

Time Heals All Wounds?

Mathematical Models of Epithelial and Dermal Wound Healing

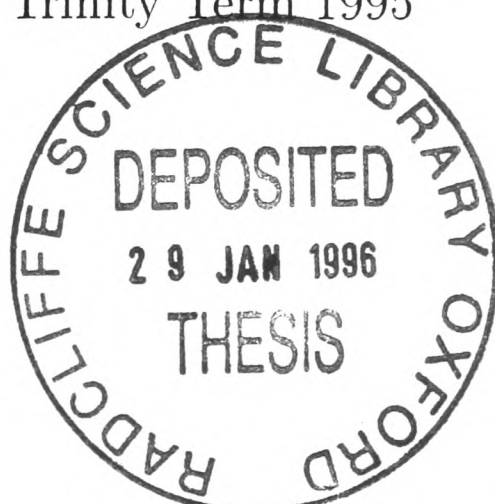
Paul David Dale

Lincoln College
Oxford



*A thesis submitted in partial fulfilment of the requirements for
the degree of D. Phil. at the University of Oxford*

Trinity Term 1995



*Dedicated to my Mother
and
in memory of my Father*

Abstract

Paul David Dale

Thesis submitted for
the degree of D.Phil.

Lincoln College, Oxford

Trinity Term 1995

Time Heals All Wounds? Mathematical Models of Epithelial and Dermal Wound Healing

The mechanisms responsible for the healing of corneal surface wounds are the subject of biological controversy. In particular, the role and source of the regulatory chemical epidermal growth factor (EGF) is an area of intense debate. In the first part of this thesis, we propose a reaction-diffusion model which focuses on the stimulus for increased mitotic and migratory activity due to secretion of EGF. A detailed numerical study of various possible models, with parameter values based on biological data, reveals that, for realistic healing times, EGF must be released by the underlying layers of the cornea, in addition to the tear film source. The model exhibits travelling wave solutions and further analysis elucidates the interaction and role of the parameters in determining the speed of healing. Furthermore, we consider the effect of topical application of EGF and investigate the effect of curvature of the eye. We show that our model is consistent with many of the key features of corneal wound healing.

Adult dermal wounds, in contrast to foetal wounds, heal with the formation of scar tissue. A crucial factor in determining the nature of the healed tissue is the ratio of collagen 1 to collagen 3, which indicates the fibril diameter. We develop a reaction-diffusion model which focuses on the stimulus for collagen synthesis due to the secretion of the different isoforms of the regulatory chemical transforming growth factor β (TGF β). Numerical simulations of the model without diffusion lead to a value of this ratio consistent with that of healthy tissue for the foetus but corresponding to scarring in the adult. The model equations evolve to waves moving into the wound, but addition of TGF β only has a transient effect on the final collagen levels. We investigate this effect by developing a caricature model. The model indicates that the main source of the fibroblasts is the underlying subcutaneous tissue and we determine key parameters which explain the difference between adult and foetal wound healing. Furthermore we make clinically testable predictions on the effects that topical application of various chemicals will have on scar formation.

Acknowledgements

I would like to express my deep gratitude to Dr Jonathan Sherratt and Dr Philip Maini, both as academic supervisors and personal friends throughout my doctoral research. Without their enthusiasm, guidance and mathematical insight, this thesis would not have been possible. It has been a great pleasure and privilege to work with them. I have also appreciated the fruitful discussions with Prof. M. W. J. Ferguson and Dr. M. Shah (University of Manchester), who helped me appreciate the complexity of the wound healing process.

I am especially grateful to my fellow graduate students at the Centre for Mathematical Biology, for their friendship, for creating such an enjoyable and productive atmosphere, and for so many cups of tea: Jane Bamblett, Debbie Benson, Wendy Brandts, Joanne Collier, Thomas Höfer, Peter Howell, Brian Hunton, Gideon Ngwa, Luke Olsen, Kevin Painter, Abbey Perumpanani, Joe Pitt-Francis and Faustino Sanchez-Garduno.

On a more personal level, I am indebted to the many close friends who have encouraged me over the past three years. In particular, I would like to thank David and Claire Gibb, for their constant love, concern, support and prayers; Phil Nicol, for his continual friendship and wit; the church family at St. Ebbe's; the boys at "ZAP!" for keeping me sane; and many more. Thank you all!

