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Cell Proliferation in Normal Epidermis*

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A detailed examination of cell proliferation kinetics in normal human epidermis is presented. Using tritiated thymidine with autoradiographic techniques, proliferative and differentiated cell kinetics are defined and interrelated. The proliferative compartment of normal epidermis has a cell cycle duration (T_c) of 311 h derived from 3 components: the germinative labeling index (LI), the duration of DNA synthesis (t_s), and the growth fraction (GF). The germinative LI is 2.7% ± 1.2 and t_s is 14 h, the latter obtained from a composite fraction of labeled mitoses curve obtained from 11 normal subjects. The GF obtained from the literature and from human skin xenografts to nude mice is estimated to be 60%.

Normal-appearing epidermis from patients with psoriasis appears to have a higher proliferation rate. The mean LI is 4.2% ± 0.9, approximately 50% greater than in normal epidermis. Absolute cell kinetic values for this tissue, however, cannot yet be calculated for lack of other information on t_s and GF.

A kinetic model for epidermal cell renewal in normal epidermis is described that interrelates the rate of birth/entry, transit, and/or loss of keratinocytes in the 3 epidermal compartments: proliferative, viable differentiated (stratum malpighii), and stratum corneum. Expected kinetic homeostasis in the epidermis is confirmed by the very similar "turnover" rates in each of the compartments that are, respectively, 1246, 1417, and 1490 cells/day/mm^2 surface area. The mean epidermal turnover time of the entire tissue is 39 days.

The T_c of 311 h in normal cells is 8-fold longer than the psoriatic T_c of 36 h and is necessary for understanding the hyperproliferative pathophysiologic process in psoriasis.

There have been many studies attempting to describe, quantitate, and relate the cell proliferation patterns of normal and diseased skin. Using mitotic indices and/or [^{3}H]thymidine labeling indices, extensive data have been obtained that provide relative approximations of cell proliferation in the germinative compartments of the tissues being studied. However, an understanding of what role the "proliferative" component plays in the pathophysiology of hyperproliferative diseases such as psoriasis requires *absolute* values for cell cycle kinetics in both normal and diseased epidermis.

In 1971 we reported preliminary values for the cell cycle in normal epidermis using [^{3}H]thymidine labeling indices and the duration of the DNA synthesis phase obtained by the frequency of labeled mitoses method [1]. Our data at that time suggested that the cell cycle of normal proliferating epidermal cells was greater than 400 h and much different than the shorter cell cycle in psoriasis of 37 h [2]. Several studies have been published since then refining cell kinetic techniques in human skin, applying the GF concept from tumor research to skin, and presenting additional labeling indices. Some reports have speculated that there is little or no difference in the cell cycles of normal and psoriatic cell without providing strong supporting data [3,4]. This report will provide additional experimental data and concepts to establish absolute values for the cell cycle kinetics of normal epidermal cells that confirm a markedly long cycle in comparison to that found in psoriatic cells. A mathematical model for epidermal cell kinetics utilizing independently derived experimental data will be presented which interrelates the kinetics of the 3 main epidermal compartments (proliferative, differentiated, and cornified).

METHODS

Labeling Indices (LI) of Normal Skin (NS) and Uninvolved Skin of Psoriatics (UnPs)

Normal skin of 23 volunteers and uninvolved skin of 14 psoriatics above the age of 40 was injected intradermally with 0.1 ml (5 μCi) [^{3}H]thymidine (New England Nuclear; sp act 6.7 Ci/mmol). One hour later, 4-mm punch biopsy specimens were obtained from nonsun-exposed sites and prepared histologically for autoradiography as previously described [2]. Biopsies were taken during daytime hours since there is ample evidence to suggest little, if any, diurnal variability in human LI [5,6]. The slides were coated with Kodak NTB2 emulsion and exposed for 2–3 weeks. A total of 1500 epidermal *basal* nuclei were counted for each specimen and the numbers of labeled basal and suprabasal cells were determined separately. The germinative LI was calculated as the number of *labeled basal* cells per 100 *basal* cells (× 100) and the total LI as all the labeled cells (basal + suprabasal) per 100 basal cells for each patient specimen. The mean germinative and total labeling indices were then determined for both NS and UnPs (Table I*A*,*B*).

