THE JOURNAL OF INVESTIGATIVE DERMATOLOGY

Vol. 59, No. 1
Printed in U.S.A.

CELL CYCLE OF NORMAL AND PSORIATIC EPIDERMIS *IN VITRO*

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ABSTRACT

Studies of growth kinetics of normal and psoriatic epidermal cells *in vitro* indicate that there is no significant difference in the rate of proliferation under these conditions. The data may also be compatible with the hypothesis that both kinds of cells replicate at similar rates *in vivo* and that earlier conclusions regarding the markedly shortened cell cycle in psoriasis were based on insufficient data regarding the size of the proliferative pool in normal steady state epidermis.

Several reports have indicated that the epidermal hyperplasia of psoriasis results from both an increase in the number of germinal cells per unit length of epidermis (1) and a significant shortening of the individual cell cycle (2, 3). Since it is still not known whether the etiologic locus of the abnormalities in psoriasis lies in the epidermis, dermis, or elsewhere, it is important to investigate further those aspects of psoriatic cell proliferative behavior that appear to make it unique. One approach is to study the behavior of cells grown *in vitro*, away from their native environment. In the present set of preliminary experiments we have determined the growth kinetics of both normal and psoriatic epidermal cells *in vitro* and have found similarities indicating that psoriatic cells may not be significantly different from normal cells. Certain aspects of the data also suggest that a reevaluation of the cell kinetics of psoriasis *in vivo* may be needed.

MATERIALS AND METHODS

All techniques were identical for both psoriatic and normal epidermis. Studies were performed on 7 day outgrowth cultures comprised of epithelial cells derived from the epidermis (4). Split thickness explants, 2 × 2 mm, were placed epidermal side up on glass coverslips in the bottom of plastic petri dishes and held in place with a clot of chick embryo extract and plasma. Cultures were immersed in Eagle's Minimal Essential Medium containing 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 U/ml) and mycostatin (80 U/ml). They were incubated at 37° C in a high humidity incubator in a mixture of 5% carbon dioxide in air.

After seven days a ring of epithelial outgrowth is present around each explant. At this point cell cycle studies were begun. Tritiated thymidine (H^3TdR-sp. act. 6.7 c/mM) was added to a final concentration of 2.0 μc/ml culture fluid. After 2 hours, cultures were rinsed through 3 baths of "cold" culture fluid and reincubated at 37° C in a fourth change of "cold" fluid. Cultures were removed at appropriate intervals and fixed in 10% buffered formalin. For radioautography, they were dehydrated, air dried, the explants removed, and coverslips containing the outgrowths dipped in Kodak NTB2 emulsion. Exposure was carried out at 4° C for 2 weeks.

Supported by Grant Number 1 PO 1CA 11536 from the National Cancer Institute, DHEW.

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RESULTS

In the Figure are plotted the curves relating percent labeled mitoses vs. time after the pulse label of H^3TdR. For normal epidermis, each point on the curve represents 6 cultures, whereas for psoriasis, each point represents only 2 cultures. Values for the cell cycle are summarized in the Table. Total cell cycle time (T_c) is taken as the interval between the peaks of the 2 successive curves of labeled mitoses (5, 6, 7). The period of DNA synthesis (S) is represented as the interval between the 50% points on the ascending and descending limbs of the first curve. The post-synthetic period (G_2) between the end of S and the beginning of mitosis is the time between the pulse and the first appearance of labeled mitoses. Mitosis (M) is the period between the pulse and 50% labeled mitoses minus G_2. G_1 is the interval between completion of mitosis and the onset of DNA synthesis for the next mitotic cycle and is calculated as the difference between T_c and the sum of S, G_2 and M.

The growth fraction (G.F.) was derived from the formula (8):

$$\text{(A)} \qquad \text{G.F.} = \frac{T_c \times \text{L.I.}}{S}$$

where T_c = length of cell cycle
L. I. = labeling index
S = duration of DNA synthesis

T_c, L.I., and S are all known from the present set of experiments. The G.F. for cultures of normal epidermal cells was also checked by continuous labeling with H^3TdR (2 μc/ml) over a period of 72 hours with sampling at regular intervals. A maximum value of 65% labeled nuclei was obtained. The mitotic index (M.I.) is the proportion of metaphases arrested in the presence of colcemid (2 μgm/ml) over a 4 hour period to total number of cells in each outgrowth and is expressed on the basis of per 1,000 nuclei (9). The labeling index was calculated as the ratio of total labeled nuclei to total nuclei.