My doctoral research was funded by a Prize Studentship from the Wellcome Trust, to whom I am most grateful. I also wish to acknowledge Lincoln College for electing me to a Senior Scholarship. Finally, my warmest thanks to my family (Mum, Mark and Karen, Sue, Louise and Katie) for their love, patience and continual support.

“He was pierced for our transgression,
he was crushed for our iniquities;
the punishment that brought us peace
was upon him,
and by his wounds we are healed.

We all, like sheep, have gone astray,
each of us has turned to his own way;
and the LORD has laid on him
the iniquity of us all.”

Contents

1	Models of Corneal Epithelial Wound Healing	1
1.1	Biological Background	1
1.1.1	Epithelial Growth Factor (EGF)	3
1.2	Mathematical Model	6
1.2.1	Estimating the Parameters	9
1.3	Tear Film Source of EGF	14
1.3.1	Linear Stability Analysis	14
1.3.2	Numerical Simulation	16
1.3.3	Conclusion	17
1.4	Improved Model	20
1.4.1	Numerical Analysis	22
1.5	The Relative Importance of Migration and Mitosis	23
1.6	Summary	28
2	The Speed of Healing	31
2.1	Introduction	31
2.2	Phase Plane Analysis	32
2.2.1	Transition Wave Speeds in Single Reaction Diffusion Equations	32
2.2.2	Travelling Waves	34
2.2.3	Numerical Evaluation of Eigenvalues	37
2.3	Analytical Expression for the Wave Speed	42

2.4	Topical Application of EGF	45
2.5	The Effect of Curvature	49
2.5.1	Formulation in Spherical Coordinates	49
2.5.2	Numerical Simulations	50
2.5.3	Analytical Expression for Speed of Waves on a Sphere	53
2.6	Summary	55
3	Analytical Solutions of the Reduced System	57
3.1	Introduction	57
3.2	Model Equation and Numerical Simulations	58
3.2.1	First Approximation	58
3.2.2	Width of the Wave in Spherical Coordinates	63
3.3	Piecewise Linear Approximation in Travelling Wave Coordinates	64
3.3.1	General Solutions in Travelling Wave Coordinates	65
3.4	Analytical Solutions - Piecewise Linear Approximations	68
3.4.1	Laplace Transform Method	71
3.5	Summary	76
4	Discussion on Corneal Epithelial Wound Healing	77
5	Scarring in Foetal and Adult Dermal Tissue	82
5.1	Biological Background	82
5.1.1	Overview of Dermal Wound Healing	82
5.1.2	Foetal and Adult Healing	86
5.1.3	Scar Formation	89
5.2	Mathematical Modelling	94
5.2.1	Derivation of Model Equations	96
5.3	Estimating the Parameters	102
5.4	Nondimensionalization	105
5.5	Summary	109

6	Temporal Model	110
6.1	Introduction	110
6.2	Linear Stability Analysis	111
6.2.1	Steady States	111
6.2.2	Linear Stability	113
6.3	Numerical Solution of the Foetal Temporal Model	115
6.3.1	Addition of $TGF\beta$	116
6.4	Adult Wound Healing	122
6.4.1	Estimating the Parameters	123
6.4.2	Numerical Solution of the Model	124
6.5	Parameter Sensitivity Analysis	127
6.6	Summary	133
7	Reaction Diffusion Equations for the Foetal Model	135
7.1	Introduction	135
7.2	Numerical Solution of the Model	135
7.2.1	Initial and Boundary Conditions	136
7.2.2	Numerical Simulations	138
7.2.3	Different Initial Conditions	143
7.3	Travelling Wave Solutions	144
7.4	Addition of $TGF\beta$	148
7.5	Summary	152
8	Caricature Models	153
8.1	Introduction	153
8.2	Basic Caricature Model	153
8.2.1	Temporal model	154
8.2.2	Numerical Solution of the Caricature Model	155
8.2.3	Travelling Waves	164

8.2.4	Asymptotic Solutions of Model Equations	166
8.3	Extensions to the Model	170
8.3.1	Haptotaxis	171
8.3.2	Robustness	173
8.3.3	Diffusion of c	174
8.4	Improved Caricature Model	178
8.5	General Caricature Model	181
8.5.1	Travelling wave analysis	181
8.5.2	Numerical Simulations	184
8.6	Summary	186
9	Discussion on Dermal Wound Healing	187
	References	193

Part 1

Corneal Epithelial Wound Healing

“My soul finds rest in God alone;
my salvation comes from him.
He alone is my rock and my salvation;
he is my fortress, I shall never be shaken.”

