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Mitotic Activity in the Oral Epithelium of the Human Female

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MITOTIC ACTIVITY IN THE ORAL EPITHELIUM OF THE HUMAN FEMALE

by

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LIFE

Joseph J. Krajewski was born March 2, 1932 in Port Chester, New York.

He moved to Toledo, Ohio and was graduated from Scott High School in 1949. From 1949 to 1952 he attended the University of Toledo.

In September, 1952 he entered the College of Dentistry at Ohio State University, Columbus, Ohio and received the degree of Doctor of Dental Surgery in June, 1956.

From 1956 to 1962 he served with the United States Army Dental Corps and engaged in private practice.

He entered Loyola University, Chicago College of Dentistry in July, 1962 for graduate work in the Department of Oral Biology. Clinical training was done in the Department of Periodontics under Dr. Anthony Gargiulo. He also participated in the United States Public Health Service Teaching-Research Training Program for two years under Dr. Harry Sicher.

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CHAPTER I

INTRODUCTION

Mitotic activity has been studied in experimental animals and the formulation of a mitotic index, the number of cells in mitosis per 1000 cells, has been determined for these animals under various environments.

Henry's findings (1952) in rabbit mitotic activity initiated the use of this experimental tool for work on the oral epithelium.

The work of Marwah (1956, 1960) established the mitotic index of the attached gingiva in the human male in different age groups (25 to 35, 50 to 75), the older group showing a significant increase in mitotic activity. The presence of inflammation increased the mitotic index.

Bullough (1943) reported changes in the epidermal mitotic index of female mice that were correlated with hormone changes in the estrus cycle.

This study will attempt to determine the mitotic index of the attached gingiva in the "normal" human female, age group 20 to 35.

The resultant index will be instrumental in establishing the female mitotic index for different age groups, during different periods in the menstrual cycle, and under varied environmental and pathological conditions.

CHAPTER II

REVIEW OF THE LITERATURE

The quantitative study of mitotic activity was initiated by Minot (1908) when he observed that there was a variation in the rate of mitotic activity in different tissues of the rabbit embryo. He was also the first to describe the mitotic index, the number of cells in mitosis per 1000 cells.

A. Mitotic Activity in Different Tissues

Human Studies

Thuringer (1924, 1928) recorded the earliest human mitotic counts. Using adult tissue he found one mitosis per 2414 cells (MI .41) on the scalp, one per 378,325 cells (MI .0026) on the skin of the leg, and one mitosis per 268,275 cells (MI .0037) on the ear.

Cooper and Schiff (1938) recorded a mitotic index of 4.6 on an 8 day human prepuce. In 1939 Cooper recorded a 3.8 mitotic index on the prepuce of a newborn.

In 1949 Andrew and Andrew while examining the adult anticubital region epidermis recorded a .13 mitotic index.

Katzberg (1952) found the abdominal skin index varied with the age, having a range of .23 to .76 .

Pinkus (1952) found a 1.59 index on the forearm flexor surface of a 46 year old human. Table I.

Animal Studies

Studies on easier to obtain animal specimens have been done on various tissues. These are summerized on Table II. The mitotic index was shown to vary with the animal, the area studied, and the age.

B. Mitotic Rhythm

The Fortuyn-van Leyden study in 1916 on various tissues of young kittens showed a variance in the rate of mitotic activity during different times of the day. Peak activity was recorded from 10:30 PM to 2:00 AM whereas 10:30 AM was found to be the time for minimal activity.

The time of peak mitotic activity is not standard for all animals or all tissues. Henry (1952) found the maximal activity in rabbit buccal mucosa occurring at 1:00 PM while the minimal occurred at 10:00 PM.

A recent study by Scheving (1957) on human epidermis has shown the peak activity to occur at 12:00 M (midnight) and 4:00 PM.

Findings are summarized on Table III.

TABLE I

Mitotic Index- Human

Age	Region	Mitotic Index	Author	Year
Adult	Epidermis-scalp	.41	Thuringer	1924
Adult	Epidermis-leg	.0026	Thuringer	1928
	-ear	.0037		
8 day	Epidermis-prepuce	4.6	Cooper and Schiff	1938
Newborn	Epidermis-prepuce	3.8	Cooper	1939
Adult	Epidermis-anticubital	.13	Andrew and Andrew	1949
Adult	Epidermis-abdomen	.23 to .76	Katzberg	1952
46 years	Epidermis-forearm	1.59	Pinkus	1952

