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Proliferation in murine epidermis after minor mechanical stimulation
Part 1. Sustained increase in keratinocyte production and migration

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Abstract. It was our objective to obtain an insight into the details and dynamics of the cell proliferative changes following minor barrier disruption, the mechanisms of recovery, and their regulation.

Hair of the dorsal area of DBA2-mice was removed and the epidermis was tape stripped. Tritiated thymidine was injected into groups of mice at daily intervals thereafter. Labelling and nuclear densities were measured at several time intervals later in the various epidermal strata to characterize cell production and cell fluxes through the tissue.

A dramatic proliferative response was observed at 24 h when the labelling density increased more than sixfold in the basal layer. Labelled cells rapidly appeared in suprabasal layers within a few hours in large quantities while this process took over 2 days in normal skin. Some cycling cells were also found in the suprabasal layer (pulse labelling at 24 h) in contrast with the controls. The cellular flux through the suprabasal layers was drastically (20-fold) increased and the transit time was shortened. Although the nuclear density in the basal layer showed only moderate changes it increased four-fold in the suprabasal layer within 5 days. A kinetic model analysis suggested that the cell cycle time of proliferative cells dropped from a normal value of about 200 h to less than 12 h post tape strip. After 7 days, the proliferative activation still persisted, even though at 3 days post tape strip the stratum corneum had been re-established.

Hence, a mild mechanical alteration with removal of some parts of the cornified layer in mouse backskin epidermis triggers a huge proliferative response with

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massive overproduction of cells that lasts at least 7 days. Our findings suggest that the re-establishment of the cornified layer does not immediately shut down cell proliferation and that more complex, slower (long-term) regulatory processes are involved.

INTRODUCTION

The cellular and regulatory organization of the epidermal epithelium is still not fully understood. A popular paradigm is based on the concept of a few reproductively active epithelial stem cells and a pedigree or lineage of transient proliferative cell stages which ultimately give rise to nonproliferating, differentiated, mature, functional keratinocytes (Potten *et al*., 1982; Loeffler *et al*., 1987; Potten & Loeffler, 1987; Clausen & Potten, 1990; Loeffler & Potten, 1997; Watt, 1998). In the steady state epidermis of the DBA2 mouse, the proliferative stem and transient proliferative cells are located in the basal cell layer, beneath a stack of maturer keratinocytes and corneocytes, where they exhibit a rather slow turnover with cycle times in the order of 100–200 h (Potten *et al*., 1982, 1985; Potten & Wichmann, 1983; Loeffler *et al*., 1987). Non-proliferating keratinocytes originate in the basal layer and migrate to the suprabasal layers where they eventually form the cornified layer. Such a 'pedigree' or cell lineage concept seems to be consistent with a large bulk of cell kinetic and organizational data for interfollicular murine epidermis in normal and hairless mice (e.g. Potten *et al*., 1974, 1982, 1985; Clausen *et al*., 1981, 1984, 1986; Potten & Bullock, 1983; Loeffler *et al*., 1987; Potten & Loeffler, 1987; Aarnaes *et al*., 1990, 1993; Kirkhus & Clausen, 1990, 1992).

Despite its merits, this pedigree concept is incomplete as it does not yet incorporate the concept of the regulatory control processes. Little is known about how cell proliferation is controlled and whether or not the structure of the putative pedigree can change on demand. Perturbation studies using ionizing radiation (e.g. Hegazy & Fowler, 1973a, b; Al-Barwari & Potten, 1976) and ultraviolet light (e.g. Al-Barwari & Potten, 1979) have provided interesting details; these were based on the fact that the perturbation damaged the proliferative cells, thereby possibly altering the pedigree and the proliferative behaviour therein.

In order to obtain further insight into the intraepithelial cell kinetic control systems we have used a perturbation that caused less direct damage to the basal cells involving a local and mild mechanical irritation. Previous reports showed that detachment of parts of the cornified layer by topical tape stripping had stimulatory proliferative effects (Pinkus, 1951, 1952; Hennings & Elgjo, 1970; Hamilton & Potten, 1972, 1974; Potten & Allen, 1975; Clausen & Lindmo, 1976; Brown & Habowsky, 1979; Boezeman *et al*., 1987) leading to epidermal hyperplasia. The data available so far only provided a limited insight into the degree and duration of changes in cell production and cell migration associated with this perturbation. Therefore, our objective was to examine in detail how epidermal cell production and cell flux change at various times after tape strip using labelling techniques and, in follow-up studies, how the label propagates through the tissue, and whether we could find further support for the pedigree concept. The data set presented hereafter and in an accompanying publication (Barthel *et al*. 2000) is a comprehensive study and analysis of post tape strip reactions in murine epidermis.

MATERIALS AND METHODS

Experimental procedures

Male DBA2 mice 7–8 weeks of age were used for the experiments. Animals at this age have the hair follicles in the back skin in the resting (telogen) phase of the hair growth cycle (Hamilton *et al*., 1974). The dorsal area of an anaesthetized mouse was prepared by clipping the hairs, removing the stubble with a depilatory cream (Immac, Anne French, London, UK), washing and drying the surface of the skin, and then carefully applying and removing adhesive tape (Sellotape, Dunstable, UK) four times, each time along the opposing longitudinal axis of the dorsal area. After applying the tape and before removal, the tape was gently pressed with the fingers along the natural contours of the mouse (without pinching). This process provided a mechanical stimulation of the epidermis, hereafter referred to as 'tape stripped' epidermis. The extent and dynamics of the damage to the stratum corneum is illustrated in Fig. 1. The experiments were performed according to the following time schedule.