Psalm 62: 1-2

Chapter 1

Models of Corneal Epithelial Wound Healing

1.1 Biological Background

The cornea is a transparent structure forming the anterior part of the fibrous tunic of the eye. It serves a refractive function while maintaining a tough, chemically impermeable barrier. The epithelium constitutes 10% of the cornea and consists of 3 layers; the superficial cell layer, the wing layer and the basal layer (Figure 1.1). The basal cells are the only cells to undergo mitosis (cell division), and the resulting daughter cells move sequentially through the cell layers, changing shape to form wing cells before becoming superficial cells, and finally entering the tear film which washes the cells away. The cytoplasm of the basal cells has a large number of glycogen granules, representing a source of stored metabolic energy to be used during the healing process.

We use corneal wound healing as a prototype for the general process of epithelial wound healing because there is a large quantity of specific data readily available and the processes involved in corneal wound healing are of particular clinical interest. For example, a keratectomy operation involves making incisions into clear cornea to remove fibrovascular growth that is causing abnormality of corneal curvature. A key aspect of this operation is the final curvature of the corneal surface, and this has been investigated in the kinematic model of Kwok (1991). Our approach

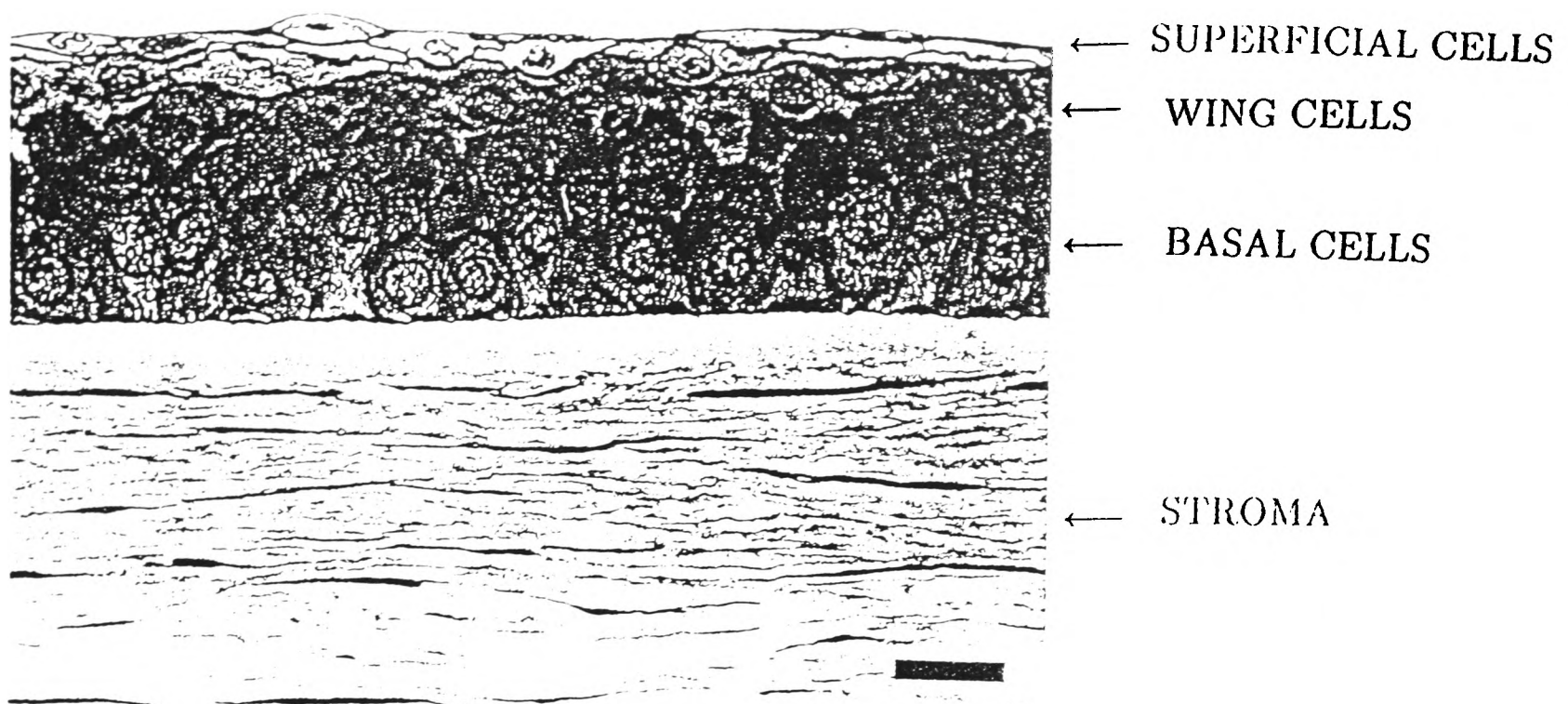


Figure 1.1: Epithelial cell structure showing the three layers; basal cells, wing cells and superficial cells. The stroma is a supportive structure consisting of collagen fibrils. Scale bar is $20\mu m$.

concentrates on the underlying cell biology, rather than on such detailed geometric considerations.

Corneal epithelial wound healing involves a combination of unimpeded cell migration, cell-to-substrate adhesion and unrestrained cell mitosis. Immediately after wounding, the damaged cells adjacent to the wound edge lose surface microvilli and within 1 hour the basal cells flatten indicating their impending motion (Brazell *et al.*, 1991). After a lag phase of approximately 6 hours, epithelial cells migrate, via lamellipodia (Kuwabara *et al.*, 1976), into the area of defect. (A cell length is approximately $10\mu m$ (Chan *et al.*, 1991)). No mitosis is noted in the vicinity of the wound initially but cell proliferation near the wound edge appears to push the whole epithelium towards the wound, providing an additional population of cells and resulting in the formation of an epithelial plug (Kitazawa *et al.*, 1990). In a normal corneal strip wound the healing rate is approximately $64\mu mhr^{-1}$ and is largely independent of wound size (Crosson *et al.*, 1986). Contact inhibition causes cessation of

