Summary

Mouse dorsal, ear, tail and foot epidermis are compared according to their tissue architecture and cell kinetics. Cell proliferation is expressed in terms of the daily volume of keratin replaced. The stratum corneum may be organized into vertical columns of squames, which may have minimal overlap as in dorsum and ear, or maximal overlap as in tail. Individual areas are adapted to their function both in squame fine structure and rate of cell replacement. The surface keratin loss/replacement rate is at its highest in foot and tail, and lowest in ear and dorsum. Observations on hairless mouse dorsum are also included.

Introduction

The occurrence of a well established vertical organization in mammalian epidermis was first reported by MacKenzie (1969) who showed that, after suitable treatment with mild alkali to induce swelling, the cells of the stratum corneum in mouse dorsum and ear were arranged in characteristic vertical columns. This work was extended further using other methods (MacKenzie, 1970; Christophers, 1970, 1971a, b; Goerttler, Reuter & Stahmer, 1973) and more recently investigated at the ultrastructural level (Allen & Potten, 1974) and from a cell kinetic viewpoint (Potten, 1974), resulting in the subdivision of mouse epidermis into a series of Epidermal Proliferative Units (EPU), one to each column of differentiating corneocytes and fully differentiated squames. Although most of the skin in mice exhibits an obviously stacked organization (body and ear skin) there are regions which do not, namely plantar and tail epidermis. Various possibilities of squame organization exist and for clarity these are presented diagrammatically in Fig. 1.

Stacked epidermis

Here the cornified cells or squames are arranged in vertical columns as shown in Fig. 1. There is both a small overlap between adjacent squames and also a regular alternation of overlapping squames. Thus the term 'stacked' refers to squames which are columnar, regularly alternate, and with minimal overlap.
An obvious variation of the stacked arrangement is one in which the overlap is increased, until the configuration shown in Fig. 1B is achieved, i.e. columnar, regularly alternate with maximum overlap.

![Diagram](image)

Fig. 1. Diagram of squame organization in vertically sectioned epidermis. A, stacked epidermis. The squames are clearly arranged in vertical columns. The edges of the squames from adjacent columns both overlap and interdigitate with their neighbours in a precise manner. B, form of stacked epidermis which is similar in arrangement to (A) but where there is maximal rather than minimal overlap of adjacent squame columns. C, completely non-stacked arrangement, with variable overlap between horizontally adjacent squames.

**Non-stacked epidermis**

In non-stacked epidermis there are no vertical columns of squames, and there is a variable amount of overlap with irregular alternation (Fig. 1C). In the diagram there are apparent gaps in the stratum corneum, but in the tissue these would be filled by the squames above and below being in contact. Because of the random nature of this arrangement small regions within the stratum corneum may appear to
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Table 1. Measurements on various sites of mammalian epidermis

<table>
<thead>
<tr>
<th>Site</th>
<th>Organization</th>
<th>No. of epidermal cell layers</th>
<th>Keratinized cell size</th>
<th>Total thickness of keratinized cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nucleated (A)</td>
<td>Keratinized (B)</td>
<td>Diameter, μm (C)</td>
</tr>
<tr>
<td>Dorsum</td>
<td>Stacked</td>
<td>4.0</td>
<td>6</td>
<td>34.6 (28-31)</td>
</tr>
<tr>
<td>Ear</td>
<td>Stacked</td>
<td>4.5</td>
<td>11</td>
<td>25.3 (19-30)</td>
</tr>
<tr>
<td>Foot</td>
<td>Non-stacked</td>
<td>12.0</td>
<td>Pad 50</td>
<td>24.5 (20-33)</td>
</tr>
<tr>
<td>Tail</td>
<td>Stacked (overlapped)</td>
<td>6.5</td>
<td>32</td>
<td>34.5 (25-31)</td>
</tr>
<tr>
<td>Hairless dorum</td>
<td>Partially stacked</td>
<td>4.5</td>
<td>7</td>
<td>30.0 (17-36)</td>
</tr>
</tbody>
</table>

(A) and (B) counted from 40 random points on sections.  
(D) measured from 30 cells in electron-microscope profile (range shown in parentheses).  
(C) measured from 20-40 cells in electron-microscope profile, 1-μm sections; or sheet preparations, with the overall mean presented; and the range for 1-μm sections in parentheses.  
(E) Surface area formula for hexagonal correction.
possess an ordered arrangement but this is always highly localized both vertically and horizontally.