TABLE II

Mitotic Index- Animals

Animal	Age	Region	Mitotic Index	Author	Year
Mice	1-7 days	Abdominal Skin	9.8	Carleton	1934
Mice	28 days	Abdominal Skin	0.8	Blumenfeld	1939
Mice	30 days	Ear Skin	1.4	Cooper and Franklin	1940
Mice	10 days	Foot Pad	25.2 Basal 27.0 Spinous	Cowdry and Thompson	1944
Mice	60 days	Interscapular Skin	2.0	Gluckman	1945
Mice	42-56 days	Ear Skin	1.69	Knowlton and Hempelmann	1949
Mice	42-56 days	Ear Skin	0.75	Knowlton and Widner	1950
Cat	Adult	Foot Pad	2.37	Thuringer	1939
Rat	300 days	Abdominal Skin	0.58	Andrew and Andrew	1949
Rabbit	100 days	Oral Mucosa	5.1	Henry	1951

TABLE III

Mitotic Rhythm

Animal	Peak Mitotic Activity	Author	Year
Cat	10:30 PM to 2:00 AM	Fortuyn-van Leyden	1916
Rabbit	1:00 PM	Henry	1952
Human	12:00 M and 4:00 PM	Scheving	1957

J. Factors Influencing Mitotic Activity

Extrinsic and intrinsic environmental factors have been shown to influence the rate of mitotic activity.

Carleton (1934), when examining the effect of light upon plant growth, observed that cell division progressed at a greater rate in the dark. Continuous exposure to light suppressed cell division.

Blumenfeld (1939), in a study of mitosis in the epidermis of the albino rat, speculated that the early morning increase was related to a high body temperature at that time. In 1943 he showed that regenerating epidermis had double the amount of activity that was present in normal tissue.

Injections of colchicine, which depressed mitosis, were first used in mice by Cowdry and Thompson in 1944. Bullough, W. in 1947 found that colchcine stopped mitosis at metaphase.

Bullough, W. (1949) found mouse ear epidermis to have a higher mitotic index in the first fourth of life, a decrease in the second fourth, an increase in the third or middle age period, and finally a decrease in old age.

Bullough, W. (1952) found the following factors increased the mitotic index in animals:

1. sleep or rest
2. increased blood sugar level
3. increased intracellular oxygen tension
4. injection of starch in saline
5. injection of starch and phosphates

6. injection of sugar and phosphates

7. injection of glycogen

8. injection of testosterone and estrogen

He found that the following factors produced a decrease:

1. less food

2. excessive muscular exercise

3. cold

4. injection of phloridzin (interferes with the process of phosphorylation)

5. injection of insulin into non-diabetics

6. decreased blood sugar level

7. castration

8. decreased intracellular oxygen tension

9. small amounts of iodoacetate and floride

10. active cell movement

11. cyanide (interferes with phosphorylation in the cytochrome system and prevents oxidation)

12. malonate (interferes with the oxidation of succinate to fumarate in the Krebs cycle)

13. increased osmotic pressure

14. mechanical injury

15. ultra-violet light

16. X ray and beta radiation

17. poisons of the tryptoflavine or radiomimetic group (disturbs nucleoprotien metabolism)

18. narcotics (interferes with dehydrogenase)

19. nitrogen mustard (interferes with hexogenase)

20. hydroquinone (interferes with glycolysis)

21. glucocorticoid hormones

22. cortisone (after estrogen)

Bullough, W.(1943,1947), in a study of female mouse epidermis, observed the greatest mitotic index in pro-estrus followed by a gradual decrease. The lowest mitotic index was recorded at the first day of diestrus.

The mitotic index was increased with the injection of estrogens. Prolonged or excessive injections had an opposite effect.

D. Studies of the Oral Mucosa

In 1952, Henry found a variation of mitotic rhythm in the mitotic activity of the rabbit oral mucosa. The greatest index, 7.2, was found at 1:00 PM, the least at 10:00 PM.

Mutley (1953) injected estrogen into young rats, young mice, and one year old mice, then observed the supporting structures of the molars. Old mice showed an initial proliferation of the gingival papillae. This proliferation soon subsided then pockets were formed.

Wentz (1952) showed no correlation with age or sex and the thickness of the oral epithelium. No mitotic counts were done.

Halberg (1954) showed an inhibition of mitotic activity in the connective tissue of the interdental papillae of rats which were treated with large doses of cortisone. A night high and day low in activity was

observed.

In 1954, Muhlemann found a night low and day high in retromolar pad mitotic counts in rats.

Mitotic activity of fibroblasts in the periodontal ligament space increased when abnormal force was exerted on the teeth (Macapanpan, 1954).

Marwah and Weinmann (1955) found a sex difference in oral epithelial cells in the human. Seventy-five per cent of females showed the presence of a nuclear sex particle in cells of the oral epithelium.