cessation of cell movement where opposing cell fronts meet squamous cells (Klyce *et al.*, 1988). By 3 to 4 days after wounding, the epithelial plug regresses and soon the new overlying epithelium becomes even with the adjacent old epithelium (Figure 1.2). The healing times depend crucially on the wound size; for a typical wound radius of 2mm, the healing time is about 37 hours (Crossen *et al.*, 1986).

The stimulus for the increase in mitotic and migratory activity is uncertain but absence of contact inhibition, change in cell shape and the presence of specific cell reagents such as endogenous growth factors (including Epidermal Growth Factor and Transforming Growth Factor- α) are all important.

1.1.1 Epithelial Growth Factor (EGF)

EGF was discovered by Cohen (1961) in the submaxillary gland of male mice. It is a large polypeptide for which receptors are expressed by almost all cell types but most abundantly on epithelial cells (Green & Couchman, 1984). A very similar protein to EGF is transforming growth factor- α (TGF- α), which also binds and activates the EGF receptor (Cofley *et al.*, 1987). For a general wound, topical application of EGF has been shown to stimulate re-epithelialization in a dosage-dependent manner (Nishida *et al.*, 1990). Moreover, although a number of different growth factors play overlapping and synergistic roles during wound healing, most studies suggest that the EGF / TGF- α family is the main regulator of epithelial repair (Martin *et al.*, 1992). Experiments also show that fibronectin, a multifunctional adhesive protein which promotes cell-substrate adhesiveness for epithelial cells, stimulates epithelial migration. However, the results suggest that, unlike fibronectin, EGF need not be present once the epithelial cells have recognized its signal (Nishida *et al.*, 1990). For this reason, we focus here on the role of EGF as an agent promoting wound healing.

For corneal wounds, the source of EGF is a vital and controversial question and we briefly discuss three possibilities: the tear film, autocrine production by epithelial cells and the underlying stroma. The exact nature of the source term is essential

