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Changes in the Maturation of Epithelial Cells in the Mucous Membrane of the Cheek After Therapeutic X-Ray Radiation in Patients with Head and Neck Carcinoma

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CHANGES IN THE MATURATION OF EPITHELIAL CELLS IN THE MUCOUS MEMBRANE OF THE CHEEK AFTER THERAPEUTIC X RAY RADIATION IN PATIENTS WITH HEAD AND NECK CARCINOMA

BY

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LIFE

Lawrence Philip Chase was born March 4, 1928 in Boston, Massachusetts.

In early years he attended Harwich Elementary Schools, Harwich High School and New Bedford Vocational High School. In 1946 he enlisted in the United States Navy, served two years of active duty and received an honorable discharge upon completion of his military service. He returned to Harwich High School and graduated in June of 1949.

In September of 1949 he enrolled in Boston College, the next year transferring to Tufts College where he received his Bachelor of Science Degree in 1953. He enrolled at Loyola University Dental School, Chicago College of Dental Surgery, in September of 1953. Four years later he graduated with the Degree of Doctor of Dental Surgery, in June of 1957.

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In May of 1958 he married Ellen I. Duddy in Maywood, Illinois. He is presently a member in the Dental Corp. of the United States Air Force Reserve--attached to the 640th

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He began graduate training in Oral Surgery in the Department of Oral Anatomy of the Graduate Division, Loyola University, in July of 1958.

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CHAPTER I

INTRODUCTION

During the treatment of head and neck cancer normal mucosa of the oral cavity has been subjected to X ray radiation. The clinical manifestations of the radiation reaction to skin is epidermitis, the reaction to the oral mucosa being epithelitis. Any change in the oral mucose before clinical manifestations appear is not known. This paper will report disturbances in maturation of oral buccal epithelium due to radiation by using the exfoliative cytological technique introduced by George N. Papanicolaou.

CHAPTER II

REVIEW OF THE LITERATURE

The literature reviewed for this thesis includes: (A) Exfoliative Cytology and its Applications; (B) The Keratinization Process; (C) General and Specific Staining Characteristics of Cells; (D) General and Specific Effects of Ionizing Radiation to the Human. An attempt will be made to discuss the four topics in the above order, however, a certain amount of overlapping should be anticipated by the reader.

A. EXFOLIATIVE CYTOLOGY

Montgomery (1951) conducted a study including 75 individuals with clinically healthy mouths. The group was composed of 25 children, 25 adults and 25 elderly individuals. Smears were taken from specific areas of the mouth. He made the classification of the cells taken in the smears as simple as possible by placing the cell types into three major catagories, blue, red and yellow, using Papanicolaou's technic. The blue cells were the non-keratinized cells, the red cells were the cells in varying stages of keratinization, and the yellow cells were the completely keratinized cells. Since there was oftentimes a mixture between the colors, cells were classified by

the color predominating in the cytoplasm. Variation was not only seen regarding staining characteristics of both nucleus and cytoplasm, but also in the shapes and sizes of the nuclei and cytoplasms. Montgomery's results indicated no particular correlation between age and sex, and the distribution of cell types in different parts of the mouth. He was able to combine the data in one table, showing mean percentage distribution of the blues, reds and yellows for each area of the mouth. Different areas of the mouth showed definite patterns of distribution. Montgomery's data regarding the cell distribution in the cheek shall be the base line in this study.

Montgomery's Findings for the Cheek:

	Blue Cells	Red Cells	Yellow Cells
% Distribution	51.5	43.8	4.7
Standard Deviation	26.6	23.8	12.3

Montgomery and Von Haam (1951) conducted a study of patients with carcinoma of the oral mucosa. The values Montgomery derived from his study on normal mucosa were compared with the values obtained from normal appearing areas in mouths of oral cancer and found no significant difference between the two. However, when they compared diseased areas

with normal areas a significant difference was found. All thirteen patients studied demonstrated significant differences between the diseased and normal areas and in all but two of them, the number of blue cells in the diseased areas was less than in the corresponding normal areas. The most frequent characteristic of malignancy in the oral cavity is an abnormally large nucleus and a disturbed nuclear-cytoplasmic ratio. Hyperchromatism was not as reliable in diagnosing a malignant lesion because of its frequent absence. A thickened nuclear membrane appeared less frequently than hyperchromatism. Abnormally large nucleoli were frequently found in the smears (13 out of 15). In the cytoplasm the most diagnostic sign of malignancy was an altered behavior in staining; cells that morphologically were identical with the blue cells stained differently, namely pink or orange.

In discussing the diagnostic value of intraoral cytology, Silverman, Becks and Farber (1958) noted some technical points of value. When taking oral smears one must remember that the smear taken could easily be contaminated by cells from other areas of the mouth. Also, once a smear is properly prepared and fixed, drying will not significantly alter the cellular detail when stained.

"In spite of inconsistencies, a relationship appeared to exist

between cytoplasmic cornification and local functional irritation...in accordance with Miller, Montgomery, Orban and Weinmann," The degree of cornification here was determined by cellular morphology as well as by the staining reaction. The immature basal cells are recognized as spherical cells with centrally placed nuclei which are consistently basophilic. The cytoplasm stains green or blue. In the process of keratinization the cells flatten, the cytoplasm becomes progressively transparent and the nucleus contracts to a small pyknotic mass of chromatin. Cornified cytoplasm is acidophilic and may stain pink, yellow or orange in a non specific fashion. Cells of intermediate maturity are termed precornified cells and are recognized as slightly flattened cells, and show some degree of nuclear contraction. Pre-cornified cells are usually basophilic, although cellular morphology is more reliable as a criterion of maturity than the staining reaction. No cellular or nuclear abnormalities were observed in the smears obtained from the series of patients who were considered to have normal mucosa. In general, lesions due to chronic irritation showed predominantly mature squamous cells, whereas smears taken adjacent to deep ulceration produced immature basal cells. The criterion for malignancy used by the authors was: 1. enlarged nuclei, 2. variation in nuclear size and shape, 3. increased nuclear-cytoplasmic ratio (nucleus increases in size with no

proportionate increase in cytoplasmic size), 4. multiple and prominent nucleoli, 5. hyperchromatism, 6. abnormal chromatin pattern and distribution, 7. discrepancy in maturation in groups of malignant cells.

Pomeranze and Stahl (1953) in supporting cytologic methods noted that in four positive diagnoses of malignancy by cytologic means, the biopsy reports had revealed no malignancy. This occurrence was explained by the fact that the smear included a greater area of the tumor and not merely a small piece of it as did the biopsy. They also noted that cytodiagnosis is an effective method for diagnosing cancer in its preinvasive stage.

Further efficacy of the oral smear was established in 1958 by Sandler and Stahl. They found agreement between smear and biopsy findings in 92% of 51 oral lesions studied. They cited two cases in which the smear and biopsy findings were not in agreement. The smears were positive for malignancy but the first biopsies were negative. When repeat biopsies were taken, the biopsies revealed positive findings for malignancy.

B. KERATINIZATION PROCESS

Weinmann (1940) observed that most of the studies concerning the mechanism of keratinization have been done on the skin.

Of the many investigator's works that Weinmann discussed, the opinions regarding keratinization were varied. Cowdry (1932) felt that the distance of the blood supply was a determining factor in the process of keratinization. It may be due to the lack of certain essential elements or an inability to eliminate waste products. Many of the following authors considered keratohyalin of the granular layer and elidin of the stratum lucidum play an important role in the formation of keratin. Mac Leod (1899) considered keratohyalin to be a separation product of cellular protoplasm and elidin a further product of keratohyalin. Mertschnigg, Posner, Selhorst etc. (1890) felt that keratohyalin was elicited from parts of the nucleus and Rabl (1897), Rosenstadt (1918), and Martinotti (1915) believed it was from cytoplasmic structure. However, there appeared to be relatively common agreement that the completely keratinized cell is devoid of a nucleus upon microscopic examination. Whether this apparent absence of a nucleus is due to an absolute absence or merely an inability to react to stain appears to be a point of conjecture. Waldeyer (1882) and Weidenreich (1901) considered keratinization a process of degeneration, while Hoepke (1927) and Patzelt (1926) believed that substances completely keratinized do not stain with Gentian violet, but substances in the process of keratiniza-

tion readily take on this stain. Weinmann (1940) in his study of the cells from the cheek found a few cells which were indicating degrees of keratinization, but did not find any completely keratinized. He felt that the epithelial cells in this area were sloughed off before they were able to produce keratin. Further in Weinmann's discussion he stated that cornification is a protective phenomenon from a teleological point of view. Another point which he brought out was the fact that keratinization may be explained as a reaction of squamous epithelium to different stimuli and irritations.

