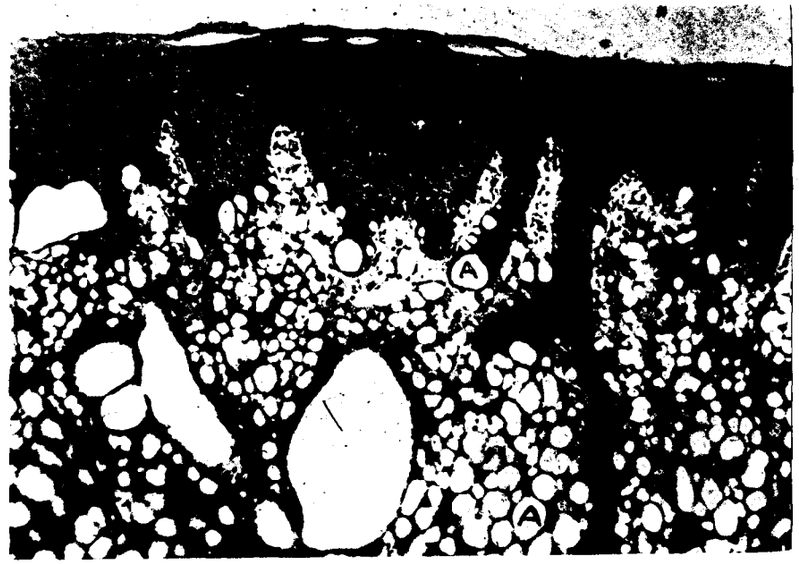

Figure 1

Plate XIII

Figure 1. Photomicrograph of oral epithelium. X 250
Note attached gingiva 30 minutes after the application of 30% hydrogen peroxide. Only a few oxygen bubbles remain in the underlying connective tissue. Shortly after, the tissue is completely void of the oxygen bubbles.

Abbreviation:
A. - Oxygen bubbles in connective tissue.

Figure 1

Plate XIV

Figure 1 and 2. Photomicrographs of oral epithelium. X 430
This tissue has been exposed to 30% hydrogen peroxide for 30 days. Note many cells in all phases of mitosis.

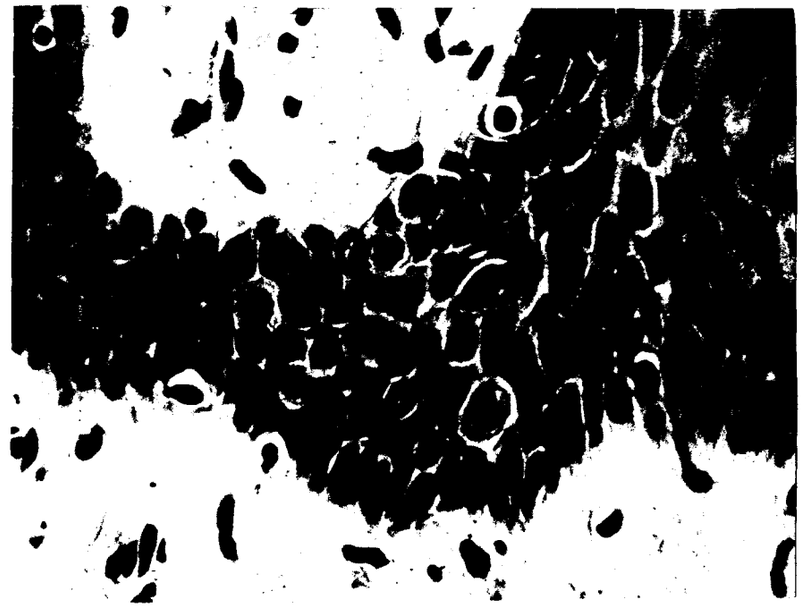

Figure 1

Figure 2

Plate XV

Figure 1. The range distribution of mitotic index in both age groups of the control specimens.