Tape stripping was performed at time 0. Groups of animals were pulse labelled by an intraperitoneal injection of 1.85MBq (50 μCi) of methyl-labelled tritiated thymidine (^{3}HTdR), with a specific activity of 248GBq (6.7 Ci) mmol^{-1} (NEN, Brussels, Belgium) in 0.2 ml at different times following tape stripping (immediately, 6, 24, 48, 72, 120, 168 h). At regular intervals ranging from 1 to 148 h after each of these label injections subgroups of at least four animals were sacrificed and the dorsal skin was removed, and either fixed (Carnoy's), embedded and sectioned; or placed in 0.6% acetic acid at 4 °C for 24 h prior to fixation (Carnoy's) for the preparation of epidermal sheets, at a range of times after labelling. One cohort of control animals had no tape stripping, but were labelled, and groups of animals were sacrificed at varying times after the tritiated thymidine injection (1, 24, 48, 72, 96, 120 h). In total 272 mice have been used. The bioavailability of thymidine only continues for approximately 20 min due to its rapid incorporation into S-phase cells and its degradation on passage through the liver. This therefore constitutes a 'flash labelling' technique and the fate of these labelled cells with the passage of time can be studied. All experiments were performed within the Regulations of the Animals (Scientific Procedures) Act, 1986 (UK).

Fixation for both sections and epidermal sheets was in Carnoy's fixative for 30 min followed by storage of the sample in 70% ethanol. After fixation, epidermal sheets were prepared (from the acetic acid treated tissue) by separation from the dermis under a dissecting microscope, and the epidermis placed on a microscope slide with the cornified layer down and the basal layer uppermost. Autoradiographs of sections and sheets were prepared by dipping in K5 emulsion (Ilford Limited, Mobberley, UK) diluted 1 : 1 with distilled water. Exposure times varied according to the nature of the experiment (sections or sheets) and according to the time after labelling at which the group of animals were sacrificed. Dilution of autoradiographic grains as a consequence of the labelled cells dividing can be compensated for by doubling of the autoradiographic exposure time. This approach was adopted in some of the appropriate experimental samples. Sections were stained with haematoxylin and eosin, and epidermal sheet preparations were stained by the Feulgen reaction.

For the sheet preparations, an area of known dimensions was defined and the total number of unlabelled and labelled basal cells was recorded. At least four areas were counted from each mouse and, since each area consisted of approximately 250 basal cells, at least 1000 total basal nuclei were counted per mouse, maintaining a constant optical focus to avoid counting suprabasal nuclei. From these measurements we derived the basal cell density (BCD) expressed as the number of cells per mm^2 and the labelling index (LI) of the basal layer as the number of labelled cells relative to the total number of basal cells.

