Virchows Arch B (1987) 53:286–300

Virchows Archiv B

Epidermal cell proliferation

II. A comprehensive mathematical model of cell proliferation and migration in the basal layer predicts some unusual properties of epidermal stem cells

M. Loeffler[1], C.S. Potten[2], and H.E. Wichmann[3]

[1] Medizinische Universitätsklinik I, Joseph-Stelzmann-Str. 9, D-5000 Köln 41, Federal Republic of Germany
[2] Paterson Institute for Cancer Research, Christie Hospital & Holt Radium Institute, Manchester M20 9BX, UK
[3] Institut für Umwelthygiene, Auf'm Hennekamp, D-4000 Düsseldorf, Federal Republic of Germany

Summary. The clustering of ^{3}HTdR labelled cells in the epidermal basal layer and their changes with time have been modelled mathematically and cannot be adequately fitted by an earlier model of the cell kinetic organisation of the skin. A more refined model analysis was performed based on Monte Carlo computer simulations of cell layers which take cell division, cell aging and lateral as well as vertical cell migration into account. A large variety of hypothetical scenarios was tested to see if each could provide a fit to the clustering data. The analysis provides further support for the concept of a cell kinetic heterogeneity with a stem-transit-postmitotic differentiation scheme. In the best overall model scheme three transit divisions are predicted but unlike in the earlier model it is now postulated that postmitotic cells can be produced at all stages in the lineage rather than only at the end of the amplification scheme. Most important, the model predicts that stem cells and most of the transit cells differ in the way they process ^{3}HTdR label. Grain dilution is an important mechanism to explain the fate of some labelled cells in the tissue, but on its own it can only consistently explain the data if the stem cells have a very low labelling index (LI $\leq$ 1%) which implies a very short biologically unreasonable S-phase. If a higher LI (longer S-phase) is assumed for the stem-cells other mechanisms must be predicted to explain the lack of large clusters and the increase in time of the singles. The selective segregation of chromosomes at mitosis is one such mechanism. However, on its own a large number of cells would have to behave in this way (i.e. both stem and T_1 cells). If combined with other assumptions such as some grain dilution this selective segregation may be restricted only to stem cells. In addition the model allows cell production and migration rates to be estimated and the analysis can be related to the EPU-concept. Indeed the model itself would tend to automatically generate an EPU like structure. The model quantitatively reproduces LI, PLM, CL and clustering data.

Key words: Epidermis – Autoradiography – Cell communication – Cell division – Theoretical model – Clustering of cells

Offprint requests to: C.S. Potten at the above address

Introduction

The changes with time in the proportion of isolated (singles) and clustered labelled cells (pairs, triplets etc.) in the epidermal basal layer over the first 24 h after pulse labelling have been presented and analysed recently (Loeffler et al. 1986a). The changes observed over longer time intervals are presented in the accompanying paper (Potten et al. 1987). Here we analyse these data with various mathematical models.

A recent model analysis of the changes in labelling index (LI) with time, percent labelled mitosis (PLM) and continuous labelling (CL) data provided evidence for the existence of discrete transit cell subpopulations in the basal layer i.e. a cell kinetic heterogeneity (Potten et al. 1982). The basic assumptions and conclusions inherent in this former model were that; all cells in S-phase can be labelled by ^{3}HTdR; label is transferred to both daughter cells on division; a labelled stem cell reproduces a labelled stem cell; grain dilution or loss of label can be neglected; a maximum of 20% of the cells are stem cells (clonogenic fraction $<$20%); at least two proliferating transit cell populations are present; the system is in steady state.

The best fit to the data was obtained with a model having the following properties (hereafter called the reference model): 1. An hierarchical organisation with five subpopulations (stem, 3 transit and 1 postmitotic), 2. A cell cycle time decreasing with the degree of differentiation or maturation (180 h for stem cells and the first transit generation, 90 h for the two subsequent transit cells, 45 h residence time for postmitotic cells), 3. An extremely short S-phase duration in stem cells (2–3 h) and a long S-phase duration in transit cells (9–21 h).

After 300 h the LI had dropped by at least a factor of five below the initial value. It was concluded that this final level is related to the initial stem cell-labelling. The short S-phase duration concluded for stem cells was necessary if other more speculative assumptions were to be avoided.

New data on the clustering of labelled cells should permit a further testing of these predictions. Here we present a new mathematical model based on the analysis of individual cells arranged, according to a certain geometry, to examine the clustering data. These data could not be examined within the framework of our previous model because it could only consider pools of cells rather than individual cells and their neighbourhood relationships.

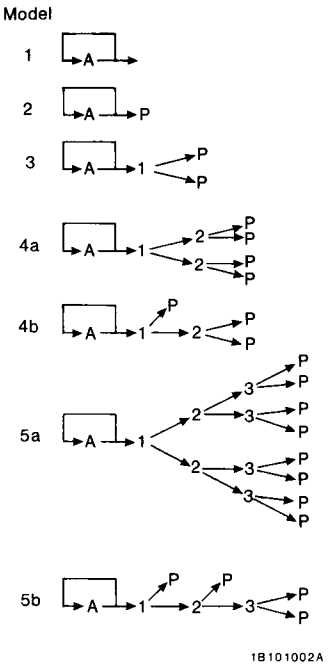

Fig. 1. Possible schemes for the epidermal organisation. A stem cells; 1, 2, 3, transit cells; P, postmitotic cells. Model 5a was suggested in a previous paper (Potten et al. 1982) and will be referred to as reference model. Models 1–3 were already rejected in this analysis on the basis of LI and PLM data

Model justification and mathematical representation

A model of epidermal proliferation has to take several things into account. For each topic we summarize the biological *a priori* knowledge under heading (i), we then describe the basic model assumptions for the reference model under (ii), the mathematical simulation technique under (iii) and any alternative scenarios under (iv).

Cell arrangement and cell hierarchy. A number of arguments suggest that the skin may be composed of epidermal proliferative units (EPU) which have a roughly hexagonal shape when viewed form the surface containing about 10 basal cells (Potten 1974). Estimates on the clonogenic fraction indicate that at maximum 30% of all cells in the basal layer are stem cells with a more likely value below 15% (Potten and Hendry 1973; Potten et al. 1983). About 50–60% of the basal layer cells are assumed to be proliferative. In a previous analysis (Potten 1976; Potten et al. 1982) the existence of at least two distinct proliferating transit cell populations was predicted (see Fig. 1 model 4a or 5a).

The model cell layer is considered as a two dimensional matrix of hexagonal cells (see Fig. 2). For a realistic simulation the matrix includes 32 × 32 cells. Other cells like Langerhans cells are not considered. Stem cells are regarded as non-migratory. In the reference model, 3 transit populations are assumed in a symmetric branching process (model 5a in Fig. 1).

The cell positions in the layer are represented by a two-dimensional time dependent matrix $A(i, j/t)$ (i refers to the column, j to the row number). The values of $A(i, j/t)$ code information about the generation type (A, T1, T2, T3, M) at position (i, j) at time t. Therefore a simulation has to start with an initial distribution of stem cells (A) on an empty matrix. For this purpose the matrix can be subdivided into subunits of 10 cells (see Fig. 2). At least one stem cell is positioned in each subunit. Depending on the clonogenic fraction assumed further stem cells were added to randomly selected subunits. Stem cells perform assymetric divisions producing one new stem cell and one proliferating transit cell (T1). Several transit generations follow (T2, T3) before post-mitotic cells (P) are formed.

Other division schemes are also considered as displayed in Fig. 1. For convenience we call models 4a, 5a "symmetric" and models 4b, 5b "asymmetric" hierarchies.