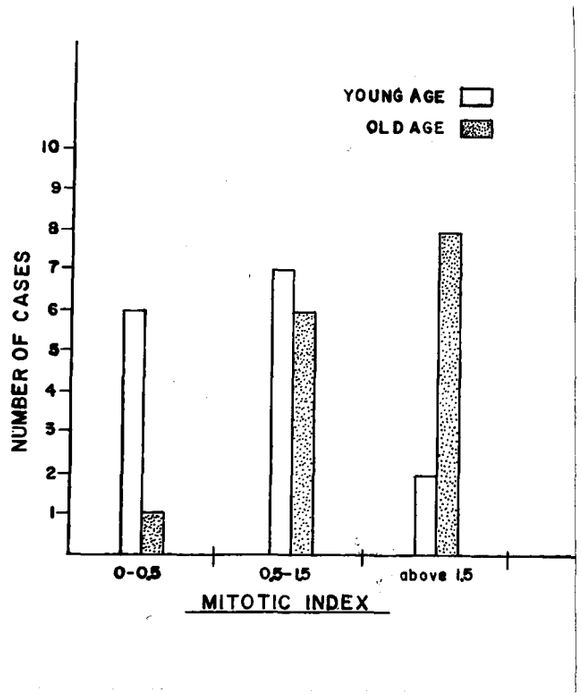

Figure 1

Plate XVI

Figure 1. The range distribution of mitoic index in both age groups of the experimental specimens.

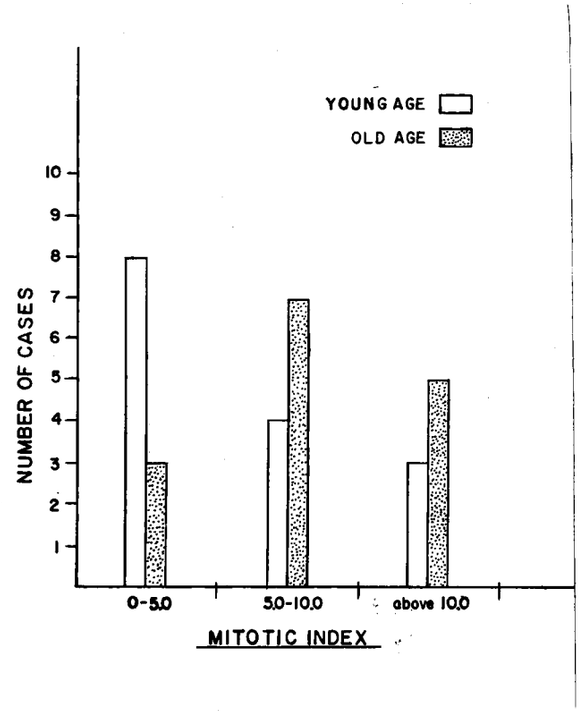

Figure 1

Plate XVII

Figure 1. Comparison of mitotic index in both age groups and specimens.

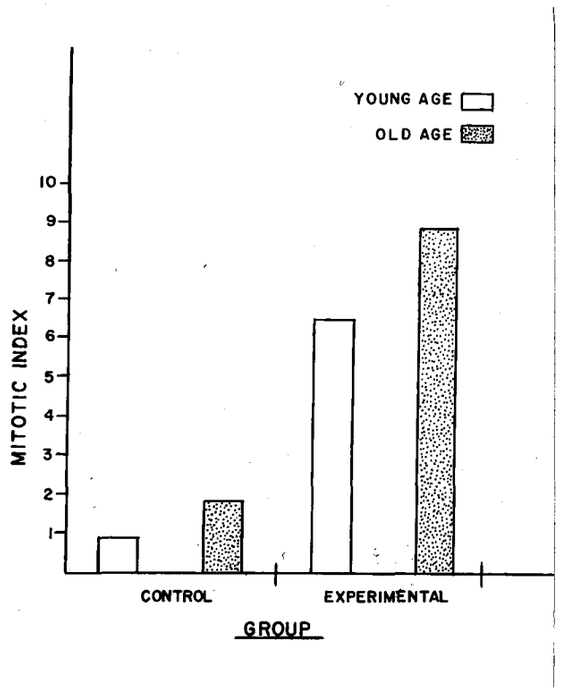

Figure 1.

Plate XVIII

Figure 1. Comparison of cell density in both age groups and specimens.