In 1956 they studied the attached gingiva in the human male and recorded a mitotic index of .98 in the 25 to 35 year old group as compared to 1.56 in the 50 to 78 year old group. Specimens were collected from 2:00 PM to 4:00 PM. There was no correlation with cell density or keratinization. Chronic inflammation (1960) showed an increase of mitotic activity in 50 per cent of the cases observed in both age groups.

Gargiulo's findings in 1961 supported the previously established male mitotic index for both age groups.

In 1963 Silberkweit studied inflammed gingiva and found a mitotic index of .514 in boys (age 5-13) and .575 in girls (age 5-13).

No studies have been done on the mitotic activity in the oral epithelium of the human female.

CHAPTER III

METHODS AND MATERIALS

Biopsy Procedure

The biopsy specimens for this study were obtained from the attached gingiva in areas which appeared to be clinically free from inflammation. The extent of the area used in most cases covered the attached and marginal gingiva of two teeth.

Twenty-four human females were selected for the study, each being in the age group of 20 to 35 years and 15 days from the onset of menstruation. All biopsies were obtained at a standard time (2:00 PM to 4:00 PM). Medical histories were obtained from each patient. Those having a history of recent pregnancy, treatment for gynecological problems, and under present medical treatment or using any medicaments were eliminated from the study.

A topical anesthetic, Ganaiden, was applied to the alveolar mucosa in the approximating area. Infiltration anesthesia (Xylocaine, 1/60,000 epinephrine) was used to prepare the area prior to incision.

The area selected was incised with an Orban gingivectomy knife. The tissue was then carefully removed from the teeth and washed in room temperature water to remove the blood. The specimen was immediately immersed in Zenker's formalin solution for fixation.

A periodontal surgical dressing was applied to the wound and allowed to remain in place for five days. Analgesics were not needed and not

used. No patient discomfort was observed or recorded.

Specimen Preparation

The tissue specimen was fixed in the Zenker's formalin solution for eight hours after which time it was washed in cold tap water for 24 hours. After embedding in paraffin, 6 micron sections were made which were cut at right angles to the surface of the specimen.

Staining was done with hemotoxylin and eosin.

Analysis Procedure

Random sections were selected from each specimen. The section was placed on a projection microscope, magnified 100 times, and a tracing made of the entire projected epithelium. Varification of the magnification was determined by projecting a disk micrometer.

The attached gingiva, free from inflammatory cells, was marked on the tracing. The area was examined microscopically (high dry 500x) for cells undergoing mitosis.

Cells undergoing mitotic division were recorded on the tracing. The phase of mitosis, plane of division, and location in the epithelium were recorded in chart form.

Phase of Mitosis: Established standards for phases of mitosis were used.

1. Prophase - the chromosomes become darkly stained and move to the middle of the cell, nuclear membrane loss
2. Metaphase- the chromosomes align themselves along the equatorial plate

3. Anaphase - the daughter chromosomes separate from one another

4. Telophase- the division of the cell body into two daughter cells

Plane of Division: The plane of division was recorded as either perpendicular or parallel to the basement membrane.

Location of Division in the Epithelial Layers: Each cell in mitosis was recorded in its relationship to the layers of the epithelium.

1. Basal cell layer
2. Spinous cell layer
3. Granular cell layer
4. Keratin layer

Cell Population: The total surface area of the attached gingiva was measured using a surface planimeter, calibrated in square centimeters. A small area was selected, measured, and the number of cells found in this sample area was recorded. By extrapolation the number of cells in the entire attached epithelium was determined.

Mitotic Index: The number of cells in mitosis per 1000 cells, the mitotic index, was determined through recording of mitotic cells versus total cell population in selected sample areas. The ratio of mitotic cells per 1000 cells was then mathematically determined.

Cell Densities: Sample areas were selected and the number of cells per 100 square micron areas were recorded.

Additional Recordings: The degree of keratinization, suprapapillary thickness of the epithelium, height of the basal cell layer, and the presence or absence of a granular cell layer were measured using a disc

micrometer.

CHAPTER IV

FINDINGS

A. Mitotic Index

The mitotic index (number of cells in mitosis per 1000 cells) was determined for each specimen utilizing the area of the attached gingiva.

The indices ranged from 0.12 to 1.48 and averaged 0.74.

The standard deviation (S.D.) is 0.151. Eleven (11) out of twenty-four (24) are within one standard deviation. Seventeen (17) out of twenty-four are within two standard deviations. See Table IV.

89,722 cells were observed for the presence of mitotic activity.

B. Cell Density

Cell density, the number of cells per $(100u)^2$, averaged 54 and varied from 31 to 89. See Table V.

C. Differential Counts of Phases in Mitosis

The phases of mitosis (prophase, metaphase, anaphase, and telophase) were tabulated as to their frequency of occurrence. The following proportion existed 5 : 3.5 : 2 : 2 .