Cell cycle parameters. Post-mitotic cells do not divide. Based on an analysis of LI and CL data it

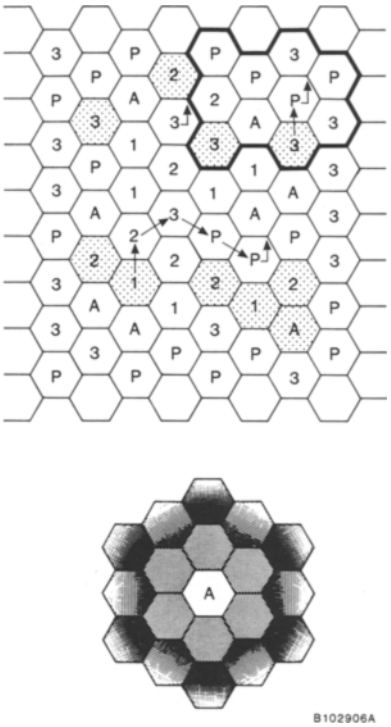

Fig. 2. (upper panel) schematic representation of the model matrix. Hexagonal cells are arranged on a lattice. Symbols A, 1, 2, 3, P identify the type of cell (see Fig. 1). The arrows begin at three cells that will divide in the next step and point to the oldest of their neighbours. The sequences of arrows end at the cell which is the oldest local cell. This cell leaves the layer (arrows at right angles) and all other cells will move laterally according to the sequence of arrows to fill the space. The stipled cells indicate labelled cells. In this sheet 10 labelled cells are present: 4 (40%) as singles, 2 (20%) as a pair and 4 (40%) as a quadruplet. After the next step in the model sequence 3 mitoses will have occurred and the labelled cells will be as follows: 3 (25%) as singles, 2 (17%) as a pair, 3 (25%) as a triplet and 4 (33%) as a quad. For one of the cells that will divide there is no older neighbours and hence one daughter on division will immediately leave the layer. The heavy solid line delineates the contour of a 10 cell epidermal proliferative unit (EPU) as used in the model in some simulations shown below. In these cases cell movement can not cross the indicated boundaries. *Lower panel.* For an evaluation of the average spread of cell clones (see Table 2) the cell shells around stem cells can be defined. The stipled cells indicate the inner shell around a stem cell, the hatched shading the second shell and the dotted shading the third shell. For both the hatched and dotted shells there will be a certain probability that the cell belongs to another cell clone and hence the uneven shading

was concluded that most of the proliferating cells have a cycle time of about 80–100 h (Potten 1975; Potten et al. 1982) with a minority having 180–200 h. Estimates of the S-phase duration show circadian variations. From PLM analyses an average of 5–6 h for labelling at 15.00 h and 3 h at 3.00 h was concluded (Potten et al. 1985). An analysis of clustered labelled cells suggested 7–10 h for labelling at 15.00 and 3 h for labelling at 3.00 h (Loeffler et al. 1986a). All these values are average numbers. Taking cell kinetic heterogeneity into account individual cell generations may have quite different S-phase durations. For the T_{G2+M}-phase a number of estimates also exists from PLM or cluster analysis ranging from 2 to 6 h (Potten et al. 1985; Loeffler et al. 1986a).

Reference model: The stem cells and T1-cells have been given an average cell-cycle time of 180 h. The other transit-cells cycle every 90 h. Steady state age distributions are assumed. Circadian variations in S-phase are taken into account by using corresponding T_S parameters. According to our previous analysis (Potten et al. 1982) the S-phase durations for experiments begun at 15.00 h are 2 h for the stem cells, 6 h for T1 and 9 h for T2 and T3 cells and 3 h, 9 h and 21 h for the 3.00 h experiments respectively. The time for T_{G2} is fixed at 2 h for stem and 1 h for transit cells. T_M is assumed to be 2 h for all cells.

The status of a cell at position (i, j) is represented by the matrix $B(i, j/t)$. The numerical value indicates how remote the cell is from its next division (e.g. if $B(5,9/t)=6$ a cell in column 5 and position 9 has 6 h to go until it divides). The model is iterated at an increment of 1 h. Every hour the cell-cycle age index is reduced by 1 as cells approach mitosis ($B(i, j/t+1)=B(i, j/t)-1$ if $B(i, j/t)>1$). If two cells are born each is given a new generation age index $A(i, j/t)$ (see above) and a new cell-cycle index $B(i, j/t)$. To account for a cell kinetic variance prospective T_{G1} and T_S times are selected at random for each new cell within a certain range. A 30% variance is generally assumed in G1 and S-phase, and no variance is assumed for G2 and M-phase.

Alternative calculations have been performed with different cell-phase durations. The cell cycle time of A- and T1-cells was varied from 90 h to 300 h. For T2- and T3-cells the 90–100 h value for T_c was not changed. In Table 1 the ratio of T_c^A versus T_c^T is used as a classification parameter varying from 1 to 3. For S-phase durations a wide range of possibilities was tested. They can in principle be subdivided into those which correlate with a high or low overall LI (corresponding to the 3.00 or 15.00 h data) and into sets of values assuming little, or much, labelling in the stem cell population.

Cell displacement. Little is known about cell movement within the epidermal basal layer. The orientation of mitotic figures suggests, that at least 80% of all cell-divisions take place within the layer

Table 1. Examples of Model scenarios tested

Model[a]	SEG[b]	GD[c]	T_C^A/T_C^T [d]	LI_o^A/LI_o^T [e]	Comparison with cluster data[f]						Overall[g] fit	Figure
					set	S	P	T	Q	LC		
Reference case												
5a	no	no	2[a]	1/10[f](2/23[k])	15.(3.)	−	=	=	+	+	no	3a, b
Variation of one parameter												
5b	no	no	2[a]	1/10[f]	15.	−	=	+	=	+	no	3c
4a/b	no	no	2[b]	1/10[g]	15.	−	+/−	+	=/+	=	no	×
5a	no	halving	2[a]	1/10[f]	15.	−	+	+	=	=	no	3d
5a	no	yes	2[a]	1/10[f]	15.	(−)	=	+	=	=	no	3e
5a	A	no	2[a]	1/10[f]	15.	−	=	+	+	=	no	
5a	A,T1, T2	no	2[a]	1/10[f]	15.	=	=	=	=	=	good	3f
Variation of two or more parameters without segregation												
5a	no	yes	2[b]	1/10[f]	15.	−	=	+	=	(+)	reas.	−
5a	no	yes	2[b]	2/23[k]	3.	=	−	+	=	+	no	−
5b	no	yes	2[b]	0.5/9[j]	15.	(+)	=	=	=	=	good	4a–f
5b	no	yes	2[b]	1/22[l]	3.	=	=	=	=	=	good	4a–f
4a	no	yes	3[c]	2/18[p]	3.	=	=	+	=	=	reas.	−
4a	no	yes	1[d]	0.5/9[i]	15.	−	=	=	+	+	no	−
5b	no	yes	2[b]	5/9[m]	15.	−	=	+	+	=	no	6b
With segregation												
5a	A	yes	2[a]	1/10[f]	15.	−	=	+	=	=	reas.	−
5b	A	yes	2[a]	6/20[o]	3.	=	=	+	=	=	good	−
5a	A	yes	2[b]	5/4[m]	15.	−	=	+	=	(+)	no	−
5b	A, T1	yes	2[b]	5/9[m]	15.	=	=	=	=	=	good	5a–f
5b	A, T1	yes	2[b]	12/11[n]	3.	=	=	=	=	=	good	5a–f
5a, 5b	A, T1, T2	yes	2[b]	5/4[m]	15.	=	=	=	=	=	good	−
5b	A, T1	yes	1[d]	5/5[h]	15.	=	=	=	=	=	good	−
4a	A	yes	1[d]	5/5[h]	15.	−	+	+	=	=	no	6e
4a	A, T1	yes	1[d]	5/5[h]	15.	=	=	=	=	=	good	6f

[a] Code according to Fig. 1

[b] SEG cell stages were chromosomal segregation is assumed named according to Fig. 1

[c] GD Grain dilution usually a median of 25 grains is assumed in the beginning with a coefficient of variance of 1.0. Halving: Pure Grain halving to each daughter cell; yes: Grains are distributed at random according to a hypergeometrical distribution

[d] ratio of cell cycle times of stem cells and transit cells A, T1, T2, etc. have the following cycle times. a: 180, 180, 90, 90 h; b: 180, 90, 90, 90 h; c: 300, 300, 100 h; d: 100, 100, 100, 100 h

