Models of epidermal wound healing

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SUMMARY

The spreading of cells across the surface of an epidermal wound enables epidermal migration to be studied independently of the wound contraction that occurs in deeper wounds. In particular, the stimulus for the increase in epidermal mitosis during would healing is uncertain. Our modelling suggests that biochemical regulation of mitosis is fundamental to the process, and that a single chemical with a simple regulatory effect can account for the healing of circular epidermal wounds. The model results compare well with experimental data.

1. BIOLOGICAL BACKGROUND

The process of wound healing is conventionally divided into three stages: inflammation (blood-clot formation, influx of leucocytes and wound debridement), wound closure and matrix remodelling in scar tissue. In a full-depth wound, that is when both the dermis and epidermis are removed at injury, the second stage is accomplished by the process of epidermal migration, in which epidermal cells spread across the wound, and wound contraction, in which the main body of the wound contracts causing the wound edges to move inwards. In an epidermal wound, wound closure is entirely due to epidermal migration; thus epidermal wounds provide an opportunity to study this process independently of wound contraction.

The mechanism of epidermal migration is only partly understood. Normal epidermal cells are non-motile. However, in the region of the wound, they undergo marked phenotype alteration ('mobilization') that gives the cells the ability to move via lamellipodia (Clark 1988). The main factor controlling cell movement seems to be contact inhibition (Irvin 1984; Bereiter-Hahn 1986). However, chemotaxis and contact guidance may also be involved (Clark 1988).

As a whole, the spreading sheet has one or two flattened cells at the advancing margin, whereas the epidermis behind this is between two and four cells deep. Two mechanisms have been proposed for the movement of the sheet. In the 'rolling mechanism', the leading cells are successively implanted as new basal cells: they assumed an oval or cuboidal shape and become embedded in the wound surface. Other cells roll over these (see Krawczyk 1971; Winstanley 1975; Ortonne *et al.* 1981). In the 'sliding mechanism', on the other hand, the cells in the interior of the sheet respond passively to the pull of the marginal cells. However, all of the migrating cells have the potential to be motile: for example, if a gap opens in the migrating sheet, cells at the boundary of this develop lamellipodia and move inwards to close the gap (Trinkaus 1984). Though the morphological data of mammalian epidermal wound healing is convincingly explained by the rolling mechanism (Stenn & DePalma 1988), unequivocal evidence is lacking, whereas the sliding mechanism is well documented in simpler systems such as amphibian epidermal wound closure (Radice 1980).

The wound edges are not the only source of migrating cells. Unless the full thickness of the dermis is removed at injury, sebaceous glands, sweat-gland ducts and the outer root sheets of hair follicles are also sources (Rudolph 1980). Indeed Winter (1972) found that in shallow wounds in domestic pigs, over 90% of new epidermis was from epidermal appendages within the wound. In such cases the wound may be regarded as a series of micro-wounds extending from one appendage to the next (and thus about 1 mm across).

After injury there is no immediate increase in the rate of cell generation above the normal mitotic rate found in epidermis; epidermal migration is essentially a spreading out of the existing cells. Indeed, the mitotic rate remains the same throughout the healing process at sources within the wound area, that is, at remnants of epidermal appendages (Winter 1972). However, soon after the onset of epidermal migration, mitotic activity increases in a band (about 1 mm thick) of the new epidermis near the wound edge, providing an additional population of cells at this source (Bereiter-Hahn 1986). The greatest mitotic activity is actually at the wound edge, where it can be as much as 15 times the rate in normal epidermis (Winter 1972); activity decreases rapidly across the band, going outwards. The stimulus for this increase in mitotic activity is uncertain. Various factors have been proposed.

1. Absence of contact inhibition, which certainly applies to mitosis as well as to cell motion (Clark 1988).

2. Change in cell shape. Epidermal migration causes the existing epidermal cells to become more flat, and this tends to increase their rate of division (Folkman & Moscona 1978).

3. Presence of a mitotic activator, or absence of an

inhibitor. One of the uses of mathematical modelling is to suggest which features of the mechanism involved in wound healing are the most important. Here we consider the role of each of these possible stimuli.