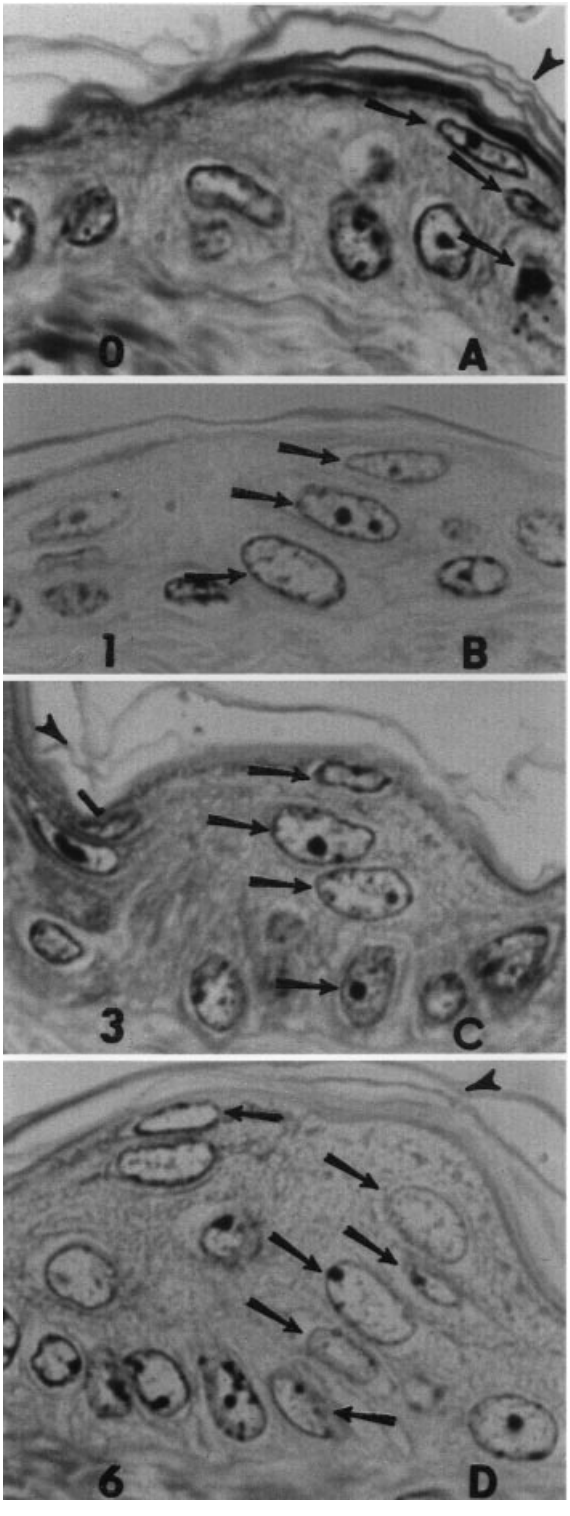

Figure 1. Thin sections (approximately 1 μm) of resin embedded mouse back skin stained with toluidine blue. (a) control skin (no tape stripping), showing stratum corneum (arrow head) and about three nucleated cell layers (arrows). (b) 1 day after tape stripping. Most of the stratum corneum has been removed. (c) 3 days after tape stripping. Some stratum corneum has been re-established. The epidermis is now thicker by about one cell layer. (d) 6 days after tape stripping. The stratum corneum is re-established and the epidermis is thicker, with up to 6 nucleated cell layers. N.B. The appearance of the epidermis is somewhat variable due ot the inherent variable nature of the tape stripping. The fields shown are representative. Magnification $\times$ 1700.

Sections cut perpendicular to the surface of the epidermis show the basal layer as a line of cells and the suprabasal cells, with their larger surface area and, hence, lower number per unit length, could also be observed in the distal portion of the epidermis. Generally, there were three nucleated suprabasal layers and, thus, labelled and nonlabelled cells in four layers (including the basal layer) could be counted in a region of epidermis with approximately 1000 basal cells. From these counts on sections, the label density (LD) could be calculated in each layer. Whereas the LD in the basal layer is equivalent to the LI obtained from sheets and is defined by the ratio of the number of labelled cells to the total number of basal cells, the LD in the suprabasal layers 1, 2, and 3 is defined as the ratio of the number of labelled cells in each of layers 1, 2, 3 to the total number of cells in the basal layer. The concept of LD as opposed to LI has been introduced to facilitate comparisons between the levels of labelling in the different layers at a given time. Labelling densities in adjacent layers then can be compared and pooled. By definition, LI and LD are only identical for the basal layer. The relative cell density (RCD) in the suprabasal layers 1, 2, and 3 could be determined from the number of cells (nuclei) in the suprabasal layers 1, 2, and 3 divided by the total number of cells in a corresponding length of the basal layer. From BCD (sheets) and RCD (sections) the suprabasal cell density (SCD, suprabasal cells per mm^2) could be estimated by multiplying BCD by a factor equal to the sum of the RCDs in SL1, 2, and 3. In addition to these parameters, the thickness (number of layers) of the epidermis was measured from the sections by counting the number of cell layers at several points (ranging from 3 to 16) along the section. In general, all of the quantitative measurements from the experiments were calculated for each animal. All data are shown as the means with standard errors of the mean, calculated from groups of equally treated animals.

A simple model of cell kinetics

A simple model of cell kinetics was used to obtain an estimate of cell fluxes and for the average turnover time of cells in the basal layer. Considering a segment of epidermis with a given area, 'A'

Let b the number of cells in the basal layer (BL);
s the number of cells in the suprabasal layers 1–3 together (SL);
bl the number of labelled cells in the basal layer;
and sl the number of labelled cells in the suprabasal layers.

The basal cell density is given by

$$BCD = \frac{b}{A}$$

and the labelling densities in BL, SL, and the total epidermis by

$$LD(BL) = \frac{bl}{b} \quad LD(SL) = \frac{sl}{b} \quad LD(total) = \frac{bl + sl}{b}$$

Within the framework of the model it was useful to relate the numbers of labelled cells, bl and sl, to the area, A. Therefore the labelling densities per area (LDA) are defined by

$$LDA(BL) = \frac{bl}{A} \quad LDA(SL) = \frac{sl}{A} \quad LDA(total) = \frac{bl + sl}{A}$$

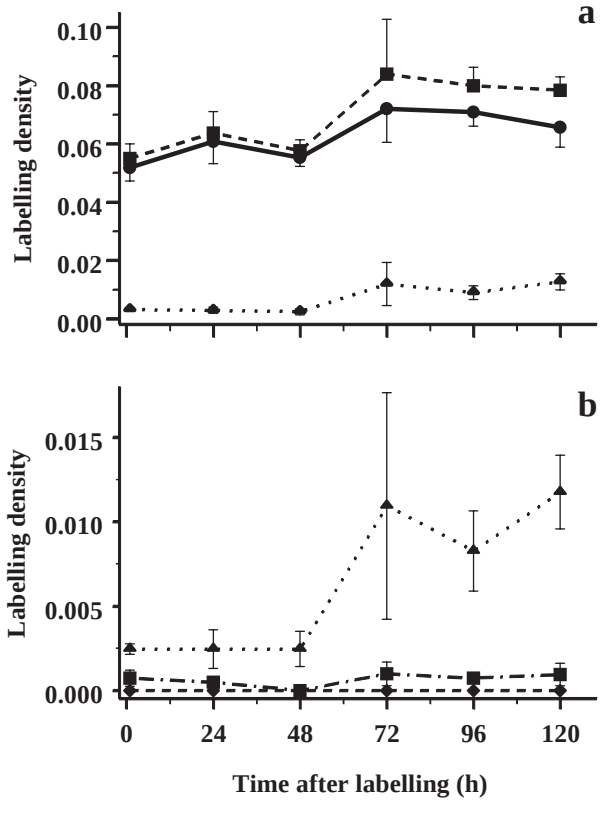

Figure 2. Changes in labelling densities (see text) in the various layers of control epidermis (number of labelled cells in a layer/number of total cells in basal layer) at various times after tritiated thymidine flash labelling. (a) labelling density in basal layer (●, solid line), pooled for suprabasal layers 1–3 (▲, dotted line), and pooled for suprabasal plus basal layers (■, dashed line); (b) labelling densities in the individual suprabasal layers 1 (▲, dotted line), 2 (■, dot & dashed line), and 3 (◆, dashed line). Error bars: mean ± SEM.