[e] Initial label index of stem cells and transit cells, percentage of cells labelled. Practically this was introduced by assuming certain S-phase durations for A, T1, T2, etc. f: 2, 6, 9, 9 h; g: 2, 9, 9 h; h: 5, 5, 5, 5 h; i: 1, 8, 8 h; j: 1, 8, 8, 8 h; k: 3, 9, 21, 21 h; l: 2, 20, 20, 20 h; m: 9, 6, 5, 5 h; n: 22, 12, 12, 12 h; o: 10, 18, 18, 18 h; p: 5, 18, 18 h

[f] Comparison of model calculations with data is classified by =: if the fit is good; +: if the model curve is higher than the data (bad fit); −: if the model curve is lower than the data (bad fit); set: either the 3.00 h or 15.00 h labelling data. S = Singles, P = Pairs, T = Triplets, Q = Quadruplets, LC = Large clusters. Models similar to 5a and 5b but with an extra transit generation are also possible good fits to the data

(Iversen 1968). Therefore a certain amount of lateral cell-displacement must be expected. No information is available on whether this cell displacement is a random or selective process. Iversen et al. (1968) proposed the concept that the oldest neighbour of a dividing cell should be removed and Potten (1976) presented a model which also suggested such an age dependent migration. There is some debate in the literature as to whether the cells that leave the layer are already postmitotic (Potten 1975) or are G1-cells which emigrate at a certain age and become postmitotic thereafter (Iversen 1968). Burns and Tannock (1970) suggest a random loss process from a presumptive Go-phase. Nevertheless a 50–70 h residence time for this last cell stage is generally assumed in all models. Per day 9–12% of the basal layer cells are regenerated which is equivalent to 1.0 cell being produced per EPU per day (Potten et al. 1983). Once a cell leaves the basal layer it can no longer be detected autoradiographically and so is lost from our analysis.

In the model, cell displacement is dependent on mitotic activity and a selection criterion based on local neighbourhoods (see Fig. 2). It is assumed

that a dividing cell selects the oldest of the six immediate neighbour cells which is then displaced. Age is used in the sense that a T3-cell is older than a T2-cell, but also that a T3-cell in S-phase is older than a T3-cell in G_1-phase (i.e. age measures the entire time passed since the origin of a cell from the stem cell). If two equally old neighbours exist one is selected at random. This local age dependent selection process then continues to select the position for the first displaced cell. This algorithm is repeated as long as older neighbour cells can be found (Fig. 2). If the selected cell is itself the oldest cell in its vicinity this determines its migration out of the layer. In general these cells will be postmitotic cells, but under certain circumstances T3-cells may be forced out of the layer (Fig. 2). Once a target cell is selected to leave the layer the other cells move into the "holes" created and continue such displacements until the free space is next to a possible dividing cell. In general it is assumed that this migration is not restricted exclusively to an EPU although this assumption can be waived if required (see IV).

The values of the matrices $A(i, j/t)$ and $B(i, j/t)$ are clearly changed when a new cell is placed at position (i, j). The algorithm is straightforward.

As an alternative mechanism the cell displacement can be assumed to be restricted to an EPU. The selection criterion then is restricted to the oldest neighbour cells within the confines of an EPU.

Labelling index. The set of LI data used for testing the model was presented in a previous analysis (Potten et al. 1982). The percentage of labelled cells (LI) after 25 microCi ^{3}HTdR was measured by the classical autoradiographic technique counting at least 4000 cells in each time sample (4 mice).

We assume that all cells in the S-phase can be equally labelled and that there is no synchronisation of cells entering the S-phase. In the reference model it is assumed that dilution of label is negligible, that both daughter cells are labelled after each division and that label can only be lost from the layer through differentiation.

Whether a cell is labelled or not is described in the matrix by $C(i, j/t)$. At time $t=0$ all cells in the S-phase are labelled by assigning the value "labelled" (0 else). To identify cells in the S-phase the matrices A and B are consulted. The Labelling Index at position (i, j) at time t is defined as the percentage of labelled cells.

Clusters. In a given autoradiograph of a two dimensional sheet of cells one can measure the clustering of labelled cells (Loeffler et al. 1986a; Potten et al. 1987). We define an isolated labelled cell as a *single* if it is totally surrounded by unlabelled cells (see Fig. 2). A *pair* is defined as two, a *triplet* as a cluster of three, and a quadruplet as a cluster of four adjacent labelled cells. A *large cluster* contains more than 4 labelled cells. These clusters change with time after labelling (see accompanying paper). After an initial drop to low values the singles demonstrate a continuous increase back to the values obtained immediately after labelling. In contrast, pairs which appear after the first mitosis disappear in the course of time to reach a low plateau level. Triplets exhibit an intermediate peak while quadruplets and large clusters disappear after about 200 h.

In the model the percentage of labelled cells present as singles, pairs, etc. can be determined. All labelled cells are counted and it is determined to which type of cluster each labelled cell belongs.

The whole cell layer is scanned for labelled cells. If one is found an algorithm looks for further labelled cells in its vicinity. If none is found it is scored as a single labelled cell, if a second labelled cell is found in the vicinity the programme searches for a further labelled cell. The results are expressed as the percentage of labelled cells (PLC) present as singles, pairs etc.

Fate of label. It is clear that succesive cell division leads to grain dilution and that given time more and more cells will fall below the 4 grain detection threshold. From the data analysed here the median grain count after labelling ranges between 20 and 30 with a fairly broad distribution. After 150 h the median grain count is still above 12. After 335 h it has dropped considerably (median of 6) suggesting that about 50% (at most 65%) of the labelled cells become undetectable. It seems plausible to assume a stochastic process for the distribution of grains so that there is a certain chance for unequal grain distributions. Recently a special hypothesis was proposed which suggested a selective chromosomal segregation which would generate unequal grain distributions in the daughters of stem cell division (Cairns 1975). At the second division after labelling one daughter cell would receive all the remaining label while the other would become completely unlabelled. So far convincing proof does not exist but a number of experimental observations do seem to support the idea (Potten et al. 1978).

In the reference model none of these mechanisms was assumed.

Therefore any of these mechanisms can be considered as alternative scenarios.

Grain dilution. A theoretical initial grain distribution is generated that is comparable to the experimental data with a medium grain number of 23. At each division the grains are assigned to the daughter cells. In one case each daughter cell received exactly half of the maternal grains. In another scenario a stochastic process was assumed where the grains are distributed independently of each other (i.e. at random) to the two daughters according to a binomial distribution. In order to simulate these cases the model matrix is used to record the number of grains over each cell. If this value is above 3 the cell is counted as a labelled cell. During cell division the grains are then distributed to the daughters randomly. Reutilisation phenomena are not considered.

Segregation. Selective or non-random chromosomal stem cell segregation implies that after the second stem cell division in a steady state system the initially labelled DNA strands will no longer be found in the stem cells but only in the T1-cell. In theory the same could hold true for transit-cells. If they underwent two divisions the first division would result in the usual grain dilution while the second division (now two transit generations down the pathway) would result in an unequal grain distribution with one labelled and one unlabelled cell.

Simulation. Using the assumptions listed above the model was operated for 200 h before a labelling experiment was simulated. This eliminated any initial random effects. For each time-step the whole layer is scanned for mitotic cells. The algorithm starts with the top left cell of the layer and proceedes row by row. Once a mitotic cell is found the displacement process is performed as described above. The scanning procedure then continues down to the last cell. Thereafter all cells age by one hour and the whole process is repeated. For all subsequent model evaluations at least 10 sheets of 1040 cells are simulated. The simulation was performed on a CYBER 76M machine.

Results

Numerous model calculations have been undertaken to test several hypotheses concerning the behaviour of clustering of labelled cells in the epidermal basal layer. Data are shown as open (3.00 h) or closed (15.00 h) circles and the model calculations as a solid line.