**MATERIALS AND METHODS**

Male 7- to 8-week-old DBA-2 (PAT) mice were used for most studies. Adult pigmented mice homozygous for hairless (hr hr) were also used in some cases. At least 3 separate areas from 3 mice were used for each site.

**Ultrastructural methods**

For scanning electron microscopy, the various tissues were cut into approximately 3-mm squares and fixed in buffered glutaraldehyde followed by osmium tetroxide (see below). The samples were then dehydrated through ascending concentrations of acetone, transferred to the pressure vessel in absolute acetone, and critical-point dried according to the method of Porter, Kelly & Andrews (1972). Briefly, the method comprises several flushes of liquid CO₂ to remove the acetone, and infiltrate the tissue, followed by the raising of the temperature past the critical point to approximately 38 °C, and a release of the gaseous CO₂ over 1-2 min. The samples were then coated with silver, and examined in a Cambridge S4-10 Stereoscan.

For transmission microscopy, the tissues were prepared as described previously (Allen & Potten, 1974) which, briefly, includes fixation in buffered 2% glutaraldehyde followed by 1% osmium tetroxide for 1 h each, ethanol dehydration, propylene oxide infiltration, and embedding in an Epon-Araldite mixture. Material was examined in an AEI EM801A microscope.

**Cell kinetic methods**

Cell production rate (k) was determined using the relationship

\[ N \cdot L_1 = k \cdot T_s \]

\[ N \cdot M_1 = k \cdot T_m \]

where \( N \) is the number of basal cells and \( L_1 \) and \( M_1 \) are the labelling and mitotic indices and \( T_s \) and \( T_m \) are the durations of the DNA-synthetic phase and mitosis respectively. Average values were obtained from the literature (Allen & Potten, 1974; Potten, 1974; Potten & Allen, 1975a; Christophers, Wolff & Laurence, 1974; Christophers & Laurence, 1973; Blenkinsopp, 1968; Grube, Auerbach & Brues, 1970; Tvermyr, 1969, 1972; Hegazy & Fowler, 1973; Sherman, Quastler & Wimber, 1961; Laurence, 1973) for \( L_1, M_1, T_s \), and \( T_m \) and were combined with some data of our own where \( L_1 \) and \( T_s \) were measured for the various tissues over a 24-h period (Potten, 1975a, b). \( N \) can be expressed in terms of 100 basal cells (from the % LI), or per EPU. In the latter cases the number of basal cells per EPU corneocyte has to be known. This number can be counted directly from epidermal sheet preparations of ear and dorsum (Potten, 1974) and can be deduced from measurements of cornified cell surface area and basal cell surface area for foot and tail. (For a more complete consideration and review of skin cell kinetic data and their interpretation see Potten, 1975a, b.)

**Measurements of epidermal cell size**

The diameter and thickness of stratum corneum cells were measured in the light microscope, using semithin (1-μm) resin and paraffin (6-μm) sections, in the EM and also using scanning electron-microscope micrographs. Measurements were made from 1-3 mice using 20-40 random points on sections of squame profiles (excluding obviously tangential sections).

For both qualitative and quantitative studies using sectioned material the centre of a squame was identified in dorsum and ear by the presence of nuclear profiles in the granular and spinous layers. These differentiating cell nuclei are centrally positioned as can be seen in sheet preparations (Allen & Potten, 1974; Potten, 1974). In tail and foot epidermis this does not occur but the large numbers of squames make valid scoring rather simpler than the small numbers in ear and dorsum stratum corneum. Care was taken to use identical fixation, dehydration and embedding procedures which were assumed to result in similar dehydration, shrinkage and superficial keratinized cell loss phenomena, thus enabling valid relative cell size and number measurements to be made. This was substantiated for cell numbers by paraffin and frozen section counts.
RESULTS