Obviously,

$$LDA = LD * BCD$$

is correct for BL, SL, and the total.

In order to obtain a crude estimate of cell production and cell migration we assumed that cell division only takes place in the BL and, at least for a period after flash label, labelled cells migrate only from BL to SL. Then the numbers of labelled cells vary with time according to the following model equations:

$$\frac{d}{dt}BL = r \cdot BL - q \cdot BL \tag{1}$$

$$\frac{d}{dt}sl = q \cdot bl \tag{2}$$

where r and q are the rates of cell production and cell migration, respectively. Dividing each equation by the area A and taking the sum of both equations one obtains

$$\frac{d}{dt} LDA(total) = r \cdot LDA(BL) \qquad \text{from (1) + (2)}$$

$$\frac{d}{dt} LDA(SL) = q \cdot LDA(BL) \qquad \text{from (2)}$$

The equations imply that one can directly obtain the average temporal changes of LDA(total) and LDA(SL) from experimental data by a linear regression analysis and the initial value of LDA(BL) for each sequence of measurements following labelling at different times post tape strip. This permits the calculation of estimates for the parameters r and q.

RESULTS

Control group with no tape stripping

Control animals were injected with tritiated thymidine and groups of mice were sacrificed at various times up to 5 days following tritiated thymidine injection. Thus, in this experiment the time course for the labelling density changes reflects the processes of cell proliferation and migration in the skin in steady state (Fig. 2). The initial labelling was essentially found only in the basal layer where approximately 5% of the cells were labelled. There was a gradual increase over the next 3 days in the basal LD which reached a maximum value of about 7% on day 3. Labelled cells began to appear in the suprabasal layers on day 3. During the first day after labelling the basal cell nuclear density decreased from an initial value of $13\,000 \pm 800$ nuclei/mm^2 to $11\,170 \pm 600$ nuclei/mm^2 but returned to the initial level after the third day. The suprabasal cell density varied in a similar way with an initial value of 4850 ± 560 nuclei/mm^2. None of these changes were statistically significant ($P > 0.05$).

The flash labelling density and cell densities at various times after tape stripping

Seven separate experiments were conducted by pulse labelling groups of animals immediately, 6, 24, 48, 72, 120 and 168 h after tape stripping. For each of these experiments subgroups of four animals were sacrificed at various times (40 min to 48 h) after labelling. Initially, only those subgroups are considered where the animals were sacrificed 40 min after the labelling. Figure 3 shows the large changes seen in the fraction of cells that are in S-phase, i.e. pulse labelled, at each time after tape stripping. There was an initial refractory period of approximately 6 h and a subsequent rapid increase in the pulse label density (LD), particularly in the basal layer. The pulse LD of the basal layer fell gradually over the period of a week, but even 7 days after tape stripping the basal cell labelling index was still higher than the control value by a factor of 2. With a slight delay, labelling in the suprabasal layers increased substantially during the course of this experiment (Fig. 2b).

During the first day after tape stripping the basal nuclear density (BCD) remained almost constant at $13\,000 \pm 800$ nuclei/mm^2 to $12\,300 \pm 1300$ nuclei/mm^2 and increased over the following three days to $16\,900 \pm 1000$ nuclei/mm^2, i.e. to 130% of the starting level. Subsequently, the BCD decreased to $14\,800 \pm 620$ nuclei/mm^2 at day 7 (Fig. 4a, solid line). The suprabasal cell density which started from a level of 4850 560 nuclei/mm^2 decreased for the first 6 hours after tape stripping to 3860 ± 130 nuclei/mm^2 but then increased very significantly reaching a maximal level of $24\,800 \pm 2400$ nuclei/mm^2, i.e. over five-fold of the initial level on day 5 post tape strip. For the last two days the suprabasal cell density decreased to $11\,800 \pm 1700$ nuclei/mm^2 (Fig. 4a, broken line). A more detailed description of the time course is given in Fig. 4(b), suggesting that there is a successive increase in cell