Determination of DNA Synthesis (S) Phase in Human Normal Skin Using Frequency (%) of Labeled Mitoses (FLM) Method

In 11 normal (nonpsoriatic) subjects multiple sites of skin were injected intradermally with 0.1 ml (5 μCi) of [^{3}H]thymidine (sp act 6.7 Ci/mmol). Sequential 4-mm punch biopsies were taken at 3-h intervals 1–33 h after injection and autoradiographs were exposed for 1–2 weeks. An average of 78 mitoses were counted for each epidermal biopsy specimen from multiple sections or parts thereof that showed adequate [^{3}H]thymidine labeling.

The mitoses were scored as labeled or unlabeled in order to get the fraction (%) of labeled mitoses. The data were plotted in a standard manner and the t_s was calculated as the interval between the 37% points on the ascending and descending limbs of the FLM curve [2]. The FLM data were also submitted for computer analysis (by Dr. M. L. Mendelsohn, University of California, Livermore, California).

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Abbreviations:
CV: coefficient of variation
FLM: frequency of labeled mitoses
GF: growth fraction(s)
LI: labeling index(ices)
N_c: total number of germinative (proliferative) cells
N_s: number of cells in S phase
NS: normal skin
SA: epidermal surface area
SL: epidermal surface length
SM: stratum malpighii
T_c: duration of germinative cell cycle
t_s: duration of DNA synthesis
TT: transit time(s)
UnPs: uninvolved skin of psoriatic

TABLE I. *Labeling indices of nonpsoriatic and psoriatic subjects*

Patient	Total basal cells counted	Labeling indices: Germinative % basal labeled cells / total basal cells	Labeling indices: Total % total labeled cells / total basal cells
	A. Normal epidermis of nonpsoriatic subjects		
101	1500	1.7	4.1
102	1500	2.1	5.9
103	1500	1.1	2.7
104	1500	2.2	5.5
105	1500	2.5	2.9
106	1514	.9	2.4
107	1500	1.7	5.2
108	1500	4.2	7.9
109	1420	4.2	7.6
110	1510	2.3	4.2
111	1538	2.5	3.5
112	1542	2.7	4.7
113	1561	3.9	6.3
114	1462	1.5	4.1
115	1469	3.5	5.7
116	1475	3.5	5.3
117	1403	2.8	3.7
118	1477	2.6	4.2
119	1314	0.7	1.8
120	1467	3.0	5.2
121	1440	1.9	2.6
122	1354	4.8	5.8
123	1451	5.0	8.4
Average (± SD)		2.7% ± 1.2	4.8% ± 0.4
	B. Uninvolved (normal-appearing) epidermis in psoriatic patients		
201	1545	3.8	7.0
202	1608	7.0	4.7
203	1500	4.8	3.5
204	1354	4.7	13.2
205	1451	5.3	8.3
206	1697	3.9	6.8
207	1646	3.0	7.7
208	1264	3.6	7.8
209	1486	6.6	13.7
210	1326	4.1	9.3
211	1608	3.4	6.3
212	1307	3.8	4.8
213	1467	5.0	8.7
214	1454	4.0	7.7
Average (± SD)		4.2% ± 0.9	8.1% ± 2.6

Quantitation of Germinative and Differentiated Cells in Normal Epidermis

Four-millimeter punch biopsies were obtained from the skin of 11 normal subjects from which microphotographs were analyzed for the following parameters: the number of basal (first-row) cells; the number of second-row (immediately suprabasal) cells; the number of cells above the second row up to the stratum corneum (spinous and granular layer cells) and defined as the stratum malpighii (SM) cells; and a measurement of the surface length of the epidermis in millimeters above the cells counted. All cell counts were normalized by conversion to number of cells per mm surface length (SL).

Since both labeled and mitotic cells are found in the second row of normal epidermis, it must be presumed that some portion, if not all the cells in that row, are part of the proliferative population. In order to determine what portion of second-row cells are proliferative, the ratio of basal cells (all considered as proliferative cells) to second-row "proliferative" cells in normal epidermis is related to the proportion of *labeled* cells in each row. The following working assumptions are made: (1) that as in other multilayer proliferative tissues, i.e., psoriasis or tumors, all labeled cells are behaving kinetically in a homogeneous manner, and (2) that the proportion of labeled cells to proliferative cells and G_0 "resting" cells is the same in the second row (or part thereof) as in the first row. Based on the determination of the number of second-row cells that are proliferating (the remaining cells being differentiated) together with all the first-row cells, the size of the proliferative and viable (noncornified) differentiated cell compartments can be calculated.