DISCUSSION

One must always be cautious about interpreting events *in vivo* from data obtained *in vitro*. How-

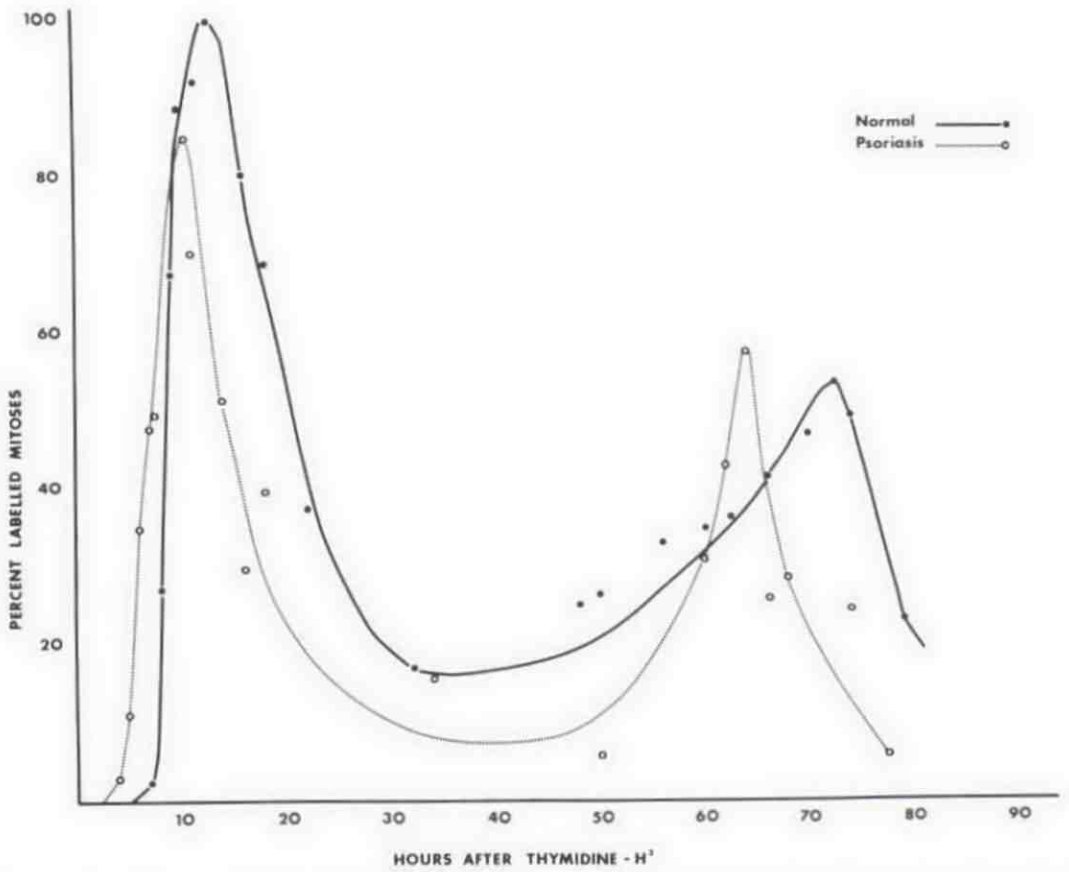

FIGURE. Graph showing percent labeled mitoses vs. time afte pulse label with tritiated thymidine. Normal and psoriatic epidermal cells *in vitro* are compared.

TABLE

Comparison of cell cycle of normal epidermis and psoriasis in vitro and in vivo

	In vitro		*In vivo* (1, 2, 3)	
	Normal epidermis	Psoriasis	Normal epidermis	Psoriasis
T_c	59 hrs	53.5 hrs	308 hrs	37.5 hrs
G_1	39 hrs	40 hrs	284 hrs	24 hrs
S	11.5 hrs	6.5 hrs	16 hrs	8.5 hrs
G_2	7 hrs	4 hrs	6–8 hrs	4 hrs
M	1.5 hrs	3 hrs	1 hr	.3 hrs
L.I.	105 ± 22/1000	40/1000	52 ± 15/1000	227 ± 24/1000
M.I.	25 ± 5/1000	18–20/1000	8.6/1000	23/1000
G.F.	54%	25%	?	?

ever, the close similarity in T_c for normal and psoriatic epidermal cells *in vitro* suggests that it might be useful to reevaluate certain aspects of the pathogenesis of psoriasis as it is thought to occur *in vivo*.

The epidermal hyperplasia of the psoriatic disease process may be due to at least 2 factors: 1) an absolute increase in the number of germinal cells/unit length of epidermis based on direct counting methods (1), and 2) a tenfold shortening of the generation time from 308 hours in normal epidermis to only 37.5 hours based on autoradiographic studies using H^3TdR in psoriasis (2, 3). Comparison of the M.I. and L.I. *in vivo* indicates that indeed, in psoriasis, more cells are dividing. Mitoses are seen not only in the basal layer, but also in the 2 layers immediately above. The length of the epidermis per centimeter is increased due to upward proliferation of dermal papillae (1). That the total number of proliferating cells is greater seems indisputable. But what about events within the population of di-

viding cells? Do the results obtained *in vitro* give any indication that cell proliferation kinetics of normal epidermis may be different than the reported data suggests?