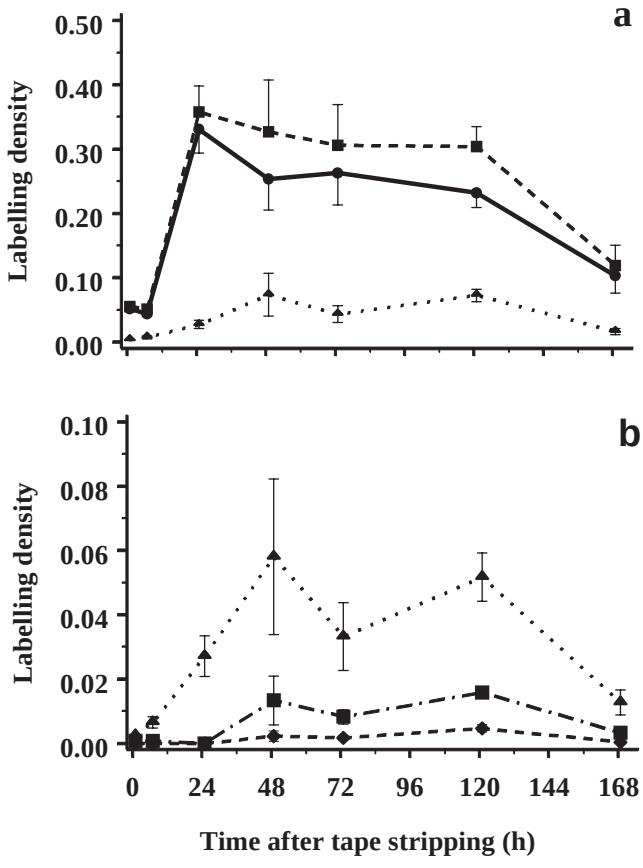

Figure 3. The flash labelling density values obtained at different times after stimulation of the epidermis by tape stripping for the basal layer, suprabasal layers and total labelling (symbols as for Figure 1). The data shown here represent the initial labelling values for the subsequent time course studies.

densities in the different suprabasal layers for up to 120 h and subsequently a parallel decline. Somewhat differently, the mean epidermal thickness increased from a reference level of 3.4 ± 0.1 cell layers to a range from 4.1 to 5.7 cell layers and remained at an elevated level 7 days after tape stripping (data not shown, but see Fig. 1).

Time course of changes in labelling density and cell density following pulse labelling at a fixed time after tape stripping

Here, we consider the time course of labelling densities and cell densities following pulse labelling at different times post tape stripping. The most dramatic changes were seen in the experiment where label was injected at 24 h post tape stripping and followed up for an additional 48 h. Figure 5 shows different time patterns of basal and suprabasal LD. The basal LD, starting from a relatively high level of 0.33 0.04, increased over a period of 24 h, then reaching a flat maximum with a level of 0.78 ± 0.02, i.e. 2.4-fold the initial value (see Fig. 2). Thereafter the basal LD decreased slowly. In contrast, the overall suprabasal LD, starting from an initial level of only 0.03 ± 0.01, increased over a period of 30 h reaching a maximum level of 0.98 ± 0.24, i.e. 33-fold the initial value and more than the maximum level in the basal layer

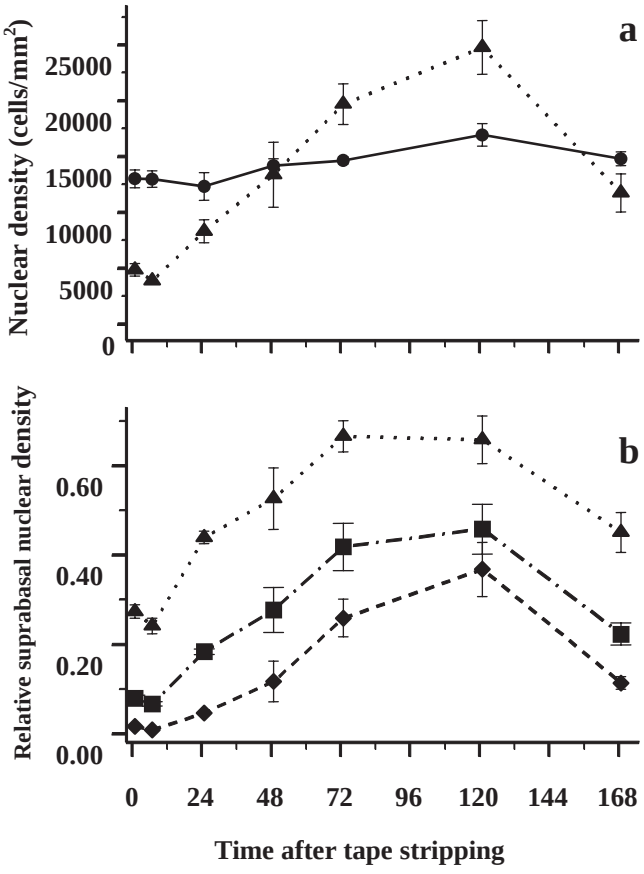

Figure 4. Changes in cell densities after tape stripping based on counts of cell nuclei. (a) nuclear density in basal layer (●, solid line) and suprabasal layers (▲, dotted line); (b) relative cell density (number of cells in suprabasal layer/number of cells in basal layer) in suprabasal layers 1 (▲, dotted line), 2 (■, dot & dashed line), and 3 (◆, dashed line).

(Fig. 5a). Furthermore, a sequence of increases of labelled cells from basal to suprabasal layers (Fig. 5a) and from SL1 to SL3 (Fig. 5b) was observed. These findings suggest a wave of labelled cells travelling rapidly from the basal layer to the suprabasal layers 1,2, and 3. The overall (basal and suprabasal layers) LD increased over a period of 30 h from an initial level of 0.36 ± 0.04 by a factor of 4.8 to a maximum of 1.72 ± 0.20, indicating that within 30 h the cells of the epidermis ran through at least two proliferation cycles. This suggests that 24 h after tape stripping the cell cycle time of the epidermis has shortened to at most 15 h.