To develop a kinetic interrelationship among the 3 epidermal cell compartments, it is necessary to know the total number of cells per mm^2 surface area (SA), the proliferation rate, and/or transit times of each compartment. Two-dimensional counts of proliferative and differentiated cells are not adequate alone to estimate the total number of cells/mm^2 SA because of large differences in cell sizes in the compartments. A 3-dimensional approach is therefore necessary using measurements of cell diameters obtained from microphotographs of normal skin. The diameters of normal germinative cells were measured by 2 techniques that gave similar results; (a) the epidermal-dermal length was divided by the number of basal cells therein to give an average diameter of each cell; (b) micrometer measurements of cells using cell boundaries. Since differentiated cells change in size as they move distally from the proliferative area, differentiated cells in vertical columns were measured by micrometer to obtain the average diameter of these cells. In 3 normal subjects an average cell diameter was obtained from 15 columns. Based on the average diameter and the number of cells in each compartment as seen 2-dimensionally, the total number of cells/compartment/mm^2 SA was calculated.

Growth Fraction Experiments in Normal Human Epidermis

The GF represents that portion of a proliferative cell population that is actually cycling. Any noncycling cells may include G_0 cells, nonkeratinocytes, or postmitotic keratinocytes (see below). Direct measurement of the GF is best obtained by continuous [^{3}H]thymidine exposure for the duration of T_c, generally a period of several days, and then quantitating the percentage of labeled cells in the proliferative compartment. In an effort to get an estimate of the GF in human skin, a pilot experiment was developed using xenografts in nude mice. Normal skin from 2 nonpsoriatic subjects was transplanted into BALB/c *nu/nu* mice. Two weeks after transplantation, Alza osmotic diffusion pumps containing [^{3}H]thymidine (sp act 20 Ci/mmol) were placed in the peritoneal cavity of each mouse. The pumps delivered 1 μCi of [^{3}H]thymidine per hour and were changed weekly. Biopsies were taken after 3 weeks of continuous infusion to obtain labeling indices.

Cell Cycle Duration Analyses

The T_c can be calculated by the stage-duration method [2,7] which requires the experimentally derived: t_s (from the first FLM peak) and the germinative LI.

The equation used can be written in several ways. To relate directly to the experimental data the following equation was used in prior publications:

$$\frac{N_s}{t_s} = \frac{N_c}{T_c} \qquad \text{(Eq 1)}$$

where N_s equals the number of cells in S phase and N_c the total number of germinative (proliferative) cells.

Eq. 1 assumes a GF of unity and a rectangular age distribution.

With the advent of the GF concept and data on the GF for normal skin, the equation can be rewritten as:

$$T_c = \frac{t_s \times GF}{LI} \qquad \text{(Eq 2)}$$
$$(LI = N_s/N_c)$$

RESULTS

Labeling Indices of Normal Skin and Uninvolved Skin of Psoriatics

The average LI of normal skin was 2.7% ± 1.2 (SD) with a range of 0.7–5.0% (Table I*A*). For uninvolved skin of psoriatics, the mean LI was 4.2% ± 0.9 (SD) with a range of 3.0–7.0% (Table I*B*). The average LI for the uninvolved skin was 1.5 times greater than the NS LI and significantly different ($p < .001$), as determined by the Student *t*-test. The total LI (which includes labeled basal and suprabasal cells per 100 basal cells) was 4.8 ± 0.4% and 8.1 ± 2.6% in normal and uninvolved skin, respectively.

Duration of DNA Synthesis by FLM Method

A single-wave FLM curve was obtained with a peak of 75% at 15 h after [^{3}H]thymidine injection. From the FLM curve measured at the 37% points, the duration of the S phase is 14 h and duration of the g_2 phase tg_2 is 10 h (Fig 1). A second peak

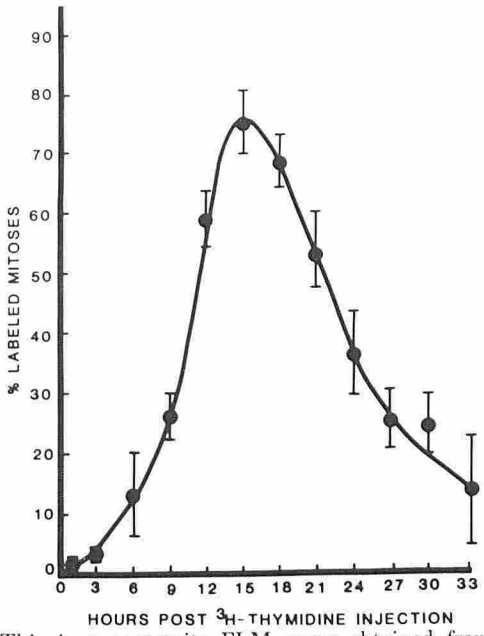

FIG 1. This is a composite FLM curve obtained from multiple biopsies in 11 normal subjects at 3-h intervals. Each *point* and *bar* represents the mean and SEM (see text).

could not be obtained within the time course of the study.