2. SIMPLE MODELS FOR EPIDERMAL WOUND HEALING

We begin with a very simple model and later improve it as necessary. As a first step we disregard factor (3) and consider a model for epidermal cell density in which biochemical effects are constant. The epidermis is sufficiently thin that we can consider the wound to be two-dimensional. This is a reasonable assumption as we consider wounds whose linear dimensions are of the order of centimetres, as compared with the thickness of the epidermis, which is about 10^{-2} cm (Odland 1983). The model consists of a conservation equation for cell density (n) per unit area, which we give first in words and then in detailed mathematical form. We explain and expand on the various terms below.

$$\begin{aligned}\text{Rate of increase} &= \text{cell migration}\\ \text{of cell density} &\quad + \text{mitotic generation},\\ \partial n/\partial t &= D\nabla\cdot[(n/n_0)^p\nabla n]\\ &\quad + sn(1-(n/n_0)).\end{aligned} \tag{1}$$

Here D, p and s are positive parameters whose values we discuss below, and n_0 is the unwounded cell density. We use a diffusion term to model cell migration as diffusion is movement down cell density gradients, and thus reflects the contact inhibition controlled migration. The mathematical representation of the diffusive flux we use is cell density dependent. It incorporates simple linear Fickian diffusion, by setting the parameter $p = 0$. In this case we have the Fisher equation, familiar from Fisher's (1937) model for the spatial spread of an advantageous gene, discussed, example, in Murray (1989). However, the representation in (1) also enables us to investigate the effect of nonlinear diffusion by taking $p > 0$: biologically, contact inhibition can give rise to such nonlinearities. We take cell growth to be reasonably described by a logistic form; s is a positive constant related to the maximum mitotic rate. We consider here only wounds without remnants of epidermal appendages, so that the appropriate initial condition is $n = 0$ inside the wound, with boundary condition $n = n_0$, the unwounded cell density, at the wound edges.

We can clarify the relative roles of the parameters by rendering the equation dimensionless; our scalings are discussed in the Appendix. We solved the dimensionless form of equation (1) numerically in a radially symmetric circular geometry and compared the numerical solutions with data from Van den Brenk (1956), one of the more careful experimental studies of such epidermal wounds. In this study the full thickness of epidermis was removed from a circular region (1 cm in diameter) in the ears of rabbits. In particular, care was taken not to leave behind any hair follicles (which would act as foci for re-epithelialization as described above), so that our model with no internal sources of epidermal cells is appropriate. The change in wound radius with time was recorded. To capture the concept of 'wound radius' for our model, we take the wound as 'healed' when the cell density reaches 80% of its unwounded level.

The dimensionless equation involves only two parameters, the power p and the dimensionless diffusion coefficient D^* ($= D/sL^2$, where L is the initial wound radius). For various values of p, we did a best fit analysis for D^*: there is no experimental data from which the dimensional diffusion coefficient D can be determined. The fit with the data improves as p increases: figure 1 shows the numerically calculated decrease in wound radius with time compared with the data for linear Fickian diffusion ($p = 0$) and for nonlinear diffusion with $p = 4$. To calculate the