Time course of changes in label cell density at different times after tape stripping

The changes in labelling density per area (LDA) with time following label injection at 6, 24, 48, 72, 120 and 168 h after tape stripping are shown in Fig. 6 for the epidermis as a whole (b) and separately for the basal (a) and suprabasal (c) layers. At all times studied, and most notably from 24 h onwards there was a rapid increase in basal labelling, suprabasal labelling, and total labelling density. The slopes of the ascending portion on these graphs are related to the rate of entry of cells into mitosis, i.e. to cell birth rates and to the rate of migration of cells from basal to suprabasal layers. The steepness of the slopes decreases progressively with

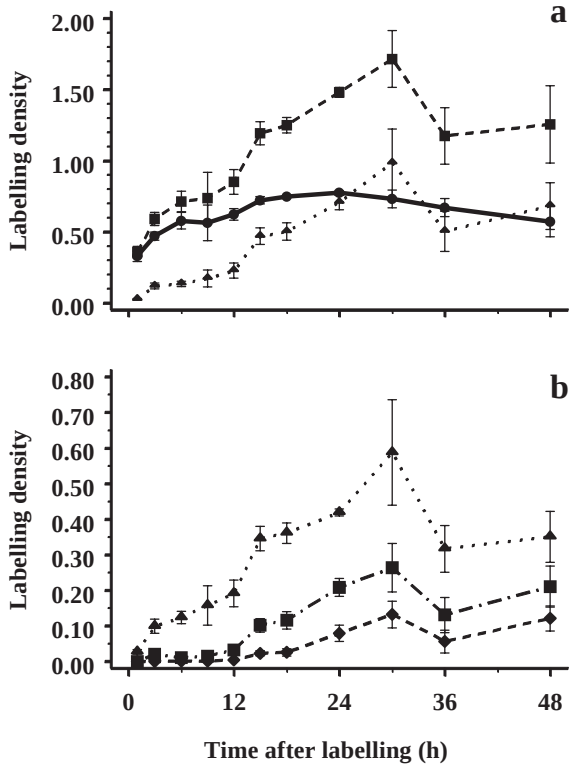

Figure 5. Labelling densities in the basal and suprabasal layers at different times after flash labelling 24 h after stimulation by tape stripping. Data shown are for the basal, suprabasal and total labelled cells (symbols as shown in Figure 1).

time after tape stripping, but the slopes are clearly much steeper than in control epidermis even 7 days after the tape stripping stimulation.

A simplified kinetic model analysis of the label density data

Some quantitative insight into the kinetic features of the tape stripped epidermis can be obtained by means of the simple mathematical model which was described in the materials and methods section. The model is based on the following assumptions.

Table 1. Estimates of basal layer cell production rates and migration rates from the basal to the suprabasal layer (mean ± SE) using a simple kinetic model of cell production and migration

Time post tape-strip (hours)	Basal cell production rate, r ($hours^{-1}$)	Cell migration rate BL → SL, q ($hours^{-1}$)	Average cell turnover time (hours)
0	0.0044 ± 0.0016	0.0017 ± 0.0006	228 ± 85
24	0.153 ± 0.032	0.103 ± 0.022	6.5 ± 1.4
48	0.076 ± 0.024	0.040 ± 0.014	13.1 ± 4.1
72	0.103 ± 0.021	0.048 ± 0.013	9.7 ± 1.9
168	0.119 ± 0.036	0.032 ± 0.011	8.4 ± 2.5

r, number of new cells produced per hour per basal cell; q, number of cells entering the suprabasal layer per hour per basal cell