The computer analysis of these FLM data gives a t_s of 12.5 h with a coefficient of variation (CV = .50) and tg_2 of 11.7 h (CV = .45). The coefficient of variation is defined as the standard deviation expressed as a percentage of the mean. The computer analysis in effect uses the higher 50% level on the FLM curve, and thus a slightly smaller t_s and a longer tg_2 is obtained.

Quantitation of Germinative and Differentiated Cells in Normal Epidermis

In normal epidermis, the population of viable nucleated cells is distributed as presented in Table II. The data were experimentally obtained over an average surface length of 0.256 mm for each specimen and then extrapolated to a length of 1.0 mm. The ratio of first-row cells to second-row cells is 1.46 with substantially more cells in the first row as expected. The number of first-row cells is 33% of the total nucleated cell population which is similar to the 31% found by Pinkus [8].

Penneys et al [9], in a detailed 3-dimensional study, found that in normal skin approximately 32% of all labeled cells are found in the second (suprabasal) row and the remaining 68% are found in the first (basal) row. Pinkus also found that in normal epidermis the distribution of mitoses was 63% in the basal row, 34% in the suprabasal row, and 3% (1 out of 33 mitoses) in higher rows [8]. Therefore, based on the criteria of labeled cells and mitotic cells, the proliferative pool of epidermal cells resides in the first row of basal cells and in at least a portion of the second row.

Using the ratio of labeled cells in rows 1 and 2 obtained from Penneys et al [9] and the mean number of cells found in the first row (Table II), the size of the proliferative compartment in the second row can be calculated from the following equation:

$$\frac{\text{labeled cells in 2nd row}}{\text{labeled cells in 1st row}} = \frac{\text{\# of 2nd-row "proliferative" cells/mm SL}}{\text{\# of 1st-row cells/mm SL}} \quad \text{(Eq 3)}$$

$$\frac{32}{68} = \frac{X}{184}$$

Number of second-row "proliferative" cells = 86.6

Since a total of 120 cells/mm are found in the second row then 72% (86.6/120) of that row contains proliferative cells based upon the distribution of [^{3}H]thymidine labeled cells in the study of Penneys et al [9]. The remaining cells in the second row are presumed to have become differentiated cells without yet leaving the second row.

The germinative cell compartment therefore includes all of the first row and 72% of the cells in the second row. When extrapolated to a 1 mm SL, there are then 184 cells in the basal row plus 86 cells in the second row to give a total germinative cell population of 270 cells/mm SL (Fig 2). Since the epidermal-dermal length undulates, it is longer than the surface length which is why there are 184 basal cells × 10.1 μm in diameter for 1 mm SL. The differentiated cell compartment consists of all viable cells above the second row (234), plus 28% of the second row (34), for a total of 268 cells/mm SL (Table II).

In normal epidermis, the average diameter of a germinative cell was found to be 10.1 μm ± 0.84 (SD) and the differentiated cell diameter was substantially larger at 16.2 μm ± 2.4. The variation for the differentiated cells is greater as expected when grossly viewing these cells histologically.

With 270 germinative cells per mm SL and each cell having an approximate diameter of 10 μm, the number of germinative cells under 1 mm^2 SA calculates to 27,000. This can be visualized in Fig 2 as a schematized proliferative cell population under an epidermal surface area of 1 mm^2 with 100 compartments, 10 μm deep by 1 mm wide, each containing 270 cells. Likewise the number of differentiated cells/mm^2 SA is calculated as 17,000. Bergstresser and Taylor [10], using a direct cell counting technique, obtained a value of 47,000 cells/mm^2 for the entire viable epidermis, almost identical to the 44,000 value found here.

