— J. of Korean Maxillo-Facial Radiol. Vol. 13, No. 1, 1983 —

RADIOAUTOGRAPHIC ANALYSIS OF CHANGES IN DIFFERENT PHASES OF CELL KINETICS IN MURINE ORAL MUCOSA

CHANG SUCK PARK

Department of Radiology, Osaka Dental University

Dong Soo You, D.D.S

Dept. of Radiology, College of Dentistry, Seoul Nation University

CONTENTS

ABSTRACT

The age related changes in the life cycle of the progenitor cell population of murine oral epithelia was studied. Using radioautographic methods which have been adopted in previous cell cycle studies, the age-related changes of different phases in renewing cells of the palatal, buccal and lingual mucosae were determined. The results confirm published findings on cell cycle changes of epithelia with aging and illustrated further that mitotic phase which has hither to been considered stationary, also changes with aging. The major parts revealed by this study are as follows: 1) The basal progenitor cells in different regions of oral mucosa have different generation times. 2) The basal cell cycle time increases as a function of aging and the region most affected by aging appears to be the epithelium of the cheek. 3) The phases of the cell cycle affected by the process of aging are in increasing order of magnitude: M-, S- and G_1-phase. 4) The age related change in the number of DNA synthesizing basal progenitor cells occurs at two age periods. Between 1 and 12 months of life it decreases, while from 12 to 20 months it increases.

Key Words:

Radioautography, ^{3}H-thymidine, Mouse, Oral mucosa, Aging, Cell cycle

INTRODUCTION

Aging is a multifactorial process which results in a progressive deterioration of biologic integrity over time, and which is manifest in the loss of physiologic function, resiliency to stress and regenerative capacity. As an individual passes from birth to old age, the oral epithelium undergoes a continuous differentiation and modification as many generations of epithelial cells continuously pass through the phases of mitosis, differentiation, maturation, senescence, death, cornification and desquamation. This continuous process of cellular exhaustion and decline

at the upper surface is balanced by progenitor cells that must survive to retain the required capacities to meet the challenge. Is it just a speculation that these intrinsic capacities become increasingly limited with the aging of the individual?

Studies of Loeb and Haven ('29)[95] and Ortiz Picon ('33)[102] initiated the attempt to understand more about the nature of physiologic regeneration of epithelia during aging. According to these and other subsequent studies (Bullough, '49[14]; Meyer, Amarjits, Marwah and Weinmann, '56[100]; Marwah, Weinmann and Meyer, '60[96]). it was found that mitotic activity in epidermis and oral epithelia increases with age. More recently, it was reported that synthesis time, duration of interphase and the generation time of the dorsal tongue epithelium are increased with age (Toto and Dhawan, '66[155]; Barakat, Toto and Choukas, '69[5]).

The present investigation was planned to first study the cell proliferation and population kinetics in the epithelia of the murine oral mucosa, to determine the alterations in cell cycle parameters that occur during the process of aging and to develop answers to such question as:

1. Do basal progenitor cells in different regions of oral mucosa have the same generation time?
2. Do these cells change in generation time as function of aging, and it so, what region or regions will be most affected by aging?
3. What particular phase (or phases) in the cell cycle is affected by aging process?
4. Is the labeling index (number of cells in DNA synthesis shortly after ^{3}H-thymidine injection) constant during aging?

REVIEW OF THE LITERATURE

A. Introduction

Enclosed in the structural component of certain tissues of adult organisms is a specific population of cells that possess an unlimited growth potential. Such a continuously proliferating group of cells have the ability to replenish older or worn-out cells that are removed from the original population on a continuous basis. Thus, in the stratified squamous epithelia of the epidermis and oral mucosa, constant replacement of desquamating cells is achieved by the production of new cells arising from mitosis.

Since the discovery of mitosis or karyokinesis in epidermis (Flemming, 1884)[45] and its detailed evaluation (Waldeyer, 1888)[159] the presence of mitotic figures has been regarded as evidence for physiological regeneration. With the criterion in mind Bizzozero (1894)[11] classified the fully grown tissues of the body as (1) tissues with transient elements, (2) tissues with stable elements, and (3) tissues with perennial elements. He also indicated that in spite of the abundance of mitoses in cell populations that continue to multiply throughout the life span, tissues show no increase in size and number of cells, implying that the addition of new cells is balanced by a constant loss of old cells. A cell population under such conditions was said to be in a steady state (Leblond and Walker, '56[87], Lipkin, '71[91] and Fabrikant, '72[41]). Among others, these

renewing cells are present in lymphatic tissues (Bertalanffy, 51)[8], intestinal epithelium (Leblond and Stevens, '48)[86], epidermis (Storey, '49)[140] and oral mucosa (Henry, Meyer, Weinmann and Schour, '52)[61].

Adopting Bizzozero's work, Lebond, Greulich and Pereira ('64)[85] classified cell populations in rat tissues of different ages based on the amount of uptake and retention of ^{3}H-thymidine (^{3}H-TdR), while Gilbert and Lajtha ('65)[54] subdivided tissues into compartments. Cleaver ('67)[28] designated a dynamic steady state model with a small "stem cell compartment" feeding a "dividing transit compartment" which in turn feeds a "simple transit compartment ". In stratified squamous epithelia, stem cells and dividing cells represent resting cells (G_0 phase of Quastler, '63[118], and Lajtha, '63[80]) and cycling cells whereas differentiating (spinosum, granular and cornified) cells exemplify a simple transit compartment.

B. The Site of Cell Proliferation

From the time of the discovery of mitosis in pig snout epidermis by Flemming (1884)[45] the exact location of cell division in epthelium has been the subject of dispute and discussion among the epithelial biologists. In cat palmar and plantar epidermis, guinea pig and rat epidermis, and human skin, cell division reportedly takes place in the basal layer or lower Malpighian laher (Thuringer '24[151], '28[152] and '39[153], Loeb and Haven '29[95], Blumenfeld '43[12], Leblond and Walker '56[87], Laurence and Randers Hansen '71[83]). Several investigators have suggested cell division also takes place in the spinous layer of epidermis (Cowdry and Thompson '44[32], Cooper, Thuringer and Katzberg '51[30], Bullough and Laurence '64[17]), human interdental gingival epithelium (Gargiulo '59[48]), rat vaginal epithelium (Bertalanffy and Weinmann '60[9], Young '68[166]), and ear epidermis (Carter '53[25], Young '68[166]). Still others have demonstrated that in mouse epidermis and rat esophageal epithelium mitoses are found exclusively in the basal layer (Bertalanffy '60[9], Leblond et al. '64[85], Iverson, Bjerknes and Devik '68[69], Smart '70[135]).

When a labeled precursor, i.e. ^{3}H-thymidine is made available to stratified squamous epithelium, progenitor cells in S-phase utilize the exogenous thymidine while replicating their nuclear DNA. The labeled epithelial cells, shortly after ^{3}H-thymidine administration, represent a random population of stem cells which are in preparation for division at that particular moment. However, it has been demonstrated that fewer cells actually divide than would be expected from the number of labeled cells, suggesting that either all of the DNA or some part of genetic material can be renewed without subsequent cellular division (Pelc and Gahan '59[108], Pelc '64[107]).

The location of labeled epithelial cells, shortly after ^{3}H-thymidine injection, can also be accounted for the site of cell proliferation. Studies of human epidermis (Epstein and Maibach '65)[39], gingival epithelium of marmosets (Skougaard '65a[132]), rat palatal and lingual epithelium (Amano '66[2], Sharav and Massler, '67[129]) and mouse tongue epithelium (Sharav '66)[128] have shown the labeled cells to be present both in basal and suprabasal layers. However, Shoenheider (60)[127] and Glass and Goepp ('74a, and b)[55,56] found all labeled epithelial cells in

dorsal epithelium of mouse tongue to be restricted to the basal layer.

Investigations by Silberkweit, Soni and Hayes ('64)[59] showed the greatest mitotic activity in inflammed gingival epithelium of children to occur in the deep third of the spinous layer. Soni, Silberkweit and Hayes ('65)[137] reported that mitoses in human gingival epithelium were seen in both the basal layer and different strata of the spinous layer. In gingival, lingual and palatal epithelia, as well as dorsal ear epidermis of rodents, mitotic cells were demonstrated to be confined to be basal layer (Dhawan '64[36], Cameron '66[19], Cutright and Bauer '67[34], Barakat, Toto and Choukas '69[5], Cameron '70[20]).

In a study of human gingival epithelium and of rat oral epithelia, Loe, Karting and Hara ('72)[94] did not rule out the possibility that mitosis was to be found only in the basal layer. They stated that in the oral epithelium cell division was confined to the three deepest cell layers. Other investigators, however, have clearly indicated the site of cell division in murine epidermis and oral epithelium to be in the basal layer (Gelfant and Candelas '72[52], Potten '75, '79[114,115]).

C. The Epithelial Stem Cell Population

Stem cells are pluripotential or unspecialized cells with unlimited growth and division capability, giving rise to further generations of daughter cells. Some of the daughter cells maintain the proliferating cell fraction while others differentiate into a variety of cell types that, depending upon the nature of cell population, perform various functions. The stem cells of stratified squamous epithelium, while being self maintaining through sequential division, provide further generations of transitional or differentiated cells destined to abandon the cycle and enter a maturation pathway as keratinocytes; they then become corneocytes, ultimately exfoliate and die.

Stem cells are interspersed throughout the stratum basale in a random manner co-existing with a heterogeneous population of cells unrelated to keratinocyte producing stem cells. Such cells include Langerhans cells (Langerhans 1868[82], Rowden, Lewis and Sullivan '77[125], Rodriguez and Coarsi '78[124]), Merkel cells (Merkel 1875, Fortman '74[46]), melanocytes and dark basal keratinocytes (Klein-Szanto '77[73]).

The proliferating stem cell population is a pool of identical cells comprised of cycling and noncycling subpopulations. The concept of noncycling or resting cells was first proposed by Lajtha ('63)[57], Quastler ('63)[118] and Gelfant ('63)[50]. Lajtha studied the pattern of DNA synthesis and mitosis in rat liver after partial hepatectomy. He showed that with an appropriate stimulus some cells could be recruited from and out of resting state during the G_1 (post mitotic) phase. As a result he introduced the G_0 concept. Studies by Gelfant ('63)[50] of mouse ear epidermis showed yet another resting period. He reported a small proportion of stem cells that could be activated to enter mitosis. This prompted Gelfant to introduce the "G_2 blocked" concept.

After the preliminary introduction of the idea that cells can move out of the cycle for limited time periods while still capable of reactivation (Lajtha '63[79], Quastler '63[118], Gelfant '63[50]), some investigators introduced elaborate schemes in describing the cell cycle and produced addi-

tional new terminology. Epifanova and Terkikh ('69)[38] suggested the resting stages of R_1 and R_2 which cells might enter while passing through the mitotic cycle. Smith and Martin ('73)[136] proposed "A state" and "B phase" while DeMaertelaer and Galand ('75)[35] introduced "G phase" and "C phase". Some proposed even further subdivision of cell cycle phases (Temin '71[144], Radley, Hodgson and Koschel '76[120]).

The existence of G_2 resting phase of Gelfant ('63)[50], where cells could be triggered to re-enter the cycle, has recently been the subject of dispute (Sauerborn, Balmain, Goerttler and Stohr '78[126])

There are also some conflicting opinions regarding the distribution of dividing stem cells in various epithelia. Flemming (1884)[45] reported mitotic figures in the Malpighian layer of pig snout epidermis to be found in localized groups. Sheving ('59)[130] observed cellular division in human epidermis to occur frequently in localized regions or "nests". Studies of mouse oral epithelium (Cameron et al. '65)[23], hamster cheek pouch (Gibbs and Casarett '72)[53], human interdental papillae (Kittler and Mieler '69)[76], and rat palatal, lingual and gingival epithelia as well as human gingival epithelium (Loe et al. '72)[94] have shown that, in germinal layers of such epithelia, mitoses occur in groups. Aggregations of mitoses in oral epithelia were also termed "clusters" (Henry et al. '52[61], Glass and Goepp '74a[55], Lin, Goepp and Sewell '77[89]). However, Waugh and Ritchie ('63)[47] held the opinion that mitotic figures have a random distribution throughout the epidermal basal layer.

Leblond et al. ('64)[85]. Pereira and Leblond ('65)[110], and Leblond et al. ('67)[84] examined the nature and mechanism of daughter cell transfer in stratified squamous epithelium. These workers showed that either one, both or none of the daughter cells could be transfered and, furthermore, the disengagement of cells from the progenitor cell compartment is a random event. They concluded that the addition of new cells by mitosis in the basal layer results in an increase of population pressure and consequently cells are forced out in a random manner. Such conclusion was also reported by Bullough and Laurence ('64)[17] in their study of mouse ear epidermis.

D. Progenitor Cell Cycle in Renewing Epithelia

Introduction. For tissue growth and maintenance throughout the body, at one time or another, there is an uninterrupted succession of cells that are produced from pre-existing cells. In each period of production cells go through a life cycle in which they grow by doubling all their structural elements and functional capabilities.

The gradual progression of events through the cell cycle is usually assessed by observing what is happening in the nucleus. The two major processes readily identifiable are DNA replication followed shortly by cell division.

The Cell Cycle. During the lifespan of certain cell populations there exists and series of consecutive and recurring metabolic events which are essential for the maintenance of a predetermined

number of cells in tissues with transit elements. This series of metabolic progressions constitutes four phases which have collectively been known as the mitotic or cell cycle.

Howard and Pelc ('50,'51a,'51b)[63,64,65] using ^{32}P and Pelc and Howard ('52)[109] using ^{35}S, in bean root and mouse testis, demonstrated DNA synthesis to be confined to a discrete portion of the interphase (as opposed to mitotic phase) during which the genetic material is duplicated. It was demonstrated in plants (Taylor, Woods and Hughes '57)[143], in bacteria (Painter, Forro, Jun and Hughes '58)[104], and in animals (Hughes, Bond, Brecher, Cronkite, Painter, Quastler and Sherman '58)[67] that the deoxyribonucleoside thymidine (TdR) labeled with tritium (^{3}H) is exclusively incorporated into DNA during its synthesis (Richard and Estborn '51[123], Amano, Messier and Leblond '59[3], Cronkite, Bond, Fliedner and Rubini '59[33]). The incorporation of ^{3}H-TdR was shown to occur during the short period of its availability since it is absorbed by tissues and broken down after injection (Hughes et al. '58[67], Potter, '59[117], Cleaver '67[29]),The work of Howard and Pelc ('53)[66] showed that the mitotic cycle was sub divided into four periods: Mitosis (M), presynthesis period (G_1), the period of DNA synthesis (S) and pre-mitotic period (G_2).

Measurement of S-Phase from Metaphase Curve. The original work of Howard and Pelc ('53)[66] prompted widespread attempts to measure the duration of the cell cycle periods in a variety of tissues. Some also worked out detailed descriptions to define the various phases of the mitotic cycle (Mazia '61, '63[98,99], Bullough '65[16]).

The duration of the cell cycle and its phases can be determined by a variety of methods (Watanabe and Okada '67[160]). The most widely used procedure for studying the kinetics of renewing cell populations in mammals is the one that was introduced by Quastler and Sherman ('59)[19]. It consists of labeling stem cells in S period by a single pulse exposure to (3H-thymidine and in observing the cohort of labeled cells as it passes through mitosis.

Principle. The experiment begins after ^{3}H-TdR administration at zero time. Only cells in S period become labeled. The objective is to observe the movement of a block of labeled cells through a fixed point (mitotic phase) at various time intervals (Quastler and Sherman '59[119] Wolfsberg '64[164], Cameron '71[21], Steel, '77[139]).