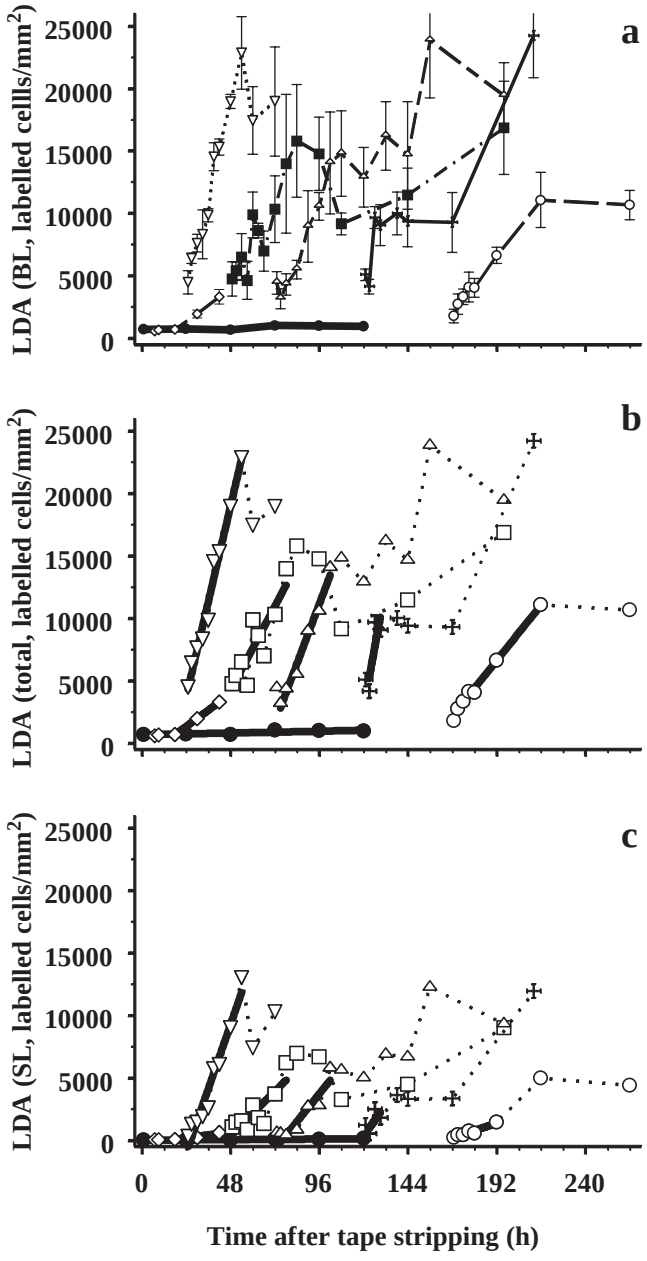

Figure 6. Labelling densities per area (LDA, labelled cells per mm^2 of epidermis) in the various layers of the epidermis after tritiated thymidine flash labelling at different times after stimulation by tape stripping (▽ 6 h, ◇ 24 h, □ 48 h, △ 72 h, + 120 h, ○ 168 h). The initial point for each curve corresponds to the time of labelling after tape stripping (see Figure 2). The control unstimulated data are shown for comparison (—●—). (a) for basal layer, (b) for basal and suprabasal layers together, (c) for suprabasal layers. The initial slopes of the LDA-time plots in (b) and (c) are used to estimate the time derivatives of LDA in the whole epidermis and in suprabasal layers depending on the time of flash labelling, which, together with the first values of the LDA-time plots in (a), are needed to estimate the rates of cell production, r and of cell migration, q (see Table 1).

The increase of total labelling density with time is due to cell production, which is assumed to take place only in the basal layer. The increase in labelling in the suprabasal layers is only due to cell emigration from the basal layer. Additionally, it is possible that a fraction of the new cells remains in the basal layer and causes an increase in the basal cell density. Therefore, the model refers to the labelling density per area (LDA). The LDA can be calculated from the data by taking the product of labelling density and basal cell density. The time derivatives of LDA in the whole epidermis and in SL, as required from the model, are estimated by the slopes of the regression lines of the ascending portions of the time curves (Fig. 6b, c). The model equations provide a means to obtain estimates for the cell production rate (r) in the basal layer and for the rate of cell migration from the basal to the suprabasal layer (q). Table 1 gives the estimates for these quantities. The reciprocal value of r can be used as a rough estimate of the cell turnover time. The table shows that the basal cell production is increased by a factor of 20–30 over the entire period of observation. The true increase is likely to be underestimated as the model disregards any cell production taking place in the suprabasal layers.

DISCUSSION

This detailed and comprehensive cell kinetic study was undertaken to investigate the proliferative response of the epidermis following mild mechanical irritation by tape stripping. This perturbation did not appear to inflict direct damage to the proliferative or nonproliferative keratinocytes *per se*, but it definitely caused disruption of the cornified barrier. Despite this mild irritation the system responded with a very rapid and dramatic stimulation of epidermal proliferation which apparently started in the basal layer and subsequently spread to the suprabasal layers. The cellular density data showed a remarkable five-fold overshoot of cells in the suprabasal layer while the basal layer showed only a 30% increase in cell density. Follow up studies of ^{3}HTdR-label injected at various time intervals after tape strip revealed a large increase in cellular turnover in the basal layer. Using a simple kinetic modelling approach we estimated that the average cell turnover time had shortened to about 6–13 h from an estimated 200 h in the normal steady state. Likewise the cell flux leaving the basal layer per time unit had approximately increased by a factor of 20. Somewhat unexpectedly the regulatory processes had not switched off the proliferative burst after 7 days despite a tremendous overproduction of suprabasal cells and recovery of the stratum corneum and the barrier function.

These findings could suggest a role for the integrity of the stratum corneum in the intraepithelial proliferative control processes, which takes a number of days to reduce the raised levels of proliferation back to those observed prior to perturbation. The rapid removal of the cornified layers apparently induces a highly significant and long lasting proliferative stimulus. Further studies have to clarify when and how the switching off occurs, which is not completed by day 7. Our findings are consistent with the concept of a cellular pedigree with a rather fixed number of transient cell divisions which cells have to go through before they enter a nonproliferative state. If, in such a concept, the cell cycle activity is accelerated and many more cells are being produced in the basal cell layer per unit time, then the lack of space in the basal layer will lead to an overspill into the suprabasal layers, where the cells complete their pedigree program. Our observations are consistent with this concept. Proliferation was not confined to the basal layer indicating that the attachment to the basal layer membrane does not solely determine proliferation. However, recent studies have shown that, at least in some circumstances, epidermal stem cells may express high levels of β_1-integrin, which mediates attachement to the basement membrane (Watt, 1998; Zhu *et al.*,

1999) and that this may be a prerequisite for stem cell control. A further study showed that cells with high levels of β_1-integrin contained only a few cells in S-phase (putative stem cells, with a long cell cycle time), whereas those cells with lower levels of β_1-integrin contained more S-phase cells (transit cells) (Jensen *et al*., 1999). The proliferative cells we observed in the suprabasal layers are probably transit cells completing their pedigree program.