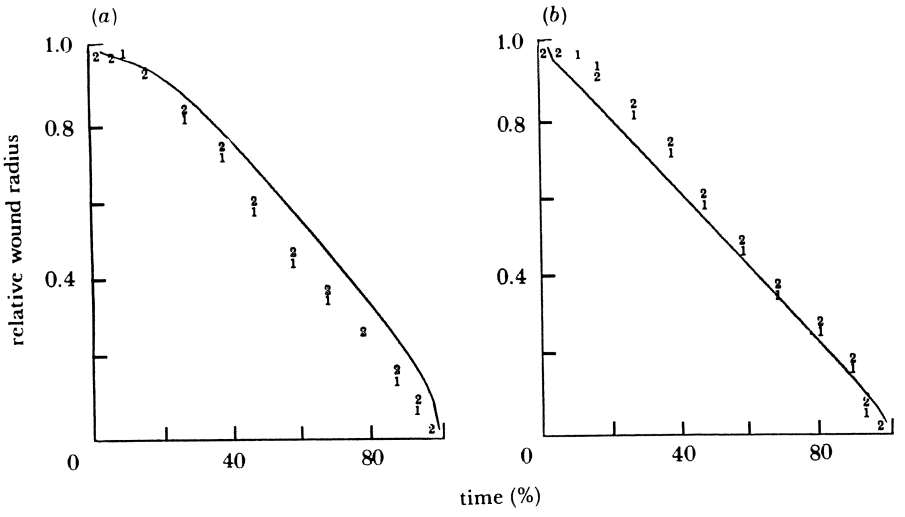

Figure 1. Numerically calculated decrease in wound radius with time from equation (1) as compared with the data, denoted by 1 and 2 (Van den Brenk 1956). (*a*) Linear Fickian diffusion ($p = 0$); (*b*) nonlinear diffusion with $p = 4$. Time is expressed as a percentage of total healing time, as this is how Van den Brenk's data is presented. The dimensionless diffusion coefficient $D^*(= D/sL^2) = 10^{-3}$. With the values of the mitotic parameter as (*a*) $s = 8\times10^{-6}\ s^{-1}$, (*b*) $s = 4\times10^{-5}\ s^{-1}$, and with $L = 0.5$ cm this gives the cell diffusion coefficient (*a*) $D = 2\times10^{-9}\ cm^2\ s^{-1}$ (*b*) $D = 10^{-8}\ cm^2\ s^{-1}$.

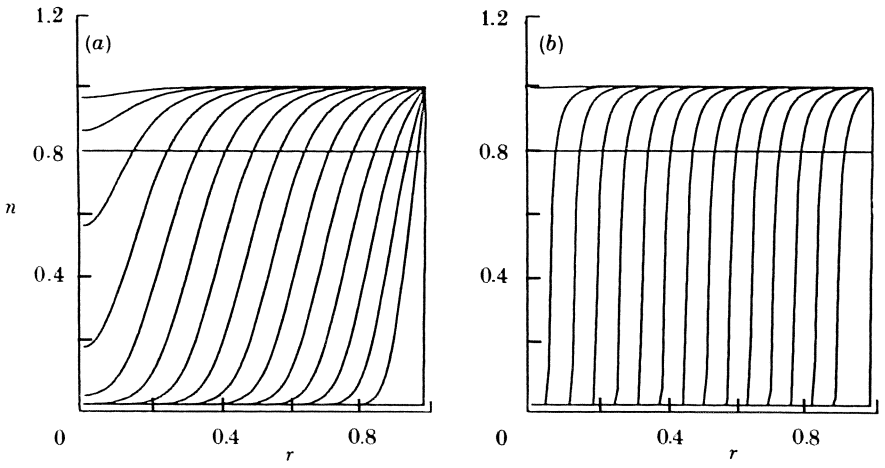

Figure 2. Cell density (n) as a function of radius (r) at a selection of equally spaced times, from equation (1). (*a*) Linear Fickian diffusion ($p = 0$); (*b*) nonlinear diffusion with $p = 4$. The dimensionless diffusion coefficient is $D^* = 10^{-3}$ as in figure 1.

dimensional values for the diffusion coefficient we have to estimate the parameter s. The maximum mitotic rate per cell is $s/4$, which we take as the reciprocal of the cell-cycle time. This varies between species but is typically about 100 hours (Wright 1983), so we take $s = 0.04\ \mathrm{h}^{-1}$. This gives a diffusion coefficient of about $3 \times 10^{-9}\ \mathrm{cm}^2\ \mathrm{s}^{-1}$, which is not biologically unreasonable.

The model solutions lack the well-documented 'lag phase followed by linear phase' that characterize experimental studies (see, for example, Snowden (1984)), although their overall form is the same. Figure 2 shows n against the radius r at a selection of equally spaced times. As expected intuitively, the form of the solutions is of a front of epidermal cells moving into the wound. The speed of the front can be estimated by measuring, visually from the graph, the distance moved by the solution per time interval. This gives speeds of about $6 \times 10^{-2}\ \mathrm{mm\ h}^{-1}$ for linear diffusion and $9 \times 10^{-3}\ \mathrm{mm\ h}^{-1}$ for nonlinear diffusion. These compare with the speed $8.6 \times 10^{-3}\ \mathrm{mm\ h}^{-1}$ found in Van den Brenk's study.

Motivation for an improved model

The models presented above fail to capture a key aspect of epidermal wound healing, namely the crucial 'lag phase followed by linear phase'. This lag phase is of several days duration, and is thus too long to be accounted for simply as a response time following injury: this phenomenon must be captured by the model solutions. The overall fit with the data is only moderately good in both cases, although for nonlinear diffusion the 'speed' of healing compares well with experiment. Further, in the case of nonlinear diffusion, the details of the initial movement of the front depend on the exact mathematical form of the initial condition for the cell density. We found this by careful numerical investigation based on the analysis of Lacey *et al.* (1982). As an exact initial condition cannot be dictated biologically, this is a further shortcoming of such a model.