Normal dorsal epidermis

The organization in normal DBA-2 mouse dorsal epidermis has already been extensively reported (Allen & Potten, 1974) and can be briefly described as follows: the stratum corneum is stacked, and this stacking organization can be traced in the electron microscope to the basement membrane. The nucleated layer of the epidermis is 4 cells thick, with an average of 6 squames in the stratum corneum (Fig. 2). The squames are joined only by a modified type of attachment, the squamosome (Allen & Potten, 1975) which is a peripheral band observed in both tangential-peripheral profiles (Allen & Potten, 1974) and cell surface replicas (Allen & Potten, 1975). This peripheral ring joins the edges of each squame to the edges of squames from neighbouring columns above and below (Fig. 3). Scanning electron micrographs of the surface of dorsal skin show the small overlap of adjacent squames, their roughly hexagonal outline, and smooth surface (Fig. 4). The average thickness of a newly keratinized basal cornified cell in dorsum is 0.21 μm whereas the superficial squames are thinner (0.07 μm) (Table 1 and Fig. 2). This change is restricted to dorsum and its significance is unclear. In terms of surface area, dorsal and tail squames are the largest of the entire epidermis (Table 1).

Ear epidermis

Mouse ear epidermis is also stacked (Fig. 5), with a characteristic hexagonal squame pattern (Fig. 6) but is thicker overall than dorsal epidermis, having 4–5 nucleated cell layers and on average 11 cornified cells. The squames themselves are shorter in profile, being about two thirds the diameter of a dorsal squame, and also are thicker (approximately twice) and have a slightly rougher surface than dorsal squames (Table 1 and Fig. 6). Again as with dorsal skin the edges of the squame columns can be extended down to the basement membrane to delimit the EPUs supplying the cells into the column of squames. Considering both the increase in thickness and number of squames, the total thickness of keratinized cells of ear is approximately 8 times that of dorsum (Table 1). No significant differences in size or organization between epidermis from normal mouse ear and from hairless mouse ear were noted.

Figs. 5, 6. Normal mouse ear epidermis.

Fig. 5. Vertical section through ear epidermis showing 2 nucleated granular cells, and the stratum corneum. The stratum corneum is thicker than dorsal skin (see Table 1), having 14 squames in this section, the squames themselves maintain a more constant thickness, are stacked in vertical columns and show the same regular interdigitation (arrowed) as dorsal skin. × 5200.

Fig. 6. Scanning electron micrograph of ear epidermal surface, showing roughly hexagonal squame columns and their minimal overlap. × 2652.
Foot epidermis

Mouse foot epidermis can be divided into 2 distinct regions, the general sole surface (plantar) and the pads which are the load-bearing area. In the pad region the thickness of the stratum corneum may be as many as 50 cell layers (Fig. 7) which are non-stacked, i.e. completely lacking in vertical organization. The stratum corneum possesses the appearance of being relatively highly compressed with no space between vertically adjacent squames as may be seen in similar preparations of dorsal skin. It is possible that the separation seen in dorsum (prepared in the same way) may occur in sample preparation; however, it is only in dorsum with its peripheral squamosomes that such a separation would be possible. The squames in foot are short and thick and have a very crenellated surface linked by multiple individual electron-dense regions of contact of desmosomal dimensions (Figs. 7, 8, 10, 11 and Table 1), instead of the squamosomes, which are a peripheral band. Supplementary to the regions of adhesion in foot (Fig. 11), there are also numerous irregular ridges over the surface of the squames which interlock to provide further resistance to shearing. These ridges are visualized best in slightly oblique sections (Fig. 8) and are also visible in surface view (Fig. 9).

The plantar region in foot is in most respects identical to the pad areas, but has a slightly thinner stratum corneum (Table 1) with an apparently slightly less closely applied squame attachment (cf. Figs. 7 and 10). At the surface (Fig. 12) the overall appearance is much more irregular, probably due to the lack of abrasion in comparison with the pad region.

Tail epidermis

Mouse tail epidermis possesses the largest squames of the entire mouse epidermis, being of similar thickness to those in foot epidermis, but having a diameter similar to dorsal epidermis so that the volume is approximately twice that of any other squame (Table 1). The total number of keratinized cells is approximately the same as in plantar skin, but the thickness of the nucleated cell zone of the epidermis is only 6-5 cells on average compared to 12 cells in foot. The tail squames themselves are smooth and flat (Fig. 13) with desmosome-type contacts, and lack the

Figs. 7-9. Mouse plantar epidermis.