Pearse's (1951) difficulty in demonstrating cystine in other than hair was experienced also by Barnett and Seligman (1954) because they could not demonstrate disulfides in the stratum Malpighii. Barnett and Seligman (1954) found similarities in the sulfhydryl content of the stratum Malpighii of the buccal mucous membrane and the same stratum of the adjacent epidermis of the mucocutaneous junction. They were able to demonstrate sulfhydryl groups in varying amounts in all epithelia. It is interesting to note at this time that Eisen, Montagna, and Chase (1953) found that protein bound sulfhydryl groups are revealed in greatest abundance in tissues undergoing keratinization, particularly in parakeratotic regions.

Barnett (1953) felt that the sulfhydryl and disulfide containing proteins contributed to the architecture of certain epithelial cells as in keratin. He did not share completely the beliefs of Giroud and Leblond (1951) that keratinization involves the oxidation of sulfhydryl to sidulfide groups.

Glycogen appears to play a role in keratinization as hinted by Papanicolaou and Traut (1943). Montagna, Chase and Hamilton found that the glycogen content of a cell diminishes as keratinization progresses.

Orban (1953) described the buccal mucosa as being typically non-keratinized. The cells on the surface of this epithelium are being constantly desquamated.

Rothman's (1954) concepts of keratinization center around recent trends to study the keratinization process by cytochemical techniques. He states that the only consistent difference between keratin and cell protein is the higher content of cystine in keratin. The keratin cystine content is associated with a decrease in sulfhydryl containing amino acids. His observations are supported by Mirsky and Anson (1936)

> "In denatured but unhydrolyzed protein the number of sulfhydryl and disulfide groups detectable is equivalent to the quantity of cysteine and cystine found in the hydrolyzed protein. Mirsky and Anson 1934-35".

Pearse (1951) was cognizant of the search for a histochemical reaction for keratin. He knew that the sulfur contained in keratin is in the form of cystine, and that the disulfide bond of cystine is very reactive to oxidizing agents. Once oxidation takes place the reaction products can be identified. It seemed logical to him that the starting point would be in tissue sections oxidized by different reagents. Dempsey, Singer and Wislocki (1950) noted an increased basophilia of the tissue proteins after oxidation with periodic acid. The oxidation of sulfhydryl and disulfide groups into sulfonic acids and these being strongly dissociated caused an increased basophilia. This brought about the possibility of demonstrating oxidation products of cystine by means of dilute Methylene Blue at low pH levels. Pearse (1951) observed that the diagnosis of acidophyl proteins as found in keratin is difficult except in hair. He suggested some reasons for this difficulty. Structures other than hair contain less cystine, their sulfur is largely in the form of cysteine (sulfhydryl) and cysteine is much more easily oxidized than cystine.

C. GENERAL AND SPECIFIC STAINING CHARACTERISTICS OF CELLS

False staining reactions may be prevented by the fixative used. De Robertis, Nowinski and Saez (1948) stressed the importance of fixation to preserve the chemical and morphologi-

cal characteristics of tissue. Thus, the living characteristics may be studied at optimal accuracy. Fixatives cause a certain amount of shrinkage and one should always be cognizant of this fact so that interpretation of what is seen after fixation is not misleading. Ziskin, Kamen and Kittay (1941) stained their smears as did Weinmann (1940) with the Ernst-Gram stain, and determined varying degrees of keratinization by the ability of the structures to react to Gentian violet or Safranin. Papanicolaou, Traut and Marchetti (1948) pointed out that a poorly fixed specimen will present a distorted histological picture, Papanicolaou and Traut (1943) found greater transparency in the stained cells and good color differentiation by using stains in 95% alcohol, rather than using stains in an aqueous solution. This result was probably explained by the fact that the stains used have a low solubility in alcohol. While studying post-radiation smears, Papanicolaou and Traut (1943) found that the healed lesion did not shed malignant cells. From this fact they felt that post-radiation cases could be followed to see that elimination of the tumor had taken place. They saw that the vaginal epithelium under certain stimuli, such as radiation, showed potentialities for keratinization. In discovering the characteristics of exfoliated cells in the vaginal smears the

non-keratinizing cells are basophilic in their staining characteristics. A purplish color in the basal cells is due to the glycogen content of the cells. As the cells mature, their staining characteristics tend toward the pink or tan colors. These keratinized cells are strongly acidophilic and their nuclei have either disappeared or they are pyknotic. Their original nuclear size is usually indicated by a persistent perinuclear vacuole. The keratinized cells being strongly acidophilic, show a predisposition for the orange G stain.

The work of Papanicolaou (1942) described color of the keratinizing cells as varying from red to orange and the non-keratinized cells staining green or blue-green.

Montagna (1952) felt that the cells of the stratum germinativum are intensely basophilic when sections of skin are stained with basic dyes. The keratohyalin granules stain intensely, but the ground cytoplasm in this layer stains lightly. The stratum lucidum and stratum corneum stain weakly. It was presumed that this basophilia was due to ribonucleic acid. However, basophilia may be induced by oxidation of sections of skin. This basophilia may be attributed to the sulfur content of the proteins. Oxidation of disulfide in keratin and of sulfhydryl groups in the stratum germinativum might conceivably lead to formation of sulfonic acids, re-

sponsible for the induced basophilia. Ascorbic acid in the stratum germinativum might mediate the transformation of sulfhydryl groups in the same layer to disulfide groups in the keratinized area.

D. EFFECTS OF IONIZING RADIATION

Gerstner (1950) felt that exposure of the whole body or of a large part of it to sufficient amounts of penetrating ionizing radiation induces in man the radiation syndrome. Clinical course and outcome of radiation syndrome are determined in essence, by two factors: dose and individual susceptibility.

Mavor (1923) claimed that perhaps the most striking of the biological effects of x rays is their effect on the chromatin of the nucleus. An organism may be rendered sterile and yet retain all of its vital characteristics.

Moss (1959) mentioned that degenerative diseases are limiting factors in radiotherapy, as they are in surgical therapy. A patient's reaction locally may be altered by his physical status. He also stated that the amount of oxygen contained in the body tissues is an important factor in determining his radiosensitivity. Increased dissolved oxygen will normally increase sensitivity and a decrease of same will reduce sensitivity.

Coutard (1922) noted that the buccal epithelium was replaced more rapidly than the epidermis and that it would be expected that the buccal epithelium would desquamate sooner than the epidermis. Coutard found that with a given dose of radiation the mucosal epithelium would desquamate in twelve days. The epidermis being subjected to the same dose would desquamate in two or three weeks. The mucosa in turn heals in two or three weeks and the epidermis requires five to six weeks for healing.

Friedman and Nosh (1939) were concerned with the rhythm of radiation effects. Part of their study was concerned with the biologic effects on the human skin and mucosa using copper filters of varying thicknesses (0.25, 0.5 and 2.0 mm.). Another part dealt with the normal variations of range of radiation epithelitis and epidermitis. The day of onset of epithelitis is fairly consistent, occurring around the tenth day. The thicker the filter used tended to forestall the occurrence of second degree epithelitis or to negate its appearance completely. The wave length if short renders the roentgen less biologically effective for epithelitis or epidermitis. It is observed that the epithelitis produced by roentgen rays filtered with 2.0 mm copper filter, when compared with that of 0.5 mm copper filter, appears later,

reaches a second degree intensity later and has a wider range of variation. To summarize, the time of onset of the epithelitis and epidermitis due to radiation from different qualities of roentgen rays is in inverse ratio to the wave length. The existence of a rhythm of radiation effect was first suggested by Coutard (1935) who integrated his observations into a phenomenon called periodicity, where in the skin, mucosa and tumor each experienced specific cyclic variation in their response to protracted external radiation. The bombardment of human tissues with protracted, fractionated doses of radium or roentgen rays evokes three phenomena: 1. Direct cellucidal effect - destructive phase; 2. Healing phase; 3. Periodicity. The direct cellucidal effect is most active during the early destructive phase of the treatment period, occupying the first seventeen to twenty-one days of treatment. The exact limits of the destructive phase are rather difficult to define as they depend upon many controllable and uncontrollable factors such as: 1, The size of the daily dose; 2. The individual susceptibility; 3. The quality of radiation; 4. The Daily exposure time; 5. The frequency of the treatment. Elaborating on the individual susceptibility the extent and duration of the destructive phase is dependent upon individual idiosyncracy. Summarizing the findings of Friedman's and

Rosh's study 2h% of their group of 126 patients, exhibited unusual reactions. The reactions were of the hyposensitive and hypersensitive individual. Frequency of the treatment has its influence. Interruptions in regular treatment not only weaken the effect of the dose, but also result in greater individual variations. In protracted, fractionated irradiation, the destructive phase is cumulative. Its effects starting slowly, manifested by a lag of seven to fourteen days at which time reactions commence. This production of destructive effects initiates a reaction on the healing process. The healing process is slower in starting than the destructive process, but soon accelerates in power so that it finally overwhelms the destructive process. The healing phase now begins. The healing phase in the mucosa commences on the thirty-fifth day, but varies between the twenty-sixth and forty-second day. A picture of the delicate mechanism can only be obtained when the reactions are mild, as a consequence of small daily doses or when the patient is hyposensitive. If the incident dose is larger, or the patient is hypersensitive the reactions are intense producing massive, unselective destruction which is not repaired, but crudely replaced by fibrous tissue. The healing phase is thus dependent upon the size of the dose, individual susceptibility, and the quality

of radiation. The existence of recurring cycles of increased cellucidal effects at thirteen days intervals, has not been proved. Friedman and Rosh observed a type of periodicity, which seems to result from alteration of the destructive process with the healing process.