The implications of this from a modelling viewpoint are that biochemical mediators are fundamental to the process of epidermal wound healing and must be taken into account. We consider now a reaction–diffusion system that incorporates biochemical regulation into the model mechanism.

3. BIOCHEMICAL REGULATION OF MITOSIS AND AN IMPROVED MODEL

There is experimental evidence, which we now briefly review, for production by epidermal cells of chemicals that inhibit mitosis and also of chemicals that stimulate it. The former are 'chalones', a generic term for inhibitors of cell proliferation that are produced by the cell types on which they act. Although the term itself has acquired a somewhat bad reputation (Iversen 1985), the evidence for such inhibitory growth regulators is now considerable.

There are two established epidermal chalones, which act at different points in the cell cycle. Their chemical properties are summarized in Fremuth (1984, pp. 37–38). Experimental work to investigate dose–response relations has shown a general increase in inhibitory effect with dosage (see, for example, Hondius-Boldingh & Laurence (1968); Iversen (1978); Marks (1973)) although beyond this it is inconclusive, which Iversen (1981) attributes to the fact that one has to use skin extracts as the chalones are not yet available in pure form.

Turning to epidermal growth activators produced by epidermal cells themselves, evidence for these is provided in recent work by Eisinger *et al.* (1988*a* (an *in vivo* study); 1988*b* (an *in vitro* study)). In the *in vivo* study, an extract derived from epidermal cell cultures was found to increase the rate of epidermal migration when applied, on a dressing, to wounds in pigs. In the *in vitro* study, the same extract was found to increase the growth rate of cultures of epidermal cells. It is unclear whether the chemical(s) causing activation of mitosis in these studies are growth factors that have already been characterized or 'new' growth factors.

There is also recent evidence that the production by epithelial cells of the chemical SPARC ('secreted

protein acidic and rich in cysteine') increases following wounding. Because SPARC affects the proliferation rate of cells, this is another possible activator (Mason *et al.* 1986; Engel *et al.* 1987; Sage *et al.* 1989).

Improved model

This model consists of two conservation equations, one for the density per unit area (n) and one for the concentration (c) of the mitosis-regulating chemical. We consider two cases, one in which the chemical activates mitosis and the other in which it inhibits it. As before the epidermis is sufficiently thin that we consider the wound to be two dimensional. The model system is as follows; we explain below what the various terms mean biologically:

$$\begin{array}{l}\text{rate of increase} \\ \text{of cell density}\end{array} = \begin{array}{c}\text{cell} \\ \text{migration}\end{array} + \begin{array}{c}\text{mitotic} \\ \text{generation}\end{array} - \begin{array}{c}\text{natural} \\ \text{loss}\end{array}$$

$$\partial n/\partial t = D\nabla^2 n + s(c)\cdot n\cdot(2-(n/n_0)) - kn \tag{2}$$

$$\begin{array}{l}\text{rate of increase of} \\ \text{chemical conc.}\end{array} = \text{diffusion} + \begin{array}{c}\text{production} \\ \text{by cells}\end{array} - \begin{array}{c}\text{decay of} \\ \text{chemical}\end{array}$$

$$\partial c/\partial t = D_c\nabla^2 c + f(n) - \lambda c. \tag{3}$$

Here D and D_c are the diffusion coefficients for cells and chemical, respectively, n_0 is the unwounded cell density, and λ and k are positive constants. As in the previous models, we use a diffusion term to model contact inhibition controlled cell migration. We use linear Fickian diffusion for both cells and chemical: the good agreement with experimental data, described below, suggests that any nonlinearities in the diffusive spread of epidermal cells are not fundamental to the healing process.