Failure of the reference model

Taking the cell cylce parameters from our earlier 'reference' model one can show that the behaviour of the singles (Fig. 3a), quadruplets and large clusters (Fig. 3b) cannot be adequately explained although the fit for LI, PLM and CL data is good (see Table 1). Two major reasons contribute to this failure. First, if a stem cell is labelled in the reference model, it will always generate a labelled daughter stem cell. Therefore, stem cell label can never disappear out of the model layer.

Second, transit cells divide three times before they leave the layer. A T1 cell which is labelled produces eight labelled postmitotic cells after three divisions. Thus, large clusters will originate in the neighborhood of initially labelled stem cells or T1 cells, while in the rest of the basal layer the label will have disappeared. The reference model therefore predicts that after a long time there will be few large foci of labelled cells in an otherwise unlabelled layer. However, the opposite pattern is actually found. The reasons for this bad fit are examined in detail by testing which of the basic assumptions must be modified to obtain a good overall fit.

Alternative assumptions

Several possibilities can be considered which might result in a better fit. These are considered one at a time (see Table 1).

Change of one parameter

Cell migration. Lateral movement of cells within the basal layer would break up large clusters of cells. In the model, migration is the consequence of cell division. A selection mechanism decides which cell in the layer has to leave if a new cell is born. Between the site of the new cell and the 'hole' cells migrate. One obvious selection process is based on the age of the cells. If this is adopted in the reference model, there is an improvement in so far as larger clusters are indeed reduced. However, this does not result in a significant increase of the singles with time. The cell migration may be sufficient to break up large clusters into smaller ones, but it is not able to effectively separate these smaller clusters into singles. All other migration processes that were tested including some with unrealisticly high migration rates could not improve the situation. It was interesting to note that restricting the migration process to an EPU of 10 cells also does slightly improve the situation.

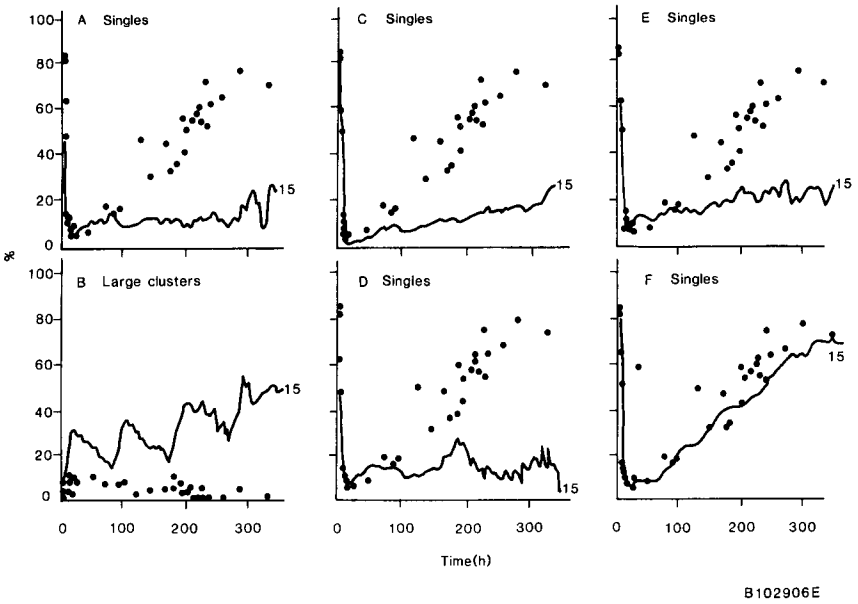

Fig. 3A–E. Rejectable model simulations. Data in all subsequent figures are presented by open circles (3.00 h labelling) or closed circles (15.00 h labelling). Model calculations are shown by the solid lines. The symbol at the right end of the lines indicate to which set of data they should be compared. For the parameters used see Table 1. **A–B** The set of parameters used in the reference model for simulating LI, PLM and CL data fails to explain singles, and large clusters (model 5a, $T_c^{A,1} = 180$ h, $T_c^{2,3} = 90$ h, no loss of label). **C** The same parameters used with an asymmetric division scheme (model 5b) does improve the situation for large clusters (not shown) but not for the singles. **D** Introducing exact grain halving into the reference model 5a induces only a very slight improvement for the singles. **E** There is a slightly better improvement in the singles fit if stochastic grain halving is assumed. The higher clusters generate reasonable fits. **F** Introducing selective chromosomal segregation in A, T1 and T2 cells leads to good fits of the singles. The model, however, seems biologically unacceptable because of the high amount of segregation postulated

The effect is primarily to restrict the choice of cells that could be selected to leave the layer. Hence, more frequently a T2 or T3 cell will be selected to leave the layer. Consequently the symmetric division scheme of reference model 5a is curtailed to something like Fig. 5b which results in slightly smaller clusters (see below). Thus the failure of the reference model was not due to the insufficient cell migration.

Changing the cell cycle parameters. In the reference model stem cells were predicted to have a very low labelling index compared with transit cells. A change to a pattern with similarly high LI's in all the compartments indeed results in a slight improvement of the clustering pattern. But in this case the level of the final LI will be unacceptably high. While a change of the average cell-cycle times (e.g. shortening of T1 and A-cell-cycle times) also does not improve the overall pattern, the variance of T_c does have an influence on the cluster curves (reference model: 10%). First, a larger variance leads to a dampening of any pronounced peaks. This effect is particularly beneficial for PLM curves where a larger variance produces the observed pattern of a first pronounced PLM peak and subsequent fluctuations at low levels. Second, the cell cycle variance influences the initial clustering directly after labelling. If the variance is small many sibling cells originating from the same mother will have progressed fairly simultaneously through the cell-cycle so that the chances of them labelling simultaneously, thereby generating an initial pair, is fairly high. The large percentage of singles observed after labelling suggests that the cell cycle variance is large enough to desynchronize the cells so that sibling cells do not commonly enter S-phase together. This is further confirmed by a statistical argument. If one takes the initial labelling index as a probability for an individual cell to be labelled independently from others one can calculate the statistical chance of producing singles, pairs and triplets on a hexagonal lattice (see Appendix). One obtains exactly the number of singles and pairs that are observed. This confirms that cell cycle variance should be considerably larger than the S-phase variance. In the model simulations shown below we generally assume a 30% coefficient of variation for the G_1-phase duration, and 20% for the S-phase duration. However, none of the changes in cell cycle parameters alone can generate satisfactory fits for all the cluster data.

Model schemes. In the previous analysis (Potten et al. 1982) models 1–3 (see Fig. 1) were rejected because they could not generate the necessary heterogeneity expressed by the measured LI, CL and PLM curves and because none of them is compatible with a clonogenic fraction of less than 20%. Model 5a with three transit compartments and a symmetric division scheme was favoured (=reference model). However, it produces large clusters. Model 5b in contrast effectively reduces the number of larger clusters and quads but does not improve the fit to the singles (Fig. 3c). Similarly models 4a and 4b produced fewer large clusters but did not provide satisfactory fits for the singles if no other parameters were changed (Table 1).

Grain dilution and DNA segregation. The above analysis so far reveals that three key aspects of the data are difficult to reconcile: the rapid fall in LI, the reappearance of singles, and the absence of many large clusters. Based on theoretical arguments we then postulated that this pattern should be achievable if one assumes that stem cells are prevented from labelling or loose label after labelling. Two possible processes were tested which might achieve this low level of stem cell labelling.

Grain dilution. First, simulations were performed with an initial median grain distribution of 23 grains per labelled cell and a precise grain halving on division. The result was still unsatisfactory for singles (Fig. 3d) but promising for quadruplets and large clusters (see Table 1). Somewhat better results for singles (Fig. 3e) and pairs were obtained if a stochastic distribution of grains to the daughter cells was simulated according to a binominal process. Starting with a realistic initial grain distribution one can then calculate that a certain percentage of cells falls below the three grain threshold: 3% immediately after labelling, 7% after 1 division, at most 20% after two divisions and less than 65% after 3 divisions. Thus, grain dilution is an effective mechanism to remove label after the second division thereby preventing the formation of large clusters and producing some singles. However, within the reference model a good fit to the data still was difficult to achieve (Table 1).