Graham and Graham (1953) felt that the clinical stage of the sensitization response was the only useful aspect of this disease and could be used only as a prognostic index. They found a cytological examination of the non-malignant epithelial cells of the vagina during the course of radiotherapy of value. It will distinguish the patient with a good response from that of one with a poor response after only 1000 r of roentgen ray therapy or after a single radium application. Cytoplasmic vacuolization of the basal cells, red granules, and increase in the density of the cytoplasm indicates an increased sensitivity to ionizing radiation. With the sensitivity response present, Graham and Graham observed a 66% cure rate and in its absence they observed only an 18% cure rate. Sensitization response and cornification do not appear to bear any relationship to one another.

Stuhman (1943) noted that in order to produce an erythema of human skin, two factors are considered: the thickness and the pigmentation present. An intensity which may evoke an

erythema in a thin skinned blond would show little or no visible effects in a thick skinned brunette. He felt it was difficult to distinguish the changes in the epithelial cells of the skin brought about the changes in the subcutaneous vascular bed. The basal cells or Malpighian layer seemed to be most sensitive and show vacuoles, pyknosis, and lack of staining power after large doses of radiation. The greatest secondary factor in skin changes following exposure to radiation is the modification of the cell nutrition by capillaries. It is the engorgement of the capillaries of the skin that was identified with the erythema. Ionization takes place fundamentally in the cell. The x-ray energy passing through a cell wall, contents, and nucleus, if absorbed will start many changes. The magnitude of the radiation is the most important factor in determining these changes.

Young (1957) felt that since tissue contains considerable portions of water, a logical starting point to study the effects of radiation on biological material is in water which contains simple solutes or pure water. In non-oxygenated water, x ray radiation produces little hydrogen peroxide or hydrogen. In oxygenated water, x ray radiation produces hydrogen peroxide. Solutes dissolved in non-oxygenated water are oxidized or reduced, however, oxidation reactions pre-

ponderate. Many free radicals are produced by ionizing radiation and although a material may be suspended in an aqueous medium, and be unaffected by the radiation, it may be subject to attack by these free radicals produced. This would naturally be a secondary effect of the radiation. In general, the dose of radiation required to produce a given effect is very much dependent upon the oxygen tension. If oxygen is lacking, the dose must be increased two or three times that required for normal oxygen tension. If the oxygen tension is increased above normal, no appreciable dosage reduction, as compared to that dosage for normal tension, is necessary. Since malignant tumors are considered relatively avascular, increasing the oxygen tension here would be beneficial in treating the tumor; and the surrounding normal tissue would not be further affected by the existence of increased oxygen tension. Fossberg and Klein (1954) noted that one of the few biochemical facts upon which there is general agreement is the reduction of ribosenucleic acid production due to radiation. When cells of a complex organism as the human are irradiated the reactions which follow are not only dependent upon the dose but also the effects caused by the irradiated and non-irradiated tissue. Young cites the mucous membrane as being a little more sensitive to radiation than the skin.

Friedman (1942) felt that the changes in the gastrointestinal tract are well established as early as four days after exposure to radiation. Changes such as edema, swelling and vacuolization of both cytoplasm and nucleus, or disintegration of cells with nuclear pyknosis or karyorrhexis, have been described.

Warren (1942) said that one of the most striking clinical effects of radiation is the change induced in cutaneous blood vessels, first apparent as an erythema. The changes occurred in the endothelium and supporting tissues of the walls. If the endothelium was not killed, proliferation often recurred to the point of obliteration of the capillaries. In the veins and arteries subintimal fibrosis, with collagen often showing some degree of hyalinization, resulted in the thickening of the wall at the expense of the lumen. The media was also thickened by large, sometimes branching fibroblasts with abundant collagen. Warren continued by saying that the vascular damage is of fundamental importance, because of the lowering of the resistance and the reparative powers of all the tissues, the blood supply of which has been impaired. As a rule, the smaller capillaries do not recover if the dose of radiation has been sufficient to produce acute radiodermatitis. The larger the blood vessel, greater is the chance of repair and

maintenance of patency. The dilatation seen may be reversible, but with doses greater than 500 to 700 r at 200 Kv it is not normally reversible. Very early telangectasia was recognized as a sequel to cutaneous exposure to radiation. Unna (1883) ascribed this early telangectasia to deep obliterating endophlebitis.

Rhoades (1948) felt that his group's findings indicated that vascular changes are probably secondary to changes in the connective tissues surrounding the vessels.

Warren (1943) noted that erythema is the first clinical evidence of irradiation of the skin. There appeared to be two distinct phases. The first phase develops within hours or a few days after the exposure to radiation and disappears after a day or two. From ten to twenty-eight days later the reddening of the skin again appears and is usually, as it fades, followed by some degree of permanent pigmentation. Pigmentation is usually not a result of the initial erythema. The early effects are thought to be caused by direct irradiation injury to those skin cells etc. The later effects are brought about by changes in the vascular bed in the corium and intercellular tissues and to some extent, changes in the epithelial cells. Ellinger's (1941) findings substantiated the description of the second phase described by Warren (1943). He stated

that from the tenth to the twelfth day after exposure capillary dilatation occurs in varying degrees but dilatation becomes persistent from about the twelfth to the sixtieth day.

Halkin (1903) stated that the first deleterious effect noted from either roentgen rays or radium was that on the skin and its appendages.

del Regato (1939) felt that irradiation of the salivary glands and the modifications in the secretion of the saliva which result therefrom, should be considered as one of the factors in the production of dental lesions. In effect, the salivary glands, notably the submaxillary glands, have always been irradiated in all of these patients and there has been a variable diminution in the quantity of saliva secreted. Although dental lesions are noted in patients who have a deficient salivary secretion, they also are seen in qualitative rather than quantitative alteration in the secretion of saliva could result after radiation. Some patients developed an intense radioepithelitis while such doses in other patients did not evoke this condition. It is an index of the particular susceptibility of certain patients whose vasculoconnective tissue and their salivary secretion are equally modified.

Since there are important similarities between oral and vaginal epithelia, Graham's (1947) observations seem pertinent

Graham (1947) cited some of the normal characteristics of the vaginal epithelial cell so that one could fully appreciate the changes in the cells. The cells from the deepest layer of the exfoliating vaginal mucosa are small, round and have large basophilic nuclei. In the layer more superficial cells have a larger cytoplasm and may be vacuolated, but the nuclei are normal in appearance. The next layer or layers are composed of precornified cells. These cells are abundant, lightly staining, and the cytoplasm transparent. The size of the cytoplasm and nucleus is greatly increased here. The edges of the cytoplasm may be wrinkled and folded over on itself. The superficial layer is composed of cornified cells. Here the nucleus is small, pyknotic and acidophilic in its staining reaction. As in the precornified layer folding and wrinkling are common. Granulation of the cytoplasm is seen in this layer. In Graham's study the patients were placed in two categories. One category showing distinct radiation reaction in the cells and the other category showing little or no change due to radiation. In the vaginal smear the first effect is seen in the normal cell. The patients in this study received 6000 r at 12 Kv or 200 Kv.

A. Non-keratinized cell changes

1. Initially the non-keratinized cells lose their

characteristic round appearance and become elongated. The nucleus is still a normal vesicular nucleus.

2. Later a change in staining reaction is seen in the basal cells. The change is from blue or blue-green to a brownish hue, probably an early sign of degeneration.