At the earliest interval, labeled mitoses may not be observed. These unlabeled mitoses are cells that were in G_2 phase at the time of isotope injection. In this relatively short period the labeled block of interphase cells moves through the premitotic gap and enters M-phase to appear as labeled prophase. The time elapsed between addition of ^{3}H-thymidine and observation of first labeled mitoses corresponds to the time required for a cell (or a group of cells) to pass from the end point of S- phase to the beginning of mitosis (Figure 2,a) or the duration of G_2-phase (Quastler and Sherman, '59[119], Cattaneo, Quastler and Sherman, '61[26], Baserga and Weibel '69[7]). The percentage of labeled mitotic nuclei continues to rise indicating the gradual exit of labeled cells that were either at an earlier or a later moment of the synthetic period when becoming labeled. The number of labeled mitoses will soon reach a saturated point of 100 percent or close to it, presenting the last unlabeled metaphase nuclei (Figure 2, b). This represents the

maximum time for those cells already in premitotic gap period that require to reach mitosis (Quastler and Sherman '59[119]), Peckham and Kiekhofer '62[106]). After a plateau the maximal labeling level starts to decline until the minimum of labeled mitoses is approached equaling inversely the number of unlabeled mitotic nuclei (Figure 2, c). The starting point of the descending limb of the curve represents cells that were in G_1-phase at the time of ^{3}H-thymidine administration and as a result, after entering DNA replication period, did not become labeled and proceeded through S and G_2 periods to cell division (Figure 2, d). The shape of the downward curve will be skewed which is an indication of the variability in DNA synthesis (Painter and Drew '59)[103]. The average duration of DNA synthesis time is estimated from the plotted percent labeled mitoses curve (Figure 3) by measuring the intervening time from the 50% point on the ascending limb (indicating the location of the front edge of the cohort of labeled mitotic cells), to the 50% point on the descending limb (representing the tail edge of the cohort of labeled mitotic cells) of the curve (Wimber '63[162]), Gelfant '63[50]), Cameron '66[72]), Cleaver '67b[28]), Flaxman and Chopra '72[44]), Thilgratnam and Main '72[145]), Goodwin, Hamilton and Fry '74[57]), Aherne, Camplejohn and Wright '77[1]). The duration of mitosis is measured from the first appearance of labeled mitosis, to the point where all mitotic nuclei appear labeled (Quastler and Sherman '59[119]), Cleaver '67b[27]), Thrasher, Berg and Hauber '71[148]).

Duration of progenitor cell cycle can be estimated by using different methods. One method is to measure peak to peak duration on metaphase curve which provides the mean cycle duration (Quastler and Sherman '59[119]), or to measure the distance on the ordinate between 50% points of the first and the second ascending limbs (Cleaver '67b[29]), Lipkin '71[91]); Aherne et al. '77[1]). In certain cell populations, such as oral epithelium, jejunum and pulmonary adenoma, damping or minimal rise of a successive curve (or curves) has been reported (Toto and Dhawan '66[155]), Lipkin and Bell '68[92]), Aherne et al. '77[1]). Such decline in the size of succeeding waves in proliferating populations due to variations in behavior and progression of the labeled cells around the cycle (Cleave '67b[29]), Lipkin '71[91]). According to Aherne et al. ('77)[1]) there is an inverse relationship between the elapsed time from injection of ^{3}H-thymidine label and the degree of damping in the curve.

Another procedure for calculation of average cell generation time is by the following formula:

$$T_g = \frac{S}{LI}$$

Where is the average time for the geminative cell cycle which equals the ratio of the average S-phase duration to the index of DNA synthesis (Quastler and Sherman, '59[118]), Lesher, Fry and Kohn '64[18]), Skougaard and Beagrei, '62[134]), Cameron and Greulich, '63[24]), Cameron '64,[18]) Thrasher and Greulich '65[150]), Lipkin '65[90]), Hwang, Cronkite and Tonna '66[68]). The calculation is based on the following assumptions: 1) the cell population is in a steady state and the entire population participates in the proliferating activity, 2) the cell population is asynchronous, and 3) the labeling index is exclusively determined from the number of cells synthesizing DNA at the time of injection and shortly therafter - prior to entering mitosis (Young '62[165]), Skougaard '65b[133]), Thrasher '66[146]), Baserga and Weibel '69[7]).

The DNA synthetic index is defined as the proportion of germimal cells labeled shortly after

injection of ^{3}H-TdR before any labeled nuclei have had a chance to divide (Weinstein and Frost '70[161]). This index is also used as an indicator of the proliferative rate or the relative size of the progenitor population in the S-Phase (Thrasher '66)[146]. Although the rate of cell proliferation can be determined by the ratio of dividing and non-dividing cells or the mitotic index (Storey and Leblond '51[141], Leblond and Walker '56[87]). The radioactive labeling index has several advantages: 1) labeled cells are easily indentifiable, 2) the labeled cells are more numerous than mitotic cells, and 3) the duration of synthetic phase is 7-10 times longer than the mitotic phase, suggesting that the sensitivity of the labeling index for estimation of progenitor cell cycle as well as tissue turnover is approximately 7-10 times more sensitive (Cameron and Greulich '63[24], Thrasher and Greulich 65b[150], Thrasher '66[146]).

The duration of presynthetic time, G_1 is obtained by subtracting the sum of the duration of S-phase, G_2-phase and M-phase from the generation time T_gC (Quastler and Sherman '59[119], Peckham and Kiekhofer '62[106]).

E. Duration of Various Phases of the Cell Cycle

There have been a larger number of investigation using different methods to study cell proliferation and population kinetics in mammalian tissues. Prior to the introduction of ^{3}H-thymicine into biological research (Taylor et al. '57)[143], growth rate and turnover time of a cell population in stratified squamous epithelium, was estimated by measuring mitotic index and duration of mitosis in untreated tissues (Leblond and Walker '56[87], Meyer et al. '60[101]). Using colchicine or vinblastine sulfate as mitostatic agents was another method of providing information on mitotic duration and frequency of cell division (Henry et al. '52, Trott and Gorenstein '63[157], Randers Hansen '66a[121] and b[122], Karring and Loe '72[71]). Several isotope-labeled precursors e.g., ^{32}P-phosphate, ^{14}C-adenime, and ^{14}C-formate (Lajtha '54a[77], '54b[78], Lajtha, Oliver and Ellis '54[81]) had been used. However, none had the advantages of ^{14}C- or ^{3}H-labeled thymidine.

The most widely used procedure for determination of T_s and other phases of the cell cycle in epidermis and oral epithelia is pulse labeling with ^{3}H-thymidine (see D). Another useful methods to determine the duration of DNA synthesis is the double labeling technique using ^{3}H-TdR and ^{14}C-TdR (Pilgrim and Maurer '62[111]; Wimber and Quastler '63[153], Baserga and Lisco '63[6]). For more information to derive cell cycle data the reader is referred to Watanabe and Okada ('67)[160]. Table 3 outlines the cell cycle paramaters and turnover time in epidermis and oral epithelia using different methods.

F. Age Related Changes

The proliferative compartment of stratified squamous epithelium is a continuously dividing population of cells that unceasingly cycle from one mitosis to the next throughout the lifespan of an individual. Since the process of organismic senescence demands certain inevitable modification which occur to the living substance in the course of life, i.e., from the time of fertilization until natural death, the effect of such chronological alterations would almost certainly influence

the regeneratively capacity of the epithelial stem cell population as a whole.

Studying the effect of various inner and outer environmental factors on the proliferative ability of the guinea-pig ear epidermis, Loeb and Haven ('29)[95] reported a lower mitotic activity in the very young and a gradual decrease in the proliferative activity of the epidermis in the adult animal with increasing weight and age. Ortiz Picon ('33)[102] questioned if physiologic regeneration of epithelia actually slowed down with age. He reported a 200 percent increase in the rate of cell division in mouse epidermis at six months in comparison to one month. Eljiri ('37) counted the number of layers of epidermal cells in young and old cadavers. He stated that with advancing age the number of layers diminishes on the head and face and increases on the dorsal surface of the arm, head and foot. After measuring the diameter of pre-and postfixed pieces of epidermis taken from young and old individuals and comparing their qualitative changes, Evans, Cowdry and Nielseon ('43)[40] noticed a greater amount of shrinkage with fixation in the young skin as compared to the old.

There are also changes that occur in the configuration of the epithelium-connective tissue interface during aging. It has been observed that, in older individuals, there is a reduction in height and number of epithelial rete pegs causing as flattening out of the boundary between dermis and epidermis (Hill and Montgomery, '40[62], Cowdry, Cooper and Smith, '47[31], Thuringer and Cooper, '50[154]; Cooper, Thuringer and Katzberg, '51[30]; Southwood, '55[138], Papa and Klig- '64[105], Montagna, '65).

Age related changes of the morphology of the epithelium-connective tissue interface of oral mucosa has also been studied (Shklar, '66)[131]. These examinations of the palatal, gingival, buccal, lingual and lip mucosa have shown that in senile human oral mucosa there is a reduction in height and number of epithelial ridges. Loe and Karring ('77)[93] studied the morphology of the epithelium-connective tissue interface of the gingiva in individuals varying in age from 7 to 77 years. Their essential finding was that in young individuals the interface displays primarily connective tissue ridges whereas in older individuals it is characterized by connective tissue papillae.

Studies of human gingiva have shown the epithelial cell density to decrease with age (Meyer, Marwah and Weinmann, '56[100], Marwah, Weinmann, Meyer, '60[96], Gargiulo, Wentz and Orban, '61[49]). However, the size of the cell population in rat oral epithelia has been reported to be constant during aging (Randers Hansen, '66a[121], Karring and Loe, '73).

Investigations of abdominal skin from individuals between the ages of 2 days to 77 years led Cooper at al. ('52)[30] to conclude that the number of epidermal cells in mitosis increases from birth through the fourth decade and then remains relatively stationary. In a study of age and mitotic activity in mouse ear epidermis, Bullough ('49)[14] indicated that during the immature age (1-3 months) mitotic rate is high but is reduced during the mature age (3-12 months). During the middle age (13-18 months) the mitotic rate increases. In senility (end of middle age to death) it is again reduced.

In human gingival epithelium (Meyer et al., '56[100], Marwah et al., '60[96], Hayes, Silberkweit, Soni and Simpson, '64[59], Soni, Silberkweit and Hayes, '65[137]) and rat epidermis (Bertalanffy, Pusey and Abott, '65)[15] mitotic index was shown to increase with age, whereas Kiljunen ('56)[74] reported a decrease of mitotic index in aging rat epidermis. Studies in oral epithelium

of colchicinized rats have also produced contradictory results. According to Randers Hansen ('66a and b)[121,122] a significantly higher mitotic activity was found in old rats, while Karring and Loe ('72)[71] reported no difference in mitotic activity between young and old rats in spite of a longer duration of mitotic phase in 15-month-old rats compared to the ones that were 3-months-old.

In radioautographic studies of rat lingual and palatal epithelia, Sharav and Massler ('67)[129] reported a decrease in synthetic index and a possible decrease in epithelial turnover between 2 and 9 months followed by an increase in radioactive labeling index between 19 and 27 months. Thrasher ('71)[147] observed that in the mouse esophageal epithelium, the percentage of labeled basal cells declines almost two-fold with age. Toto and Ojha ('62)[156], Dhawan and Toto ('65)[37], and Toto and Dhawan ('66)[155] reported the S-phase in 60 day old mice to be 8 hours, while in 300 and 400 day old mice it appeared to be 10 hours. In a follow up study of cell renewal in 600 day old mice, Toto and his co-workers found the duration of S-phase in oral epithelia to be 12 hours (Barakat et al., '69)[5]. In comparison to the previous results these authors concluded that the S period, G_2 and basal cell generation time (T_gC) of the oral epithelium increases as the physiologic regeneration will slow down with age.

It was been observed by several investigators that with increased age there is a reduction in the thickness of the human epidermis (Thuringer and Cooper, '50[154], Southwood, '55[138], Papa and Kligman, '64[105], Bertalanffy, '65[10]). However, Andrew ('55) stated that in old rat epidermis no change in the thickness is observed. Cooper et al. ('51) concluded that young human epidermis is relatively thick, and that with advancing age it undergoes atrophy and is reduced to a few layers of cells. Histological and clinical studies of oral mucosa among the elderly revealed a thinner surface epithelium, a loss of elasticity, a tendency to hyperkeratosis, and a delayed healing response (Shklar, '66[131], Massler, '56[97]).

There are only a few electron microscopic observations of the aged epidermis. Mitchell ('69), in a study of chronically damaged epidermis, reported a decrease in overall amount of tonofilaments, keratohyalin granules and rough endoplasmic reticulum. According to an electron microscopic study of protected senile epidermis by Nagy and Janner ('70b) minimal cell irregularity, vacuolization of epidermal cells and minimal widening of intercellular space was found. Senile unprotected skin showed disturbances of the dermo-epidermal junction with duplication of the basement membrane and intracellular vacuolization of epidermal cells (Nagy and Janner, Zoa). The fine structural organization of the epidermis, dermal-epidermal junction and papillary dermis from exposed and unexposed sites of the elderly people was compared to the organization of similar regions of young people by Lavker ('79). He observed no alterations in the differentiation products associated with keratinization process and stratum corneum had the same thickness in young and old. However, in senile epidermis he found a relatively flat dermal-epidermal junction devoid of the microplications of basal cells into the dermis which he concluded to be an indication of a tissue less resistant to shearing force.

In split skin preparations Montagna and Carlisle ('79) studied the epithelial-connective tissue boundary using the scanning electron microscope. They found the underside of the epidermis in progressively older people to become increasingly flattened out and in very old persons to

appear as a flat boundary.

MATERIALS AND METHODS

A. Preparation of Animals

Experimental Animals

Throughout this investigation a total of 244 male inbred mice of Balb/C strain were utilized. Mice were chosen because of their: 1) rapid growth, maturation and aging in a span of two to two and one half years; 2) small body size and weight which require a small amount of radioactive precursor per animal. Only male mice were used, since the hormonal effects of estrous cycle in females could add a variable influencing the rate of mitosis (Swann, '58[142], Bullough, '62[15]). The mice were kept in air conditioned animal quarters at The University of Michigan, School of Dentistry, with the regular lighting schedule of 12 hours of dark and 12 hours of light. All mice were fed Purina Mouse Chow and tap water *ad libitum*. The animal-room temperature was maintained at 22-23°C. Since temperatures above 26°C stimulate mitotic activity in certain epithelia (Gelfant, '62)[15]. The animals were undisturbed except for daily cleaning and replenishing of food and water.

Age Groups

In order to determine the effects of age on the germinative cell cycle of oral epithelium, four age groups were selected: 4 weeks, 12 weeks, 12 months and 20 months. These ages in the mouse are comparable in man to: 1) first few years (growth and development); 2) around the age of 20 (young); 3) around the age of 45 (mature adults); and 4) at the age of 65 to 70 years where the individual is close to the end of the normal life expectancy.

Experimental Time Intervals

In order to determine the number of cells in DNA synthesis and to estimate other phases of the cell cycle after the initial pulse label at zero hour, animals in groups of three (first in groups of two and later individually) were sacrificed at 15 and 30 minute, and 1, 2, 4, 6, 8, 12, 16, 20, 25 and 30 hours after the precursor injection.

Radioactive Precursor

Thymidine (methyl-^{3}H)[1], at a dosage of 1 μCi/gm body weight was administered via intraperitoneal injection. The labeled precursor was selected as a tracer for this study because: 1) Thymidine is incorporated only into the pyrimidine, thymine, of deoxyribonucleic acid (Ham-

1. Specific activity – 6,700 mCi/m mol, New England Nuclear, 549 Albany Street, Boston, Mass. 02119

mersten, Richards and Saluste, '50)[58]. When a cell is about to divide it doubles its content of DNA and in so doing takes into its nucleus various DNA precursors. As one of these precursors, ^{3}H-thymidine is incorporated in the nuclear DNA. During mitosis and cell division an equal quantity of DNA is passed on to each daughter cell and with tritium added to the thymidine the radioactive content is also equally divided between daughter cells (Taylor, Woods and Hughes, '57)[43]. 2) In stratified squamous epithelium the incorporation of ^{3}H-thymidine exclusively occurs in the germinative cell population comprised only of cells in stratum basale. 3) The relatively long half-life (12.4 years) permits the use of this nucleotide in studies which require extended time periods (Pillinger, Hentges and Blair, '61)[112]. 4) The low energy of electrons emitted by tritium travel only a micron or so and will allow high radioautographic resolution and precise localization of the tracer in individual cells (Johnson, Haymaker, Rubini, Fliender, Bond, Cronkite and Hughes, '60[70]; Feinendegen, '67a[42]). 5) Since ^{3}H-thymidine is incorporated into newly synthesized DNA for only a small part of the S phase, the various phases of the generation cycle can be studied quantitatively by counting the appearance of labeled and unlabeled mitotic figures at different times after presentation of the precursor.