A total of sixty-three (63) mitotic figures were recorded in this study. The percentage of mitotic phases was as follows:

Prophase	40%
Metaphase	29%
Anaphase	16%
Telophase	16%

TABLE IV

Mitotic Index

Specimen No.	Mitotic Index	Deviation
1.	0.69	- 0.05
2.	0.79	0.05
3.	1.16	0.42
4.	0.48	- 0.26
5.	0.73	- 0.01
6.	0.31	- 0.43
7.	0.52	- 0.22
8.	0.12	- 0.62
9.	0.52	- 0.22
10.	0.67	- 0.07
11.	0.86	0.12
12.	0.42	- 0.32
13.	1.19	0.45
14.	0.84	0.10
15.	1.30	0.56
16.	0.69	- 0.05
17.	0.75	0.01
18.	1.48	0.74

TABLE IV (cont.)

Mitotic Index

Specimen No.	Mitotic Index	Deviation
19.	0.75	0.01
20.	0.63	- 0.11
21.	0.91	0.23
22.	0.78	0.04
23.	0.57	- 0.17
24.	0.50	- 0.24

TABLE V

Cell Density

Specimen No.	Average Cell Density $(100u)^2$	Average Mitotic Index
1.	77	0.69
2.	89	0.79
3.	54	1.16
4.	38	0.48
5.	52	0.73
6.	42	0.31
7.	45	0.52
8.	76	0.12
9.	55	0.52
10.	31	0.67
11.	35	0.86
12.	46	0.42
13.	60	1.19
14.	41	0.84
15.	48	1.30
16.	54	0.69
17.	47	0.75
18.	56	1.48

TABLE V (cont.)

Cell Density

Specimen No.	Average Cell Density $(100u)^2$	Average Mitotic Index
19.	62	0.75
20.	55	0.65
21.	65	0.91
22.	59	0.78
23.	57	0.57
24.	62	0.50

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

Cells in mitosis were found in the basal cell layer and deeper layers of the stratum spinosum. A total of sixty-three (63) mitotic cells were recorded. Thirty-five (35) per cent were found in the basal layer and sixty-five (65) per cent in the lower prickle cell layers.

The plane of division was determined for cells in late metaphase, anaphase, and telophase. Plane determination is difficult to ascertain in prophase and early metaphase. They were not included in this part of the study.

Perpendicular division was noted in 18 cases (47%) ; parallel division in 20 cases (53%). See Tables VI, VII, VIII, IX, and X.

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

A keratin layer was present in all cases but two (92%). The width varied from 4.0 to 36.0 microns with an average of 13.41 microns. See Table XI.

The suprapapillary epithelial thickness varied from 54.0 to 324.0 microns, with an average of 155.79 microns.

The height of the basal layer averaged 7.99 microns and varied from 4.5 to 15.0 microns. See Table XI.

A granular cell layer was present in 14 specimens, 58%.

TABLE VI

Location of Mitosis

Specimen No.	Mitotic Index	Total No. of Cells in Mitosis	Location of Mitosis	
			Basal Cell Layer	Prickle Cell Layer
1.	0.69	5	2	3
2.	0.79	5	2	3
3.	1.16	5	2	3
4.	0.48	2		2
5.	0.73	2		2
6.	0.31	1		1
7.	0.52	2		2
8.	0.13	1	1	
9.	0.52	2	1	1
10.	0.67	1	1	
11.	0.86	3	1	2
12.	0.42	2	1	1
13.	1.19	2		2
14.	0.84	2	1	1
15.	1.30	4		4
16.	0.69	2	1	1
17.	0.75	2	2	
18.	1.48	5	2	3

TABLE VI (cont.)

Location of Mitosis

Specimen No.	Mitotic Index	Total No. of Cells in Mitosis	Location of Mitosis	
			Basal Cell Layer	Prickle Cell Layer
19.	0.75	3	1	2
20.	0.63	2	2	
21.	0.91	5	1	4
22.	0.78	3		3
23.	0.57	1		1
24.	0.50	1	1	

TABLE VII

Phase of Mitosis and Layer Location

Specimen No.	Mitotic Index	Basal Cell Layer			
		Prophase	Metaphase	Anaphase	Telophase
1.	0.69		2		
2.	0.79				2
3.	1.16		1	1	
4.	0.48				
5.	0.73				
6.	0.31				
7.	0.52				
8.	0.12	1			
9.	0.52	1			
10.	0.67	1			
11.	0.86			1	
12.	0.42	1			
13.	1.19				
14.	0.84	1			
15.	1.30				
16.	0.69		1		
17.	0.75		2		
18.	1.48	1	1		

TABLE VII (cont.)