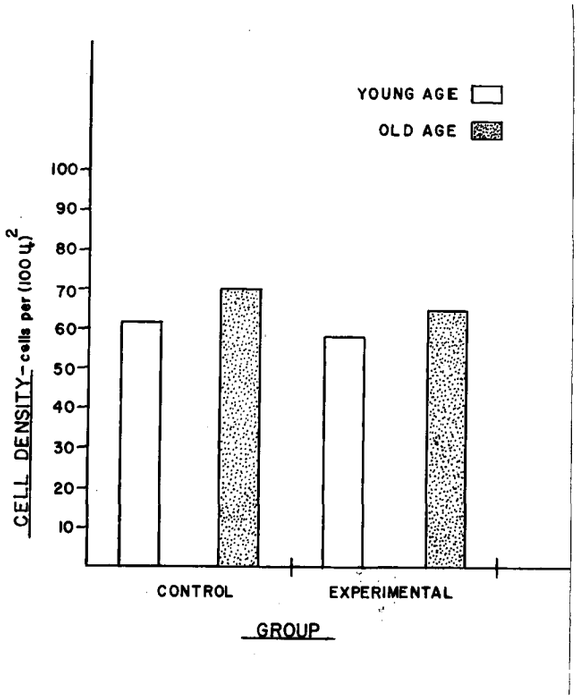

Figure 1

IX APPENDIX

Table A

Cell Densities

Area Measurements, Cell Counts-Total Cell Population, and Mitotic Index of Control Young Age Group

Specimen No.	Age	Planimeter Measurement in sq. cm.		Cell Count in Sample Area	No. of Cells Per $_2$ (100u.)	Computed No. Cells in Total Epith.	No. of Mitotic Figures	Mitotic Index
		Total Epithelium	Sample Area					
MC-1	22	8.09	0.48	316	66	5322	3	0.56
MC-2	22	8.29	0.44	383	87	7221	8	1.11
MD-1	23	4.80	0.61	405	66	3187	1	0.31
MD-2	23	11.66	0.87	387	44	5188	1	0.19
ME-1	23	9.74	0.56	305	55	5318	0	0.00
ME-2	23	7.40	0.78	417	53	3952	1	0.25
MF-1	23	6.03	0.44	286	65	3919	0	0.00
MF-2	23	5.91	0.59	332	56	3317	2	0.60
C-1	23	8.67	0.54	363	66	5031	9	2.01
MA-1	27	7.82	0.56	356	64	4973	2	0.40
MA-2	27	7.57	0.69	503	74	5587	4	0.72
MB-1	27	7.71	0.31	237	70	5582	4	0.68
MB-2	27	4.51	0.48	194	62	2819	5	1.80
A-1	27	13.23	0.58	393	68	8936	5	0.59
A-2	27	10.63	0.47	150	28	2947	5	1.40

Table B

Cell Densities

Area Measurements, Cell Counts-Total Cell Population, and Mitotic Index of Control Old Age Group

Specimen No.	Age	Planimeter Measurement in sq. cm.		Cell Count in Sample Area	No. of Cells Per cm^2 (100u)	Computed No. Cells in Total Epith.	No. of Mitotic Figures	Mitotic Index
		Total Epithelium	Sample Area					
MN-1	49	7.24	0.63	373	59	4286	4	0.93
MN-2	49	9.65	0.18	179	99	9592	6	0.62
MP-1	50	7.30	0.46	295	64	4679	9	1.90
MP-2	50	13.58	0.36	264	73	9895	7	0.70
R-1	53	15.73	0.34	142	84	13,502	10	0.74
MO-1	56	7.53	0.47	327	69	5218	8	1.50
MO-2	56	10.12	0.25	170	68	6868	3	0.44
MM-1	58	5.23	0.34	254	75	3907	3	0.76
MM-2	58	12.64	0.53	357	67	8479	8	0.95
Q-1	58	9.42	0.55	235	43	4022	10	2.40
Q-2	58	9.40	0.37	131	35	3328	8	2.30
ML-1	60	9.42	0.37	225	61	5727	18	3.10
ML-2	60	7.14	0.32	277	87	6176	18	2.90
MK-1	63	9.02	0.32	227	71	6395	23	3.40
MK-2	63	6.36	0.45	402	89	5679	16	2.80

Table C

Cell Densities

Area Measurements, Cell Counts-Total Cell Population, and Mitotic Index of Experimental Young Age Group