Fig. 7. Vertical section through the stratum corneum of a pad. This section has approximately 21 squame profiles, but is by no means the full thickness (see Table 1). The squames are highly compressed (except for the uppermost 2 or 3, which are presumably detaching) and joined by numerous desmosomal-like joints (arrowed and see Fig. 11). There is no evidence of vertical stacking of the squames. $\times 8290$.

Fig. 8. Oblique vertical section through pad region of sole. This section shows that there are numerous interlocking ridges on the surface of the squames, presumably adding extra mechanical strength. $\times 12570$.

Fig. 9. Scanning electron micrograph of the pad region of mouse sole. The edges of the individual squames clearly reveal the irregular overlapping, and the ridged nature of the squame surface is also visible. $\times 1650$. 

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interdigation of surface ridges found in foot. Although no organization is apparent in vertical sections through the stratum corneum of tail (Fig. 13), scanning electron micrographs of the surface show that the squames overlap like tiles on a roof (Fig. 14). Individual areas may also show an extremely well defined organization, suggesting in fact that the overall arrangement of squames in tail may also be columnar, but in this case, with the columns maximally overlapped, as shown in profile in Fig. 18. A model of this arrangement is easily produced by superimposing 2 hexagonal grids, in such a way that there is maximal overlap of hexagons. The edges of the overlying hexagons produce a characteristic 'Y' in the centre of each underlying hexagon, a feature often observed on individual tail squames (Fig. 15). Thus it appears that tail squames are in fact stacked, but with maximal overlap, as opposed to minimal overlap in dorsum and ear.

Hairless mouse dorsal epidermis

Compared with normal mouse dorsum, the number of cell layers in the hairless epidermis was not significantly different (Table 1) but the squame thickness in hairless mouse was greater, so that total thickness of the keratinized cells was some 5 times that in haired mouse (cf. Figs. 2 and 16).

The stacking in hairless mouse dorsum was variable. In some regions the stratum corneum was random (Fig. 16), but occasionally there was more complete end alignment of squames (Fig. 17). The surface of the epidermis as shown in scanning preparations also showed some areas of stacking (Fig. 18). Other reports have indicated a more regular stacking in hairless mouse epidermis (Raknerud, Hovig & Iversen, 1971; Raknerud, 1974).

Cell kinetics

The cell production rates \( k \) (based largely on published labelling or mitotic index data and estimates for the length of \( S \) and \( M \)) for the various epidermal sites are shown in Table 2. These rates have been expressed in terms of cells produced per cornified cell per day (column 1) (using measurements or estimates for the ratio of basal cells to cornified cells or EPUs). These estimates or measurements are: 10.6, 9.7, 20, 25 and 11 for dorsum, ear, foot, tail and hairless dorsum, respectively (our own data and Allen & Potten, 1974; Potten, 1975a; Christophers & Laurence, 1973). The production rates can also be expressed, using the thickness of cornified cells, as the volume of keratinized cells produced per unit area per day (columns 3 and 4 of

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Fig. 10. Vertical section through plantar region of mouse epidermis (between pads). There is no evidence of stacking, but the stratum corneum is thinner and less compressed than in the pads, being some 20 squames thick in this region. × 7730.

Fig. 11. Vertical section through the plantar region of mouse epidermis, showing the numerous desmosome-type joints between adjacent squames. × 46480.

Fig. 12. Scanning electron micrograph of an area of sole epidermis between pads. In contrast to the pads the surface appears unabraded, although the squames are still completely overlapping and have a ridged surface. × 1650.
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Table 2). Expressed relatively, the rate of keratinized cell production and hence replacement of the keratin barrier can be seen to be fastest in foot, and slowest in dorsal skin (see Fig. 19). As the overall thickness of the stratum corneum is maintained more or less constant, the rate at which keratin is produced reflects the rate of loss of the squames from the surface. Thus the loss/replacement rate in the plantar region is approximately 14 times that in dorsal skin, and 1.5 times that in tail. Ear and haired mouse dorsum have rather similar rates, but hairless dorsum is some 3 times faster than haired dorsum. This is perhaps to be expected as the dorsal epidermis alone in hairless mouse has to fulfill the same function as epidermis plus pelage in haired mouse.