Selective segregation. Another effective mechanism to prevent formation of large clusters is a transfer of all label to one daughter cell while the other daughter cell remains unlabelled. *Selective chromosomal segregation* could be considered to occur in different proportions of the basal cells. It could be restricted only to the stem cells or could also include T1 and T2-cells (see Table 1). In all other compartments a random grain dilution process was assumed to take place. Selective segregation acting only on A cells or on A and T_1 was insufficient to improve the reference model (Table 1) without other additional assumptions. Segregation in A, T1 and T2 added to the reference model generated a perfect fit to all the cluster data and both the LI and PLM data (Fig. 3f).

Thus, it was not possible to obtain a fit using the parameters of the reference model by individually changing either the mechanisms of cell displacement, the cell-kinetic parameters, the hierarchical organisation or the levels of grain dilution. Only a high level of selective chromosomal segregation as a single change was successful. This, however, required a large percentage of the cells to be selectively segregating their DNA which is biologically doubtful. Therefore, a subsequent analysis was performed to test whether combinations of the above alternative assumptions would provide a good fit while maintaining the level of selective segregation to a minimum.

Combined solutions

About 400 different model scenarios were tested. A selective-survey of the results is given in Table 1 and Figs. 4–6. Optimal fits to the experimental data were obtained by the following model arrangement.

Low LI in stem cells – No chromosomal segregation. A few sets of assumptions could be identified which generated good fits to the data without assuming any selective chromosomal segregation (Table 1). The basic feature here is that the stem cells must be largely unlabelled. Their labelling index has to be about 10 times smaller than for transit cells. Good fits can also be obtained if stem cells are not labelled at all. One of the best fits obtained is shown in Fig. 4 which involves a model 5b with a T_c^A of 180 h, and a T_c^T of 90 h. In order to match the 15.00 h data 0.6% of the *A* cells and 9% of the *T* cells were assumed to be labelled, while 1% of the *A* and 22% of the *T* cells were labelled for the 3.00 h data. (An LI of 1% with a cycle time of 180 h means a T_S value of 1.8 h.) Only grain dilution was also assumed to occur. Other models (5a, 4a, 4b) did not generate good fits to either set of data because they generated peaks of triplets which are absent from the data.

A large LI in stem cells with selective chromosomal segregation. The more cell stages that are assumed

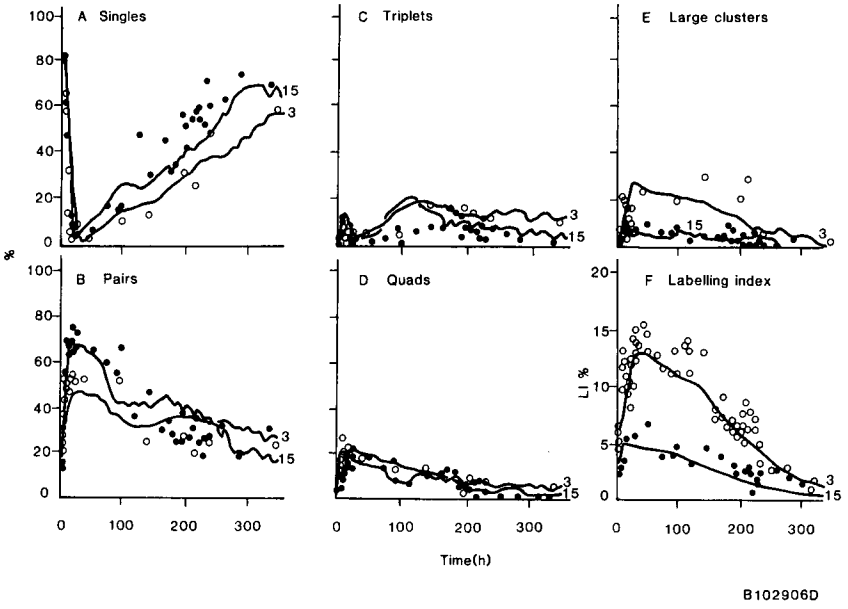

Fig. 4. Model fit with low LI in stem cells. Here we assume a low labelling index in epidermal stem cells, an asymmetric division scheme (model 5b), $T_c^A = 180$ h, $T_c^{1,2,3} = 90$ h, $T_s^A = 1$ or 2 h, $T_s^{1,2,3} = 9$ or 22 h (for 15.00 h or 03.00 labelling) and a binomial grain dilution process. The fit of the model curves to the data is satisfactory. Segregation is not assumed

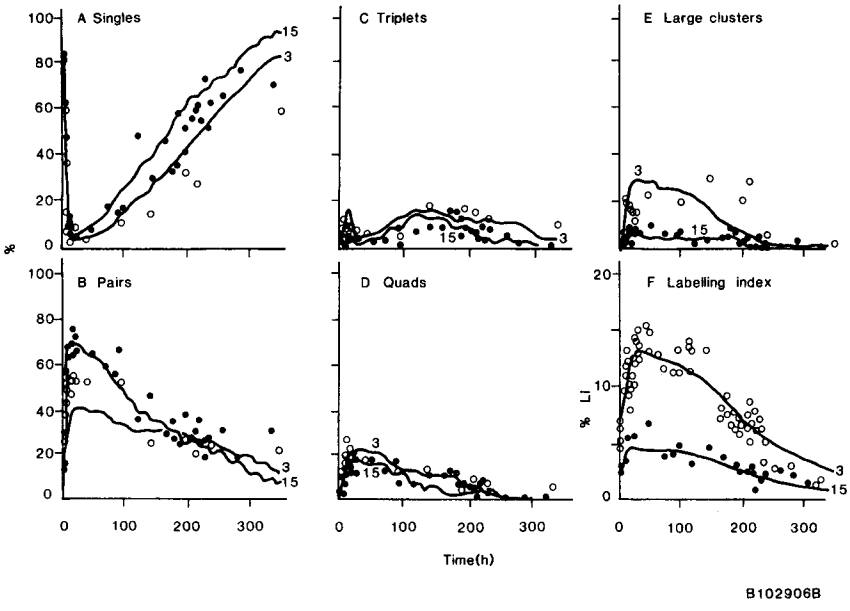

Fig. 5. Model fit with high LI in stem cells. Here we also assumed an asymmetric division scheme, $T_c^A = 180$ h, $T_c^{1,2,3} = 90$ h and $T_s^A = 9$ or 18 h and $T_s^{1,2,3} = 5$ or 15 h (for 15.00 or 03.00 h labelling). In stem cells and T1-cells selective chromosomal segregation is assumed and a binomial grain dilution on T2 and T3 cells. The fit is satisfactory

to exhibit selective chromosomal segregation the broader is the range of other parameters that lead to satisfactory fits to the data. If it is assumed that only stem cells selectively segregate their DNA models 5a and 5b generate a good fit only if the fraction of labelled stem cells remains fairly small (similar to the case just described). If the LI in stem cells is assumed to be comparable to that of the transit cells (5–15%) selective segregation in both the A and T1 cells must be assumed. Figure 5 shows examples with a good fit with 5% LI for A and T1 cells for the 15.00 h data and a 12% LI for the 3.00 h data. (An LI of 5% with a cycle time of 180 h means a T_S value of 9 h.) Figure 6a gives a picture of the corresponding PLM. Figure 6b shows the failure of the fitting exercise if selective segregation is ignored in this particular case. If a shorter cycle time of *A* cells is assumed good fits can be obtained (Table 1) and the fraction of cells assumed to be selectively segregating could be reduced from about 20% to 15%. In general, the more cell generations that are assumed to exhibit selective chromosomal segregation the easier it is to generate good fits with higher (more reasonable) levels for the initial LI of stem cells.