Route of Administration of Labeled Precursor

The method of administration of ^{3}H-thymidine in the present study, was an intraperitoneal injection, which requires the diffusion of the labeled precursor through the peritoneal membrane into the circulation. This route of administration is preferably used in studies of cell population kinetics with ^{3}H-thymidine (Feinendegen, 67b[43]; Tuohimaa, Segal and Koide, '73[158]). Tritiated thymidine is absorbed so rapidly after I.P. injections that more than 85% of the isotope is removed at 15 minutes, more than 90% at 30 minutes and more than 96% at 1 hour after the injection (Post, Huang, Hoffman and Mimeyev, '63)[113].

During injection of ^{3}H-thymidine extreme care was taken to avoid a reflux of the isotope solution following the withdrawal of the needle. In order to minimize the effects of diurnal cycle on DNA synthesis and mitotic activity (Allen, Smith and Garnder, '37[31]; Blumenfeld, '43[12]; Bullough, '49[14]) all injections were given at 10:00 a.m.

B. Histological Procedures

Anesthesia

All mice were anesthetized via an I.P. injection of 3.5% solution of chloral hydrate (1 ml per 100 gram body weight) in saline. A depilatory agent was used to remove hair on the mice head prior to fixation in order to avoid trapped air bubbles among facial hair and to provide better availability and penetration of fixative, dehydration solution and in particular embedding material.

Fixation

Animals were sacrificed at designated time intervals with an intracardiac perfusion of 10% neutral buffered formalin (Table 1). Immediately following the vascular perfusion, heads were removed and immersed in the same fixative for 49 hours and then washed and decalcified for 4 weeks in 0.5 M sodium ethylenediamine tetraacetate (EDTA) under constant agitation. The pH of decalcifying solution was maintained at 7.2 by adding 5.0 M citric acid monohydrate solution. Heads were then washed in running water and subsequently dehydrated in ascending concentrations of ethyl alcohol, cleared with xylene, followed by vacuum infiltration and embedding in paraffin. Blocks of paraffin were systematically numbered, mounted and sectioned with a rotary microtome and serial sections cut at 8 to 10 μm in thickness.

Selection of Sections

All sections were made from identical regions of oral cavity at a region about 2 millimeters anterior to the eyes. From each mouse head 24 serial sections were cut. One out of every five sections were selected in order to avoid double counting of those nondividing or dividing nuclei which were included in more than one section. These sections were then mounted (in sets of four) on glass slides pre-coated with subbing solution made up of:

A.	pure gelatin	1.5 gm
b.	chromium potassium sulfate	0.15 gm
c.	distilled water	300 ml

Preparation of Radioautographs

Tissue sections were deparaffinized by passing them through several changes of xylene and hydrated by passage through descending series of alcohol followed by complete hydration in distilled water.

Prior to coating with emulsion, slides were placed in water bath to bring their temperature to 45°C. Slides were then coated with a thin layer of nuclear tract emulsion (Kodak NTB-3) in total darkness. This was accomplished by heating the bottles of emulsion in a water bath preheated to 45°C and dipping the slides into this emulsion according to a routine procedure modified from Messier and Leblond ('57). The slides were then placed on end on drying rack at 60° position with tissue side up and allowed to dry in a preheated (45°C) oven.

After coating and drying, the slides were placed in regular slide boxes along with a dessicant (several capsules of Drierite per box) to maintain a dry atmosphere during the entire exposure time. The boxes were then sealed with adhesive tape, wrapped in lead sheaths and aluminum foil and packed with brown paper. The boxes were stored at 4°C for three weeks. At the end of the exposure period the slides were removed from cold storage and allowed to reach room temperature prior to development. The developing of radioautographs was accomplished by carrying out the following procedure in total darkness:

a.	Distilled water wash	5 minutes (18°C)
b.	Kodak D-19 developer	5 minutes (18°C)

c. Kodak stop bath 30 seconds

d. Kodak acid fixer 10 minutes (18°C)

e. distilled water wash 30 minutes (18°C)

The slides were then stained through the emulsion with hematoxylin and eosin.

C. Procedure for Determination of Cell Kinetic Parameters

Cell Identification

The radioautograms were examined under oil emersion (with total magnification including occular lens equal to 400X) with a Zeiss Photomicroscope. Regions of oral mucosa selected for this study were epithelia from the palate, cheek and the dorsum of tongue. Since cellular outlines were indistinct, nuclei, rather than cells themselves, were counted. Before a cell was considered as being labeled it had to meet the following criteria: 1) The grain had to be clustered over nuclei but was well above the negligible background count for an equivalent area of unlabelled tissue. 2) Five grains or more must be located over nucleus (interphase or mitotic).

Mitotic nuclei and stages of mitosis were recognized based on the following standards: 1) Prophase was identified by condensation and visibility of the chromosomes, the cell's tendency to assume a spherical form and an increased in the size of the cells. 2) Metaphase was recognized by gathering and arrangement of the chromosomes along the equatorial plate and the absence of nuclear membrane. 3) Anaphase was characterized by the separation and divergence of the two chromatids of each chromosome and their migration toward opposite poles of the spindle. 4) Telophase was identified by the division of cytoplasm into two sister cells.

The number of labeled and unlabeled basal epithelial cell nuclei per an average of 100 mitoses for each time interval were recorded (Gelfant, '66[51]; Barakat et al., '69[5]). Owing to the difficulty of identifying prophases and telophases in heavily labeled cells, only metaphases and anaphases were scored. In case a doubt existed as to whether a labeled cell belonged to the basal layer, the cell was recorded as a basal cell.

Microscopic Adjustment

In order to make sure that none of the nuclei were counted more than once and demarcate the desired region of the tissue in a rectangle, the position of the position of the reflecting system of the photomicroscope was set on position III which shows the rectangle used for photographic references. The first microscopic field was selected, then successive fields were observed and cell counts were made in one field after another. After counting one field, using a hand tally counter, the slide was moved one field to the right until the left side of the window (rectangle) bordered the last counted field. Such procedure was repeated until the other side of the section was reached.

Optically, the silver grains are often out of focus with respect to one another so that the microscope must be delicately racked up and down in order to count every one of them. The

microscope was first focused on the uppermost plane of the radioautographic emulsion to observe those silver grains belonging to the most superficial group of basal cells in the tissue section. Such nuclei belonged to those epithelial cells that were to be scored. Likewise, to avoid scoring of false positive mitoses which might be situated at a low level that would have had no bearing on grain localization, each labeled mitotic figure was examined by focusing the microscope up and down, and making a subjective judgement pertinent to this problem. All cell counts were made with a bright-field oil immersion objective.

Analysis of Kinetic Parameters

Introduction. In any renewing cell population of an adult organism there must exist a balance between the rate of cell production and the rate of cell loss. Some basal daughter cells remain undifferentiated and act as a source for new cells to replenish a continuously diminishing and differentiating and exfoliating cell population (spinous, granular and horny cells). The epithelial cell proliferation is morphologically comprised of four well-defined phases: S, G_2, M and G_1 phase (Howard and Pelc, '50[63], '51a[64], and b[65]).

Percent Labeled Mitoses (PLM) Curve and Measurement of Cell Cycle Phases. The basis of PLM or FLM (fraction of labeled mitoses) curve is the pulse labeling of cells by ^{3}H-thymidine in the S-phase and the subsequent radioautographic detection of the fraction of cells labeled in mitotic phase at different times after the pulse. The percentage of three groups of animals (each group constitutes individual animals sacrificed at designated time intervals) for each age group were counted and statistical analyses were performed. These percentages can then be plotted in a curve against time after the initial isotope administration at zero hour. The abscissa representing the sacrifice time intervals-periods at which cell counts were made; the ordinate indicating percent of labeled mitotic cells (Figures 1 and 2). The S-phase was determined by the extrapolation of the coordinates of the curves at 50% labeling of mitotic figures (Quastler and Sherman, '59[119]; Toto and Ojha, '62[156]; Cameron and Greulich, '63[24]; Brown and Berry, '68[13]). The time interval between the administration of ^{3}H-thymidine and appearance of the first labeled metaphase corresponds to the minimal time required for a cell to pass from the end of S-phase to division plus the time required for passage through prophase (Quastler and Sherman, '59[119]; Baserga and Wiebel, '69[7]). The interval of time between the first appearance of labeled mitoses and when they attain maximum labeling (up to 100%) is an estimate of the mean duration of mitosis (Quastler and Sherman, '59[119]; Cameron and Greulich, '63[24]; Thrasher, '65[150]).

The DNA Synthetic Index and Estimation of Progenitor Cell Turnover Time. Labeling index is the relative proportion of progenitor cells which have utilized ^{3}H-thymidine at an instant in time (Cameron and Greulich, '63)[24] In order to obtain a more reasonable representative number of labeled cells for statistical comparison for each age goup, labeled cells at 15, 30 and 60 minutes were scored and later pooled. For determination of the progenitor cell cycle duration, this index was divided into the mean duration of the S phase (Quastler and Sherman, '59[119]; Kisieleski, Baserga and Lisco, '61[75]; Peckham and Kiekhofer, '62[106]).

Mitotic Counts. The mitotic counts were obtained by scoring at least 3,000 basal cells (counts

for all three animals per age groups) for each experimental time interval in a given area.

Statistical Analysis

Model Used. Three points were picked on the upward swing and three points on the groups (4 weeks, 12 weeks and twelve months) time intervals of 1, 2 and 4 hours on the ascending limb and intervals of 6, 8 and 12 hours on the descending limb were chosen. For the last age group (20 months) since the ascending limb had a linear nature only two time intervals of 1 and 2 hours were selected, while on the descending limb intervals at 8, 12 and 16 hours were picked. In each region we assumed:

$$P = \gamma + \beta t + \alpha t^2 + \varepsilon$$

where *alpha, beta, gamma* are parameters determining the shape of the fitted quadratic curve (Figure 3b) to be estimated and *e* is a random error.

Justification. 1) We were unwilling to assume a functional form which would relate p (percent) to t over the whole range of t. 2) Using linear interpolation for two points surrounding p = .50 line may lead to a systematic bias in estimating $t_{.5u}$ and $t_{.5d}$ if the time curve relating p to t were not linear (there is strong evidence that it is not linear -- see graphs). 3) Using quadratic interpolation seems to be a good approximation to underlying functional relation in regions containing $t_{.5u}$ to $t_{.5d}$ (in several cases in a region containing $t_{.5u}$ a linear relation p = *alpha* + *beta*t + *e* seemed to suffice).

Problems. To fit the model above and to apply the usual statistical theory, it requires that the variance of measurements at each time point be constant over time points and that successive observations be independent. The second assumption was met since different animals were used at different time intervals; however, assumption of homogeneity of variance did not hold.

Adjusted Model. In order to adjust for lack of homogeneity, the model was fit to p^* = arc sine $(\sqrt{p})$ resulting in a model of the form.

$$\text{arc sine}(\sqrt{p}) = \gamma + \beta t + \alpha t^2 + \varepsilon$$

This model was fit to the data. Furthermore, we obtained the usual least square (maximum likelihood) estimators of *alpha, beta, gamma* and the estimated variances and covariances of these estimators.

As a result $t_{.5u}$ and $t_{.5d}$ were estimated by solving the following equation for t.

$$\text{arc sine}(\sqrt{.5}) = \hat{\gamma} + \hat{\beta} t + \hat{\alpha} t^2$$

Where *alpha, beta* and *gamma* are the estimated values of *alpha, beta* and *gamma* respectively. The estimator of $t_{.5u}$ and $t_{.5d}$ was given by:

$$t^{.5} = \frac{-\hat{\beta} \pm \sqrt{\hat{\beta}^2 - 4\hat{\alpha}(\hat{\gamma} - \text{arc sine } \sqrt{.5})}}{2\hat{\alpha}} = g(\hat{\alpha}, \hat{\beta}, \hat{\gamma})$$

The variance and standard errors of these estimators were obtained by expanding the above expressions for $t_{.5}$ in a Taylor series about the true values of *alpha, beta* and *gamma* (including only the constant and linear terms). Thus,

$$v(\hat{t}_{.5}) = \left(\frac{\partial g(\alpha\beta\gamma)}{\partial \alpha}\right)^2 v(\hat{\alpha}) + \left(\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \beta}\right)^2 v(\hat{\beta})$$

$$+\left(\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \gamma}\right)^2 v(\hat{\gamma}) + 2\left(\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \alpha}\right)\left(\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\lambda})}{\partial \beta}\right) Cov(\hat{\alpha}, \hat{\beta})$$

$$+ 2\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial g}\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \gamma} Cov(\hat{\alpha}, \hat{\beta}),$$

$$+ 2\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \beta}\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \gamma} Cov(\hat{\beta}, \hat{\gamma}),$$

Where $\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \alpha} = \frac{\hat{\beta}}{2\hat{\alpha}^2} \mp \frac{D + 2\hat{\alpha}(\hat{\gamma} - .785398)}{2\hat{\alpha}^2 D^{1/2}}$

$\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \beta} = -\frac{1}{\hat{\alpha}} \pm \frac{\hat{\beta}}{2\hat{\alpha} D^{1/2}}$ $\qquad$ $\frac{\partial g(\hat{\alpha}\hat{\beta}\hat{\gamma})}{\partial \gamma} = \pm\frac{1}{D^{1/2}}$

Where $D = \hat{\beta}^2 - 4\hat{\alpha}(\hat{\gamma} - .785398)$

then we estimated $t_{.5d} - t_{.5u}$ by $\hat{t}_{.5d} - \hat{t}_{.5u}$

and its variance by $v(t_{.5d}) + v(t_{.5u})$.

Approximate 95% confidence intervals for $t_{.5d} - t_{.5u}$ were given by:

$$\hat{t}_{.5u} - \hat{t}_{.5d} \pm 4\sqrt{v(\hat{t}_{.5d}) + v(\hat{t}_{.5u})}$$

We have used ± 4 standard deviations for these intervals rather than the usual ± 2. Since the latter requiring that the underlying distribution of the estimators is normal with known variances. In our case the exact distribution of the estimators were unknown and the variances were estimated via a Taylor expansion. It is believed that doubling the length of the interval more than compensates for these differences and yields consecutive intervals.

In the few cases (ascending limb of the curve in 20 month old animals) where a linear relation was assumed, the same procedures as above were used with the exception that $t_{.5u}$ is estimated by

$$\hat{t}_{.5u} = \frac{\text{arc sine } (\sqrt{.5}) - \hat{\alpha}}{\hat{\beta}}$$

$$\text{and } v(\hat{t}_{.5u}) = \frac{(.785398 - \hat{\alpha})^2}{\hat{\beta}^4} v(\hat{\beta}) + \frac{1}{\hat{\beta}^2} v(\hat{\alpha}) + 2\frac{.785398 - \hat{\alpha}}{\hat{\beta}^3} Cov(\hat{\alpha}, \hat{\beta})$$

RESULTS

Results of the Cell Cycle Studies

A. Position and Distribution of Labeled and Mitotic Nuclei

The examination of radioautograms at the hour interval after ^{3}H-TdR injection revealed that all labeled cells were observed to be confined to the basal layer. There was also no particular pattern of distribution of mitotic nuclei. They were most often seen to be dispersed randomly

among the basal cells. Although statistical test for randomness was not performed, the existance of mitotic clusters was not expected and none were found. In tongue epithelium mitotic cells occurred in groups of about two.