3. The subsequent changes are in the nucleus of the basal cells. Nuclei may appear as one small pyknotic dot or karyorrhexis may occur. These changes generally take place from the tenth day of treatment and increase throughout the course of treatment.

4. Beginning on the twelfth day there is a tremendous increase in the cell size, up to four times. The nucleus is more finely granular than normal. The total size of the cell approximates that of the precornified cell. The cytoplasm here does not have the true transparency of a precornified cell and the nucleus is much too large for a normal precornified cell.

5. The non-keratinized cells show abnormal vacuolisation of the cytoplasm which is marked after the fifteenth day. The vacuoles almost fill the cell.

6. Bizarre cell shapes are next in appearing. The shapes vary from dumb-bell shapes, elongated forms, and tadpole forms. The nuclei of these forms are evenly granular and large or

pyknotic and small. They are not hyperchromatic.

B. Changes in the pre-keratinized cells

1. The first change to appear is increase in size of two or three times a normal sized precornified cell. This change occurs at the fifteenth day. The ratio in size of the cytoplasm to the nucleus remains unchanged.

2. Initially the only change in the nuclei is an increase in size, but soon the chromatin structure begins to show degenerative changes. The nuclei become dark, wrinkled and show little structural detail. Despite the abnormal size of the nucleui, they have a pyknotic appearance. The nuclear borders are very irregular. The nucleus may fragment and appear as dark particles. Multiple nuclei for a cell are not common.

3. The cytoplasm shows degenerative changes at this time. The appearance of very fine fibers which may occur in concentric circles around the nucleus or run from the nucleus to the cytoplasm periphery. Vacuolization is a very prominent feature.

4. Next in appearance are pre-keratinized cells of odd shape around the eighteenth day. Besides elongation and tadpole shapes some may have extensions resembling pseudopodia.

5. The last change to occur in the precornified cells is the appearance of polymorphonuclear leukocytes in the cells

around the twentieth day.

C. Changes in the keratinized cells

1. Very little change takes place in the nuclei of these cells because they are pyknotic.

2. The pre-keratinized cell may not always have a basophilic staining nucleus, it may be acidophilic. The nuclear shape is the distinguishing characteristic between the pre-keratinized and keratinized cells.

3. The cytoplasm undergoes the above mentioned changes of the precornified cells: increased in size, fiber appearance vacuolization, multimorphs and the appearance of polymorphonuclear leukocytes.

CHAPTER III

MATERIALS AND METHODS

The human subjects included in this study vary in race, age, sex, diagnosis, general health, therapy period, number of treatments, and to a slight degree in the characteristics of the x ray therapy. The subjects were distinguished by being assigned a number beginning with 100 and increasing by multiples of 100 until the thirteenth patient's number was 1300.

A. SUBJECTS

#100 Negro male 55 years of age.

Squamous cell carcinoma of the soft palate with a 6 month history of pain and swelling. General condition was good. From the first day of therapy until the last, 40 days elapsed. A 10 by 10 cm. port was used. During the first 6 days of treatment he received 100 r in air in 4 minutes on the skin each day. From the 7th to the 24th treatment he received 150 r in air on the skin each day. Total dose: 4284 r on the skin including back scatter and 4216 r to the buccal mucosa.

#200 Caucasian male 41 years of age.

Squamous cell carcinoma of the soft palate. Patient first noticed lesion 9 months ago, and approximately one month ago patient became aware that his palate was per-

forated. General physical condition was fair. 29 days elapsed from the initial treatment until the last. An 8 by 10 cm. port was used. The patient received 24 treatments of 150 r in air in 6 minutes per day. Total skin dose including back scatter was 4366 r on the skin and 4320 r to the mucosa.

#300 Caucasian male 83 years of age.

Squamous cell carcinoma on the anterior two-thirds of the tongue. A tender lymph node could be palpated in the left upper cervical area. The patient noticed a sore on his tongue with some pain. Therapy lasted for a period of 21 days. A 6 by 8 cm. port was used. The patient received 15 treatments in all. For the first 4 treatments he received 200 r in air in 8 minutes on the skin each day. Beginning with the 5th treatment he received 250 r in air in 10 minutes until the 15th day of treatment. Total dose on the skin including back scatter was 4295 r and 4118 r to the mucosa.

#400 Negro female 65 years of age.

Squamous cell carcinoma of the left lip and the lower portion of the buccal mucosa on the same side. This was a recurrent tumor along a surgical scar. An attempt had been made, 2 months previously, to remove the tumor surgically. Cervical metastases were present. The lesion was painful. General physical condition was fair. Therapy lasted for a

period of 20 days. A 10 by 10 cm. port was used. The patient received 15 treatments of 250 r in air in 10 minutes on the skin each day of treatment. Total dosage on the skin including back scatter was 4725 r and to the mucosa was 4650 r.

#500 Caucasian male 71 years of age.

Squamous cell carcinoma of the right tonsil with metastases to the neck. Dysphagia for several months. General physical condition was poor. Therapy extended over a 10 day period. A 10 by 12 cm. port was used. The patient received 5 treatments and died. For the first 3 treatments he received 150 r in air in 6 minutes on the skin each day. For the 4th and 5th treatment the patient received 250 r in air in 10 minutes. The treatment was administered every other day for the 5 treatments. The patient received a total of 1260 r on the skin including back scatter and 1187 r to the mucosa.

#600 Caucasian male 72 years of age.

Squamous cell carcinoma of the buccal mucosa, and the soft palate. Patient noticed a sore in his mouth of 3 months duration. General physical condition was fair. Therapy extended over a 47 day period. An 8 by 10 cm. port was used. The patient received 15 treatments every other day of 200 r in air in 8 minutes on the skin each day of treatment. Total dose to the skin surface including back scatter was 3720 r and

3700 r to the mucosa.

#700 Caucasian male 53 years of age.

Squamous cell carcinoma of the soft palate extending to the anterior and posterior pillars and the tonsillar fossa. Patient noticed a sore throat and a swelling in his neck of seven months duration. General physical condition was good. Therapy extended over 43 days. The patient received 16 treatments every other day. Through a 10 by 15 cm. port the first 6 treatments were 200 r in air in 8 minutes each treatment day on the skin. From the 7th to the 10th treatment he received 250 r in air in 10 minutes each day. For the 11th and 12th treatments he received 150 r in air in 6 minutes each day. From the 13th to the 16th treatment he received 200 r in air for 8 minutes each day. Total dose to the skin surface including back scatter was 4257 r and 4224 r to the mucosa.

#800 Caucasian male 59 years of age.

Squamous cell carcinoma of the floor of the mouth. Patient noticed pain in his mouth, especially when eating. The patient's general physical condition was fair, however the patient was considered a chronic alcoholic. The patient became very sensitive to the therapy. The therapy was halted after 21 treatments. Therapy extended over 34 days. Therapy was administered through a port of 10 by 12 cm. The patient

received 100 r in air in 4 minutes on the skin each day for the first 8 treatments. The next 4 treatments were 150 r in air in 6 minutes each day. The next 7 treatments were 175 r in air in 7 minutes each day. The last 2 treatments were 150 r in air in 6 minutes each day. Total skin surface dose including back scatter was 4000 r and to the mucosa 3718 r.

#900 Caucasian male 60 years of age.

Squamous cell carcinoma of the right hypopharynx involving the pyriform sinus. Dysphagia for six months. Patient's general physical condition was good. Therapy extended over a period of 40 days through an 8 by 10 cm. port. The patient received 13 treatments every other day. It consisted of 150 r in air in 6 minutes each day on the skin. Total skin surface dose was 2423 r including back scatter and 2340 r to the mucosa.

#1000 Caucasian male 66 years of age.

Squamous cell carcinoma of the hypopharynx involving pyriform sinus on left side. Patient began to experience difficulty in swallowing one month ago. General physical condition was poor. Patient died before the end of therapy. The therapy extended over a period of 40 days through a 7 by 9 cm. port. A total of 11 treatments was given on alternate days. The treatments were 200 r in air in 8 minutes on the

skin each day of treatment. The patient received a total dose on the skin including back scatter of 2764 r and 2643 r to the mucosa.

#1100 Caucasian male 51 years of age.

Squamous cell carcinoma of the floor of the mouth. The patient experienced soreness in the right side of his mouth for six months. General physical condition was fair. Therapy extended over a period of 21 days. The patient received 16 treatments through a 6 by 8 cm. port at 150 r in air in 6 minutes on the skin for the first 8 treatments. From the 9th to the 16th treatment he received 250 r in air in 10 minutes each day. Total dose including back scatter to the skin surface was 3872 r and to the mucosa 3712 r.