Tape strip induced epidermal hyperplasia is a well known phenomenon whose mechanisms are still not fully resolved. The first reports date back Pinkus (1951, 1952) and Christophers & Braun-Falco (1967). Some early cell kinetic analyses on mice were performed by and Iverson *et al*. (1968) and Hamilton & Potten (1972, 1974). Clausen & Lindmo (1976) and Clausen & Thorud (1980) demonstrated a very rapid kinetic reaction in the basal layer with an early temporary block of the transition of cells to M-phase and high labelling and mitotic indices on the subsequent 2 days in nude mice. The authors concluded that a shortening of the cell cycle time is likely to be the underlying mechanism. Other investigations of tape strip induced hyperplasia showed similar increases in label indices or mitotic indices (Boezeman *et al*., 1987; Hashimoto *et al*., 1995a, b). However, to the best of our knowledge, no kinetic flux measurements were performed nor were they related to location within the tissue. Removal of the stratum corneum by tape stripping results in the removal of the cornified cell barrier. The understanding of the short-term effects of barrier removal has been much improved in recent years. There is a rapid recovery of barrier function and the prevention of transepidermal water loss (TEWL) within a time scale of a few hours. This process implies secretion of lamellar bodies, and production of further lamellar bodies and lipids to re-establish a functional cornified envelope (Menon *et al*., 1992; Ekanayake-Mudiyanselage *et al*., 1998). It is assumed that intraepithelial calcium levels are involved in the regulation of these phenomena. Following tape strip, the intraepidermal calcium gradient collapses probably due to a wash out effect from the stratum granulosum (Menon *et al*., 1992). All of these processes seem to be linked to the degree of transepidermal water loss and can partly be prevented by occlusion of the lesion (Denda *et al*., 1996). The external addition of calcium is reported to have inhibitory effects on the recovery of the barrier function. All processes involved with barrier homeostasis appear to operate on a time scale of a few hours.

In contrast to these processes the mechanisms controlling cell production and differentiation are less well understood. With regard to signalling mechanisms, calcium levels (Menon *et al*., 1992) and IL1α (Denda *et al*., 1996) are considered as candidates to trigger proliferation, but both would only show a dynamic behaviour on a very short time scale, which would raise doubts as to whether they can be the regulatory signals themselves. Recent observations have reported a differential response of keratin expression in the basal and suprabasal cell layers during tape strip induced hyperplasia (Ekanayake-Mudiyanselage *et al*., 1998). In the basal layer expression of keratins K5 and K14 was reduced whilst, in the suprabasal layer, K1 expression was reduced and K10 expression was enhanced. Furthermore, Ekanayake-Mudiyanselage *et al*. showed that K6, K16, and K17 were expressed 12 h after acetone-induced barrier disruption. K6 was detected (by immunohistochemistry) in the basal and lower spinous layers, whereas 24 h after barrier disruption K6 expression was found in all nucleated epidermal layers. We interpret this pattern as supportive of our pedigree concept. If the pedigree concept holds, then keratin expression would be correlated with the location of a cell within the pedigree. During a hyperproliferative phase, the cells in the basal layer will become, on average, less mature and more immature cells will spill over to the suprabasal layer. Thus, we would suggest that there might be a major shift in the quantity and locations of keratin expressions during that phase.

The molecular mechanisms of the regulatory processes involved in activation and inactivation of hyperproliferation post tape strip remain to be resolved. We speculate that an intraepithelial diffusible inhibitory signal that controls proliferation may be involved. Such a substance may be produced by the epithelial cells and accumulate in the epithelium if the stratum corneum is intact, thereby producing a rather slow cell turnover in the normal steady state. A destruction of the epidermal barrier may open channels through which the factor leaks out, leading to a reduction of its concentration in the epidermis and, subsequently, to a proliferative stimulation. Recovery of the concentration of the factor may require a rather long time period (some days) and an intact stratum corneum. At present we are performing a systematic analysis of such scenaria using a simulation model of epithelial cell kinetics. This model is aimed at describing the cell kinetics in the normal steady state and after perturbations like tape stripping, ionizing irradiation and ultraviolet light exposure using one comprehensive set of assumptions and parameters. It will be an extension of the model presented by Loeffler *et al*. (1987), but including regulatory processes.

The model used in this paper to analyse cell fluxes is rather simple in nature and was designed to give a rough estimate of the dynamic parameters. Detailed information on the changes in the cell cycle and the duration of the cell cycle phases cannot be provided by this type of experiment. Consequently, a double labelling experiment (^{3}HTdR and BrdUrd) was performed in a separate experiment and analysed by a new statistical procedure which accounts for nonsteady state situations (Barthel *et al*., 2000). The above findings of an accelerated cell turnover are confirmed by this additional analysis suggesting an S-phase duration of 3–5 h and a shortening of the average normal steady state cell cycle time of 150 h to 12–15 h between days 1–3 post tape strip. Further characterization of the tape stripped tissue using immunochemistry is underway to provide greater detail about the cellular differentiation process.

To our knowledge the present study is the most comprehensive cell kinetic analysis of cell production in normal and tape stripped skin to date. We conclude that post tape strip hyperplasia is due to a strong and long lasting activation of the cell cycle in the epidermis, probably maintaining the cellular pedigree of stem and transient cell generations during the response.

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