B. Duration of Cell Cycle and Its Phases

For a quick and over all evaluation of the data, duration of DNA synthetic phase for all regions and age groups was primarily estimated by simple linear interpolation between time points constructing the percent labeled mitoses curves from the raw data. These results were then compared to the values for the same parameter obtained through the quadratic model that was fit to the original data. It was then realized that the results were in many cases similar and in others there was a small variation.

C. Kinetics of the Labeled Mitotic Figures for 4 Week Old Age Group

The plot of the percentage of labeled mitoses (PLM) versus time for the epithelium of the palate (P), the epithelium of the cheek (C) and the epithelium of the tongue (T) is shown in Figures 5, 6 and 7. Labeled mitoses were first seen at one hour after ^{3}H-TdR administration, rose rapidly and approached a maximum of 99 percent, 99 percent and 99.5 percent for P, C and T respectively at 4 hours (Table 2). By twelve hours it fell below the 50% level down to 15% in P, 12.6% in C and 16% in T. It then reached zero at the time interval of sixteen hour for C, twenty hours for P and Twenty-five hours for T.

Duration of the Various Phases of the Cell Cycle. From the behavior of the ascending limb of PLM curves in Figures 4, 5 and 6, and using the principle depicted in Figure 1a, it is possible to estimate the duration of G_2 period. Since labeled mitotic figures (LMF) did not appear at 30 minutes and were first seen at the one hour interval, it could only be estimated that LMF could have appeared sometime between 30 and 60 minutes. This gives us a post synthetic period of 45 minutes for P, C and T. Duration of M phase was estimated from the time it took labeled mitotic nuclei to go from the point of first appearance at the one hour interval to a maximum percentage at the four hour interval (see Figure 1b). The mitotic period appeared to be three hours for P, C and T. There is a strong possibility that mitotic period is less than 3 hours since several additional intervening time intervals between 2 and 4 hours could have shown a better estimate for M-phase.

The values obtained for the duration of S period were derived from the model curve as the following:

Palate

$\hat{t}_l = 1.79$

$\hat{t}_u = 8.33$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 6.54$

CI = 5.80, 7.28

SE = .18

where t_1 is the estimated lower limit of time reflected on the abscissa from the intercept of

ascending limb by 50% line. $\hat{t}_u$ is the estimated upper limit of time on the abscissa from the intercept of descending limb at 50% point. CI is the confidence interval of S phase duration. SE for standard error of t.

Cheek

$\hat{t}_l = 1.86$

$\hat{t}_u = 8.03$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 6.17$

CI = 5.26, 7.08

SE = .22

Tongue

$\hat{t}_l = 1.76$

$\hat{t}_u = 8.32$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 6.55$

CI = 5.98, 7.12

SE = .14

The decimal points of the time periods (t) in hours for the three regions were then further converted to the nearest one half or one hour. The values obtained for the mean duration of the S-phase were 6½, 6 and 6½ hours for P, C and T respectively.

Epithelial basal cell generation time (TgC) was estimated from the equation:

$$T_gC = \frac{TS}{LI} \qquad (I)$$

where Ts is the average DNA synthesis time, and LI is the DNA synthetic index or the ratio of labeled basal cells to the total number of cells in the basal layer. In order to have a better estimate of the number of basal cells that were synthesizing DNA at, and shortly after, the time of ^{3}H-TdR injection, the mean value of LI for time periods of 15, 30 and 60 minutes was calculated (Table 3, 4, and 5) and was found to be .134 ± .058, .189 ± .092 and .146 ± .044 for P, C and T, respectively (.058, .092 and .044 are equal to one standard error of the means). The total generation time for epithelial basal cells in palate, cheek and tongue were calculated to be 48, 35 and 45 hours. The duration of presynthetic time (T_{G1}) was obtained by subtracting the sum of the synthetic time, postsynthetic time and mitotic time from the total generation time:

$$T_{G1} = T_gC - (T_S + T_{G2} + T_M) \qquad (II)$$

The postmitotic or presynthetic time periods were found to be 38 hours for palatal epithelium, 25.5 hours for cheek epithelium, and 35 hours for lingual epithelium.

The estimates of the duration of various phases of the oral epithelial cell cycle in 4 week old mice are represented in Table 6.

D. Kinetics of the Labeled Mitotic Figures for 12 Week Old Age Group

According to PLM curves seen in Figures 7, 8, and 9, labeled mitotic nuclei (LMN) begin to appear at one hour interval and pass the 50% level before the time interval at two hours. They

reach a maximum of 100% in tongue, 99.5% in palate and 99% in cheek at four hours (Table 7). LMN then continued to decline steadily and reached a zero percentage at sixteen hours for cheek, at twenty hours for palate and at twenty-five hours for tongue.

Duration of the Various Phases of the Cell Cycle. The appearance of LMN and the time they reached their maximum level was identical to the pattern observed in the previous age group and consequently the transit times for G_2 and M were the same. The postsynthetic time of 45 minutes and mitotic period of 3 hours were calculated for P, C and T by a similar procedure (Figures 7, 8 and 9).

The T_S values of the model curve for the 12 week old age group were as follows:

Palate

$\hat{t}_l = 1.71$

$\hat{t}_u = 7.75$

$\hat{t}_{su} - \hat{t}_{sl} = 6.04$

CI = 5.45, 6.63

SE = .14

Cheek

$\hat{t}_l = 1.70$

$\hat{t}_u = 7.60$

$\hat{t}_{.su} - \hat{t}_{.sl} = 5.90$

CI = 5.23, 6.55

SE = .16

Tongue

$\hat{t}_l = 1.68$

$\hat{t}_u = 8.15$

$\hat{t}_{.su} - \hat{t}_{.sl} = 6.47$

CI = 5.89, 7.04

SE = .14

The estimated duration of DNA synthesis for the 12 week old age group turned out to be 6 hours in the palate, 6 hours in the cheek and 6.5 hours in the tongue. The mean values of labeling indices were .118 ± .031, .104 ± .027 and .132 ± .022 for P, C and T (Figures 5, 6, and 7). For deriving the values for cell cycle time and postmitotic time, the same procedures seen in previous age groups were followed and equations I and II were solved. The generation cycle of basal cells were 50 hours in the palate, 56 hours in the cheek and 49 hours in the tongue. The presynthetic durations in the palate, cheek and tongue were 40 hours, 46 hours and 39 hours respectively (Table 8).

E. Kinetics of the Labeled Mitotic Figures for 12 Month Old Age Group

As it is presented in Table 9, the labeled mitotic figures in 12 month old mice were first

observed one hour after injection of ^{3}H-TdR. The number of LMF continued to rise and was past the 50% level at two hours (Figures 10, 11, and 12). It reached maximum level of 98% in the palate, 99% in the cheek and 98% in the tongue at the 4 hours sacrifice time. It stayed at about this level at six hours whereas at eight hours the LMF percentage appeared close to 50%. Unlike the previous age groups, it took the labeled mitotic nuclei in the three regions 25 hours to totally evacuate the mitotic phase (Table 9). In other words, it took those very last labeled cells in S-phase about 25 hours to complete their division (Figure 1d).

Duration of the Various Phases of the Cell Cycle. Basal epithelial cells in 12 month old age group appeared to have a postsynthetic and mitotic period of 45 minutes and 3 hours respectively. These parameters were also derived from the plots of the percentage of labeled mitoses. The model curve showed the following t value and its upper and lower limits:

Palate

$\hat{t}_l = 1.67$

$\hat{t}_u = 8.32$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 6.65$

CI = 5.91, 7.39

SE = .18

Cheek

$\hat{t}_l = 1.80$

$\hat{t}_u = 8.42$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 6.61$

CI = 5.8, 7.43

SE = .20

Tongue

$\hat{t}_l = 1.83$

$\hat{t}_u = 8.19$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 6.36$

CI = 5.80, 6.91

SE = .13

The calculated estimates for the duration of S phase from the above values were 6½ hours for all three regions (Figures 10, 11, and 12). The DNA synthetic indices obtained where .063 ± .022, .052 ± .025 and .064 + .02 for P, C and T (Tables 3, 4, and 5). The total generation time, $T_g C$, and the duration of postmitotic time, T_{G1}, was estimated from equations I and II. The values obtained were 105 hours, 127 hours and 98 hours for cell cycle duration and 95 hours, 117 hours and 88 hours for postmitotic gap period in palate, cheek and tongue respectively (Table 10).

F. Kinetics of the Labeled Mitotic Figures for 20 Month Old Age Group

The flow of labeled cells through mitosis and the fraction of labeled mitoses figures at 12

time intervals are shown (Figures 13, 14, and 15; Table 11). Unlike any other age group the percentage distribution of the labeled mitotic figures in the oral epithelia of 20 month old mice showed a sharp and linear rise after the one hour interval and reached maximum level of 100% in tongue, in palate 98%, and in cheek 97%, whereas the decline of the fraction of LMF was rather slow and fell close to 50% at the 12 hours interval. In the epithelium of palate and cheek it reached zero level after 25 hours, while in the tongue it was after 30 hours when the LMF had cleared out of the mitotic phase.

Duration of the Various Phases of the Cell Cycle. The first labeled mitotic figures were seen at the 1 hour interval and the duration of G_2 showed a similar time as the previous age groups. The mitotic duration was estimated as the time from the appearance of the first LMF (at 1 hour interval) until the labeled fraction reached a maximum level (at 2 hours interval, a value of 1 hour. However, based on the assumption that labeled mitotic figures, could have appeared at some time between 15 and 30 minutes, a better estimation for M-phase would be one and ¼ of an hour (Table 12).

According to the model curve the t values for S-phase in different regions of the oral cavity were:

Palate

$\hat{t}_l = 1.42$

$\hat{t}_u = 12.53$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 11.11$

CI = 10.25, 11.97

SE = .21

Cheek

$\hat{t}_l = 1.45$

$\hat{t}_u = 13.32$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 11.87$

CI = 9.54, 14.20

SE = .58

Tongue

$\hat{t} = 1.40$

$\hat{t}_u = 12.64$

$\hat{t}_{.5u} - \hat{t}_{.5l} = 11.24$

CI = 10.38, 12.09

SE = .21

The estimated values for S-phase after conversion of decimal points were 11 hours in palate, 12 hours in cheek and 11 hours in tongue. The labeling indices for P, C and T (Table 3, 4, and 5) were .095 ± .020, .111 ± .026 and .122 ± .03 respectively. The equation I was then solved by entering the available values for S-phase and indices of labeling. $T_g C$ for different regions of old oral mucosa were 117 hours in palate, 107 hours in cheek and 92 hours in tongue. Solving

the equation II, postmitotic periods for P, C and T were 104, 93 and 79 respectively (Table 12).

If the data derived for the cell cycle parameters in the oral epithelium of the different age groups is compared, the following results are evident (Tables 16, 17, and 18). The duration of postmitotic phase (G_1-phase) has a continuous rise in the palatal epithelium while in the epithelium of the cheek and the tongue it declines slightly. The increase in the mean duration of the cell cycle with age is mainly due to a corresponding change in the duration of the G_1-phase. In order to determine the type of change in G_1-phase and T_gC period over the four age groups, a quadratic curve was fit through the various time points (Figures 19 and 20). The results indicated that there is a significant linear and quadratic effect for cheek while in palate and tongue no significant duration (G_2-phase) in all age groups appears to be constant. Throughout the first three age groups, the duration of S-phase remains constant, while in old animals (20 months) DNA synthetic period is doubled. Such age related change is further substantiated through statistical analysis. Confidence intervals of Ts for 4, and 12 weeks and 12 months overlap but disjoint with that for 20 months and provide evidence of a significance. It is also noticed that the duration of M-phase in the first three age-groups remains constant, while in 20 month old animals it gains a faster rate of about 50% (Figure 18).

G. Labeling Index

The percentage of labeled basal cell nuclei in various regions shortly after ^{3}H-TdR injection is given in Table 13. The highest percentage of labeling index was found in 4 week old mice oral mucosa, i.e. 13.4 in palate, 18.9 in cheek and 14.6 in tongue. In 12 week old and 12 month old age groups it continuously declined, but it rose again in 20 month old age group. In order to determine whether there is a linear or quadratic effect in labeling index between the age groups averaged over the first three time intervals, an analysis of variance was performed. Since the variance within the 4 week old age group was significantly greater than the variances within the other three age groups, the analysis of variance was performed using only the last three age groups. This test showed that the variation between age groups was greater than within each group (palate, $F = 43.2$; cheek, $F = 53.8$; and tongue, $F = 74.8$). The F statistic was highly significant in all of the regions, $p = .0001$ (Table 14). Further statistical analysis of the data revealed that the changes between the age groups had a significant quadratic effect(Table 15) while in the palate, a linear effect also existed.

DISCUSSION

A. Age Related Changes in the Progenitor Cell Cycle

Comparisons of the mean duration of the synthetic phase along with the generation cycle time between the various age groups revealed that the epithelial covering of murine oral mucosa

undergoes a dramatic and progressive change in the rate of keratinocyte production. The studies described suggest that one effect of aging on oral mucosal epithelium (P, C and T) is a gradual increase in cell cycle time (Tables 16, 17, and 18), which is somewhat balanced (or offset) by a progressive decline in the number of proliferating cells in the S-phase as the animals age (Table 13), regardless of its slight increase from 12 to 20 months of age (Table 15 and Figure 19).

The analyses of DNA synthetic activity showed a significant decrease throughout the age groups. The continuous decline was significantly evident between 4 weeks and 12 months. That the percentage of labeling index is reduced is in agreement with the results of age related changes reported in the esophageal epithelium (Thrasher, '71[148]; Cameron, '72[22]), duodenal epithelium (Thrasher and Greulich, '65a[149] and b[150]), lingual epithlium and epidermis (Cameron, '72)[22]. whereas similar studies of oral epithelium (Toto and Ojha, '62[156]; Dhawan and Toto, '65[37]; Barakat et al., '69[5]) and duodenal epithelium (Lesher et al., '61)[88] have indicated no change in the index of labeling during aging.

The present study also revealed a moderate increase of 3.1% in the number of DNA synthesizing basal cells of the epithelium of the palate, 5.9% of the epithelium of the cheek, and 5.8% of the epithelium of the tongue at 20 months of age. Such recovery and increase was also reported in the oral epithelium of rats between the ages of 9 and 27 months (Sharav and Massler, '67)[129].

Other age related changes in the life cycle of the basal progenitor cells are a faster rate of about 50% in the duration of cell division which turns out to be simultaneous to a lengthening of the duration of S-phase in the senescent animals. The latter is a confirmation of the results of others (Toto and Ojha, '62[156]; Dhawan and Toto, '65[37]; Barakat et al., '69[5]; Lesher et al., '61[88]).

The changes in cellular proliferation is also reflected in the mean duration of the cell cycle. Cell cycle time is at its shortest in 4 weeks of age while in 20 months it extends considerably. This is mostly due to an increase in the G_1-phase of the cell cycle and is in agreement with the finding of others (Toto and Ojha, '62[156]; Dhawan and Toto, '65[37]; Barakat, '67[4]; Thrasher, '71[148]).

Despite the fact that the S-phase period increased in the senescent group, the values for the percentage of labeled cells participating in DNA synthesis suggests that the number of proliferating cells in the stem cell compartment had almost doubled between 12 and 20 months. It was also remarkable to note the occurrence of a simultaneous fluctuation in the duration of the progenitor cell cycle. In fact, such fluctuation was characterized by an increase in the duration of postmitotic period in all regions at 12 months, followed by a decrease at the age of 20 months seen only in the epithelium of the cheek and tongue.

The mechanisms which result in the development of such events can only be speculated. As the animal ages there is a reduction in the growth rate which might in turn be the result of a change in the internal environment. Reduction of the bodily growth has a more apparent effect in tissues with a continuous renewal rate like epithelial proliferating populations. Thus it is possible that when the epithelial growth rate indicated by the fraction of labeled cells or by the duration of the germinative cell cycle reaches a certain threshold, it activates the physiological state of the tissue and reestablishes a new rate of growth. Furthermore, the shortening of the

mitotic phase in the senescent group might be regarded as a compensatory process of the part of the basal progenitor cells to step up the production of new cells and offset the delay of the arrival of cells in the synthetic period.