We now justify the mathematical forms we have used for the four reaction terms. The decay of active chemical with time is typically governed by first-order kinetics, which we model by $-\lambda c$. In the absence of the other terms on the right-hand side of the equation this gives an exponential decay with time. The rate of chemical production by cells is a function of n, which must equal zero when $n = 0$ (when there are no cells, nothing can be produced by them) and must equal λc_0 when $n = n_0$ so that the unwounded state is a steady state. Here n_0 and c_0 are the unwounded cell density and chemical concentration, respectively. Further, the function, $f(n)$ say, must reflect an appropriate cellular response to injury depending on whether the chemical activates or inhibits mitosis. Thus the qualitative form of the function $f(n)$ in the two cases is as shown in figure 3. For computational purposes we take simple mathematical forms that conform to these requirements, and these are given in the Appendix.

Natural cell loss is due to the sloughing of the outermost layer of epidermal cells, and we take the rate as proportional to n, say kn. Finally, for the rate of (chemically mediated) cell division we require that when $c = c_0$, the unwounded state, the sum of this term and the previous one is of logistic growth form, $kn(1-n/n_0)$. Thus we model this term with $s(c)\cdot n\cdot(2-n/n_0)$, where $s(c)$ reflects the chemical control of mitosis with $s(c_0) = k$. Thus $s(c)$ has the qualitative form shown in figure 4. For a chemical activator, a decrease of $s(c)$ to $s(0)$ for large c is included because it is found experimentally (Eisinger *et al.* 1988*a*). However, the numerical solutions below show that this decrease has little effect, as $c < 2.5$ throughout these solutions, whereas the maximum value of $s(c)$ occurs at $c = 40$, as explained in the Appendix. In both cases we require $0 < s_\infty < s_{\max} = hk$, say, and we take $s_\infty = k/2$. Again we take simple mathematical forms satisfying these criteria (see the Appendix).

It is possible to estimate the parameters λ and k from experimental data. We estimate λ in the case of a chemical inhibitor by using experimental data on chalones. Brugal & Pelmont (1975) found a decrease in the proliferation rate in intestinal epithelium during the 12 h after injection with epithelium extract. Hennings *et al.* (1969) were able to maintain suppression of epidermal DNA synthesis by repeated injection of epidermal extract at 12 h intervals. Based on these studies, we take the half-life of chemical decay as 12 h. Considering only the decay term in the second equation gives exponential decay with a half-life of $\lambda^{-1}\log 2$. We thus take $\lambda = 0.05$ ($\approx \frac{1}{12}\log 2$).

For a chemical activator, there is little quantitative

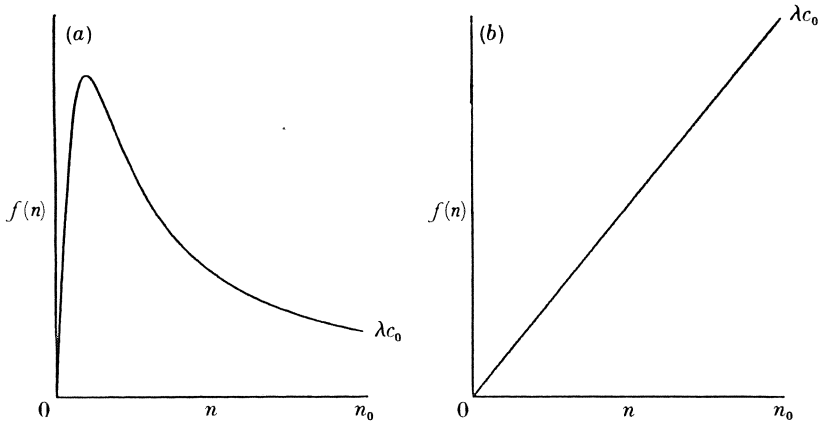

Figure 3. The qualitative form of the function $f(n)$, which reflects the rate of chemical production by epidermal cells. (*a*) Activator; (*b*) inhibitor.