#1200 Caucasian female 57 years of age.

Squamous cell carcinoma of the soft palate with left cervical metastases. Patient noticed swelling under her jaw two months ago. Three months ago the patient noticed a sore on her tongue and experienced pain in her left ear. General physical condition was poor. Therapy extended over a period of 42 days. The patient received 16 treatments through an 8 by 8 cm. port. For the first 5 consecutive treatments the patient received 150 r in air in 6 minutes on the skin each day. From the 6th treatment on, the patient received

every other day 250 r in air in 10 minutes on the skin. Total skin surface dose including back scatter was 4347 r and 4130 r to the mucosa.

#1300 Caucasian male 57 years of age.

Squamous cell carcinoma of the left tonsillar fossa. Patient experienced a sore throat for several months. General physical condition was good. Therapy extended over a period of 34 days. Through an 8 by 10 cm. port the patient received 21 treatments. The first 5 treatments were 200 r in air in 8 minutes on the skin each day. From the 6th to the 21st treatment he received 150 r in air in 6 minutes. Total dose to the skin including backscatter was 4216 r and 4080 r to the mucosa.

To eliminate information which might be misleading, the patients chosen for this study had no previous radiation therapy. Although they all exhibited a squamous cell carcinoma of the head and neck region, their buccal mucosa, from which the smears were taken, appeared healthy. All patients received the x ray radiation through a Thoreus filter, a laminated metal filter composed of tin, copper and aluminum. The aluminum portion was placed closest to the skin. The Thoreau filter is used because it minimizes erythema and its discomforts.

B. COLLECTION OF MATERIAL

1. Two smears were taken from an area of the buccal mucosa approximating the occlusal plane that clinically appeared normal. Smears were taken before therapy and then at twenty-four hour intervals until the completion of therapy.

2. The smears were obtained with wooden tongue blades.

3. The material collected on the wooden tongue blade was gently transferred onto a clean glass slide, spreading the material as evenly as possible.

4. The second smear was obtained and this material was transferred gently onto the same surface of the slide beside the first smear.

5. To prevent drying, the slide was immediately immersed into a fixative of a fifty-fifty mixture of 95% ethyl alcohol and ether.

C. STAINING TECHNIQUE, Papanicolaou, George N. (1942)

"1. Fix smears immediately (before drying) in equal parts of 95% alcohol and ether for 5 to 15 minutes. Rinse in 70% and 50% alcohol and in distilled water.

2. Stain in hematoxylin for 5 to 10 minutes. Rinse in distilled water. Rinse 3 to 4 times in 0.5% aqueous solution of hydrochloric acid. Rinse thoroughly in water. Leave for one minute in weak solution of lithium carbonate (3 drops of a saturated aqueous solution per 100 cc of water). Rinse thoroughly in water.

3. Rinse in distilled water, then in 50%, 70%, 80% and 95% alcohol.

4. Stain in the solution of OG 6 for one minute.

5. Rinse 5 to 10 minutes in each of two jars containing 95% alcohol to remove excess stain.

6. Stain in EA 36 or EA 25 for two minutes.

7. Rinse 5 to 10 minutes in each of three jars containing 95% alcohol. (Do not use same alcohol which was used for Orange G,) Rinse in absolute alcohol and xylol.

8. Mount in Clarite, Canada Balsam or Gum Damar".

N.B. Canada Balsam was used for mounting in this study.

D. COUNTS

1. The two smears of each slide were scanned and as soon as a readable and typical area was found where the cells were evenly dispersed a differential count of the three cell types was made. Only those cells in one 100X magnification field were counted per smear. The cell types were: green (non-keratinized), red (partially keratinized), and tan or yellow (completely keratinized).

2. Some cells stained two colors. A cell would have a red cytoplasm with the exception of the periphery which would be green. Cellular morphology was studied to determine which category the particular cell would be placed. If cellular morphology was not distinct, predominance of the color in the

cytoplasm was used to categorize the cell.

E. CALCULATIONS

1. The two differential counts made on each slide, one count from each smear, were added together. From this total an average per cent of each cell type was determined.

2. For each patient the per cent of each cell type of every interval counted was plotted on a graph. This information included the percent distribution of each cell type before therapy and at 24 hour intervals throughout therapy.

3. Upon examination of all the linear graphs it was noted that peaks occurred at similar time intervals in many of the patients. This time pattern of the peaks was studied. The peaks occurred on the 8th, 13th, and 32nd days in a majority of the patients. The representative values of the patients associated with the time intervals, 8, 13 and 32, were placed into tabular form. Also, representative values of the slides of all patients were placed into tabular form for the day before therapy and the last day of therapy.

4. Calculations in the tables

a. A mean per cent was determined of the total green, red and tan cell per cent in all tables. This value was calculated by summing the per cent values in three columns and dividing by the number of patients represented.

b. The variance* signified by the symbol S^2, was calculated in the following manner: In determining the variance for the green cells, the difference between the mean per cent and the per cent value of the green cells of each patient for that particular day was calculated. The individual differences were squared. The squared values were summed and divided by the number of patients represented. This procedure was repeated to determine the variance of the red and tan cells.

c. The standard deviation was calculated by taking the square root of the variance.

* The variance was calculated according to the method found in Batson's Introduction to Statistics in the Medical Sciences.

F. COMPOSITE GRAPHS

Composite graphs were made using the information obtained from the individual patient graphs. The three composite graphs made will be discussed in detail in the findings of this thesis.

CHAPTER IV

FINDINGS

A. CHARACTERISTICS OF THE SMEARS

1. The total number of smears collected for this study was 422. This figure includes the smears taken before therapy. Of the 422 smears collected on 211 slides, 14 could not be used probably because of poor fixation and, therefore, poor staining.

2. Toward the end of therapy (32nd day) fewer cells were contained in comparable fields of each smear. Average number per low powered field (100x) before the 32nd day was 57 and after the 32nd day the number of cells per low power field was 26.

3. No difficulty was experienced in counting the cell types once a typical field was selected. Although in most areas of the smear the cells were well distributed, clumping of particular cell types was observed.

B. PER CENT VALUES DURING STUDY

1. Typical per cent values

a. There was a definite decrease in the relative number of green cells (non-keratinized) cells) at the end of therapy as compared to this value before initiation of therapy.

The percentage of green cells was 64.23% initially whereas at the end of therapy in 8 out of 13 patients the green cell count was under 25%. The number of green cells increased around the 8th day to 71.34%, declined at the 13th day to 43.99 %, showed a further decline by the 32nd day to 27.29% and finally to an average percent of 24.79.

b. The relative number of red cells (partially keratinized cells) showed a definite increase at the end of therapy as compared with the value before therapy.

The red cell count was 33.10% initially, at the 8th day it was 25.59%, on the 13th day it was 49.23%, and by the end of the 32nd day it had further risen to 54.85% and its final value 55.73%.

c. The relative number of tan cells (keratinized cells) showed a marked increase at the end of therapy as compared to the initial value.

The relative number of tan cells was 2.54%, by the 8th day it had risen to 3.05%, by the 13th day it was 6.96% and on the 32nd day it was 17.56% and at the end of therapy 19.78%.

2. Exceptions to the typical values

By examining the patients' cell percentages individually there were found a few exceptions to the overall result.

a. The initial relative number of green cells in two

individuals was decidedly less than the red cells.

b. In only three instances did the final green cell number exceed the value of the final red cell number.

c. In only one instance did the initial tan cell number exceed the final tan cell value.

C. GRAPHS

1. Individual differences in the distribution of red and green cells.

The thirteen patients could be divided into 3 groups.

a. The first and largest group (8 out of 13) showed a marked decline in green cell count and a marked rise in red cell count by the 18th day at the latest, that remained fairly constant until the end of therapy.

b. The second group composed of two individuals, in contrast to the first group, showed an extended high count of greens and a low count of reds until late in therapy (32nd) day. The tan cells showed their gradual rise throughout therapy.

c. The third group, composed of two individuals, showed a drastic dip in green cell count and a concomitant red cell rise about the 13th day. Then there was a reversal of this phenomenon in three or four days to remain with a high green cell count and a low red cell count until the end of therapy.

As in all groups, the tan cells showed a rise throughout therapy.

2. Tan cell curve of all subjects

All 13 patients showed a gradual increase in tan cell numbers during therapy. The tan cell count showed two periods in the course of therapy which indicated temporary increases in number. These two significant periods occurred between the 4th and 8th days, and again and more pronounced at the 13th day. The rise which occurred between the 4th and the 8th days was present in ten out of 13 patients. That which occurred on the 13th day was present in 8 out of 13 patients.