B. Distribution and Nature of the Progenitor Compartment

The epithelium of various regions in oral mucosa was examined shortly after ^{3}H-TdR administration. During this brief period, radioactive label was present exclusively over the nucleus of a certain percentage (Table 13) of the basal cells. Therefore, only the cells in the basal layer were synthesizing DNA. In addition, the stratum basals consisted of a single row of cells which were contiguous to the connective tissue. Although the occurrence of basal cells in more than one layer has been reported (Skougaard, '65[133]; Amano, '66[2]; Sharav, '66[128]; Sharav and Massler, '67[129]), a logical explanation for such contradiction could most likely be due to the difficulty in maintaining a perpendicular pain of section to the epithelium-connective tissue boundary. In regions such as dorsum of the tongue and palatal rugae, connective tissue papillae are long and slender and are cut in a parallel plane. Consequently, some of the basal cells which were originally in contract with the basement membrane appear to be stratified in two or more cell layers suprabasally.

In stratified squamous epithelium the stem cell compartment of the basal layer is regarded as a self-maintaining population containing cells of non-identical proliferative capacity. In other words, it is believed that: 1) There is only a certain percentage of basal cells participating in the regenerative activity (Potten, '75; Sauerborn et al., '78[126]). This has been shown radioautographically by determination of values for index of labeling. 2) Basal stem cells are capable of moving out of the cycle and entering the G_0 period where they may stay for an indefinite period of time (Epifanova and Terskikh, '69[38]). 3) There is a unique population of progenitor cells that are "blocked" in G_1 and G_2 periods and enter S period or M period upon stimulation (Gelfant, '63 and '72[50,52]).

With regard to the first item, continuous labeling procedures (LeBlond et al., '67[84]; Hegazy and Fowler, '73[60]; Potten, Kovacs, and Hamilton, '74[116]) have reported that every cell of the basal layer becomes labeled which is indicative of a growth fraction of unity. When only a fraction of the basal cells are labeled, it is merely a response to the pulse label at that particular time and should not be interpreted as the proliferative fraction of the whole population. The concept that basal progenitor cells perform their reproductive duty or exercise their proliferative capacity in a periodic manner bring us to the second item. In steady state renewal systems like epithelial basal cells, it does not seem a festible biological strategy to enter a resting period or a state of "null" and retire from the mitotic cycle for an indefinite period of time.

Basal epithelial cells are structurally arranged in a single layer and are obliged to sustain the structural conformity of such layer and the overlying layers as a whole. In order to fulfill this requirement they would have to supply keratinocytes to the differentiating compartment on a regular basis and in relatively short time intervals which is in accordance to the renewal rate of the population as a whole viz, proliferating and differentiating compartments.

One can challenge the concept that cells remain in the G_1-, G_0-, or G_2-blocked states indefinitely until there is a tissue injury or other proliferative stimuli. The existance of a certain population of cells in G_1 and G_2 periods at any given time can more appropriately be indicative of a different behavior, as explained in the following paragraph.

During each and every mitotic cycle, randomly selected groups of basal progenitor cells enter a pocket or a subdivision of G_1 or G_2 phase. Thus, on a continuous basis a "reserve" group of cells, while being transient, will maintain a subpopulation of cells in postmitotic or postsynthetic periods on a permanent basis. Conceivably, a remnant of an evolutionary measure of adaptaion to unfavorable environmental or physiological conditions, the "reserve" cells by an unknown mechanism are called upon or recruited to enhance the number of cycling cells or vice versa.

The above description leads to the conclusion that the stem cell population of the stratified squamous epithelium consists of a single layer of basal cells. These cells are all homogeneous in their physiological activities. They all go through the cell cycle and they all have the same type of proliferative behavior.

SUMMARY

The age related changes in the life cycle of the progenitor cell population of murine oral epithelia was studied.

In this investigation the following observations are made:

1. The basal progenitor cells in different regions of oral mucosa have different generation times.
2. The basal cell cycle time increases as a function of aging and the region most affected by aging appears to be the epithelium of the cheek.
3. The phases of the cell cycle affected by the process of aging are in increasing order of magnitude: M-, S- and G_1- phase.
4. The age related change in the number of DNA synthesizing basal progenitor cells occurs at two age periods. Between 1 and 12 months of life it decreases, while from 12 to 20 months it increases.

REFERENCES

1. Aherne, W. A., Camplejohn, R. S., and Wright, N. A. (Eds.) *An Introduction to Cell Population Kinetics.* London: Edward Arnold Ltd., 1977. p. 34.
2. Amano, D. S. Autoradiography of Cell Proliferation and Migration in the Palatal Epithelium of the Rat. (M.S. Thesis), The University of Illinois, 1966. pp. 16-17.
3. Amano, M., Messier, B., and Leblond, C. P. Specificity of labelled thymidine as a DNA precursor in autoradiography. *J. Histochem. Cytochem., 7:153-155,* 1959.
4. Barakat, N. J. Age and Generation Cycle of Oral Epithelium. (M.S. Thesis), Illinois: Loyola University, 1967. p. 41.
5. Barakat, N. J., Toto, P. D., and Choukas, N. C. Aging and cell renewal of oral epithelium. *J. Periodont., 40:*599-602, 1969.
6. Baserga, R., and Lisco, E. Duration of DNA3 synthesis in Ehrlich ascites cells as estimated by double-labelling with ^{14}C- and ^{3}H-thymidine and autoradiography. *J. Natl. Cancer Cins., 31:*1559-1571, 1963.
7. Basergo, R., and Wiebel, F. The cell cycle of mammalian cells. *In:* International Review of Experimental Pathology. Vol. 7. G. W. Richter and M. A. Epstein (Eds.) New York and London: Academic Press, 1969. pp. 1-30.
8. Bertalanffy, F. D. The Mitotic Activity and Renewal of the Lung. (M.S. Thesis), Montreal: McGill University, 1951. p. 1.
9. Bertalanffy, F. D. Mitotic rates and renewal times of the digestive tract epithelia in the rat. *Acta Anat., 40:*130-148, 1960.
10. Bertalanffy, F. D., Pusey, V., and Abott, M. O. Mitotic rates of rat epidermis. During growth, maturity, senility, and regeneration. *Arch. Derm., 92:*91-102, 1965.
11. Bizzozero, G. An address on the growth and regeneration of the organism. *The Brit. Med. J., 1:*728-732, 1894.
12. Blumenfeld, C. M. Studies of normal and abnormal mitotic activity. III. The rate and periodicity of mitotic activity in regenerating epidermis of healing wounds in rabbits. *Arch. Path., 36:*493-498, 1943.
13. Brown, J. M., and Berry, R. J. The relationship between diurnal variation of the number of cells in mitosis and of the number of cells synthesizing DNA in epithelium of the hamster cheek pouch. *Cell Tissue Kinet., 1:*23-33, 1968.
14. Bullough, W. S. Age and mitotic activity in the male mouse, *Mus Musculus L. J. Exp. Biol., 26:*261-286, 1949.
15. Bullough, W. S. The control of mitotic activity in adult mammalian tissues. *Biol. Rev., 37:*307-342, 1962.
16. Bullough, W. S. Mitotic and functional homeostasis: A speculative review. *Can Res., 25:*1683-1727, 1965.
17. Bullough, W. S., and Laurence, E. B. The production of epidermal cells. *In:* The Mammalian Epidermis and Its Derivatives. Symposia of the Zoological Society of London. Number 12. F. J. Ebling (Ed.) London: Academic Press, 1964. pp. 1-23.
18. Cameron, I. L. Is the duration of DNA synthesis in somatic cells of mammals and birds a constant? *J. Cell Biol., 20:*185-188, 1964.
19. Cameron, I. L. Cell proliferation, migration, and specialization in the epithelium

of the mouse tongue. *J. Exp. Zool., 163:* 271-284, 1966.

20. Cameron, I. L. Cell renewal in the organs and tissues of the non-growing adult mouse. *Texas Pep. Biol. Med., 28:*203-248, 1970.
21. Cameron, I. L. Cell proliferation and renewal in the mammalian body. *In:* Cellular and Molecular Renewal in the Mammalian Body. I. L. Cameron and J. D. Thkasher (Eds.) New York: Academic Press, 1971. Chapt. 3, pp. 45-85.
22. Cameron, I. L. Minimum number of cell doubling in an epithelial cell population during the life-span of the mouse. *J. Gerontology, 27:*157-161, 1972.
23. Cameron, I. L., Gasslee, D. G., and Pilgrim, C. The spatial distribution of dividing and DNA synthesizing cells in mouse epithelium. *J. Cell Comp. Physiol., 66:*431-436, 1965.
24. Cameron, I. L., and Greulich, R. C. Evidence for an essentially constant duration of DNA synthesis in renewing epithelia of the adult mouse. *J. Cell Biol., 18:*31-40, 1963.
25. Carter, S. B. The influence of oestrone on the division of cells. *J. Endocrin., 9:* 19:29., 1953.
26. Cattaneo, S. M., Quastler, H., and Sherman, F. G. Proliferative cycle in the growing hair follicle of the mouse. Nature, 190: 923-924, 1961.
27. Cleaver, J. E. (Ed.). a. Thymidine metabolism: pathways of incorporation and degradation. *In:* Thymidine Metabolism and Cell Kinetics. Amsterdam: North-Holland Publishing Company, 1967. Chapt. 2, pp. 43-69.
28. Cleaver, J. E. (Ed.). b. Interphase replication of DNA and the cell cycle. *In:* Thymidine Metabolism and Cell Kinetics. Amsterdam: North-Holland Publishing Company, 1967. Chapt. 4, pp. 104-136.
29. Cleaver, J. E. (Ed.). c. Population kinetics in animal tissues. *In:* Thymidine Metabolism and Cell Kinetics. Amsterdam: North-Holland Publishing Company, 1976b. Chapt. 7, pp. 184-225.
30. Cooper, Z. K., Thuringer, J. M., and Katzberg, A. A. Age variations in mitotic frequency and pattern of the rete in human epidermis. *J. Geront., 6:*73-74, 1951.
31. Cowdry, E. V., Copper, Z. K., and Smith, W. Program of research on aging of the skin. *J. Geront., 2:*31-44, 1947.
32. Cowdry, E. V., and Thompson, H. C. Jr. Localization of maximum cell division in epidermis. *Anat. Rec., 88:*403-409, 1944.
33. Cronkite, E. P., Bond, V. P., Fliedner, T. J., and Rubini, J. R. The use of tritiated thymidine in the study of DNA synthesis and cell turnover in hemopoietic tissues. *Lab. Invest., 8:*263-277, 1959.
34. Cutright, D. E., and Bauer, H. Cell renewal in the oral mucosa and skin of the rat. I. Turn-over time. *Oral Surg., Oral Med., Oral Path., 23:*249-259, 1967.
35. DeMaertelaer, V., and Galand, P. Some properties of a "G_0" Model of the cell cycle. *Cell Tissue Kinet., 8:*11-22, 1975.
36. Dhawan, A. S. Renewal of Cell population in Palate and Tongue Epithelium of Mice. (M.S. Thesis), Illinois: Loyola University, 1964. pp. 22, 28-29.
37. Dhawan, A. S., and Toto, P. D. Renewal of cell population in palate and tongue epithelia of mice. *J. Dent. Res., 44:*989-995, 1965.
38. Epifanova, O. I., and Terskikh, V. V. On the resting periods in the cell life cycle. *Cell Tissue Kinet., 2:*75-93, 1969.
39. Epstein, W. L., and Maibach, H. I. Cell

renewal in human epidermis. *Arch. Derm., 92:*462-468, 1965.

40. Evans, R. E., Cowdry, V., and Nielseon, P. E. Aging of Human skin. I. Influence of dermal shrinkage an appearance of the epidermis in young and old fixed tissues. *Anat. Rec., 86:*545-565, 1943.
41. Fabrikant, J. I. (Ed.) Cell Population Kinetics. *In:* Radiobiology. Chicago: Year Book Medical Publishers, Inc., 1972. pp. 96-119.
42. Feinendegen, L. E. Physics of tritium, *In:* Tritium Labeled Molecules in Biology and Medicine. New York: Academic Press, 1967. pp. 1-14a.
43. Feinendegen, L. E. Routes of administration of the labeled precursor. *In:* Tritium-labeled Molecules in Biology and Medicine. New York: Academic Press, 1967. pp. 238-241.
44. Flaxman, A., and Chopra, D. P. Cell cycle of normal and psoriatic epidermis *in vitro. J. Invest. Derm., 59:*102-105, 1972.
45. Flemming, W. Zur kenntniss der regeneration der epidermis bein sangethier. *Arch. Mikr. Anat., 23:*148-154, 1884.
46. Fortman, G. J. Ultrastructure of the Mekel Cell in Human Oral Mucosa. (M.S. Thesis), University of Minnesota, 1974. pp. 26-58.
47. Frei, J. V., Waugh, W. O. N., and Ritchie, A. C. Mitosis: Distribution in mouse ear epidermis. *Science, 140:*487-488, 1963.
48. Gargiulo, A. W. Mitotic activity of human oral epithelium when exposed to hydrogen peroxide. (M.S. Thesis), Illinois: Loyola University, 1959, p. 25.
49. Gargiulo, A. W., Wentz, F. M., and Orban, B. Mitotic activity of human oral epithelium exposed to 30 percent hydrogen peroxide. *Oral Surg., Oral Med., Oral Patb., 14:* 474-492, 1961.
50. Celfant, S. A new theory on the mechanisms of cell division. *In:* Cell Growth and Cell Division. R. J. C. Harris (Ed.), New York: Academic Press, 1963. pp. 229-259.
51. Gelfant, S. Patterns of cell division: The demonstration of discrete cell populations. *In:* Methods in Cell Physiology. Vol. 2. David M. Prescott (Ed.), New York: Academic Press, 1966. pp. 359-395.
52. Gelfant, S., and Candelas, G. C. Regulation of epidermal mitosis. *J. Invest. Drm., 59:*7-12, 1972.
53. Gibbs, S. J., and Casarett, G. W. Spatial distribution of cells in mitotic and DNA synthetic phases of the cell cycle in hamster cheek pouch epithelium. *J. Dent. Res., 51:* 30-38, 1972.
54. Gilbert, C. W., and Lajtha, L. G. (Eds.) The importance of cell population kinetics in determining response to irradiation of normal and malignant tissues. *In:* Cellular Radiation Biology. XVIIIth Annual Symposium on Fundamental Cancer Research. Baltimore: Williams and Wilkins, 1965. pp. 474-497.
55. Glass, R. T., and Goepp, R. A. a. Spatial relationship of basal cells in the mouse tongue after radiation injury. *Rad. Res., 58:* 219-229, 1974a.
56. Glass, R. T., and Goepp, R. A. b. Movement of labeled basal cells in the mouse tongue after radiation injury. *Rad. Res., 58:*230-238, 1974b.
57. Goodwin, P., Hamilton, S., and Fry, L. The cell cycle in psoriasis. *Brit. J. Derm., 90:* 517-524, 1974.
58. Hammersten, E., Richards, P., and Salvste, E. Pyrimidine nucleotides as precursors of pyrimidines in polynucleotides. *J. Biol. Chem., 183:*105-109, 1950.
59. Hayes, R. L., Silberkweit, M., Soni, N. N.,

and Simpson, T. H. Pattern of mitotic activity and cell densities in normal gingival epithelium of children. *J. Dent. Res., 43:* 217-223, 1964.