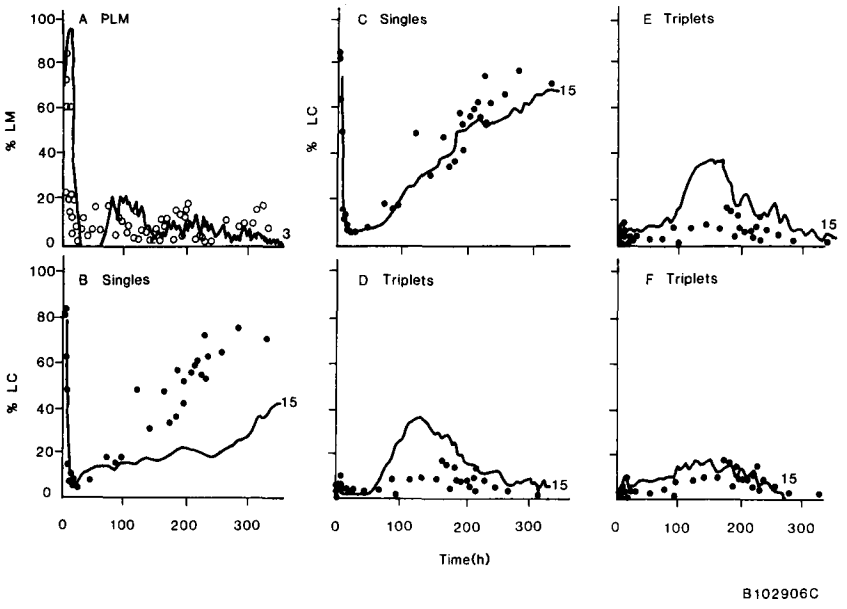

Fig. 6. Miscellaneous topics. The calculations shown in Fig. 5 are repeated with special modifications. **A** PLM curve related to Fig. 5. A similar result is obtained for the model in Fig. 4. **B** Selective segregation is excluded. The singles do not fit the data. **C** Cell migration is restricted to within the EPU which contained 10 cells. The increase in – singles becomes less pronounced in the later phase. **D** A prolongation of the average residence time of postmitotic cells to 150 hrs leads to the appearance of a triplet peak. **E** Model 4a is simulated with selective segregation only in stem cells. This generates unsatisfactory fits for the triplets. **F** The same is repeated with selective segregation assumed in both stem an T1 cells producing good fits in all cases (e.g. triplets)

Additional evaluations

The EPU concept. If migration in the optimal reference 5b model is restricted to within an EPU the increase in the singles becomes less pronounced (Fig. 6c). The main reason is that large clusters should persist in some EPU's for long times. The EPU concept with rigid boundaries tends to flatten the increase of singles and preserves islands of labelled clusters.

Residence time of postmitotic cells. Models with a large percentage of postmitotic cells ($\geq 60\%$) resulting from a long residence time (>150 h), tend to generate a worse fit particularly for the triplets (Fig. 6d).

Model 4. Models with more than 3 transit populations are in principle conceivable but would necessitate even greater levels of selective DNA segregation. Models with two transit populations tend to generate enormous peaks of triplets (Fig. 6e) which disappear only if one assumes selective segregation in both A and T1 cells (Fig. 6f), which together represent a rather high percentage of the basal layer cells for this T_2 type of model.

Stem cell clones and the EPU. Some parameters can be concluded from the model which are not directly accessible to measurement, for example the range of cell displacements and the spread of a stem cell clone (Fig. 2 and Table 2). It is apparent that the stem cell clones tend to spread over several cell positions so that a certain mixing between adjacent stem-cell clones occurs. If one relates the area covered by a stem cell clone to the proposed EPU structure it becomes apparent that with a 20% clonogenic fraction 64–76% of cells from a stem cell clone are within a 7 cell EPU and 80–91% are within a 13 cell EPU. Thus, without imposing a rigid EPU restriction to the displacement mechanism the system is self-ordering in the sense that it generates average stem cell clones that corre-

Table 2

	Model 5a[a] Shell				Model 5b[a] Shell			
	1	2	3	>3	1	2	3	>3
T1	100%	–	–	–	100%	–	–	–
T2	80%	15%	5%	–	100%	–	–	–
T3	45%	25%	20%	10%	90%	10%	–	–
P	30%	20%	20%	30%	55%	25%	15%	5%

[a] $T_C^A = 180$ h, $T_C^T = 90$ h, and a clonogenic fraction of 20% was assumed (see Fig. 2 lower panel)

spond to the EPU-concept. It is noticeable that in the model postmitotic cells can be found in the outer shells indicating that the cells migrate from the center of an "EPU" to its periphery where the cells predominantly leave the layer as was predicted earlier (Potten 1976).

Cell production rate. The number of postmitotic cells leaving the layer per day can also be estimated. In model 5a ($T_C^A = 180$ h, $T_C^T = 90$ h) about 16–18 cells are produced per day per 100 basal layer cells, while the value for the best model 5b (same T_C^A and T_C^T) ranges between 10–12. The experimentally deduced cell production of 10–15 cells per 100 cells per day (Potten 1975) is in agreement with model 5b.

Appearance of label in the suprabasal layer. The proportion of cells leaving the basal layer that are labelled can be deduced from the model. In the best fit models presented in Figs. 4 and 5 for 3.00 h labelling this percentage was 2% at day 1, it reached a maximum of 14% not before day 4 and slowly declined to less than 5% at day 15. For the 15.00 h labelling the maximum was 6% which was not reached before day 4.

Mitotic axis. Although the model generates new cells within the basal layer, there may be circumstances where a new cell is immediately positioned suprabasally. This occurs, if the dividing cell is the oldest cell amongst all its immediate neighbours. A direct vertical cell positioning of daughter cells into the suprabasal layer was obtained in our simulations after 1%, 3% or 5% of all mitoses, depending on whether the percentage of postmitotic cells in the layer was 50%, 40% or 30%. If, in addition one includes those cells that leave the basal layer in the model very shortly after mitosis (eg. within 10 h) the percentage of vertical mitoses in the model ranges between 3% and 8%. Amongst the cells leaving the layer only a minority are transit cells and not postmitotic cells which is in agreement with the fact that proliferating cells are hardly ever found in the suprabasal layer. Measurements show that the frequency of mitotic figures with a vertical axis (where one daughter cell leaves the basal layer immediately) in mouse epidermis is indeed small (5%, Wright and Alison 1984, or 6% in 32 day old mice, Smart 1970). However, preliminary unpublished data on our own mice suggest that the proportion of vertically oriented mitotic figures might be greater (15%–20%) than suggested above.

Discussion

In previous modelling, discrete heterogeneous subpopulations and an hierarchical stem-transit-postmitotic-cell division scheme was postulated (Potten et al. 1982), with a T_c of 180 h for stem and T1 cells, and 90 h for T2 and T3 cells. The S-phase durations were predicted to be very small for stem cells and longer for transit-populations. This is referred to here as the reference model.

New data were presented by Potten and Loeffler (1987) on the clustering of labelled cells which then had to be tested against the model. This proved difficult and a new model had to be designed to simulate the clustering patterns. The model presented here is a two-dimensional model which allows Monte-Carlo simulations of the epidermal basal layer. Running this model with only parameters of the reference model and various basic assumptions on the cell migration process it was not possible to generate the measured cluster patterns (see Fig. 3a–c). The previous (reference) model predicted the formation of large clusters in the vicinity of labelled stem cells and very few single labelled cells at later times. The data, however, show almost the opposite effects.

A change in several of the critical assumptions (migration-processes, different cell cycle parameters, different division schemes, and various mechanisms to remove labelled cells) were examined for their ability to correct the discrepancy. The major conclusions from this analysis are:

Division scheme. The heterogeneous composition of the basal layer with stem cells, transit-cells, and postmitotic cells is confirmed. Three transit cell generations seem to give the best fit. The best model simulations were achieved with about 40% of P-cells. These figures are quite consistent with our original model.

Stem cell properties. It was necessary to predict that stem cells exhibit a very special behaviour with respect to ^{3}HTdR labelling. Two possibilities exist:
a) If it is assumed that stem cells can be labelled with ^{3}HTdR to give a reasonable LI (>1%) one is forced to postulate a selective chromosomal segregation in both the stem cells and the T1-cells if one also assumes an asymmetric division scheme (model 5b). If a symmetric hierarchical division scheme is assumed (5a) selective segregation in the T2-cells is also required.
b) If it is assumed that stem cells can only be labelled very occasionally i.e. have very low LI values (<1%), then selective chromosomal segre-

gation is not required. Grain dilution and asymmetry in the division scheme are all that are required to reconcile the model with the data.