Phase of Mitosis and Layer Location

Specimen No.	Mitotic Index	Basal Cell Layer			
		Prophase	Metaphase	Anaphase	Telophase
19.	0.75				1
20.	0.63			1	1
21.	0.91	1			
22.	0.78				
23.	0.57				
24.	0.50	1			

TABLE VIII

Phase of Mitosis and Layer Location

Specimen No.	Mitotic Index	Prickle Cell Layer			
		Prophase	Metaphase	Anaphase	Telophase
1.	0.69	2		1	
2.	0.79	1		1	1
3.	1.16		2	1	
4.	0.48	1	1		
5.	0.73	2			
6.	0.31	1			
7.	0.52	1			1
8.	0.12				
9.	0.52	1			
10.	0.67				
11.	0.86		1		1
12.	0.42			1	
13.	1.19	2			
14.	0.84				1
15.	1.30	4			
16.	0.69		1		
17.	0.75				
18.	1.48		2	1	

TABLE VIII (cont.)

Phase of Mitosis and Layer Location

Specimen No.	Mitotic Index	Prickle Cell Layer			
		Prophase	Metaphase	Anaphase	Telophase
19.	0.75	1			1
20.	0.63				
21.	0.91	1	2	1	
22.	0.78	1	1		1
23.	0.57			1	
24.	0.50				

TABLE IX

Plane of Mitosis

Specimen No.	Mitotic Index	Total No. of Cells in Mitosis	Plane of Mitosis	
			Perpendicular	Parallel
1.	0.69	5		3
2.	0.79	5	1	3
3.	1.16	5	4	1
4.	0.48	2		1
5.	0.73	2		
6.	0.31	1	1	
7.	0.58	2		1
8.	0.12	1		
9.	0.52	2		
10.	0.67	1		
11.	0.86	3	3	
12.	0.42	2	1	
13.	1.19	2		
14.	0.84	2		1
15.	1.30	4		
16.	0.69	2	2	
17.	0.73	2	1	1
18.	1.48	5	1	3

TABLE IX (cont.)

Plane of Mitosis

Specimen No.	Mitotic Index	Total No. of Cells in Mitosis	Plane of Mitosis	
			Perpendicular	Parallel
19.	0.75	3	1	1
20.	0.63	2		2
21.	0.91	5	1	2
22.	0.78	3	2	
23.	0.57	1		1
24.	0.50	1		

TABLE X

Plane of Mitosis and Layer Location

Specimen No.	Mitotic Index	Basal Cell Layer		Prickle Cell Layer	
		Perpendicular	Parallel	Perpendicular	Parallel
1.	0.69		2		1
2.	0.79		2	1	1
3.	1.16	2		2	1
4.	0.48				1
5.	0.73				
6.	0.31			1	
7.	0.52				1
8.	0.12				
9.	0.52				
10.	0.69				
11.	0.86	1		2	
12.	0.42			1	
13.	1.19				1
14.	0.84				
15.	1.30				
16.	0.69	1		1	
17.	0.75	1	1		
18.	1.48		1	1	1

TABLE X (cont.)

Plane of Mitosis and Layer Location

Specimen No.	Mitotic Index	Basal Cell Layer		Prickle Cell Layer	
		Perpendicular	Parallel	Perpendicular	Parallel
19.	0.75		1	1	
20.	0.63		2		
21.	0.91			1	2
22.	0.78			2	
23.	0.57				1
24.	0.50				

TABLE XI

Keratin and Mitotic Index

Specimen No.	Mitotic Index	Keratin Layer	
		Average Thickness (microns)	Range (microns)
1.	0.69	11.7	9.0-18.0
2.	0.79	10.8	4.5-27.0
3.	1.16	12.0	9.0-22.5
4.	0.48	10.3	9.0-13.5
5.	0.75	none	none
6.	0.31	none	none
7.	0.52	12.7	9.0-18.0
8.	0.12	10.3	9.0-13.5
9.	0.52	27.0	22.5-36.0
10.	0.67	27.4	22.5-36.0
11.	0.86	11.2	9.0-13.5
12.	0.42	9.4	9.0-13.5
13.	1.19	7.2	4.5-13.5
14.	0.84	11.7	9.0-13.5
15.	1.30	14.4	9.0-18.0
16.	0.69	13.5	9.0-18.0
17.	0.75	9.9	9.0-13.5
18.	1.48	9.9	9.0-13.5

TABLE XI (cont.)