60. Hegazy, M. A. H., and Fowler, J. F. Cell population kinetics of plucked and unplucked mouse skin. I. Unirradiated skin. *Cell Tissue Kinet., 6:*17-33, 1973.
61. Henry, J. L., Meyer, J., Weinmann, J. P., and Schour, I. Pattern of mitotic activity in oral epithelium of rabbits. *Arch. Path., 54:*281-297, 1952.
62. Hill, W. R., and Montgomery, H. Regional changes and changes caused by age in the normal skin. *J. Invest. Derm., 3:*231-245, 1940.
63. Howard, A., and Pelc, S. R., P^{32} autoradiographs of mouse testis; preliminary observations of the timing of spermatogenic stages. *Brit. J. Radiol., 23:*634-641, 1950.
64. Howard, A., and Pelc, S. R. Synthesis of nucleoprotein in bean root cells. *Nature, 167:*599-600, 1951a.
65. Howard, A., and Pelc, S. R. Nuclear incorporation of P^{32} as demonstrated by autoradiographs. *Exp. Cell Res., 2:*178-187, 1951b.
66. Howard, A., and Pelc, S. R. Synthesis of deoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. *In: Symposium on Chromosome Breakage Heredity (suppl.). Charles C. Thomas, U.S.A., 6:*261-273, 1953.
67. Hughes, W. L., Bond, V. P., Brecher, G., Cronkite, E. P., Painter, R. B., Quastler, H., and Sherman, F. G. Cellular proliferation in the mouse as revealed by autoradiography with tritiated thymidine. *Proc. Nat. Acad. Sci. (USA), 44:*476-483, 1958.
68. Hwang, W. S. S., Cronkite, E. P., and Tonna, E. A. Cell proliferation kinetics of the internal enamel epithelium of mouse incisors. *J. Dent. Res., 45:*350-358, 1966.
69. Iverson, O. H., Bjerknes, R., and Devik, F. Kinetics of cell renewal, cell migration, and cell loss in the hairless mouse dorsal epidermis. *Cell Tissue Kinet., 1:*351-367, 1968.
70. Johnson, H. A., Haymaker, W. E., Rubini, J. R., Fliender, T. M., Bond, U. P., Cronkite, E. P., and Hughes, W. L. A radiographic study of a human brain and glioblastoma multiform after the *in vivo* Uptake of tritiated thymidine. *Cancer, 13:*639-642, 1960.
71. Karring, T., and Loe, H. The use of colchicine for the assessment of mitotic activity in rat oral epithelium. *Scand. J. Dent. Res., 80:*474-485, 1972.
72. Kauffman, S. L. An autoradiographic study of the generation cycle in the ten-day mouse embryo renal tube. *Exp. Cell Res., 42:*67-73, 1966.
73. Klein-Szanto, A. J. P. Clear and dark basal keratinocytes in human epidermis. *J. Cut. Path., 4:*275-280, 1977.
74. Kiljunen, A. Mitotic activity in normal and malignant epidermal tissue of the rat. *Acta Path. Microbiol. Scand., 112:* 14-52, 1956.
75. Kisieleski, W. E., Baserga, R. and Liscc H. Tritiated thymidine and the study of tumors. *Atompraxis, 7:*81-85, 1961.
76. Kittler, G., and Mieler, I. Der Mitosekoeffizient der gesunden und entzundlich erkrankten Interdentalpapillen. *Deutsch Zahn-, Mund- und Kieferheilkunde, 53:*19-27, 1969.
77. Lajtha, L. G. Detection of adenine carbon-14 in deoxyribonucleic acid by autoradiography. *Nature, 173:*587-588, 1954a.
78. Lajtha, L. G. Utilization of formate- ^{14}C

for synthesis of deoxyribonucleic acid by human bone marrow cells *in vitro*. *Nature, 174:*1013-1014, 1954b.

79. Lajtha, L. G. Differential sensitivity of the cell life cycle. *J. Cellular Comp. Physiol., 62*(suppl. 1):141-156, 1963.
80. Lajtha, L. G. On the concept of the cell cycle. *J. Cell Comp. Physiol., 62*(suppl. 1):143-145, 1963.
81. Lajtha, L. G. Oliver, R., and Ellis, F. Incorporation of ^{32}P and adenine ^{14}C into DNA by human bone marrow cells *in vitro*. *Brit. J. Cancer, 8:*367-379, 1954.
82. Langerhans, P. Uber die Nerven der menschlichen Hant. *Virch. Arch. Anat. Path., 44:*325-337, 1868.
83. Laurence, E. B., and Randers Hansen, E. An *in vitro* study of epidermal chalone and stress hormones on mitosis in tongue epithelium and ear epidermis of the mouse. *Virch. Arch. Abt. BuZellpath., 9:*271-279, 1971.
84. Leblond, C. P., Clermont, Y., and Nadler, N. J. The pattern of stern cell renewal in three epithelia (esophagus, intestine and testis). In: Stem Cells (Proceedings of the Seventh Canadian Cancer Conference. Vol. 7. J. F. Morgan, R. L. Noble, R. J. Rossiter, R. M. Taylor, A. C. Wallace, D. M. Whitelaw (Eds.), Oxford: Pergamon Press, 1967. pp. 3-30.
85. Leblond, C. P., Greulich, R. C., and Pereira, J. P. M. Relationship of cell formation and cell migration in the renewal of stratified squamous epithelia. *In: Advances* in Biology of Skin. Vol. V. Wound Healing. W. Montagna and R. E. Billingham (Eds.), New York: The MacMillian Company, 1964. pp. 39-67.
86. Leblond, C. P., and Stevens, C. E. The constant renewal of the intestinal epithelium in the albino rat. *Anat. Rec., 100:*357-371, 1948.
87. Leblond, C. P., and Walker, B. E. Renewal of cell populations. *Physiol. Rev., 36:* 255-276, 1956.
88. Lesher, S., Fry, R. J. M., and Kohn, H. I. Age and the generation time of the mouse deudenal epithelial cell. *Exp. Cell Res., 24:*343, 1961.
89. Lin, L., Goepp, R. A., and Sewell, A. F. Diurnal variation in epithelial cell population kinetics of young mouse tongue. *J. Dent. Res., 56:*425-436, 1977.
90. Lipkin, M. Cell proliferation in the gastrointestinal tract of man. *Fed. Proc., 24:*10-15, 1965.
91. Lipkin, M. The proliferative cycle of mammalian cells. *In:* The Cell Cycle and Cancer. R. A. Baserga (Ed.) New York: Marcel Decker, Inc., 1971. pp. 6-26.
92. Lipkin, M., and Bell, B. Cell proliferation. *In:* Handbook of Physiology. Section 6: Alimentary Canal. Vol. 5. Washington, D. C.: American Physiological Society, 1968. Chapt. 138, pp. 2861-2879.
93. Loe, H., and Karring, T. The three-dimensional morphology of the epithelium-connective tissue interface of the gingiva as related to age and sex. *Scand. J. Dent. Res., 79:*315-326, 1971.
94. Loe, H., Karring, T., and Hara, K. The site of mitotic activity in rat and human oral epithelium. *Scand. J. Dent. Res., 80:*111-119, 1972.
95. Loeb, L., and Haven, F. L. Quantitative studies on the growth of the epidermis. *Anat. Rec., 42:*217-241, 1929.
96. Marwah, A. S., Weinmann, J. P., and Meyer, J. Effect of chronic inflammation on the epithelial turnover of the human gingiva. *Arch. Path., 69:*147-153, 1960.

97. Massler, M. Tissue changes during aging. *Oral Surg., Oral Med., Oral Path., 9:*1185-1196, 1956.

98. Mazia, D. Mitosis and the physiology of cell division. *In:* The Cell. Vol. 3. J. Brachet and A. E. Hirsky (Eds.) New York: Academic Press, Inc., 1961. pp. 77-412.

99. Mazia, D. Synthetic activities leading to mitosis. *J. Cell Comp. Physiol., 62* (suppl. 1):123-140, 1963.

100. Meyer, J., Marwah, A. S., and Weinmann, J. P. Mitotic rate of gingival epithelium in two age groups. *J. Invest. Derm., 27:* 237-247, 1956.

101. Meyer, J., Medak, H., and Weinmann, J. P. Mitotic activity and rates of growth in regions of oral epithelium differing in width. *Growth, 24:*29-46, 1960.

102. Ortiz Picon, J. M. Uber Zellteilungsfrequenz und Zellteilungsrhythmús in der Epidermis der Mans. *Z. Zellforsch, 19:*488-509, 1933.

103. Painter, R. B., and Drew, R. M. Studies on deoxyribonucleic acid metabolism in human cancer cell culture (Hela). I. The temporal relationship of deoxyribonucleic acid synthesis to mitosis and turnover time. *Lab. Invest., 8:*278-285, 1959.

104. Painter, R. B., Forro, Jun, F., and Hughes, W. L. Distribution of tritium labeled thymidine in *Escherichia coli* during cell multiplication. *Nature, 181:*328-329, 1958.

105. Papa, C. M., and Kligman, A. M. The effect of topical steroids on the aged human axilla. *In:* Advances in Biology of Skin. Aging. Vol. VI. W. Montagna (Ed.) Oxford: Pergamon Press, 1964. pp. 177-198.

106. Peckham, B., and Kiekhofer, W. Cellular behavior in the vaginal epithelium of estrogen-treated rats. *Am. J. Obstet. Gynecol., 83:*1021-1027, 1962.

107. Pelc, S. R. Labeling of DNA and cell division in so-called nondividing tissues. *J. Cell Biol., 22:*21-28, 1964.

108. Pelc, S. R., and Gahan, P. B. Incorporation of labeled thymidine in the seminal vesicle of the labeled mouse. *Nature, 183:* 335-336, 1959.

109. Pelc, S. R., and Howard, A. Chromosome metabolism as shown by autoradiographs. *Exp. Cell Res., 2* (Suppl.):269-278, 1952.

110. Pereira, J. P. M., and Leblond, C. P. Mitosis and differentiation in the stratified squamous epithelium of the rat esophagus. *Am. J. Anat., 117:*73-90, 1965.

111. Pilgrim, C., and Maurer, W. Autoradiographische Bestummung der DNS-Verdopplungszeit verschiedener Zellarten van Mans und Ratte bei Doppelmarkierung miz H-3-und c-14-Thymidin. *Naturwissenschaften, 49:*544-545, 1962.

112. Pillinger, W. L., Hentges, J. J., and Blair, J. A. Tritium decay energy. *Phys. Rev., 121:*232-233, 1961.

113. Post, J., Huang, C., Hoffman, J., and Mimeyev, T. B. The replication time and pattern of liver cells in the growing rat. *J. Cell Biol., 18:*1-12, 1963.

114. Potten, C. S. Epidermal cell production rates. *J. Invest, Derm., 65:*488-500, 1975.

115. Potten, C. S. Personal communication. Aug. 1979.

116. Potten, C. S., Kovacs, L., and Hamilton, E. Continuous labeling studies on mouse skin and intestine. *Cell Tissue Kinet., 7:* 271-283, 1974.

117. Potter, V. R. Metabolic products formed from thymidine. *In:* The Kinetics of Cell Proliferation. F. Stohlmann (Ed.) New York: Grune and Stratton, 1959. pp. 104-110.

118. Quastler, H. The anlaysis of cell popula-

tion kinetics. In: Cell Proliferation. A Guinness Symposium held at the University of Dublin, Trinity College. L. F. Lamberton and R. J. M. Fry (Eds.) Oxford: Blackwell Scientific Publications, 1963. pp. 18-34.

119. Quastler, H., and Sherman, F. G. Cell population kinetics in the intestinal epithelium of the mouse. *Exp. Cell Res., 17:* 420-438, 1959.
120. Radley, J. M., Hodgson, G. S., and Koschel, K. W. Properties of Go *cells: Variations* in the proliferative response following isoprenaline. *Cell Tissue Kinet., 9:*371-377, 1976.
121. Randers Hangsen, E. Mitotic activity in the the oral epithelium of the rat. Variations according to age and time of the day. *Odont. T., 74:*196-201, 1966a.
122. Randers Hansen, E. Mitotic activity of the gingival epithelium in colchicinized rats. *Odont. T., 74:*229-239, 1966b.
123. Richard, P., and Estborn, B. Utilization of deoxyribosides in the synthesis of polynucleotides. *J. Biol. Chem., 188:*839-846, 1951.
124. Rodriguez, E. M., and Caorsi, I. A second look at the ultrastructure of Langerhans cells of the human epidermis. *J. Ultrastruct. Res., 65:*279-295, 1978.
125. Rowden, G., Lewis, M. G., and Sullivan, A. K. la antigen expression on human epidermal Langergans cells. *Nature, 268:* 247-248, 1977.
126. Saverborn, R., Balmain, A., Goerttler, K., and Stohr, M. On the existance of "arrested G_2 cells" in mouse epidermis. *Cell Tissue Kinet., 11:*291-300, 1978.
127. Schoenheider, W. A., Jr. DNA metabolism of the basal cell layer of the epithelium of the tongue in the mouse. (M.S. Thesis), Illinois: Loyola University, 1960. pp. 18, 24.
128. Sharav, Y. Age changes in oral epithelia. (M.S. Thesis), University of Illinois, 1966. pp. 21-22.
129. Sharav, Y., and Massler, M. Age changes in oral epithelia. Progenitor population, synthesis index and tissue turnover. *Exp. Cell Res., 47:*132-138, 1967.
130. Sheving, L. E. Mitotic activity in the human epidermis. *Anat. Rec., 135:*7-19, 1959.
131. Shaklar, G. The effects of aging upon oral mucosa. *J. Invest. Derm., 47:*115-120, 1966.
132. Skougaard, M. Turnover of gingival epithelium in marmosets. *Acta Odont. Scand., 23:*623-643, 1965a.
133. Skougaard, M. The renewal of the gingival epithelial cells in mice and marmosets. *In:* Cell Population Kinetics of the Gingival Epithelium. Copenhagen: Rhodos International Scientific Publishers, 1965b. p. 101.
134. Skougaard, M.R., and Beagrie, G.S. The renewal of gingival epithelium in marmosets *(Callithrix jacchus)* as determined through autoradiography with thymidine-H. *Acta Odont. Scand., 20:*467-484, 1962.
135. Smart, I.H.M. Variation in the plane of cell cleavage during the process of stratification in the mouse epidermis. *Brit. J. Derm., 82-*276-282, 1970.
136. Smith, J.A., and Martin, L. Do cells cycle? *Proc. Nat. Acad. Sci. (U.S.), 70:*1263-1267, 1973.
137. Soni, N.N., Silbrkeitt, M., and Hayes, R.L. Pattern of mitotic activity and cell densities in human gingival epithelium. *J. Periodont., 36:*15-21, 1965.
138. Southwood, W.F.W. The thickness of the skin. *Plast. Reconst. Surg., 15:*423-429, 1955.

139. Steel, G.G. (Ed.) Technique of labelled mitoses. *In*:Growth Kinetics of Tumors. Cell population Kinetics in Relation to the Growth and Treatment of Cancer. Oxford: Claredon Press, 1977. Chapt. 4, pp. 120-145.

140. Storey, W.F. Renewal time of the epidermis in the white rat. (M.S. Thesis) Montreal: McGill University, 1949.

141. Storey, W.F., and Leblond, C.P. Measurement of the rate of proliferation of epidermis and associated structures. *Ann. N.Y. Acad. Sci., 53*:537-545, 1951.

142. Swann, M.M. The control of cell division: A review. II. Special mechanisms. *Cancer Res., 18*:1118-1160, 1958.

143. Taylor, J.H., Woods, P.S., and Hughes, W.L. The organization and duplication of chromosome as revealed by autoradiographic studies using tritium labeled thymidine. *Proc. Nat. Acad. Sci. (Wash.), 43*:122-128, 1957.

144. Temin, H. Stimulation by serum of multiplication of stationary chicken cells. *J. Cellular Physiol., 78:161-170,* 1971.

145. Thilagaratnam. C.N., and Main, J.M.P. Changes in cell cycle characteristics in hamster cheek pouch epithelium during treatment with DMBA. *J. Oral Path., 1*:89-102, 1972.

146. Thrasher, J.D. Analysis of renewing epithelial cell population. *In*: Methods in Cell Physiology. Vol. II. D. M. Prescott (Ed.) New York: Academic Press, 1966. pp. 323-357.

147. Thrasher, J.D. Age and the cell cycle of the mouse esophageal epithelium. *Exp. Geront., 6*:19-24, 1971.

148. Thrasher, J.D., Berg. N.B., and Hauber, E. Natural variation in the S-phase of renewing epithelia of the pouchless oppossum, *Marmosa mitio. Cell Tissue Kinet., 4*:185-192, 1971.