DISCUSSION

Site adaptation

The function of mammalian epidermis is to provide a barrier to the environment. This barrier is obviously modified by the site, and also by the presence or absence of pelage. Further factors exist such as amount of abrasion, with plantar skin obviously being subjected to maximal abrasion, while dorsum has minimal abrasion. The main function of dorsal skin might be to provide an insulating and impervious barrier whereas foot skin provides also a mechanical barrier. These differences would be reflected by the tissue architecture. The stacked arrangement of squames in columns (dorsum) with columns joined only at their edges, forms a quilt-like structure, honeycombed with numerous individual enclosed "spaces" between squames in the same column. This presumably forms an efficient barrier to both the loss of moisture and heat. In contrast, plantar epidermis is obviously adapted to provide maximal mechanical strength and resistance to the constant abrasion that it undergoes, by having a complete overlap of squames, held together by both desmosomal attachments and interlocking ridges.

Relative production of keratinized cells

The expression of cell production rate in terms of its end product, i.e. its contribution to the keratin barrier, is considered useful. Besides its site adaptation significance it enables a distinction to be made between sites of similar appearance or turnover rates. For example, the LI of tail, ear, dorsum and even foot can at certain times of the
day be quite similar while the relative keratinized cell volume or thickness clearly differs. The relative volume of keratinized cell material produced per day can be compared for the various sites with the epidermal thickness (cell layers), i.e. nucleated basal and differentiating cells, and cornified cells (Fig. 19). The relative squame surface areas are also included, but thickness has been omitted since with the exception of dorsum there is little variation between sites (Table 1). Normal dorsum and ear epidermis have relatively low production rates, while tail and foot have high production rates with hairless dorsum intermediate.

**Cell proliferation and stacking**

It is tempting to correlate orderedness of the stratum corneum (stacking and non-stacking) with cell production rate; low production rates being linked with stacking and high rates with non-stacking. This correlation is dependent upon the parameters used to measure cell proliferation. These can be LI (i.e. basal cell DNA synthesis), MI (basal cell mitosis), or basal cell migration into the differentiating layers. LI depends on the rate of DNA synthesis, the rate of entry and exit into $S$, which may vary with the time of day and cannot be used reliably alone to separate stacked and non-stacked tissues unless average values over a 24-h period are obtained. Cell production in terms of migration into the differentiating layers can also be extremely high, but the epidermis may retain a stacked organization as seen in the initial rapid migration after minor wounding (Potten & Allen, 1975a). Later during the hyperplastic response, the epidermis becomes unstacked at a much lower migration rate. This suggests the possible necessity for a post-mitotic basal cell maturation period in a stacked system, which influences the direction of migration and extent of flattening of basal cells during differentiation. Providing cells are permitted this maturation period the proliferative activity could be relatively high and the cells will continue to stack. Thus stacking might be better correlated to basal maturation time than proliferative activity or cell loss rate.

**Origin and maintenance of thickness and organization**

There are 2 occurrences of a stacked arrangement of epidermis arising from a previously unstacked organization. They occur in the re-establishment of stacking.

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*Figs. 16–18. Hairless mouse dorsal epidermis.*

*Fig. 16. Vertical section through hairless mouse dorsal skin. The squames themselves are thicker than those in haired mouse dorsum, and have a more irregular profile. Taking the central basal cornified cell as the reference (ends marked by right-hand arrows) and comparing the incidence of squame ends over its length (left-hand arrows) the unstacked nature of the stratum corneum is clear. $\times 2100$.  

*Fig. 17. Vertical section through hairless mouse dorsal skin. In this region the squames are vertically stacked, with only one profile (marked by circle) not exhibiting its ends in a vertical line with the others. $\times 11030$.  

*Fig. 18. Scanning electron micrograph of hairless mouse dorsal skin, showing areas of both small (stacked) and large (unstacked) squame overlap (arrowed). $\times 2380$.  