D. CELLULAR CHANGES DUE TO RADIATION

1. Staining

The most frequently observed change in staining characteristics due to radiation was in the green cells. The green cells took on a considerable amount of red stain in their cytoplasm, red clumps appeared in the green cells. The clumps were granular and constituted no definite pattern.

2. Morphological changes

An increase in size of the red cells was the most frequently seen change. Both nucleus and cytoplasm increased in size simultaneously.

3. Pathological changes

No pathological changes were noted.

E. TABLES

Tables have been inserted in the following pages so that a more complete view of the results at particular time intervals may be studied.

CHAPTER V

DISCUSSION

The epithelium of the buccal mucosa is described as non-keratinized. This observation was made in histologic specimens (Orban 1953) as well as in exfoliative cytologic studies, (Weinmann 1940). Smears of the buccal epithelium approximating the occlusal plane in this study revealed 2.54% of the cells to be keratinized. This finding cannot be interpreted as being representative of the entire buccal mucosa, because this area of the mucosa often is subjected to irritations not present in other parts.

A. CHANGES IN THE EPITHELIUM DUE TO RADIATION

The most significant change in the buccal epithelium due to radiation was expressed by a decrease in green, non-keratinized cell numbers and an increase in the red, keratinizing, and tan, keratinized cell numbers. This shift in the distribution of the cells was first noted as an increase in the relative number of the tan cells between the 4th and 8th days. Around the 13th day the beginning of a decrease in the green cell numbers and an increase in the red cell number was observed. After the 32nd day, the relative number of cells selected at random in a low power, 100x, field was diminished.

The mode by which these changes due to radiation in the buccal epithelium occurred was attributable to: 1. Reduction in mitosis, 2. The oxidative effects of radiation and 3. blood vessel damage by radiation.

1. Reduction in mitosis:

Reduction in the number of mitoses by radiation is known to occur. Young (1957), Stuhman (1943), Mavor (1932). There is no reason to believe that mitosis have not been reduced in the basal cell layer of the epithelium because sufficient radiation has been administered. Green cell number reduction indicates that fewer green cells are available for exfoliation; it also indicates the non-keratinized cells capable of exfoliation are remaining in their mother tissue longer, thus aging more before desquamation. Also, if there is a reduction in the number of newly produced cells, and if this epithelium is to continue to protect the underlying connective tissue, the cells present would have to remain in site longer. The keratinization process would continue while these cells remained in site, the result being a greater number in keratinizing or keratinized desquamated cells.

2. Oxidative effects:

When a tissue is subjected to ionizing radiation, hydrogen peroxide and free radicals are produced, Young (1957).

These products of ionization produce reactions which are predominantly oxidative Young (1957). There is reason to believe that oxidation of sulfhydryl group to disulfide groups is instrumental in the keratinization of tissue, Giroud and Leblond (1951). Also, disulfide groups, cystine, are found in abundance in those tissues which are highly keratinized, Barnett and Seligman (1954). Thus, keratinization is enhanced by the addition of oxidative effects to these cells, by ionizing radiation.

3. Blood vessel damage by radiation.

Injury to blood vessels by ionizing radiation is a well established fact, Warren (1942), Unna (1889) and Rhoades (1948). In the repair of these vessels after radiation damage proliferation of the endothelial cells results sometimes in obliteration of the lumen, Wallbach (1943). Obliteration of a blood vessel would naturally diminish metabolic exchanges between the blood vessel and the tissue supplied. If the blood supply to the buccal epithelium is diminished, this could very well be a further stimulus to keratinization of its cells.

B. CELLULAR CHANGES DUE TO RADIATION

1. Probably the most significant change caused by radiation to cells was an increase in the size of the red, keratinizing cells. This was evidenced by an observable increase in

the diameter, two times its normal pre-radiation size. This observed increase in size of the red cells is substantiated in the findings of Graham (1947).

The mode by which this cell widening occurred is an indirect effect of radiation by the reduction of mitoses in the basal layer of the buccal epithelium. The reduction in mitoses reduces the number of cells which are to comprise this epithelium. A sub-normal number of cells are forced to carry on the protective function assigned to a normally greater number of cells. The only way in which the surface may be covered properly with a reduced number of cells is for these cells of the mucosa to widen. Thus, a functional adaptation expressed by an increase in the size of the keratinizing cells in the buccal mucosa has been brought about indirectly by ionizing radiation.

2. A change in the staining reaction of the green, non-keratinizing cells was also brought about by radiation. Although this change was not dramatic there was an increased frequency of green cells with red staining masses dispersed in the cytoplasm. Direct radiation effect to the green cells is thought to cause this atypical staining reaction. The observations of Stuhman (1943) state that ionization takes place fundamentally in the cell. The ionization which takes

place in the green cells could easily disturb RNA (ribose nucleic acid) synthesis, Forssberg and Klein (1954) resulting in an alteration of the staining reaction.

3. Finding no pathologic changes in the cells due to radiation was expected. The obvious reason was the fact that the cells were being collected from a clinically healthy buccal mucosa. Another reason being Papanicolaou and Traut (1943) did not find any malignant exfoliated cells when taking smears of effectively radiated malignant lesions.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Smears of the buccal mucosa of thirteen patients who had received therapeutic x ray radiation for head and neck cancer were taken before therapy and at twenty-four hour intervals during the course of therapy. Papanicolaou's method of staining was used and it permitted good differentiation of cell types. Furthermore, cytologic changes were also observed.

Only that area of the buccal epithelium approximating the occlusal plane was studied and 2.54% of the cells found before therapy were keratinized. A shift in the distribution of all cell types, non-keratinized, partially keratinized, and completely keratinized, was observed during radiation therapy. Radiation therapy caused a decrease of the green, non-keratinized cells and an increase in the red, partially keratinized and tan, completely keratinized cells.

Cytologic changes due to radiation therapy were increased size of the red cells and a staining variation of red clumps in the green cell cytoplasm. No pathologic changes were observed in the individual cells.

The changes observed in the cells of epithelium caused by radiation in this study had no observable relationship to age,

sex or race.

From the findings in this study, the following conclusions are made:

1. The concept of studying radiated buccal epithelium by exfoliative cytologic means is sound.

2. Therapeutic xray radiation causes an increase in keratinization of some of the cells in the buccal mucosa approximating the occlusal surfaces of the teeth. Further, radiation causes a change in the normal distribution of the cells in the buccal epithelium.

3. Radiation causes cytologic changes in the cells of the buccal epithelium expressed by an increase in the size of the partially keratinized cells and staining variations in the non-keratinized exfoliative cells.

TABLE I

Distribution of green (non-keratinized) cells, red (partially keratinized) cells, and tan (keratinized) cells before the initiation of therapy.

Patient	%Green	%Red	%Tan
100	31.84	66.48	1.70
200	58.53	30.89	10.56
300	87.93	11.20	.86
400	57.14	36.45	6.40
500	57.46	41.04	1.49
600	48.17	51.21	.06
700	88.09	10.31	1.50
800	87.91	12.08	.00
900	49.12	50.87	.00
1000	85.66	13.44	.34
1100	67.29	27.67	5.03
1200	35.29	61.17	3.52
1300	80.95	17.46	1.58
Mean %	64.23	33.10	2.54
s^2	412.13	393.87	8.66
s	20.30	19.84	2.94

s^2 - variance

s - standard deviation

TABLE II

Distribution at the eighth day.

Patient	%Green	%Red	%Tan
100	72.09	23.42	4.50
300	81.69	13.38	4.92
400	51.31	40.78	7.89
500	49.28	47.84	2.87
600	53.74	43.95	2.19
800	83.03	14.28	2.60
900	71.69	26.41	1.88
1000	68.75	31.25	.00
1100	84.23	15.76	.00
1200	84.24	11.84	3.94
1300	84.72	12.50	2.77
Mean %	71.34	25.59	3.05
s^2	196.83	183.42	3.31
s	14.02	13.54	1.81

TABLE III

Distribution at the thirteenth day.

Patient	%Green	%Red	%Tan
100	20.42	70.42	9.15
200	34.21	56.57	9.21
400	27.08	64.38	8.33
600	25.31	64.55	10.12
700	92.22	4.44	5.33
800	95.55	4.44	.00
900	58.82	36.27	4.90
1100	22.35	70.58	7.06
1200	20.00	71.42	8.57
Mean %	43.99	49.23	6.96
s^2	837.14	677.28	3.46
s	28.93	26.02	1.86

TABLE IV

Distribution at thirty-second to the thirty-fourth day.