149. Thrasher, J.D., and Greulich, R.C. The duodenal progenitor population. I. Age related changes in size and distribution. J. *Exp. Zool., 159*:385-396, 1965.

150. Thrasher, J. D., and Greulich, R.C. The duodenal progenitor population. II. Age related changes in size and distribution. *J. Exp. Zool., 159*:385-396, 1965.

151. Thuringer, J. M. Regeneration of stratified squamous epithelium. *Anat. Rec., 28*:31-43, 1924.

152. Thuringer, J. M. Studies on cell division in human epidermis. *Anat. Rec., 40*:1-13, 1928.

153. Thuringer, J. M. The mitotic index of the palmar and plantar epidermis in response to stimulation. *J. Invest. Derm., 2*:313-326, 1939.

154. Thuringer, J. M., and Cooper, Z.K. The mitotic index of the human epidermis, the site of maximum cell proliferation and the development of the epidermal pattern. *Anat. Rec., 106*:225, 1950. (Abstract)

155. Toto, P.D., and Dhawan, A.S. Generation cycle of oral epithelium in 400-day-old mice. *J. Dent. Res., 45*:948-950, 1966.

156. Toto, P.D., and Ojha, G.D. Generation cycle of oral epithelium in mice. *J. Dent. Res., 41*:388-391, 1962.

157. Trott, J.R., and Gorenstein, S.L. Mitotic Rates in the oral and gingival epithelium of the rat. *Arch. Biol., 8*:425-434, 1963.

158. Tuohimaa, P., Segal, S.J., and Koide, S.S. Routes of administration and uptake of tritiated nucleotides. *Histochemie, 37*:39-47, 1973.

159. Waldeyer, W. Ueber Karyokinise und ihre Beziehungen zu den Befruntungs-norgamgen. *Aarch Mikros. Anat., 32*:1-122, 1888.

160. Watanabe, I., and Okada, S.Effects of tem-

perature on growth rate of cultured mammalian cells (L5178y) *J. Cell Biol., 32*309-323, 1967.

161. Weinstein, G.D., and Frost, P. Cell proliferation in human basal cell carcinoma. *Cancer Res., 30*:724-728, 1970.
162. Wimber, D.E. Methods of studying cell proliferation with emphasis on DNA labels. *In*: Cell Proliferation. A guinness Symposium held at the University of Dublin, Trinity College. L.F. Lamberton and R. J.M. Fry (Eds.) Oxford: Blackwell Scientific Publications, 1963. pp. 1-17.
163. Wimber. D.E., and Quastler, H.A 14C-and 3H-thymidine double labelling technique in the study of cell proliferation in Trades-Cantia root tips. *Exp. Cell Res., 30*:8-22, 1963.
164. Wolfsberg, M.F. Cell population kinetics in epithelium of the forestomach of the mouse. *Exp. Cell Res., 35*:119-131, 1964.
165. Young, R.W. Cell proliferation and specialization during endochondral osteogenesis in young rats. *J. Cell Biol., 14*:357-370, 1962.
166. Young, W.G. Mitotic activity in the oral epithelium of the female rat. J. *Periodont. Res., 3*:51-62, 1968.

Table 1. Number of mice in cell cycle experiments

Age	Time intervals	Mice/time interval	Number of animals
4 weeks	12	3	36
12 weeks	12	3	36
12 months	12	3	36
20 months	12	3	36
		Total	144

Table 2. Percentage of labeled mitotic figures at different experimental time intervals in 4 week old murine oral mucosa

experimental time intervals	Percent of labeled mitotic figures		
	P	C	T
1/4	0	0	0
1/2	0	0	0
1	7.5	5.5	10.3
2	61.0	58.0	63.0
4	99.0	99.0	99.5
6	92.0	90.0	91.0
8	56.0	51.0	55.0
12	15.0	12.6	16.0
16	4.6	0	5.0
20	0	0	1.3
25	0	0	0
30	0	0	0

Table 3. The labeling index in palatal epithelium of different age groups shortly after ^{3}H-thymidine injection

Age	15m[1]	30m	60m	mean ± SD[2]
4 weeks	0.129	0.142	0.129	0.134 ± 0.058
12 weeks	0.115	0.122	0.115	0.118 ± 0.030
12 months	0.046	0.076	0.067	0.063 ± 0.018
20 months	0.098	0.100	0.088	0.094 ± 0.019

[1] Duration in minutes. [2] Standard Deviation.

Table 4. The labeling index in epithelium of the cheek of different age groups shortly after ^{3}H-thymidine injection

Age	15m[1]	30m	60m	mean ± SD[2]
4 weeks	0.149	0.181	0.238	0.189 ± 0.085
12 weeks	0.109	0.113	0.091	0.104 ± 0.025
12 months	0.041	0.049	0.067	0.052 ± 0.022
20 months	0.113	0.117	0.104	0.111 ± 0.026

[1] Duration in minutes. [2] Standard Deviation.

Table 5. The labeling index in lingual epithelium of different age groups shrotly after ^{3}H-thymidine injection

Age	15m[1]	30m	60m	mean ± SD[2]
4 weeks	0.138	0.139	0.161	0.146 ± 0.044
12 weeks	0.125	0.152	0.188	0.132 ± 0.016
12 months	0.049	0.070	0.074	0.064 ± 0.017
20 months	0.188	0.143	0.140	0.122 ± 0.024

[1] Duration in minutes. [2] Standard Deviation.

Table 6. Duration of various phases of the basal epithelial cell cycle and generation time in 4 week old murine oral mucosa

Epithelium	S (h)[1]	G_2 (m)[2]	M (h)	G_1 (h)	T_gC (h)
Palate	6½	45	3	38	48
Cheek	6	45	3	25½	35
Tongue	6½	45	3	35	45

[1] Duration in hours.
[2] Duration in minutes.

Table 8. Duration of various phases of the basal epithelial cell cycle and generation time in 12 week old murine oral mucosa

Epithelium	S (h)[1]	G_2 (m)[2]	M (h)	G_1 (h)	T_gC (h)
Palate	6	45	3	40	50
Cheek	6	45	3	46	56
Tongue	6½	45	3	39	49

[1] Duration in hours.
[2] Duration in minutes.

Table 7. Percentage of labeled mitotic figures at different experimental time intervals in 12 week old murine oral mucosa

experimental time intervals	Percent of labeled mitotic figures		
	P	C	T
1/4	0	0	0
1/2	0	0	0
1	12.0	10.9	14.0
2	63.0	67.0	65.0
4	99.5	99.0	100.0
6	90.0	89.0	91.0
8	44.0	40.0	53.0
12	12.0	8.3	13.0
16	2.5	0	5.7
20	0	0	1.4
25	0	0	0
30	0	0	0

Table 9. Percentage of labeled mitotic figures at different experimental time intervals in 12 month old murine oral mucosa.

experimental time intervals	Percent of labeled miotic figures		
	P	C	T
1/4	0	0	0
1/2	0	0	0
1	12.0	13.0	14.0
2	65.0	59.0	57.0
4	98.0	99.0	98.0
6	97.0	96.0	97.0
8	57.0	59.0	54.0
12	22.0	20.0	22.0
16	11.0	11.0	15.0
20	4.0	4.0	5.0
25	0	0	0
30	0	0	0

Table 10. Duration of various phases of the basal epithelial cell cycle and generation time in 12 month old murine oral mucosa.

Epithelium	S (h)[1]	G_2 (m)[2]	M (h)	G_1 (h)	T_gC (h)
Palate	6½	45	3	95	105
Cheek	6½	45	3	117	127
Tongue	6½	45	3	88	98

[1] Duration in hours.
[2] Duration in minutes.

Table 11. Percentage of labeled mitotic figures at different experimental time intervals in 20 month old murine oral mucosa

experimental time intervals	Percent of labeled mitotic figures		
	P	C	T
1/4	0	0	0
1/2	0	0	0
1	7.0	6.0	6.0
2	98.0	97.0	100.0
4	95.0	95.0	98.0
6	78.0	80.0	85.0
8	66.0	60.0	71.0
12	54.0	49.0	55.0
16	11.0	8.0	12.0
20	2.0	2.0	4.0
25	0	0	1.0
30	0	0	0

Table 12. Duration of various phases of the basal epithelial cell cycle and generation time in 20 month old murine oral mucosa

Epithelium	S (h)[1]	G_2 (m)[2]	M (h)	G_1 (h)	T_gC (h)
Palate	11	45	1¼	104	117
Cheek	12	45	1¼	93	107
Tongue	11	45	1¼	79	92

[1] Duration in hours.
[1] Duration in minutes.

Table 13. The percentage of labeled basal progenitor cells in murine oral epithelium during aging

Epithelium	4 weeks	12 weeks	12 months	20 months
Palate	13.4	11.8	6.3	9.5
Cheek	18.9	10.4	5.2	11.1
Tongue	14.6	13.2	6.4	12.2

Table 14. Analysis of various in the labeling index of the basal progenitor cells in murine oral epithelium during aging*

Epithelium	Source of variation	Amount of variation	df	Mean square	F-statistic	P
Palate	Between ages	0.054	2	0.0271	43.22	<0.0001
	Within ages	0.066	105	0.0006	–	–
Cheek	Between ages	0.075	2	0.0374	53.858	<0.0001
	Within ages	0.073	105	0.0006	–	–
Tongue	Between ages	0.094	2	0.0471	74.865	<0.0001
	Within ages	0.066	105	0.0006		

* Age groups compared consisted of the 12 week old, the 12 month old, and the 20 month old animals

Table 16. Mean duration of the cell cycle and its phases in the palatal epithelium of aging mouse

Age	S (h)[1]	G_2 (m)[2]	M (h)	G_1 (h)	T_gC (h)
4 weeks	6½	45	3	38	48
12 weeks	6	45	3	40	50
12 months	6½	45	3	95	105
20 months	11	45	1¼	104	117

Duration in hours
Duration in minutes

Table 15. The significance of linear and quadratic effects between 12 week old 12 month old, and 20 month old age groups

Epithelium	CI[1] for linear effect	CI for Quadratic eff
Palate	- 0.022 ± 0.015	0.086 ± 0.0
Cheek	0.007 ± 0.015	0.111 ± 0.0
Tongue	- 0.010 ± 0.014	0.124 ± 0.0

[1] CI, 95% confidence interval

Table 17. Mean duration of the cell cycle and its phases in the epithelium of the cheek of aging mouse

Age	S (h)[1]	G_2 (m)[2]	M (h)	G_1 (h)	T_gC (h)
4 weeks	6	45	3	25½	35
12 weeks	6	45	3	46	56
12 months	6½	45	3	117	127
20 months	12	45	1¼	93	107

[1] Duration in hours
[2] Duration in minutes

Table 18. Mean duration of the cell cycle and its phases in the lingual epithelium of aging mouse

Age	S (h)[1]	G_2 (m)[2]	M (h)	G_1 (h)	T_gC (h)
4 weeks	6½	45	3	35	45
12 weeks	6½	45	3	39	49
12 months	6½	45	3	88	98
20 months	11	45	1½	79	92

1. Duration in hours
2. Duration in minutes

Table 19. The linear and quadratic effects in G_1-phase between 4 week, 12 week, 12 month, and 20 month old age groups

Epithelium	Estimate of linear effect			Estimate of quadratic effect		
	Estimate	SE[1]	Att. SL[2]	Estimate	SE	Att. SL
Palate	2.0069	0.6931	0.2117	- 0.0125	0.0081	0.3654
Cheek	4.0254	0.3894	0.0614	- 0.0368	0.0045	0.0787
Tongue	2.2373	0.6397	0.1773	- 0.0188	0.0075	0.2415

[1] SE, standard error based on 1 degree of freedom.
[2] Att. SL, attained significance level for testing the hypothesis of no linear or quadratic effect.

Table 20. The linear and quadratic effects in T_gC between 4 week, 12 week, 12 month, and 20 month old age groups

Epithelium	Estimate of linear effect			Estimate of quadratic effect		
	Estimate	SE[1]	Att. SL[2]	Estimate	SE	Att. SL
Palate	1.9423	0.6729	0.2123	- 0.0113	0.0079	0.3865
Cheek	3.9578	0.3417	0.0548	- 0.0353	0.0040	0.0720
Tongue	2.1727	0.6195	0.1768	- 0.0176	0.0072	0.2491

[1] SE, standard error based on 1 degree of freedom.
[2] Att. SL, attained significance level for testing the hypothesis of no linear or quadratic effect

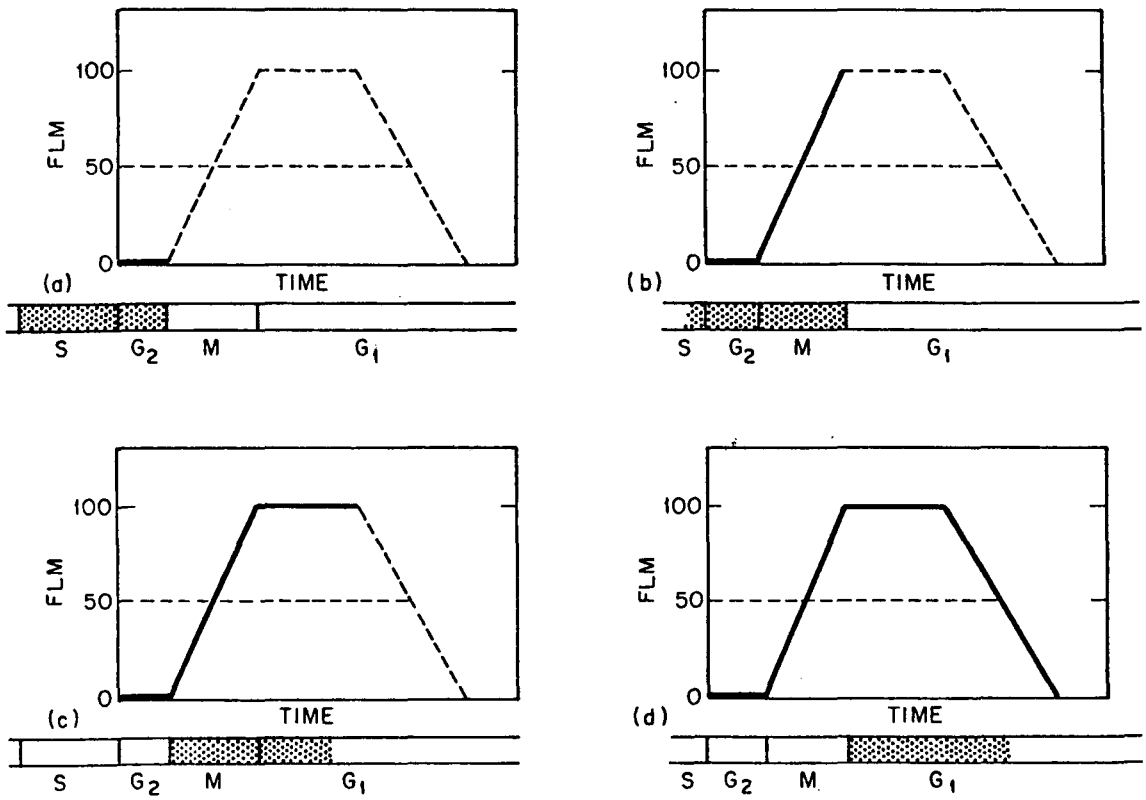

Fig. 1. Explanation of the principle for timing the periods of cell cycle. At the bottom of each curve a cohort of labeld nuclei are represented by black dots. As the block of labeled nuclei move through G_2 (a), the proportion of labeled mitotic nuclei (LMN) will increase until it reaches a maximum level or 100 percent (b). It then takes a short while for the trailing edge of LMN to clear through G_2 (c). As the leading edge of unlabeled mitotic nuclei enters M, the percentage of LMN starts to decline until all LMN have moved out of M (d) and have been replaced by unlabeled mitotic nuclei. The latter are cells which at the time of ^{3}H-thymidine injection were in G_1 period.