TABLE II. *Experimental and extrapolated data on numbers of cells in epidermal proliferative and differentiated compartments*

	Experimental data	Extrapolation to 1 mm surface length
Average number of cells in first row	47.1 ± 10.9	184
Average number of cells in second row	30.7 ± 5.6	120
Average number of SM cells	60.0 ± 20.9	234
Average surface length in mm for area examined	0.256	1.0
Numer of specimens	18	
Germinative compartments/mm SL (first row + 72% of second row)		270
Differentiated compartments/mm SL (SM cells + 28% of second row)		268

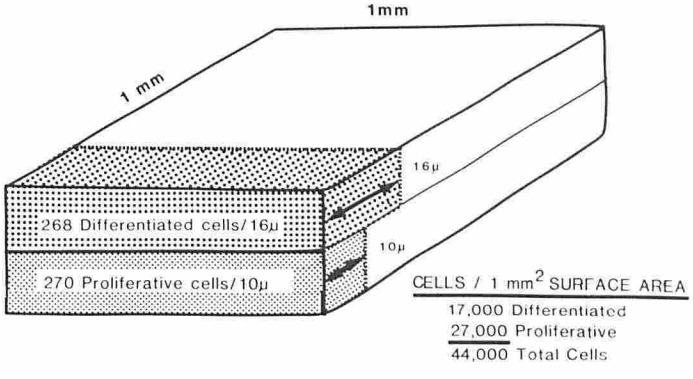

FIG 2. Viable cell populations of normal epidermis under 1 mm^2 surface area. The *front panel* shows the number of cells per compartment under 1 mm surface length and one cell diameter thick. At the *lower right* is shown the total number of cells per compartment under 1 mm^2 surface area.

Growth Fraction Experiments in Normal Human Epidermis

After 3 weeks of continuous [^{3}H]thymidine perfusion, the labeling indices (labeled basal cells/100 basal cells) for implants from 2 patients were 84.3% and 94.0%, or an average of 89.3%. The pre-implant 1 h in vitro labeling indices averaged 10.6%.

Cell Cycle Duration Analyses in Normal (Nonpsoriatic) Epidermis

The cell cycle duration T_c is calculated using Eq 2, with values of t_s = 14 h from the FLM curve and 2.7% for the normal germinative LI. For the GF the value of 60% is used based on the limited published data in human subjects [11] and the data on human skin xenografts in nude mice. The mean T_c for normal epidermal cells is 311 h. A range or SD for this value is not available since 2 of the 3 variables in Eq 2 have their own variations. The third variable, the in vivo GF, is only in 1 subject [11]. If the computer-derived t_s of 12.5 h is used the T_c would be slightly lower at 278 h. The cell kinetic literature is still controversial on the use of computer- or hand-plotted techniques to determine the t_s from FLM data. The value of T_c = 311 h based on the hand-plotted technique will be used to be consistent with other FLM studies from this laboratory in different tissues.

The calculation for T_c in normal epidermis assumes a rectangular age distribution. The concept of rectangular vs exponential age distributions (and values in between) are described by Duffill et al [12] and Appleton et al [13] and depends in part on the direction of mitoses. In two studies similar results were obtained, with the majority of mitotic figures either in a vertical axis (54%, 58%) or at an intermediate angle (12%, 18%) as opposed to horizontal (34%, 24%) [14,12]. If all mitoses were vertical then a rectangular age distribution would be obtained [12]. In normal epidermis it is likely that at least 66–76% of mitoses divide vertically, therefore producing a predominantly rectangular age distribution. In that case there would be little effect on the calculated T_c in normal epidermis, and thus a rectangular age distribution has been assumed for this study.

DISCUSSION

The results of the present study now provide a more complete picture of epidermal cell kinetics in normal skin. Using [^{3}H] thymidine labeling indices, relative approximations of cell kinetics by comparing the LI of normal and psoriatic epidermis have been obtained. However, to understand the pathophysiology of psoriasis, the absolute cell cycle values rather than the relative LI are necessary. In analyzing the germinative LI for determining absolute proliferation kinetic parameters, the number of labeled cells must be counted within the putative proliferative cell population. The basal cell row (excluding melanocytes) is generally agreed by most workers to be the germinative cell population in normal epidermis. However, we have presented data to show that while the basal row represents the major portion of proliferative cell population, there are many proliferative cells in the second row. To determine the kinetic picture of the epidermis as a whole it is necessary to include the entire proliferative compartment and not just the basal row. The presence of suprabasal proliferative cells has recently been confirmed by Van Neste et al using a combination of [^{3}H]thymidine labeling and immunoperoxidase staining of 67K polypeptide in suprabasal epidermal cells [15]. Their mean basal LI was 2.5% with another 1.7% LI of suprabasal cells, indicating that about one-third of the germinative cell activity is suprabasal, similar to the values in our study.