Patient	%Green	%Red	%Tan
100	8.94	80.50	10.16
600	17.56	67.56	14.86
700	65.11	16.27	17.60
800	.00	74.28	25.71
900	27.77	63.88	8.33
1200	64.31	11.11	24.07
1300	7.40	70.37	22.22
Mean %	27.29	54.85	17.56
s^2	604.67	816.70	46.29
s	24.58	28.57	6.80

TABLE V

Distribution at end of therapy.

Patient	%Green	%Red	%Tan
132	8.94	80.50	10.16
229	22.54	72.36	5.09
321	11.32	78.49	13.20
420	.00	77.50	22.50
510	29.68	62.50	7.81
640	.00	32.14	67.35
747	60.56	22.53	16.90
834	.00	74.28	25.71
940	31.70	58.53	9.75
1040	78.26	15.21	6.52
1121	18.89	59.48	23.62
1242	53.57	14.28	32.14
1334	6.84	76.71	16.43
Mean %	24.79	55.73	19.78
s^2	486.09	646.06	271.62
s	22.04	25.41	16.48

TABLE VI

Patient	Race	Sex	Age	Total Days	Total Treatments	Total r to Mucosa	Total r to Skin	Port Size in cm.	Sequence of Therapy	Groups
100	Neg.	M	55	32	24	4216	4284	10 x 10	E	A
200	Cau.	M	41	29	24	4320	4366	8 x 10	E	A
300	Cau.	M	83	21	15	4118	4295	6 x 8	E	A
400	Neg.	F	65	20	15	4650	4725	10 x 10	E	A
500	Cau.	M	71	10	5	1187	1206	10 x 12	S	A
600	Cau.	M	72	47	15	3600	3720	8 x 10	S	A
700	Cau.	M	53	43	16	4224	4257	10 x 15	S	B
800	Cau.	M	59	34	21	3718	4000	10 x 12	E	A
900	Cau.	M	60	40	13	2340	2423	8 x 10	S	B
1000	Cau.	M	66	40	11	2640	2764	7 x 10	S	C
1100	Cau.	M	51	21	16	3712	3872	6 x 8	E	A
1200	Cau.	F	57	42	16	4130	4347	8 x 8	S	C
1300	Cau.	M	57	34	21	4080	4216	8 x 10	E	A

Legend:

Race - Negro (Neg.), Caucasian (Cau.)
Sex - Male (M), Female (F).
Sequence of therapy - Consecutive days (E), Alternate days (S)
Groups - 1st Group (A), 2nd Group (B) 3rd Group (C)

(A) This is the largest group and one in which the green cells showed a decrease relative number and the red cells an increase at the eighteenth day. This change remained. Tan Cell numbers increased throughout the therapy.

(B) This group showed a very late decline of the green cell numbers with a concomitant late rise in red cell numbers. Tan cell numbers rose.

(C) This group showed erratic behavior. The green cell numbers decreased at the thirteenth day and rose again in three or four days to remain high for the remainder of the therapy. A compensatory curve was shown for the red cells. Tan cell numbers rose throughout therapy.

COMMENTS ABOUT GRAPH FIGURES 1, 2, and 3

Legend: % Counts Green Cells _____, Red Cells .... and Tan cells - - - -.

Group 1.

This group showed a decrease in green, non-keratinized cell numbers and an increase in red, keratinizing, and tan, keratinized cell numbers beginning the 13th day. The changes in relative green cell numbers and red cell numbers ceased at the 18th day, thus the green cell counts remained relatively low and the red counts relatively high for the remainder of the therapy. The tan cells increased gradually from the 13th day on.

Group 2.

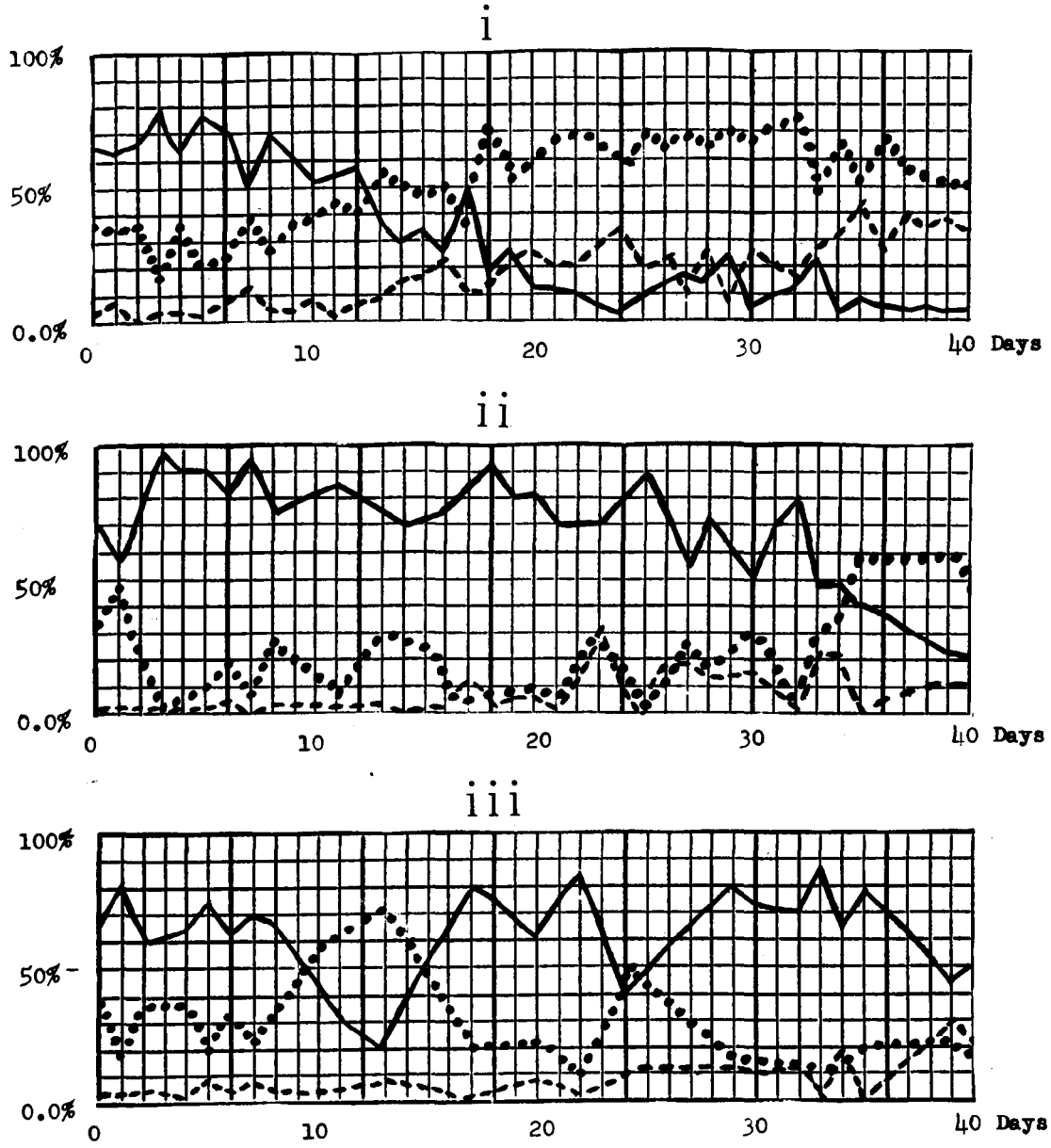
This group showed a slightly elevated pre-radiation green cell count until the 32nd day when this non-keratinized count decreased. The red cell relative numbers decreased. The tan cell numbers showed a gradual but sporadic increase throughout therapy.

Group 3.

This group showed erratic changes in distribution of the green and red cells. At the 8th day the green counts decreased and the red cell counts increased from pre-radiation values, a reversal of this occurred at the 11th day, and on the 22nd day

the green and red counts began to return to their pre-radiation values. Another reversal of green and red counts began on the 35th day. The tan cell counts were expressed by a gradual rise throughout therapy.

FIGURES 1, 2 and 3



BIBLIOGRAPHY

Barnett, R.J. 1953 The histochemical distribution of protein bound sulfhydryl groups. Jour. Nat. Ca. Inst. 13: 905-925.

Barnett, R.J. and Seligman, A.M. 1952. Histochemical demonstration of protein bound sulfhydryl groups. Science 116: 323-327.