Keratin and Mitotic Index

Specimen No.	Mitotic Index	Keratin Layer	
		Average Thickness (microns)	Range (microns)
19.	0.75	12.6	9.0-18.0
20.	0.63	12.6	9.0-18.0
21.	0.91	25.2	22.5-31.5
22.	0.78	25.2	22.5-31.5
23.	0.57	18.9	13.5-31.5
24.	0.50	18.0	13.5-22.5

TABLE XII

Mitotic Index; Measurement of Keratin Cell Layer Suprapapillary Ridge and Basal Cell Layer Thickness

Specimen No.	Mitotic Index	Measurements of Epithelial Cell Layers in Microns		
		Keratin Cell Layer	Suprapapillary Ridge	Basal Cell Layer
1.	0.69	11.7	270.3	10.2
2.	0.79	10.8	126.3	9.0
3.	1.16	12.0	185.4	7.2
4.	0.48	10.3	149.4	7.2
5.	0.73	none	165.6	7.2
6.	0.31	none	219.6	5.8
7.	0.52	12.7	147.6	8.1
8.	0.12	10.3	201.6	7.6
9.	0.52	27.0	225.0	7.6
10.	0.67	27.4	329.4	7.2
11.	0.86	11.2	100.6	7.0
12.	0.42	9.4	72.1	9.0
13.	1.19	7.2	122.4	7.2
14.	0.84	11.7	100.8	8.1
15.	1.30	14.4	153.0	9.0
16.	0.69	13.5	135.0	7.2

TABLE XII (cont.)

Mitotic Index; Measurement of Keratin Cell Layer, Suprapapillary Ridge and Basal Cell Layer Thickness

Specimen No.	Mitotic Index	Measurements of Epithelial Cell Layers in Microns		
		Keratin Cell Layer	Suprapapillary Ridge	Basal Cell Layer
17.	0.75	9.9	165.6	9.0
18.	1.48	9.9	144.0	9.9
19.	0.75	12.6	86.4	9.0
20.	0.63	12.5	84.6	8.1
21.	0.91	25.2	172.8	9.0
22.	0.78	25.2	156.6	8.1
23.	0.57	18.9	126.5	7.2
24.	0.50	18.0	117.0	7.2

CHAPTER V

DISCUSSION

This study has attempted to determine the mitotic index of normal human females in the age group of 20 to 35.

The mitotic index (the number of cells in mitosis per 1000 cells) has been used as an experimental tool to determine any changes in mitotic activity that might occur under different variables such as age, sex, and time of day.

The introduction of mitotic counts into studies of the oral epithelium by Henry in 1952 added further impetus to the role of cell division in the function of oral tissues.

The work of Marwah (1956) recorded the mitotic activity present in oral epithelium of human males. Silberkweit recently (1963) recorded the mitotic index of inflammed oral epithelium in boys and girls (age 5 to 13). A comparable study of the mitotic activity in the oral epithelium of the mature human female had not been conducted because of hormonal influences which might present standardization difficulties.

This study has endevered to eliminate many of the difficulties which may be present by standardizing the age, time of day, and period in the menstrual cycle.

Bullough's report (1943) of increased mitotic activity in mice at pro-estrus as a function of the production of progesterone

suggested that a comparable period in the human menstrual cycle be used in this study. As progesterone is not produced until puberty this may account for a lower mitotic index (.54) found in girls in the 5 to 13 year old group. This may also account for gingival proliferations common in pregnancy, a period of continued progesterone production. Orban's findings confirms the presence of increased mitotic activity in the gingiva of pregnant women.

Further studies during different times of the menstrual cycle and after menopause will help to establish the correlation of female hormones and the mitotic activity in the oral epithelium.

The mitotic index of 0.74 found in this study is significantly lower than the index of 0.98 (Marwah, 1956) found in human males of a similar age group. This signifies a definite sex difference in the mitotic index of each specimen. Eleven (11) out of twenty-four (24) indices were found to be within one standard deviation; seventeen (17) out of twenty-four (24) were within two standard deviations. This further substantiates the homogenicity of the group studied and would indicate that the mitotic index is a valid and valuable experimental tool.

A cell density of 54 cells per $(100u)^2$ recorded in this study is lower than found in comparable age group of males. The young age group recorded a cell density of 62 whereas the older male group showed an increase of cell density to 70 cells per $(100u)^2$.

This indicates that the individual cell size in this study is larger. No increase of size was observed in the intercellular spaces. The epithelial cells normally contain some glycogen. Possibly progesterone induces the storage of an abnormally high amount of intracellular glycogen. This is a normal finding of endometrium at this time.

A larger number of mitotic cells (78%) were located in the deeper layers of the prickle cell layer than were found in the studies of Pinkus (1952) and Rothman (1954) who found a 50% incidence of mitotic cells in the basal cell layer. These studies were conducted on human skin. Marwah's work (1956) on the oral epithelium agreed with the findings of Pinkus and Rothman.