Specimen No.	Age	Planimeter Measurement in sq. cm.		Cell count in Sample Area	No. of Cells Per (100u)	Computed No. of Cells in Total Epith.	No. of Mitotic Figures	Mitotic Index
		Total Epithelium	Sample Area					
LO-31	22	4.71	0.49	243	50	2336	10	4.2
LO-32	22	5.18	0.59	460	78	4037	13	3.4
LO-41	23	7.35	0.52	406	69	5057	27	5.3
LO-42	23	10.24	0.41	208	51	5192	60	11.5
LO-44	23	6.89	0.35	215	61	4037	6	1.4
LO-51	23	5.49	0.44	180	41	2245	12	5.1
LO-54	23	7.11	0.35	215	61	4365	12	2.7
D-1	23	6.88	0.25	134	54	3687	36	9.8
D-2	23	10.54	0.22	85	39	4072	91	22.3
LO-20	27	3.99	0.34	221	65	2593	13	5.0
LO-21	27	8.82	0.40	253	63	5573	23	4.1
LO-22	27	9.95	0.45	282	63	6589	68	10.3
LO-23	27	5.72	0.32	197	62	3523	13	3.7
B-1	27	8.84	0.39	206	53	4667	30	6.4
B-2	27	5.80	0.44	287	65	3781	9	2.3

Table D

Cell Densities

Area Measurements, Cell Counts-Total Cell Population, and Mitotic Index of Experimental Old Age Group

Specimen No.	Age	Planimeter Measurement in sq. cm.		Cell Count in Sample Area	No. of Cells Per (100u)	Computed No. of Cells in Total Epith.	No. of Mitotic Figures	Mitotic Index
		Total Epithelium	Sample Area					
LO-D1	49	8.36	0.36	233	65	5399	35	6.5
LO-D2	49	6.61	0.52	424	82	5387	25	4.6
LO-D6	50	6.09	0.32	187	57	3477	17	4.9
LO-C2	50	7.48	0.43	172	40	2992	40	13.3
LO-C4	53	6.72	0.40	332	83	4977	34	6.9
LO-D3	56	7.79	0.55	236	43	3342	43	12.9
LO-D9	56	4.42	0.48	346	72	3187	22	6.9
LO-C4	58	3.81	0.39	270	69	2786	43	15.7
LO-C5	58	3.82	0.35	224	64	2445	35	14.2
LO-C8	58	4.71	0.46	322	70	3297	30	9.1
LO-C9	58	5.50	0.42	269	64	3520	47	13.4
LO-A6	60	5.30	0.56	335	59	3132	18	5.7
LO-C9	60	3.42	0.36	285	79	2705	19	7.0
LO-A1	63	5.91	0.45	314	70	4119	19	4.6
LO-A5	63	7.89	0.34	219	64	5073	29	5.7

Table E

Mitotic Index-Incidence of Granular Cell Layer; Measurement of Keratin Cell Layer, Suprapapillary Ridge and Basal Cell Layer Thickness in Control Young Age Group

Specimen No.	Age	Mitotic Index	Granular Cell Layer	Measurements of Epithelial Cell Layers in Microns		
				Keratin Cell Layer	Suprapapillary Ridge	Basal Cell Layer
MC-1	22	0.56	Inc. 1 to 2	9.5	118.4	6.0
MC-2	22	1.11	Inc. 1	6.4	105.6	4.8
MD-1	23	0.31	Inc. 1	4.8	108.8	7.0
MD-2	23	0.19	Inc. 1 to 3	9.6	102.4	6.4
ME-1	23	0.00	Inc. 1	16.0	112.0	6.4
ME-2	23	0.25	Inc. 1	11.2	105.6	5.7
MF-1	23	0.00	Inc. 1 to 2	3.2	176.0	9.5
MF-2	23	0.60	Inc. 1 to 2	6.4	105.6	6.4
C-1	23	2.01	None	0.0	125.6	6.4
MA-1	27	0.40	Inc. 1	12.8	70.4	6.4
MA-2	27	0.72	Inc. 1 to 2	11.2	96.0	9.6
MB-1	27	0.68	None	8.8	96.0	6.4
MB-2	27	1.80	None	8.3	124.8	6.4

Table F

Mitotic Index-Incidence of Granular Cell Layer; Measurement of Keratin Cell Layer, Suprapapillary Ridge and Basal Cell Layer Thickness in Control Old Age Group