Barnett, R.J. and Seligman, A.M. 1954 Histochemical demonstration of sulfhydryl and disulfide groups of protein. Jour. Nat. Ca. Inst. 14:769-804.

Batson, H.C. 1956 An introduction to statistics in the medical sciences. Burgess Publishing Co. Minneapolis 15, Minnesota.

Coutard, H. 1922 Sur les delais d'apparition et d'evolution des reactions de la peau, et des muqueses de la bouche et du pharynx, provoquees par les rayons x. X-ray effects in humans, translated by Dr. J.J. Van houtte. Rend. Soc. de Biol. 86:1140-1141.

Cowdry, E.V. Literature cited: Weinmann, J. 1940. The keratinization of the human oral mucosa. J.D.R. 19:57.

del Regato, J.A. 1939. Dental lesions observed after roentgen therapy in carcinoma of the buccal cavity, pharynx and larynx. Am. J. Roent. and Rad. T. 42:404.

Dempsey, Singer and Wislocki. Literature cited: Pearse, E.A.G. 1951. The histochemical demonstration of keratin by methods involving selective oxidation. Q. J. Micro. Sc. 92:393.

DeRoberts, E.D.P. Nowinski, W.W. Saez, F.A. 1948 General Cytology. W. B. Saunders Co., Philadelphia, Pa.

Eisen, Arthur Z. Montagna, W. and Chase, H.B. 1953 Sulfhydryl Groups in the skin of the mouse and guinea pig. Jour. Nat. Ca. Inst. 14:341-353.

Ellinger, F. 1941 The biologic fundamentals of radiation therapy. Translated by R. Goss. Nordemon Publishing Co., Inc. New York.

Ernst, P. Literature cited: Weinmann, J. 1940 The keratinization of the human oral mucosa. J.D.R. 19:57.

Forssberg, A. and Klein, G. 1954 Studies on the effect of x-rays on the biochemistry and cellular composition of acites tumors. I Effect on growth protein, cell volume, nucleic acid and nytrogen content in acites tumor. Exp.

Cell Res. 6:211.

Friedman, N.B. 1942 Effects of radiation on normal tissue. Warren, Shields, IV Effects of radiation on G.I. tract, including the salivary glands, the liver and the pancreas. Arch. of Path. 34:749.

Friedman, M. and Rosh, R. 1939 The rhythm of radiation effects. Am. J. Roent. and Rad. T. 42:572.

Gay, Helen 1960 Nuclear control of the cell. Scientific American. 202:126.

Gerstner, H.B. 1958 Radiation syndrome. J.O.S. 16:413.

Giroud, A. and Leblond, C.P. 1951 The keratinization of epidermis and its derivatives especially the hair, as shown by x-ray diffraction and histochemical studies. Ann. of New York Acad. of Sc. 53:613-626.

Gladstone, S.A. 1950 Sponge biopsy in carcinoma of skin and oral mucosa. Arch. of Dermat. and Syph. 62:380.

Graham, R.M. 1947 The effects of radiation on vaginal cells in cervical carcinoma. I. Description of cellular changes. Surg. Gyn. Obs. 84:156-165.

Graham, R. and Graham, J.B. 1953 A cellular index of the sensitivity to ionizing radiation, the sensitization re-

sponse. Cancer Philadelphia 6: 2:215.

Halkin, H. 1903 Effects of x-ray radiation on the salivary glands. Arch. of Dermat. and Syph. 65:201.

Hoepke, H. Literature cited: Weinmann, J. 1940 The keratinization of human oral mucosa. J.D.R. 19:57.

Laffront, A. Literature cited: Weinmann, J. 1940 The keratinization of human oral mucosa. J.D.R. 19:57.

MacLeod, J.M.H. Literature cited: Weinmann, J. 1940 The keratinization of human oral mucosa. J.D.R. 19:57.

Marston, H.R. Literature cited: Rothman, S. 1954 Physiology and biochemistry of the skin. University of Chicago Press, Chicago, Ill.

Marston, H.R. 1952 Cobalt, copper and molybdenum in the maturation of animals and plants. Physiol. Rev. 32:66-121.

Martinotti, L. Literature cited: Weinmann, J. 1940 The keratinization of human oral mucosa. J.D.R. 19:57.

Mavor, J.W. 1932 Studies on the biological effect of x-ray. Am. J. Roent. 10:968-974.

Mertschnigg, H. Literature cited: Weinmann, J. 1940 The keratinization of the human oral mucosa. J.D.R. 19:57.

Mirsky, A.E. and Anson, M.L. 1936 Sulfhydryl and disulfide groups of proteins, III Sulfhydryl groups of native proteins-hemoglobin and the proteins of the crystalline lens. J. of Gen. Physiol. 19:451-459.

Montagna, W. 1952 Cytology of mammalian epidermis and sebaceous glands. Int. Rev. of Cyto. 1:265-304.

Montagna, W. Chase, H.B. 1951 The distribution of glycogen and lipids in human skin. J. Invs. Derm. 17:147-57.

Montgomery, P.W. 1951 A study of exfoliative cytology in normal human oral mucosa. J.D.R. 30:12.

Montgomery, P.W. and Von Haam, E. 1951 A study of the exfoliative cytology in patients with carcinoma of the oral mucosa. J.D.R. 30:303.

Moss, W.T. 1959 Therapeutic radiology. C.V. Mosby Co. St. Louis, Missouri.

Orban, B. 1953 Oral histology and embryology. C.V.Mosby Co. St. Louis, Missouri.

Papanicolaou, G.N. 1942 A new procedure for staining vaginal smears. Science 95:438-439.

Papanicolaou, G.N. and Traut, H.F. 1943 Diagnosis of uterine cancer by the vaginal smear. The Commonwealth Fund

New York, N. Y.

Papanicolaou, G.N., Traut, H.F. and Marchetti, A.A. 1948 Epithelia of womans' reproductive organs. Commonwealth Fund New York, N. Y.

Patzelt, V. Literature cited: Weinmann, J. 1940 The keratinization of human oral mucosa. J.D.R. 19:57.

Pearse, E.A.G. 1951 The histochemical demonstration of keratin by methods involving selective oxidation. Q.J. Micro. Sc. 92:393.

Pomeranze, M.J. and Stahl, S.S. 1953 A correlative study of cyto-diagnosis and biopsy. O.S., O.M. and O.P. 6:1026.

Posner, C. Literature cited: Weinmann, J. 1940 The keratinization of human oral mucosa. J.D.R. 19:57.

Rabl, H. Literature cited:

Rhoades, R.P. 1948 The vascular system. Bloom, W., Editor: The histopathology of irradiation. McGraw-Hill Book Co., New York, N. Y.

Rosenstadt, B. Literature cited: Weinmann, J. 1940 The keratinization of human oral mucosa. J.D.R. 19:57.

Rothman, S. 1954 Rhysiology and biochemistry of the skin. University of Chicago, Press, Chicago, Ill.

Sandler, H.C. and Stahl, S.S. 1958 Exfoliative cytology as a diagnostic aid in detection of oral neoplasms. J.O.S. 16:414.

Selhorst, S.B. Literature cited: Weinmann, J. 1940 The keratinization of the human oral mucosa. J.D.R. 19:57.

Silverman, S., Becks, H. and Farber, S.M. 1958 The diagnostic value of intraoral cytology. J.D.R. 37:195.

Stuhman, Otto 1943 An introduction to biophysics. John Wiley and Son, New York.

Unna, P.G. Literature cited: Weinmann, J. 1940 The keratinization of the human oral mucosa. J.D.R. 19:57.

Waldeyer, W. Literature cited: Weinmann, J. 1940 The keratinization of the human oral mucosa. J.D.R. 19:57.

Warren, Shields 1942 Effects of radiation on normal tissue, effects of radiation on the cardiovascular system. Arch. Path. 34:1074.

Warren, Shields 1943 Effects of radiation on normal tissue, XIII Effects on the skin. Arch. Path. 34:340-347.

Weidenreich, F. Literature cited: Weinmann, J. 1940 The keratinization of the human oral mucosa. J.D.R. 19:57.

Weinmann, J. 1940 The keratinization of the human oral mucosa.

J.D.R. 19:57.

Young, M.E.G. 1957 Radiological physics. Academic Press, Inc. New York.

Ziskin, D.E., Kamen, P. and Kittay, I. 1941 Epithelial smears of the oral mucosa. J.D.R. 20:386.

APPROVAL SHEET

This thesis submitted by Dr. Lawrence P. Chase has been read and approved by 3 members of the Department of Anatomy and Oral Anatomy.

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.

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Date

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Signature of Advisor