The greater incidence of prophase (40% versus 25% found by Marwah in 1956) indicates that the subject is in a period of great mitotic activity initiation. This great incidence of prophase may be due to a daily cyclical event in women at the time of ovulation. Daily variations in mitotic activity occur in many species of animals and humans (Scheving, 1957).

The suprapapillary thickness of epithelium in the gingiva in this study varied over a wide range. A five fold difference in such thickness as recorded in this study points out that this measurement could not be accepted as a function of this period in the menstrual cycle. Marwah (1956) and Gargiulo (1961) measured suprapapillary thickness but did not discuss its significance.

The keratin layer thickness is similar to that reported by Marwah (1956) and Gargiulo (1961). This is an interesting observation as there is a significant difference in these studies and ours regarding the cell density and the mitotic index. The similarity in keratin formation arising from cells having a large volume but a reduced mitotic index suggests a difference in maturation.

This study indicates that a significant difference exists in mitotic activity in the oral epithelium of females in this age group and previously studied human males. Further studies will elaborate on the influence of hormones on mitotic activity in the oral epithelium.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Twenty-four clinically normal gingival biopsy specimens were analysed to determine the mitotic index of the attached gingiva in the human female.

Systemically normal biopsy subjects were selected as to age (20 to 35 years), period of the menstrual cycle (15 days after the onset of menstruation), and time of the day (2:00 P.M. to 4:00 P.M.).

Specimens were fixed in Zenker's formalin, embedded in parafin sectioned at 6 microns and stained with hemotoxylin and eosin.

Projection drawings and planimetric measurements were used to determine the mitotic index and cell density.

89,722 cells were observed and a mitotic index of 0.74 was found. A standard deviation of .151 was recorded.

Additional measurements were recorded.

An average cell density of 54 cells per $(100u)^2$ was found.

A differential count of mitotic phases was observed (prophase 65%, metaphase 29%, anaphase 16%, telophase 16%).

Cells in mitosis were located in the basal cell layer (35%) and deeper layers of the stratum spinosum (85%).

A keratin layer was present in 92% of the cases and averaged 13.41 microns.

The basal cell layer averaged 7.99 microns in height.

A granular cell layer was present in 14 cases (58%).

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VIII ILLUSTRATIONS

Plate I

Figure 1. Diagram of a biopsy specimen.

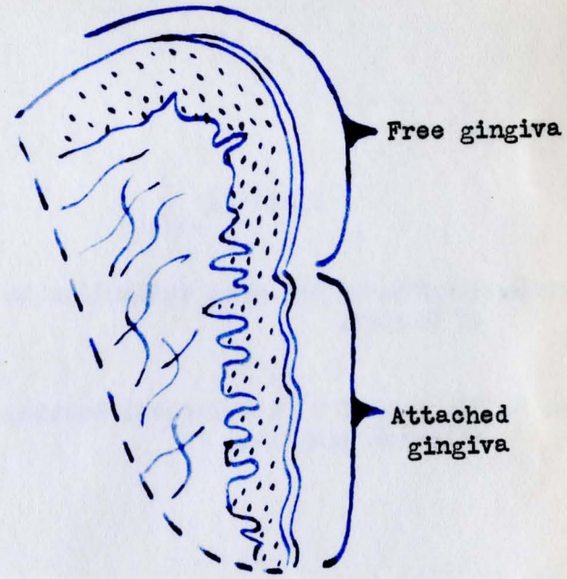

Figure 1

Plate II

Figure 1. Diagram of the oral epithelium in the area of biopsy.

Figure 2. Diagram of a histological section of the attached gingiva.