Within the limits of experimental error, it can be seen that T_c is similar for psoriatic and normal epidermal cells of the same age *in vitro*. Comparison of the data in the Table would suggest that the generation time for a normal epidermal cell *in vitro* is 1/5 that reported *in vivo*, i.e. the cell cycle is apparently speeded up, whereas for psoriasis, the cell cycle is actually lengthened somewhat when compared with T_c *in vivo*. This would seem to be a perfectly logical result understandable on the assumption that normal epidermal cells have escaped the influences that inhibit their rate of proliferation, while psoriatic cells are no longer speeded up by whatever factors cause the disease in the first place. Thus, the psoriatic cell seems not to be permanently committed to its original rate of replication. This might have been suspected anyway, since psoriasis is often spontaneously reversible, but demonstration of this fact in the experimental situation is important since it focuses our attention on the non-epidermal components of the disease as possible etiologic sites.

It should be noted that within the cell cycle, the duration of S and G_2 is shorter for psoriatic cells *in vitro* than for normal epidermal cells. This might reflect differences in the cell cycle *in vivo*. However, the determination of S and G_2 is dependent on the accuracy of the slope of the labeled mitosis curve and since many more studies were carried out in normal epidermis than psoriasis, additional data on psoriatic cultures might well alter the values.

Assuming, then, that psoriatic and normal epidermal cells have similar reproductive rates *in vitro*, what of the concept that in psoriasis *in vivo* the cell cycle is shortened from 308 to 37.5 hours? Examination of the other aspects of growth *in vitro* such as M.I., L.I. and G.F. suggests that earlier interpretations of differences in the cell cycle *in vivo* may be erroneous. Previous calculations of T_c for both normal and psoriatic epidermis *in vivo* have been based on values obtained from a single curve of labeled mitoses using the formula (2, 3):

$$\text{(B)} \qquad \frac{N_s}{T_s} = \frac{N_c}{T_c}$$

where N_s = number of cells in S phase
T_s = duration of S phase
N_c = number of cells in the proliferative pool
T_c = total cell cycle time

The value of T_c is wholly dependent on the validity of the assumption that 100% of the basal cells in normal epidermis or the 1–3 layers of cells above the basement membrane in psoriasis are in the proliferative pool (N_c). While this assumption has recently been shown to be true for psoriasis (10), the growth fraction, or ratio of proliferative to non-proliferative cells, for normal epidermis has not been determined. Unless the G.F. is known, T_c can only be reliably determined by using 2 curves of labeled mitoses as was done in the present study. Using Weinstein and Frost's data from normal epidermis *in vivo* (2, 3), it can be seen that an overestimation of N_c would lead to a mistakenly long value for T_c. Considering only normal epidermal cells for the moment, it is interesting to note that under conditions *in vitro* that supposedly might lead to an increased rate of growth, the total proliferative pool size is only about 54% of all the cells in the outgrowth†. Thus, it seems likely that at the moment of explantation an even smaller percentage of basal cells may have been in the pool.

Other evidence also indicates that under normal conditions *in vivo* a significant portion of epidermal basal cells are outside the proliferative pool. The studies of Sullivan and Epstein (11, 12) of wound healing in human epidermis have clearly demonstrated a 48 hour lag period before there is any significant rise in the number of mitoses. This is quite suggestive of results in other systems such as liver and kidney where most of the cells are outside the replicative pool in the so-called G_0 phase (13, 14). These cells normally do not divide unless appropriately stimulated. Following stimulation there is a variable lag before the onset of DNA synthesis which is followed by mitosis. If 100% of normal epidermal cells were in the replicative cycle, one would expect that, following wounding, there would be a gradual increase in the number of mitoses during the first 48 hours instead of the observed precipitous rise. The actual existence of cells in G_0 has been demonstrated in chick epidermis *in vitro* stimulated by a protein known as epidermal growth factor (15).

It has recently been demonstrated that in psoriasis *in vivo*, the entire cell population of the 3 deepest epidermal layers are germinal (10). But *in vitro*, the L.I., M.I. and G.F. are *less* than that for normal epidermis. This is consistent with our observations that outgrowths of psoriatic epidermis enlarge more slowly and do not achieve as large a size as those from normal epidermis. Rowe *et al.* (16) have made similar observations. The reasons for the slower growth are not at all clear but may be related to the observation that shortly after explantation there is much cell death in the basal layer and total necrosis 2 layers immediately above where mitoses are seen *in vivo* (17). Nevertheless, the cells that survive divide at a rate quite similar to that of the normal epidermal cells.

† The value of 54% is based on the formula (A). Continuous labeling gives a higher value (65%) but this is misleading since during this time cells in the proliferative pool have divided once thus increasing the ratio of labeled to unlabeled nuclei. Therefore, the true G.F. is most likely closer to 54%.

In summary, the cell cycle data obtained *in vitro* by us suggest that there is no significant difference in proliferative rate between a normal and psoriatic epidermal cell. It seems possible that both kinds of cells replicate at similar rates *in vivo* and that earlier conclusions that the cell cycle is shortened as much as 10 times in psoriasis were based on insufficient data regarding the size of the proliferative pool in normal steady state epidermis.

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