The difficulty with comparing LI from the literature has been that some authors have counted *all* labeled cells including suprabasal labeled cells but have used these counts only per 100 basal cells (total LI). This will provide falsely higher measures of labeling activity since at least a third of the labeled cells are outside of the arbitrarily defined proliferative population, i.e., the basal cell row [9]. Not all published LI are clear as to whether a method for a germinative or a total LI was used. The following germinative LI have been reported in normal epidermis: 2.2% [16], 2.7% (present study), and 3.8% [17]; while at least 2 other reports use "total" LI: 4.9% [18] and 5.5% [19]. Comparing other LI reports is also complicated by variables including site of biopsies, specific activity, and quantity of isotope injected, in in vivo or in vitro experiments, and duration of autoradiograph exposures. Nonetheless the range of normal LI published is relatively small. The germinative LI is necessary for the computations necessary to obtain the cell cycle duration by the stage-duration method (see below).

In the present study the germinative LI in UnPs is 4.2 ± 0.9%, approximately 50% higher than in normal skin. Other published values for UnPs are 4.9% [20] and 6.1% [6], but techniques also appear to vary slightly. The consistent small but real increase in LI of UnPs suggests that a latent indicator of the psoriatic diathesis is present in normal-appearing skin. Unfortunately the range of values in normal and UnPs overlaps and prevents the use of the LI in normal-appearing skin as a marker for the psoriatic diathesis.

The foundation of cell cycle kinetics is based on the FLM method. The optimum use of this technique allows one to determine the duration of the cell cycle (T_c) and its component parts (G_1, S, G_2, and M phases) in vivo. T_c can be measured both directly and indirectly but the FLM method is the only direct approach applicable to skin. It avoids many variables and assumptions inherent in the indirect methods requiring other [^{3}H]thymidine techniques and/or mitotic counts. With the FLM method, 2 or more waves of labeled mitoses can be found with adequate sequential specimens as the labeled cohort of cells traverses the cell cycle more than one time. If 2 FLM peaks are obtained, then the entire T_c can be measured directly as the distance between the 2 peaks [7].

The FLM method can be used in *normal* human skin to obtain only the first wave of labeled mitoses. The number of biopsies and the amount of [^{3}H]thymidine, if used intradermally, that would be required to obtain a second peak for a cell cycle estimated at over 300 h would be far beyond a reasonable or practical level in human subjects. Furthermore, the second peak in FLM studies is generally much flatter than the first peak because of desynchronization of the labeled cohort of cells. With such a long T_c, this second peak would probably be very difficult to find. The double-peaked curve in skin has been found in only one of several animal studies. Hegazy and Fowler obtained a well-defined second peak in mouse epidermis that provided a relatively long T_c of about 100 h and a T_s of 11.5 h [21].

In the present study the FLM curve in normal epidermis yields a T_s = 14 h which is a more accurate determination than previously published with fewer subjects [22]. There are no other published FLM data in human skin to our knowledge that utilize this optimum *direct* measurement for the T_s value. The T_s in normal skin has been estimated by other investigators using indirect methods that may be subject to various technical flaws. The indirect methods have usually been double-labeling experiments either in vivo or in vitro. These studies have found a *reversal* of the T_s in normal epidermis (6.6 h [23] or 7.2 h [24] vs 14 h by the direct FLM method) and in psoriatic epidermis (13.2 h or 20 h [24] vs 8.5 h by the direct method [2]). The reason(s) for the discrepancies found in the reversal of these values is not known, but experimental problems with these indirect methods as described below may be substantial. In vitro methods are subject to error by loss of physiologic conditions during the extended isotope incubations. An example of the difficulties in this technique is seen in the report by Galosi et al [25]. The thymidine LI for psoriasis found was 11.0 ± 3.5% which is substantially below the LI of 22.7% from our laboratory [2] and 21.2% by Gelfant et al [6]. Secondly, and perhaps more critically, is the derivation of T_s based on the ratio obtained from sequential 1- to 2-h incubations of [^{14}C]-

and [^{3}H]thymidine with the skin specimens. The ratio used is the combined LI of the [^{14}C]- and [^{3}H]thymidine labeled cells to the [^{3}H]thymidine labeling index alone. In the experiments by Galosi et al [25] where a T_s of 10.5 h was obtained, a 1-h incubation would only increase the combined LI theoretically by about 1% (11%/10.5 h). Considering that the SD of the LI in this experiment is 3.5%, a 1% change in the LI would not permit an accurate calculation of the T_s from a ratio of LI. Cell kinetic values using techniques that depend on grain counts have assumed that normal and diseased (psoriatic) cells are the same physiologically and biochemically [26], but this is not a reasonable assumption. Normal vs diseased cells are subject to differences in precursor pool sizes, critical enzyme levels, and local blood flow that may be present in normal and psoriatic epidermis leading to spurious results. There is evidence to suggest that the de novo pathway supplying thymidine for DNA synthesis is more important than the salvage pathway in psoriasis, with the opposite situation occurring in normal epidermis [27]. Thus one cannot assume that grain counts reflect the rate of DNA synthesis.