G_2, postsynthetic period.
M, mitotic period.
G_1, postmitotic period.

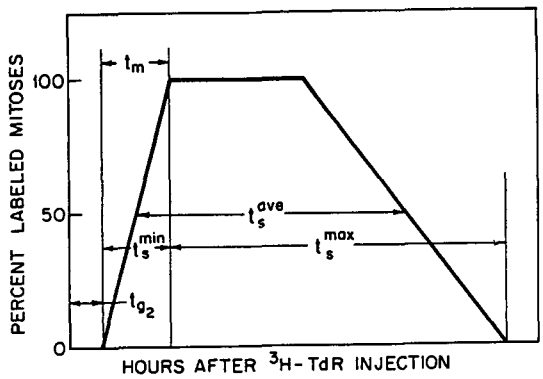

Fig. 2. A diagrammatic plot demonstrating the percentage of labeled mitosis (ordinate) as a function of the time between ^{3}H-thymidine injection and sacrifice (abscissa), and vaious time parameters.

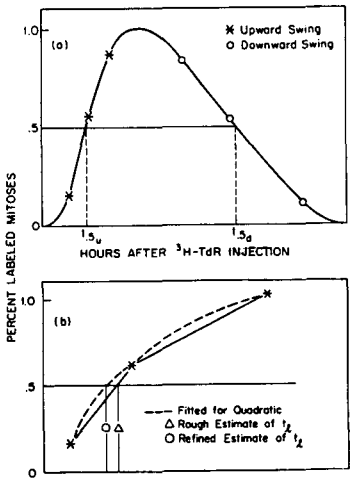

Fig. 3. (a) Hypothetical model curve. .05 intercepts on upward and downward swings of the curve show lower ($t_{.5u}$) and upper ($t_{.5d}$) values for time.
(b) Curve showing rough linear and refined quadratic interpolations.

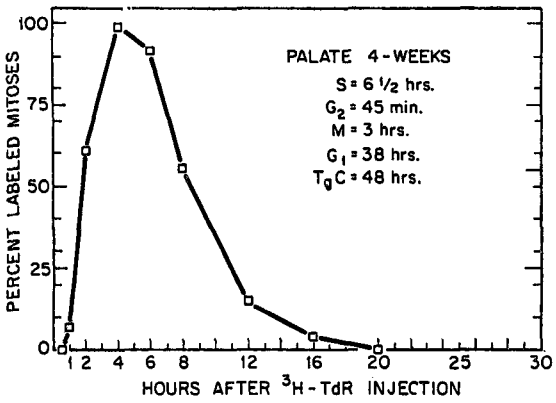

Fig. 4. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the palatal epithelium of 4 week old mouse.

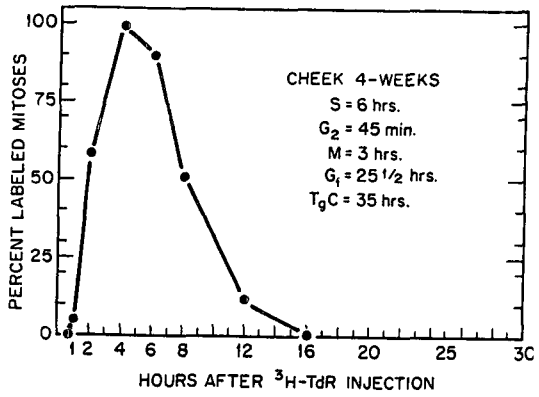

Fig. 5. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection ^{3}H-TdR in the epithelium of the check of 4 week mouse.

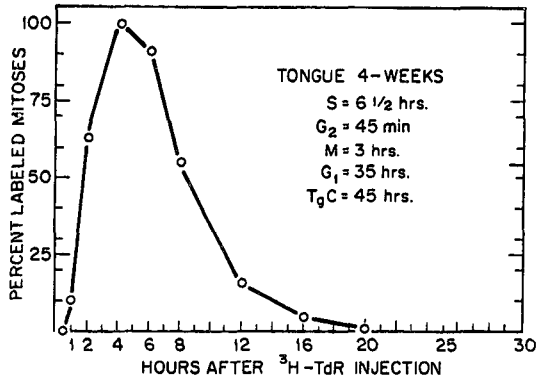

Fig. 6. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following injection of ^{3}H-TdR in the lingual epithelium of 4 week old mouse.

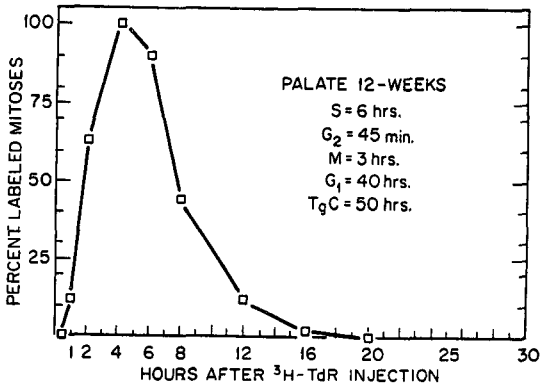

Fig. 7. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the palatal epithelium of 12 week old mouse.

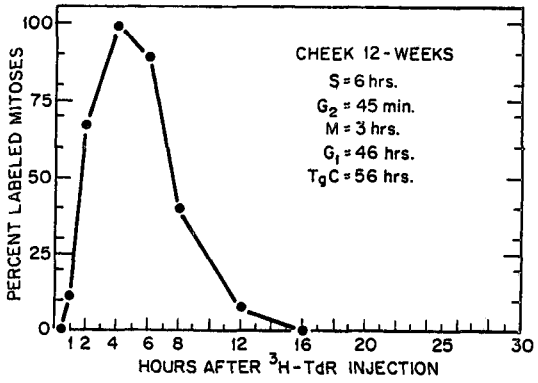

Fig. 8. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the epithelium of the cheek of 12 week old mouse.

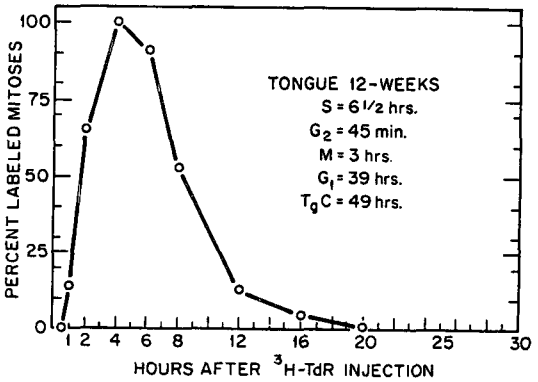

Fig. 9. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection ^{3}H-TdR in the lingual epithelium of 12 week old mouse.

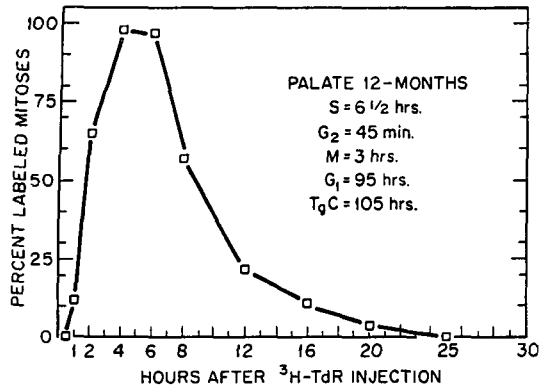

Fig. 10. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the palatal epithelium of 12 month old mouse.

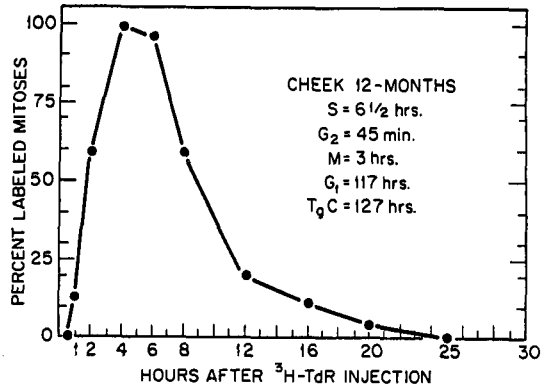

Fig. 11. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the epithelium of the cheek of 12 month old mouse.

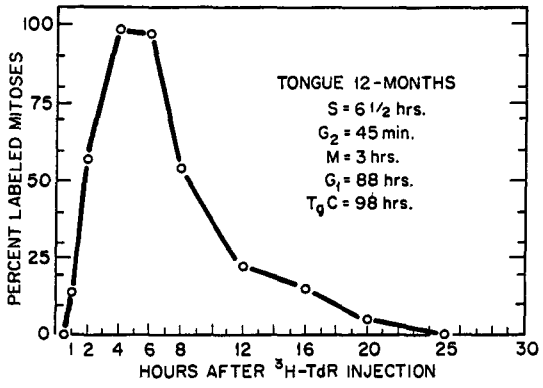

Fig. 12. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the lingual epithelium of 12 month old mouse.

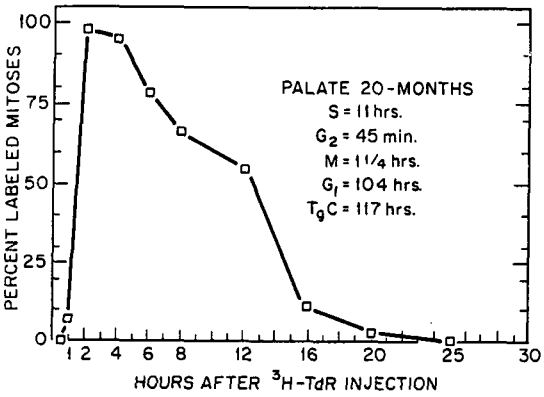

Fig. 13. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the palatal epithelium of 20 month old mouse.

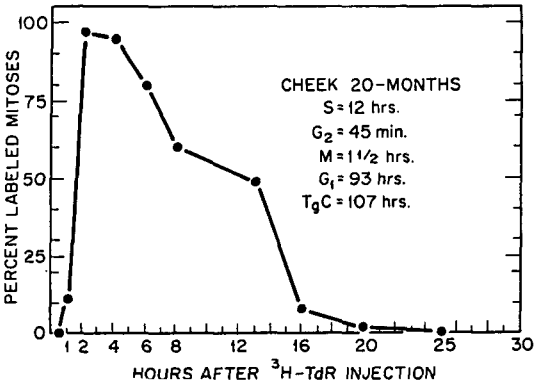

Fig. 14. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the epithelium of the cheek of 20 month old mouse.

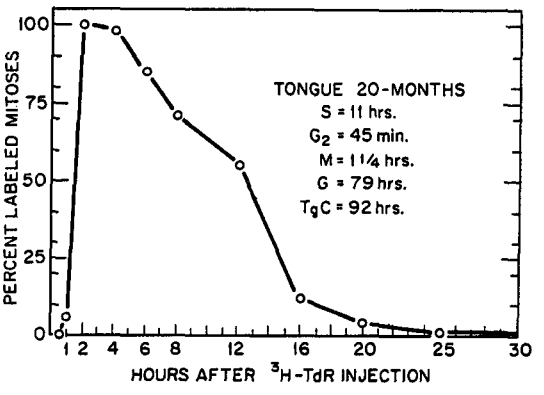

Fig. 15. Curve describing the rate of appearance and disappearance of labeled mitotic nuclei following a single injection of ^{3}H-TdR in the lingual epithelium of 20 month old mouse.

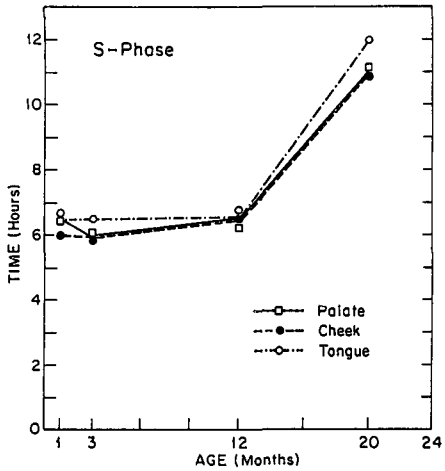

Fig. 16. Effects of aging on the duration of S-phase in the epithelium of murine oral mucosa.

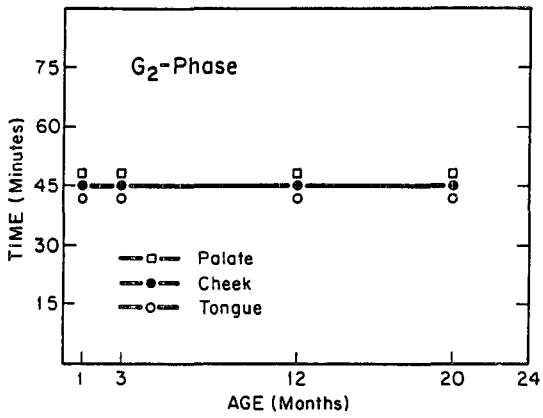

Fig. 17. Effects of aging on the duration of G_2-phase in the epithelium of murine oral mucosa.

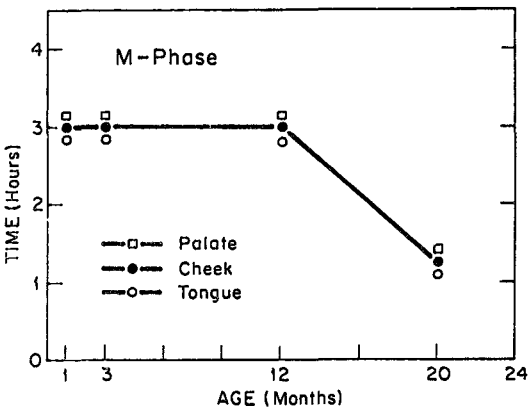

Fig. 18. Effects of aging on the duration of M-phase in the epithelium of murine oral mucosa.

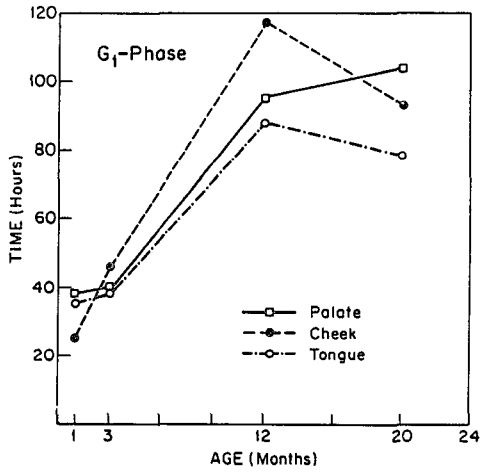

Fig. 19. Effects of aging on the duration of G_1-phase in the epithelium of murine oral mucosa.

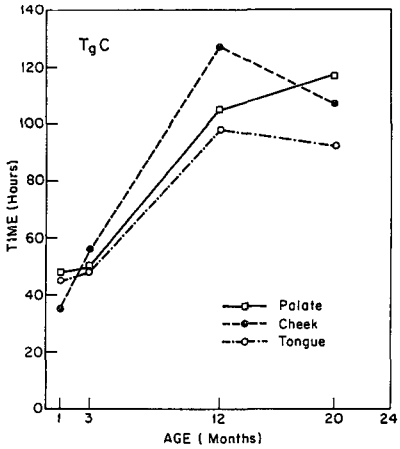

Fig. 20. Effects of aging on the duration of the progenitor cell cycle in the epithelium of murine oral mucosa.

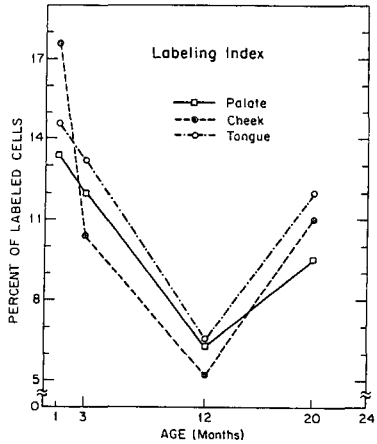

Fig. 21. Effects of aging on the percentage of labeling index of the epithelial basal progenitor cells of murine oral mucosa.