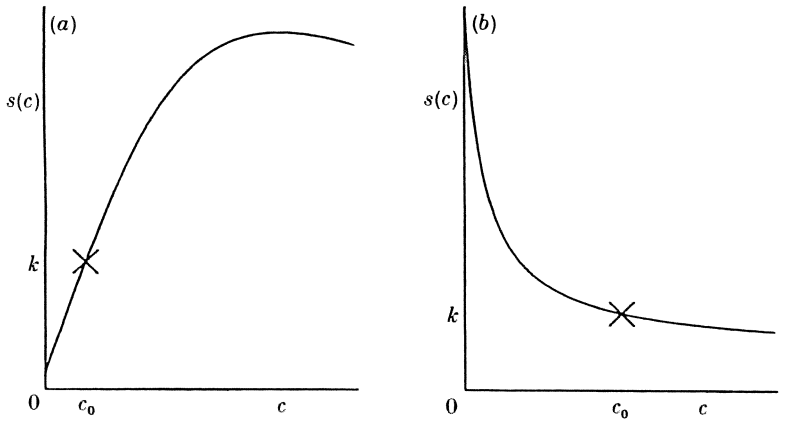

Figure 4. The qualitative form of the function $s(c)$, which reflects the chemical control of mitosis. (*a*) activator; (*b*) inhibitor. Here, c_0 represents the steady-state chemical concentration in the unwounded state, and k is a parameter equal to the reciprocal of the cell cycle time. The cross therefore marks the steady state.

experimental data. However, comparison of the work of Eisinger *et al.* (1988*a*, *b*) on chemical activators in wound healing and the clinical studies of chalone effects by Rytömaa & Kiviniemi (1969, 1970) suggests a longer timescale for the chalone activity, by a factor of about six, so we take $\lambda = 0.3$ for the activator.

The parameter k is simply the reciprocal of the epidermal cell cycle time. As above, this is about 100 h (Wright 1983), so we take $k = 0.01\ \mathrm{h}^{-1}$. The diffusion coefficients D and D_c were estimated by using a best-fit analysis, as there is no experimental data from which they can be determined. This gave values $D = 3.5 \times 10^{-10}\ \mathrm{cm}^2\ \mathrm{s}^{-1}$, $D_c = 3.1 \times 10^{-7}\ \mathrm{cm}^2\ \mathrm{s}^{-1}$ for the activator, and $D = 6.9 \times 10^{-11}\ \mathrm{cm}^2\ \mathrm{s}^{-1}$, $D_c = 5.9 \times 10^{-6}\ \mathrm{cm}^2\ \mathrm{s}^{-1}$ for the inhibitor. These are not biologically unreasonable for cells and relatively low molecular mass biochemicals.

Again we can clarify the relative roles of the parameters by an appropriate non-dimensionalization: our scalings are discussed in the Appendix. We solved equations (2) and (3) numerically, again in a radially symmetric circular geometry, and compared the numerical solutions with Van den Brenk's (1956) data. Figure 5 shows the numerically calculated decrease in wound radius with time compared with the data. As before, we take the wound to be 'healed' when the cell density reaches 80% of its unwounded level. Although best-fit analysis was necessary for the diffusion coefficients, this calculated healing profile depends crucially on the form and parameter values of the reaction terms, which have a biological basis. In figure 6 we plot the cell density n and chemical concentration c as a function of the radius r at a selection of equally spaced times. As before, a front of cells moves into the wound. With this improved model, however, there is good overall agreement with the data, and the numerical solutions exhibit the important two phases, namely a lag phase followed by a linear phase, which characterize epidermal wound healing (see, for example, Snowden (1984)). As above, the (constant) speed of the linear phase can be estimated from the graph of n against r, which gives dimensional wave speeds of $2.6 \times 10^{-3}\ \mathrm{mm\ h}^{-1}$ for the activator and $1.2 \times 10^{-3}\ \mathrm{mm\ h}^{-1}$ for the inhibitor. These compare