Table 2. Keratinized cell production rates

<table>
<thead>
<tr>
<th>Site</th>
<th>Proliferation rate (k), cells/cornified cell/day</th>
<th>Keratinized cell thickness, μm</th>
<th>Daily volume (μm³) replaced/μm³* (Daily thickness replaced/EPU)</th>
<th>Daily volume replaced/EPU, μm³</th>
<th>Proliferation rate, % total thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsum</td>
<td>1.4</td>
<td>0.21</td>
<td>0.29</td>
<td>1.0</td>
<td>226</td>
</tr>
<tr>
<td>Ear</td>
<td>0.9</td>
<td>0.57</td>
<td>0.51</td>
<td>1.74</td>
<td>212</td>
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<tr>
<td>Foot</td>
<td>6.0</td>
<td>0.68</td>
<td>4.08</td>
<td>13.88</td>
<td>1591</td>
</tr>
<tr>
<td>Tail</td>
<td>4.0</td>
<td>0.61</td>
<td>2.44</td>
<td>8.30</td>
<td>1886</td>
</tr>
<tr>
<td>Hairless dorsum</td>
<td>1.8</td>
<td>0.54</td>
<td>0.97</td>
<td>3.31</td>
<td>567</td>
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<tr>
<td>Derivation</td>
<td>$k = N_{Li} / T_s = N_{Mi} / T_s$</td>
<td>Measured</td>
<td>Column 1 * column 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Daily skin volume replacement is calculated from the proliferation rate/squame (area) and the observed squame thickness.
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Fig. 19. Comparison of keratinized cell production and relative epidermal thickness.

A, relative keratinized cell production rates for each tissue (with dorsum as unity). The units in the histogram are relative and not to scale with the lower diagram (see Tables 1 and 2).

B, relative epidermal thickness (cell layers). The 5 tissues are shown diagrammatically with their basal cornified cells at the same level. The nucleated cells are separated into basal cells and differentiating cells by a horizontal line: using the criteria that only basal cells possess the ability to divide, foot is shown to have a basal layer which is 2 cells in depth. Within the relative depths of the cornified layer, the relative sizes of the squames are shown diagrammatically as hexagons. S, stacked; PS, partly stacked; NS, non-stacked.
after post-wounding hyperplasia (Potten & Allen, 1975a) and in normal development of baby mice, where stacking does not appear until some time after birth (Allen & Potten, unpublished). Since regenerating and embryonic epidermis grows rapidly in an unstacked arrangement as does normal adult foot epidermis it would appear to be a reasonable suggestion that adult ear and dorsum are more constrained, i.e. stacked epidermis may well be a modification of a more basic unstacked situation.

In the modification from an unstacked arrangement to a stacked arrangement an extra level of control involving some phasing of neighbouring EPUs (i.e. an inter-EPU control) may well become necessary to maintain the regular interdigitation of the squames in neighbouring columns (Potten & Allen, 1975b). Thus there may be 2 levels of control acting in stacked epidermis, one which controls the overall thickness of the epidermis and a secondary phasing control for the stacking itself. Should for any reason the thickness of the stratum corneum be suddenly depleted as in minor wounding, then the overall control for thickness assumes priority leading to an unstacked arrangement until thickness is re-established when the secondary control (and stacking) may return. In unstacked epidermis therefore only a single control of overall thickness would be necessary, allowing in fact a constant response to the level of 'wear' sustained by the foot and tail epidermis. Such a control may reside in the dermis, for epidermal grafting experiments have shown that the replacement rate and overall thickness do not always stay true to their original site when placed in a new environment (Billingham & Silvers, 1967; Billingham, 1971).

Although there are large differences in total epidermal thickness in the different regions, if the turnover rate is observed relative to the thickness (Table 2) it can be seen that approximately 12% of the epidermis (range 6% for ear epidermis, 16% for hairless dorsum) is replaced daily regardless of site, again suggesting a mechanism for maintenance of some predetermined overall thickness of epidermis. The uniform thickness of the stratum corneum for a given site and the return to this thickness after injury (Potten & Allen, 1975a) suggest that the steady state replacement rates for each tissue may be fixed during late development and remain at a set rate throughout life, except for localized reactions to injury.

The authors would like to thank Mr G. R. Bennion for technical help during the ultrastructural studies and Mr F. Leigh for preparing the micrographs.

The work was supported by grants from the Cancer Research Campaign and the Medical Research Council.

REFERENCES

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(Received 30 November 1974—Revised 8 December 1975)