Migration. A local age-dependent selection criterion for the positioning of a newborn daughter cell provides an adequate mechanism to explain the selection of the P-cell in the vicinity of a dividing cell which will leave the layer. For this to work each cell only has to know the 'age' of it's immediate neighbouring cells. This generates a pattern of adjacent stem cell clones which partly overlap in their outer shells. Thus on average, an "EPU" type structure is generated as the natural consequence of the local age dependent migration process with proliferative cells in the middle and postmitotic cells in the periphery. The local age selection predicts a large percentage (over 95%) lateral and only few vertical mitoses and the occurrence of labelled cells in the suprabasal layer at a time which is in good agreement with experimental data (Iversen 1968). The frequency of lateral cell displacements associated with mitosis is fairly low perhaps 1–3 cells per mitosis. This conclusion of an age dependent selection process turns out to be very similar to the conclusion drawn from modelling studies in the crypts of the intestine (Loeffler et al. 1986b) which suggest that it might be a universal mechanism.

Cell cycle parameters. The transit-cells are deduced to have a T_c of 90–100 h while stem cells may have T_c values of 180–200 h. A cell-cycle variance of at least 30% is concluded. The S-phase durations are more difficult to deduce and will be discussed below. These are the same as predicted in our earlier reference model.

Grain dilution is not important in the basal layer for the first two cell divisions. For times up to 250 h less than 20% of the cells will have dropped below the 4 grains threshold (provided 25 micro-Ci ^{3}HTdR are given). After the third division, however, a larger proportion will not be detectable as labelled cells. This fraction, however, does not exceed 65% after 350 h. In addition a random distribution of the grains to the daughter cells seems to fit the observations much better than a fixed 1:1 distribution.

The cell production rate of the best model (5b) is 10–12% cells per day. Which is in agreement with measurements by Allen and Potten (1974).

Labelled cells can appear in the *suprabasal layer* after 1 day (about 2%) with a maximum after day 4 (3.00 h labelling). The appearance of labelled suprabasal cells is not primarily connected to a vertical mitotic axis but due mainly to postmitotic previously labelled cells leaving the layer. The lack of an early appearance of labelled suprabasal cells suggests that truely vertical mitotic figures are not very common.

We therefore conclude that we have a model that is comprehensive enough to explain most of our experimental data on epidermal cell kinetics, and that the autoradiographic cluster data were very effective in permitting a selection of hypotheses that result in good fits to the data.

Although the models discussed above take several aspects of the epidermal cell replacement into account they still oversimplify the situation in a number of ways.

Firstly, Langerhans cells are not taken into account. As these are cells with a low turnover they will usually remain unlabelled and will not affect the clusters. Therefore they could be neglected. Secondly, it was assumed for convenience that the cells have a hexagonal shape. Thirdly, it has been assumed that cell displacement is the consequence of mitotic activity. This does not necessarily imply that this is biologically correct (see Potten et al. 1984) but it is more convenient for modelling. Other concepts, eg. that mitosis is triggered by a "hole" left behind by a cell that left the layer, cannot be rejected at this stage and may produce equally acceptable fits. Fourthly, the model simulations are performed on a 32×32 lattice and finite size effects may bias the statistics.

Finally, statements made on S-phase durations should be interpreted with great caution. The values given here (eg. 9 h, 22 h) must be considered as pseudo-S-phase durations used to generate the appropriate LI in a steady state model. As no circadian mechanism was introduced into the model a synchronized cohort of cells could not be generated. In order to simulate labelling of a synchronized wave of cells we labelled more cells in the steady state model by artificially adjusting the model S-phase. This defect, however has no consequences on the simulation of the cluster data because they only depend on the number of cells initially labelled. The biologically correct S-phase durations for these mice should be taken from Potten et al. (1985) or Loeffler et al. (1986). They conclude an average of 6–8 h for the 15.00 experiment but only 4 h for the 3.00 experiment (whereas the model requires about 20 h). Two explanations for this latter discrepancy may be given. Firstly, the assumption of a strict steady state may be wrong. If many cells are labelled within a short S-phase

it must mean that there is a cell synchrony triggered by circadian rhythms. Several authors have suggested a circadian trigger to be present at the end of G1 or at the beginning of the S-phase (Wichmann and Fesser 1982; Thorud et al. 1979a, b; Aarnaes et al. 1981). Secondly, there could be a massive reutilisation of label from cells which are degraded after being killed initially by the label ("suicide") (Wichmann et al. 1987). In order to spread this label from 3–6 h to about 20 h a delayed thymidine reutilisation was suggested. Some fine structure in the initial LI and PLM curves (small extra peaks) might be explained by this process (Wichmann et al. 1987). Other studies have also suggested some delayed thymidine utilisation (Hume and Potten 1982). Longer term reutilisation phenomena have not been considered.

As mentioned above there is some debate in the literature as to whether postmitotic or G_1-cells leave the basal layer. Assuming a local age dependent selection process our model supports the former concept. Asymmetric models (e.g. model 5b) appear to be more compatible with the data. Their basic feature is that from any transit generation cells may become postmitotic and thereby perform an "age jump". These P-cells then become eligible for the selection process. The consequence of this is that the proportion of P-cells rises unless we accept a higher proliferation of stem (clonogenic) cells. The best overall fits are obtained with models based on the scheme 5b (Fig. 1) or intermediate schemes between 5a and 5b. Assuming model 5b and some grain dilution effects, the data can be fitted if we assume about 20% A-cells with either a very short S-phase and other assumptions or a larger S-phase (higher LI) in which case we have to assume some selective DNA segregation. Clearly the proportion of P-cells is related to the extent of branching in the cell lineage and it rises to 45–50% in the best fit model and as a consequence the average residence time for the P-cells will be 80–100 h. This is somewhat longer than is commonly quoted (Iversen et al. 1968; Potten 1976; Potten et al. 1982; Wright and Alison 1984) but is clearly indicated by the length of the plateau for the 3.00 h pair data (Fig. 3 accompanying paper). One further consequence of this is that the functional growth fraction in mouse epidermis is likely to be less than 0.45–0.50 particularly when other cell types are taken into account (eg. Langerhans cells).

The models favoured here predict a frequency of vertical cell division that is somewhat lower than is actually measured by scoring the orientation of mitotic metaphase or anaphase figures (3–8% versus 5–20%). The discrepancy is most likely due to a difficulty in directly comparing the model values with the data. A vertical mitotic figure does not necessarily mean a vertical cell division. Lateral cell positioning may still be possible as is suggested by the late appearance of labelled suprabasal cells. In contrast, measurements of mitotic axis involve a subjective interpretation of the appearance of cells and vertical mitotic figures could still result in both daughters lying in the basal layer in some cases. In addition some modelling situations would question the existence of a very high percentage of vertical mitoses.

Within the model 5b a situation with a high number of vertical mitotic figures could only be explained with a much smaller percentage of transit and postmitotic cells and a clonogenic fraction of up to 35% which is considered as unlikely. In order to keep the clonogenic fraction as low as 20% in such a case one would have to claim the existence of a forth transit cell stage which, however, would clearly worsen the fit to the clustering data.

Probably the most interesting aspect of this analysis is the unusual behaviour predicted for the stem cells with regard to DNA segregation. Either stem cells label to a similar extent as transit cells and then they must selectively segregate their DNA between 100 and 200 h after labelling. Alternatively stem cells exhibit a rather peculiar ^{3}HTdR metabolism (eg. have a very short S-phase) and therefore only a few are labelled or they take up little ^{3}HTdR (low LI, and/or very low grain count). A low LI may result from the following explanations:

a) The stem cells could have a large internal thymidine pool so that flooding the system with ^{3}HTdR is impossible,

b) they could have a very poor thymidine uptake mechanism,

c) the S-phase on average could be very short,

d) the stem cells could be very radiosensitive so that only a small fraction survives as a labelled cell. If this were so the model would predict some reduction in epidermal cell production after about 500 h (perhaps by 10–20%). In the small intestine the cells at the stem cells region in fact appear to be very efficient scavengers of exogenous thymidine (Chwalinski and Potten 1986) and some of the cells in the zone appear to be extremely radiosensitive (Potten 1977).