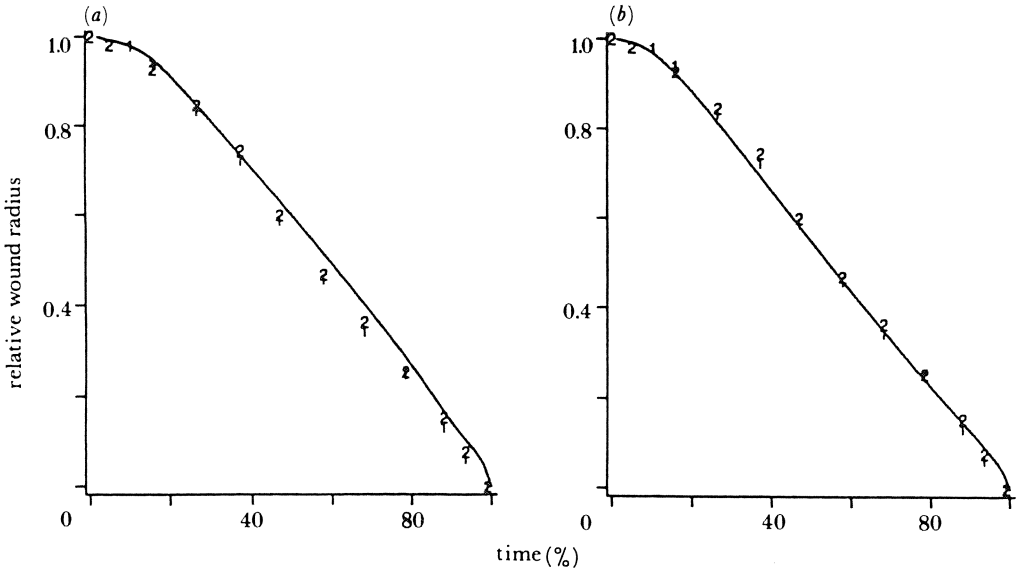

Figure 5. Numerically calculated decrease in wound radius with time from equations (2) and (3) as compared with the data, denoted by 1 and 2 (Van den Brenk 1956). As in figure 1, time is expressed as a percentage of total healing time. (*a*) Biochemical activation of mitosis; (*b*) biochemical inhibition of mitosis.

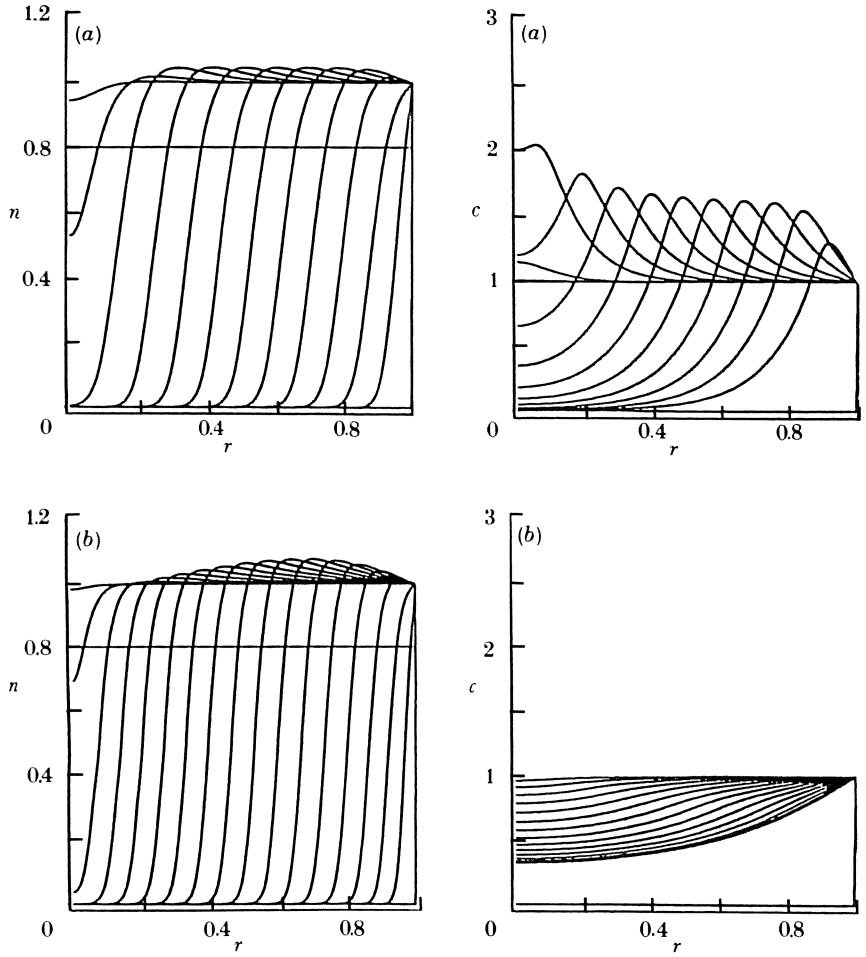

Figure 6. Cell density (n) and chemical concentration (c) as a function of radius (r) at a selection of equally spaced times, from equations (2) and (3). (*a*) Biochemical activation of mitosis; (*b*) biochemical inhibition of mitosis.

with the speed 8.6×10^{-3} mm h^{-1} found in Van den Brenk's (1956) study.