The direct method using the FLM, however, with a single isotope injection per time point follows the movement of cells through the phases of the cell cycle without appearing to influence the cell's physiology. Ideally i.v. administration of the isotopically labeled compound would be preferable to intradermal injections, but obvious restraints are present in studying benign conditions. However, when we have used multiple intralesional injections in different sites for FLM curves in human subjects, almost identical results have been obtained in FLM curves using i.v. [^{3}H]thymidine in kinetic studies of basal cell carcinoma, squamous cell carcinoma, and melanoma [28]. Single [^{3}H]thymidine injections into multiple skin sites in human subjects, each site being biopsied at a different time point, comes closest to following the experimental models where multiple small animals injected systemically with the isotope are sacrificed for each time point.

The concept of the GF includes some cells within the putative proliferation tumor cell compartment that may be in a resting (G_0) phase rather than actively cycling. It has been hypothesized that there may be a large GF in normal epidermis and that the development of the psoriatic lesion might result solely from an expansion of the GF without a change in the T_c of the normal epidermal cells [3,4]. The experimental data and calculations presented in this paper prove that this hypothesis is not correct when also including the fact that the psoriatic T_c is ⅛ that of normal [2,29].

The GF in human skin or any other human tissue is difficult to quantitate because of the legitimate concerns in subjecting patients to large doses of beta-emitting isotopes and because of the inaccessibility for sampling of tissues other than skin. The experimental techniques used to obtain GF utilize either a full FLM curve or the continuous labeling method [7]. Since a full or double-peaked FLM curve is not attainable in normal human skin as mentioned above, the continuous labeling method provides the only chance of obtaining an estimate of the GF even though there are theoretical if not real problems with this method. Enough evidence has been gathered as described below, however, to suggest that there is a relatively high GF in mammalian, including human, epidermis. In 4 GF studies of mouse epidermis, a labeling index plateau at 85% suggests that the GF approximates 85% [30]. In swine epidermis, which closely resembles human skin by histologic and kinetic characteristics [31], Archambeau and Bennett found a GF of approximately 90% after 12 days of i.v. thymidine infusion.†

There is only one study for GF in human skin in vivo in which a patient with multiple myeloma received [^{3}H]thymidine i.v. continuously for 8.5 days to examine tumor and epidermal kinetics [11]. Assuming no alterations in kinetics because of the systemic malignancy or possible prior chemotherapy, 60% of the germinative epidermal cells were labeled by 8.5 days with no other subsequent time points provided. This suggests that the GF in this one subject was at least 60%. It is very possible that had [^{3}H]thymidine been given for longer than 8.5 days, approaching the calculated T_c of 13 days, the GF may well have been greater as seen in mouse and swine skin.

A GF of at least 60% in human epidermis is consistent with experiments testing xenografts of normal human skin in nude mice. In xenograft experiments, GF of 55–60% [32], 75–80% (J. Krueger, personal communication), and 89% (present study) have been obtained. From the various experiments in mammalian epidermis done to date, it appears that the GF ranges from 55–100% and it is thus reasonable to assume that human epidermis has a relatively high GF.

The cell cycle duration for normal epidermal cells was calculated by the stage-duration method (Eq 2) using the T_s of 14 h from the experimentally determined first FLM peak, a mean LI of 2.7%, and a GF of 60%. The mean T_c is 311 h and assumes a rectangular age distribution.

Experiments to obtain a T_c of UnPs have not yet been performed. The LI of 4.2% is approximately 50% higher than in normal epidermis and a similar trend has been found by other investigators. One limited FLM study of UnPs has been attempted but only a small number of patients and biopsies were obtained [20]. In that study, a T_s of 11.4 h was derived by computer analysis, but it must be considered as a very tentative value. This value is slightly less than NS comparing the computerized analyses but is substantially longer than the T_s of 8.5 h for psoriasis [2]. No GF information is available on UnPs, which also prevents obtaining a good estimate of T_c. For a first approximation at this time, UnPs can be considered comparable in kinetic behavior to normal skin based on clinical, histologic, and LI similarities.