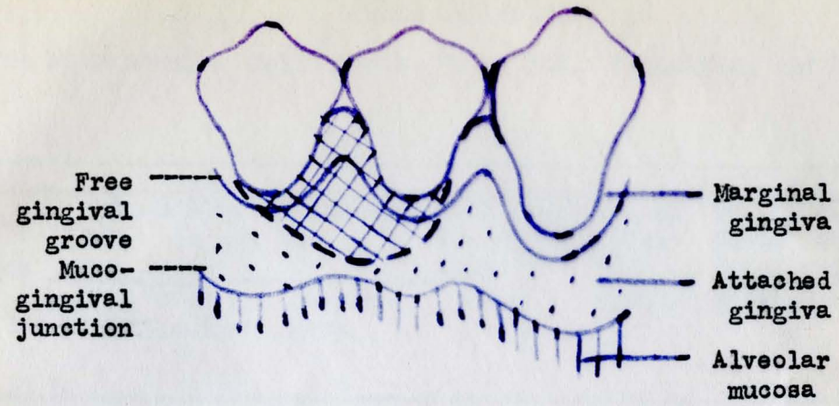

Figure 1

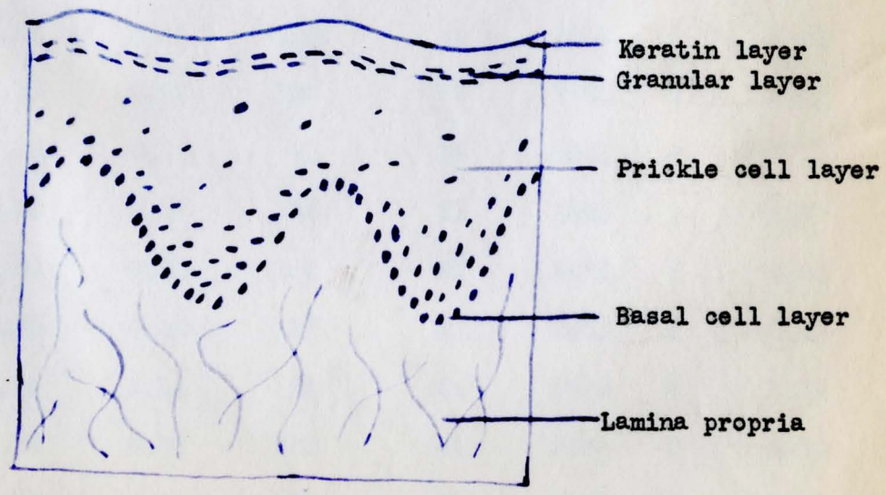

Figure 2

IX APPENDIX

TABLE A

Cell Densities

Area Measurements, Cell Counts, Total Cell Population, and Mitotic Index

Specimen No.	Age	Planimeter Measurement in sq. cm.		Cell count in sample area	No. of Cells Per $(100u)^2$	No, of Cells Total Epith.	No. of Mitotic Figures	Mitotic Index
		Total Epithelium	Sample Area					
1.	33	9.50	0.18	138	77	7283	5	0.69
2.	33	7.07	0.13	116	89	6309	5	0.79
3.	28	8.04	0.16	86	54	4321	5	1.16
4.	28	10.86	0.12	46	38	4163	2	0.48
5.	32	5.28	0.24	125	52	2750	2	0.73
6.	32	7.76	0.33	139	42	3269	1	0.31
7.	28	8.52	0.26	118	45	3866	2	0.52
8.	28	10.18	0.17	130	76	7785	1	0.12
9.	25	6.76	0.16	91	55	3851	2	0.52
10.	25	4.78	0.17	53	31	1490	1	0.67
11.	25	9.92	0.40	141	35	3472	3	0.86
12.	25	10.39	0.08	37	46	4805	2	0.42
13.	28	2.79	0.14	84	60	1674	2	1.19
14.	28	5.72	0.29	120	41	2367	2	0.84
15.	28	6.36	0.46	223	48	3083	4	1.30
16.	28	5.39	0.27	145	54	2892	2	0.69

TABLE 3 (cont.)

Measurements of

Keratin Layer, Suprapapillary Epithelium, and Basal Cell Layer

Specimen No.	Keratin Layer Thickness (microns)		Suprapapillary Epithelium Thickness				Basal Cell Layer Height (microns)	
	Range	Average	Range Cell Layers	Average Cell Layers	Range (u.)	Average (u.)	Range (u.)	Average (u.)
14.	9-13	11.7	6-8	7.0	90-126	100	4.5- 9.0	8.1
15.	9-18	14.4	8-18	12.4	108-216	153	9.0- 9.0	9.0
16.	9-18	13.5	8-10	9.2	72-180	135	4.5- 9.0	7.2
17.	9-13	9.9	9-10	9.3	126-180	165	9.0- 9.0	9.0
18.	9-13	9.9	7-12	10.2	90-216	144	9.0-13.5	9.9
19.	9-18	12.6	7-10	8.6	72-126	86	9.0- 9.0	9.0
20.	9-18	12.6	7-11	9.4	72-108	84	4.5- 9.0	8.1
21.	22-31	25.2	8-13	10.7	108-234	172	9.0- 9.0	9.0
22.	22-31	25.2	10-15	10.8	126-182	156	4.5- 9.0	8.1
23.	13-31	18.9	7-10	9.0	90-162	126	4.5- 9.0	7.2
24.	13-22	18.0	8-10	8.7	90-162	117	4.5- 9.0	7.2

APPROVAL SHEET

The thesis submitted by Joseph J. Krajewski has been read and approved by four members of the faculty of the Graduate School.

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

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

Date May 27, 1964

Anthony W. Gargiulo, D.D.S., M.S.

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Signature of Adviser