4. CONCLUSION

We have developed basic cell-reaction–diffusion models for epidermal wound healing in which the parameter values are based as far as possible on experimental fact. At this stage it is difficult to suggest specific experiments to discriminate between the activator and inhibitor models because there is insufficient experimental data, and many crucial biological details of wound healing are not known. The surprisingly good fit between the theory and data supports the view that biochemical regulation of mitosis is fundamental to the process of epidermal migration in wound healing. Further, the normal healing of circular epidermal wounds can be accounted for by the production, by epidermal cells, of either an activator or an inhibitor of mitosis.

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APPENDIX

Dimensionless form of equation (1)

To make equation (1) dimensionless, we scale the quantities as follows, where superscript (*) denotes a dimensionless variable:

$$n^* = n/n_0, \quad \boldsymbol{r}^* = \boldsymbol{r}/L, \quad t^* = st, \quad D^* = D/(sL^2).$$

Here, L is a characteristic linear dimension of the wound (the radius in circular wounds) and n_0 is the unwounded cell density. With these, the dimensionless equation is (dropping the asterisks for notational simplicity)

$$\partial n/\partial t = D\nabla \cdot (n^p \nabla n)\, n + n(1-n),$$

which is the equation solved numerically.

Functional forms of $f(n)$ and $s(c)$ in the improved model

For computational purposes we take simple functional representations conforming to the qualitative forms of $f(n)$ and $s(c)$ described above. These are:

$$f(n) = \lambda c_0 \cdot \frac{n}{n_0} \cdot \left(\frac{n_0^2+\alpha^2}{n^2+\alpha^2}\right), \text{ for the activator,}$$

and

$$f(n) = (\lambda c_0/n_0)\cdot n, \text{ for the inhibitor;}$$

$$s(c) = k \cdot \left\{\frac{2c_m(h-\beta)\,c}{c_m^2+c^2} + \beta\right\}, \text{ for the activator,}$$

where

$$\beta = \frac{c_0^2 + c_m^2 - 2hc_0 c_m}{(c_0 - c_m)^2},$$

and

$$s(c) = \frac{(h-1)c + hc_0}{2(h-1)c + c_0} \cdot k, \text{ for the inhibitor.}$$

Here α, h and c_m are positive parameters. We take $\alpha = 0.1$, so that $f(n)$ has an appropriate qualitative form. We take $h = 10$, based on data by Winter (1972) on epidermal wound healing in pigs. His study shows graphs of mitotic activity against position in the wound at various times after wounding; the maximum level of activity is about ten times that in wounded skin apart from a single measurement of about fifteen times. Finally, we take $c_m = 40$: straightforward linear analysis shows that $c_m > 38$ is necessary for the unwounded state to be unstable to small perturbations, which is a biological requirement. The functions $f(n)$ and $s(c)$ are illustrated in figures 3 and 4.

Dimensionless form of equations (2), (3)

To clarify the roles of the various parameters, we non-dimensionalize equations (2) and (3) by using a typical length scale, L (the radius, for example, in a circular wound) and typical timescale $1/k$ (the cell cycle time seems the most relevant timescale). We define the following dimensionless quantities (denoted by *):

$$n^* = n/n_0, \quad c^* = c/c_0, \quad \boldsymbol{r}^* = \boldsymbol{r}/L, \quad t^* = kt,$$
$$D^* = D/(kL^2),$$
$$\lambda^* = \lambda/k, \quad c_m^* = c_m/c_0, \quad \alpha^* = \alpha/n_0,$$
$$D_c^* = D_c/(kL^2).$$

With these definitions, the dimensionless model equations become (dropping the asterisks for notational simplicity)

$$\partial n/\partial t = D\nabla^2 n + s(c) \cdot n \cdot (2-n) - n$$

$$\partial c/\partial t = D_c \nabla^2 c + \lambda g(n) - \lambda c.$$

Here, for the activator:

$$g(n) = \frac{n(1+\alpha^2)}{n^2+\alpha^2}, \quad s(c) = \frac{2c_m(h-\beta)c}{c_m^2 + c^2} + \beta,$$

where

$$\beta = \frac{1 + c_m^2 - 2hc_m}{(1-c_m)^2},$$

and for the inhibitor:

$$g(n) = n, \quad s(c) = \frac{(h-1)c + h}{2(h-1)c + 1}.$$

These equations were solved numerically to obtain the results in figures 5 and 6.

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