The identity of the "nonproliferating cells" in the proliferative part of the epidermis where there is a GF of less than 100% is not known. It has been suggested that these cells may include: (a) nonkeratinocytes such as melanocytes and Langerhans cells; (b) postmitotic maturing keratinocytes that will soon leave the proliferative compartment; and (c) G_0 cells with the potential of reentering the active cell cycle following an appropriate signal [30]. With current techniques none of these cells with the exception of melanocytes can be detected on histologic sections.

A Kinetic Model for Epidermal Proliferation

It is now possible to develop a quantitative interrelationship among the 3 major cellular compartments of the epidermis—the proliferative, the viable differentiated (stratum malpighii), and the stratum corneum. To assure a kinetic and physiologic homeostasis in the epidermis, the rate of birth/entry, transit, and/or loss of keratinocytes in each of these compartments must be approximately the same. To create such a mathematical model the number of cells and the rate of cell movement through each compartment is necessary. Having established, in normal epidermis, the number of cells in the proliferative and differentiated cell compartments as 27,000 and 17,000/mm^2, respectively (Fig 2), it is possible to calculate the birth or transit rate of cells through each compartment. In the proliferative compartment, 16,200 cells (60% of 27,000) are actively proliferating. With a T_c of 311 h (13 days) a daily birth rate (number of proliferating cells/T_c; 16,200/13) of 1246 cells can be calculated (Fig 3). In order to maintain homeostasis in the proliferative compartment, a like number of postmitotic daughter cells would have to exit into the viable differentiated compartment presumably on a random basis.

Past studies with different techniques [18,33] have shown that there is a 10- to 14-day transit time (TT) of cells *migrating* from the proliferative cell compartment *up to* the stratum corneum, i.e., the TT through the stratum malpighii or viable

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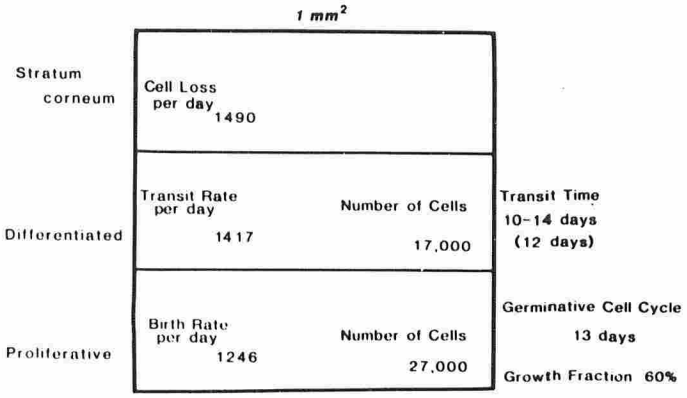

FIG 3. Cell proliferation kinetics model for normal epidermis. Birth, transit, and cell loss rates are presented for respective compartments. Cell loss/day (from Bergstresser and Taylor [10]) represents number of corneocytes desquamating daily from stratum corneum.

differentiated cell compartment. There has been some speculation as to whether this is a minimal or a realistic mean range of TT. Since many cells appear to move in tandem as a front, we believe that a mean TT of 12 days would reasonably reflect cell migration through the differentiated cell compartment. From the independent measurements of 17,000 cells/mm^2 SA and the 12-day TT for the differentiated compartment, a transit rate/day (number of differentiated cells/TT) of 1417 cells is obtained (Fig 3).

For the third epidermal compartment, the stratum corneum, Bergstresser and Taylor [10] computed the cell loss/day to be 1490 cells/mm^2 SA. The close similarities of the independently derived turnover rates in the 3 epidermal compartments (Fig 3) strongly confirm the proposed kinetic model of epidermal homeostasis. The primary significance of this model is in confirming that the T_c of normal epidermal cells is approximately 311 h and thus substantially longer than that found in psoriasis.

The turnover or renewal time of normal epidermis in its entirety can be viewed as the sum of the turnover (transit, cell cycle) times for each of the separate compartments. The TT of the stratum corneum is approximately 14 days as determined in 3 different studies [34–36]. The mean epidermal turnover time is thus 39 days, consisting of 13 days for the proliferative compartment, 12 days for the differentiated compartment, and 14 days for the stratum corneum.

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