Specimen No.	Age	Mitotic Index	Granular Cell Layer	Measurements of Epithelial Cell Layers in Microns		
				Keratin Cell Layer	Suprapapillary Ridge	Basal Cell Layer
MN-1	49	0.93	None	6.0	92.8	6.4
MN-2	49	0.62	None	9.6	115.2	6.4
MP-1	50	1.90	None	6.4	89.6	6.4
MP-2	50	0.70	None	4.0	128.0	6.4
R-1	53	0.74	Inc. 1	16.0	150.0	6.4
MO-1	56	1.50	Inc. 1 to 2	16.0	89.6	6.4
MO-2	56	0.44	Inc. 1	19.2	124.8	7.0
MM-1	58	0.76	Inc. 1	12.8	76.8	6.4
MM-2	58	0.95	Inc. 1	17.0	108.8	6.4
Q-1	58	2.40	None	16.2	144.0	7.4
Q-2	58	2.30	None	14.0	160.0	6.7
ML-1	60	3.10	None	7.0	108.8	16.0
ML-2	60	2.90	None	16.0	102.4	6.8
MK-1	63	3.40	None	12.8	99.2	9.6
MK-2	63	2.80	None	16.0	89.6	6.4

Table G

Mitotic Index-Incidence of Granular Cell Layer; Measurement of Keratin Cell Layer, Suprapapillary Ridge and Basal Cell Layer Thickness in Experimental Young Age Group

Specimen No.	Age	Mitotic Index	Granular Cell Layer	Measurements of Epithelial Cell Layers in Microns		
				Keratin Cell Layer	Suprapapillary Ridge	Basal Cell Layer
LG-31	22	4.2	None	16.0	144.0	9.6
LG-32	22	3.4	None	16.0	105.6	10.2
LG-41	23	5.3	None	16.0	144.0	9.6
LG-42	23	11.5	None	3.0	144.0	9.6
LG-44	23	1.4	Inc. 1 to 4	19.2	134.4	6.4
LG-51	23	5.1	Comp. 3	16.0	144.0	9.6
LG-54	23	2.7	Comp. 3	9.6	131.2	6.4
D-1	23	9.8	None	12.8	131.2	12.5
D-2	23	22.3	None	3.2	128.0	12.8
LG-20	27	5.0	Inc. 1 to 2	12.8	80.0	8.2
LG-21	27	4.1	None	12.8	112.0	12.8
LG-22	27	10.3	None	3.2	112.0	12.8
LG-23	27	3.7	Inc. 2 to 3	12.8	121.6	6.0
B-1	27	6.4	None	12.8	115.2	12.8
B-2	27	2.3	None	12.8	118.4	12.7

Table H

Mitotic Index-Incidence of Granular Cell Layer; Measurement of Keratin Cell Layer; Suprapapillary Ridge and Basal Cell Layer Thickness in Experimental Old Age Group

Specimen No.	Age	Mitotic Index	Granular Cell Layer	Measurements of Epithelial Cell Layers in Microns		
				Keratin Cell Layer	Suprapapillary Ridge	Basal Cell Layer
LG-D1	49	4.6	None	6.4	144.0	7.2
LG-D2	49	4.6	None	6.4	102.4	6.4
LG-D6	50	4.9	None	16.0	128.0	8.5
LG-G2	50	13.3	None	25.6	121.6	13.5
LG-G4	53	6.9	None	6.4	76.8	6.4
LG-D3	56	12.9	None	0.0	192.0	8.0
LG-D9	56	6.9	None	3.2	112.0	9.6
LG-C4	58	15.7	None	22.0	80.0	9.6
LG-C5	58	14.2	None	19.2	96.0	12.8
LG-C8	58	9.1	Inc. 1	12.8	128.0	9.6
LG-C9	58	13.4	Inc. 1 to 2	12.8	121.0	6.4
LG-A6	60	5.7	None	15.0	112.0	9.6
LG-G9	60	6.9	None	24.0	138.0	15.0
LG-A1	63	6.5	Inc. 2 to 3	22.4	144.0	12.8
LG-A5	63	5.7	Inc. 2	24.0	128.0	16.0

APPROVAL SHEET

The thesis submitted by Anthony W. Gargiulo 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 January 5, 1954

Frank M. Wentz, D.D.S., M.S., Ph.D.

Frank M Wentz

Signature of Adviser