Thus it seems fairly clear that epidermal stem cells differ from transit cells not only in their cell cycle time but in a much more pronounced way

with respect to their incorporation and or handling of ^{3}HTdR. It is possible that this might help in identifying epidermal stem cells.

The model presented here has many similarities to a model used for describing the intestinal crypt (Loeffler et al. 1986). The crypt model also predicts a local age-dependent migration process, cell heterogeneity and can be used to analyse clustering data (referred to in the crypt analysis as RUN data). The conceptual similarity between these models and their success in giving a comprehensive explanation of experimental data strongly suggests that a similar epithelial organisation exists in the intestine and the epidermal basal layer.

In summary the present model of the clustering of labelled cells in murine epidermis confirms our previous conclusion of a cellular hierarchy in epidermis. It is also deduced that epidermal stem cells differ drastically for the transit proliferative cells with respect to their ^{3}HTdR metabolism. The most intriguing speculations are that stem cells either have an extremely short S-phase duration or show selective chromosomal segregation. In addition the computer model used for this analysis gives a comprehensive quantitative representation of a large body of experimental data including LI, PLM, CL, cell production rates, clonogenic fraction, growth fraction and in addition allows a series of evaluations that cannot directly be assessed experimentally.

Acknowledgements. We like to thank Günther Michel, Wilfried Weiß and in particular Heino Falcke for their excellent help in performing the computer simulations and Stephan Gontard for preparing the manuscript. The research was supported by the Cancer Research Campaign (UK) and the Volkswagenstiftung (FRG).

Appendix

The stochastic chance to find a cluster of s labelled cells (s-cluster for short, eg. singles, pairs, triplets etc.) can be calculated on any given regular lattice if the labelling takes place at random. The formula for this probability is:

$$\text{pr(s-cluster)} = s\,\text{SUM}\ (g_{st}\, p^s(1-p)^t)$$

summed over all configurations t

$$\text{with } p = \underset{s}{\text{SUM}}\ (\text{pr(s-cluster)})$$

where $s = 1, 2, 3, \ldots$ stands for the category of clusters (1 = singles, 2 = pair); t stands for the number of neighbours of the clusters (eg. 6 for a single, 8 for a pair on a hexagonal lattice, 4 for a single and 6 for a pair on a quadratic lattice); g_{st} quantifies how many similar configurations with s and t are possible (eg. $g_{1,6} = 1$, $g_{2,8} = 3$, $g_{3,10} = 7$, $g_{3,9} = 2$ on a hexagonal lattice, $g_{1,4} = 1$, $g_{2,6} = 2$, $g_{3,8} = 2$, $g_{3,7} = 4$ on a quadratic lattice); p is the probability of labelling (i.e. LI). If one has an initial label index of 0.035 (15.00 h) or 0.07 (3.00 h) one can calculate the probabilities to find singles, pairs etc. for a hexagonal structure:

	15.00 h data		03.00 h data	
	theory	data	theory	data
pr (single)	0.81	0.84	0.65	0.67
pr (pair)	0.16	0.13	0.24	0.23
pr (triplet)	0.02	0.03	0.07	0.08

(pr = probability)

The measured data are in quantitative agreement with this analysis (see figures).

References

Aarnaes E, Thorud E, Clausen OP (1981) Model analysis of circadian rhythms in mouse epidermal basal cell proliferation. J Theor Biol 88:355–370

Allen TD, Potten CS (1974) Fine structural identification and organisation of the epidermal proliferation unit. J Cell Sci 15:291–319

Burns FJ, Tannock IF (1970) On the existence of a Go-phase in the cell cycle. Cell Tissue Kinet 3:321–334

Cairns J (1975) Mutation selection and the natural history of cancer. Nature 225:197–200

Chwalinski S, Potten CS (1986) Cell position dependence of labelling of thymidine nucleotides using the de novo and salvage pathways in the crypt of small intestine. Cell Tissue Kinet 19:647–659

Hume WJ, Potten CS (1982) A long lined thymidine pool in epithelial stem cells. Cell Tissue Kinet 15:49–58

Iversen OH, Bjerknes R, Devik F (1968) Kinetics of cell renewal, cell migration and cell loss in the hairless mouse dorsal epidermis. Cell Tissue Kinet 1:351–367

Loeffler M, Potten CS, Ditchfield A, Wichmann HE (1986a) Analysis of the changes in the proportions of clustered labelled cells in epidermis. Cell Tissue Kinet 19:377–389

Loeffler M, Stein R, Wichmann HE, Potten CS, Kaur P, Chwalinski S (1986b) Intestinal cell proliferation-I. A comprehensive model of steady state proliferation in the crypt. Cell Tissue Kinet 19:627–645

Potten CS (1974) The epidermal proliferative unit: The possible roll of the central basal cell. Cell Tissue Kinet 7:77–88

Potten CS (1975) Epidermal cell production rates. J Inv Dermatol 65:488–500

Potten CS (1976) Identification of clonogenic cells in the epidermis and the structural arrangement of the epidermal proliferative unit. In: Cairnie AB, Lada PK (eds) Stem cells of renewing cell propulations. Academic Press, New York, 91–102

Potten CS (1977) Extreme sensitivity of some intestinal crypt cells to X and gamma-irradiation. Nature 269:518–521

Potten CS, Loeffler M (1987) Epidermal cell proliferation: I. Changes with time in the proportions of isolated, paired and clustered labelled cells in sheets of murine epidermis. Virchow's Archiv (Cell Pathol) 53:279–285

Potten CS, Hendry JH (1973) Clonogenic cells and stem cells in epidermis. Int J Radiat Biol 24:537–540

Potten CS, Hendry JH, Al-Barwari SE (1983) A cellular analysis of radiation injury in epidermis. In: Potten CS, Hendry JH (eds) Cytotoxic insults to tissue. Churchill Livingstone, Edinburgh, pp 153–185

Potten CS, Hume WJ, Parkinson EK (1984) Migration and mitosis in the epidermis. Br J Dermatol 111:695–699

Potten CS, Hume WJ, Reid P, Cairns J (1978) The segregation of DNA in epithelial stem cells. Cell 15:899–906

Potten CS, Wichmann HE, Dobek K, Birch J, Codd TM, Horrocks L, Pedrick M, Tickle SP (1985) Cell kinetic studies in the epidermis of mouse – III. The percent labelled mitosis technique (plm). Cell Tissue Kinet 18:59–70

Potten CS, Wichmann HE, Loeffler M, Dobek K, Major D (1982) Evidence for discrete cell kinetic subpopulations in mouse epidermis based on mathematical analysis. Cell Tissue Kinet 15:305–329

Smart IHM (1970) Variation in the plane of cell cleavage during the process of stratification in the mouse epidermis. Br J Dermatol 82:276–282

Thorud E, Clausen OP, Aarnaes E, Bjerknes R (1979a) Circadian changes in cell cycle phase durations in murine epidermal basal cells. Chronobiol 6:163

Thorud E, Clausen OP, Aarnaes E, Bjerknes R, Elgjo K (1979b) Circadian rhythms in epidermal basal cell proliferation. Cell Tissue Kinet 12:685–686

Wichmann HE, Fesser K (1982) Influence of 3HTdR on the circadian rhythm – A model analysis for mouse epidermis. J Theor Biol 97:371–391

Wichmann HE, Franke H, Potten CS, Todd L (1987) Modelling of the influence of 3HTD on cell kinetics in mouse epidermis. Adv Math Comput Sci Med (in press)

Wright N, Alison M (1984) The biology of epithelial cell populations, vol 1, 2. Clarendon Press, Oxford, 1247 pp

Received January 6, 1987 / Accepted April 30, 1987