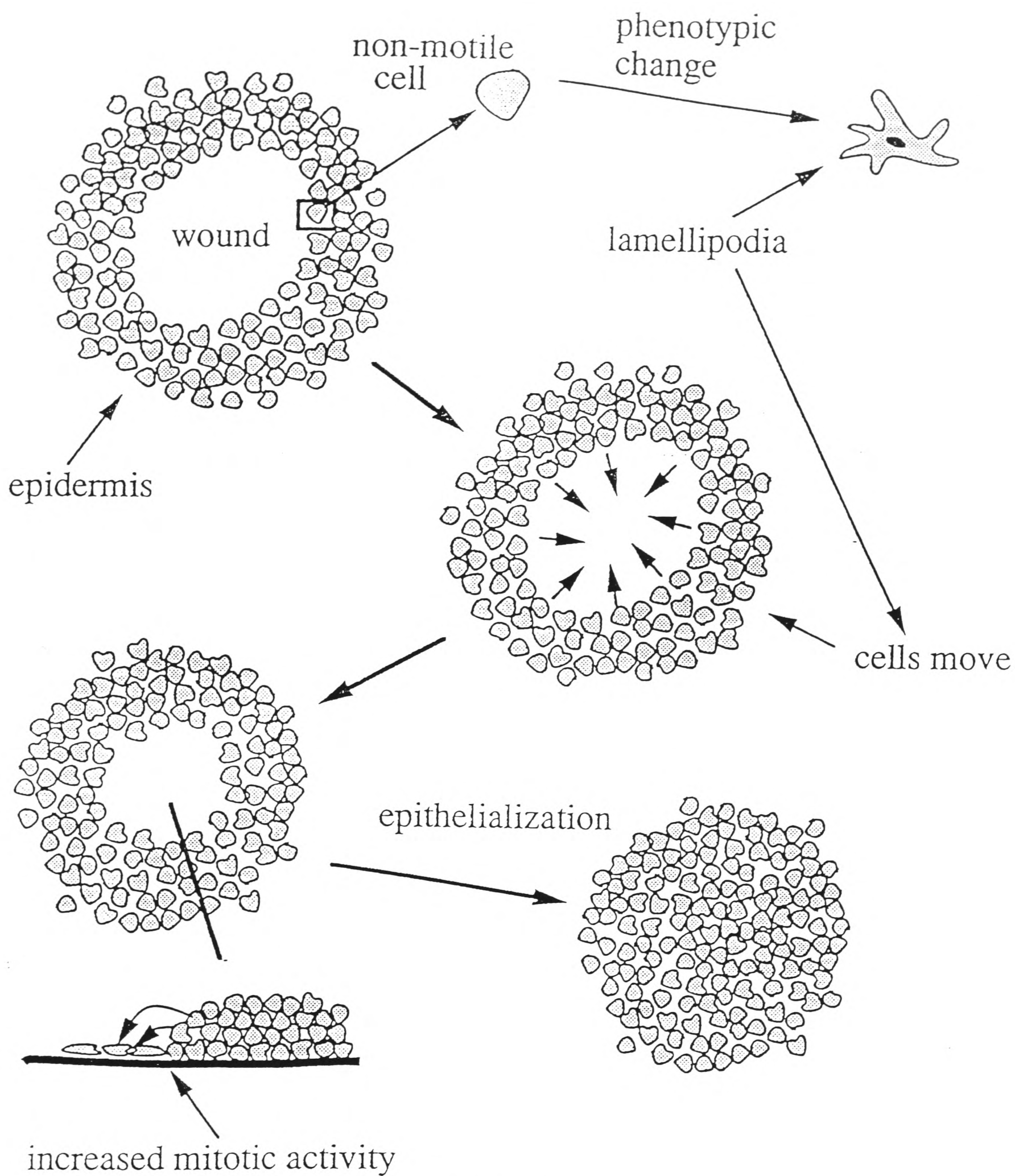


Figure 1.2: A schematic representation of the processes involved in epidermal wound healing.

to any possible predictions being made from the mathematical models. Ohashi *et al.* (1989) investigated the presence of EGF in tears using human EGF specific radioimmunoassay techniques and detected up to 4 ng/ml of EGF in reflex tears. Van Setten *et al.* (1990) improved these results by determining and comparing the concentrations of human EGF and tear fluid flow rate. Their study showed that intense reflex tearing increased the release rate of EGF into the tear fluid, but this was accompanied by a drastic decrease in the tear EGF concentration.

Coffey *et al.* (1987) suggested an auto-induction process, for a general epithelial wound. The experiments indicated that wounding by abrasion increases the detectable quantities of TGF- α by freeing bound TGF- α from the epidermis and activating a process which releases bound TGF- α from the cells. The release at the wound site of EGF-like peptides, which bind to EGF receptors, induced an enhanced TGF- α synthesis in epithelial cells to increase their rate of proliferation. This autocrine feedback process is the basis of Sherratt & Murray's (1990) model for general epidermal repair, and there is some evidence to support it. However, in the cornea, the process remains biologically unproven and the average concentration observed by Coffey *et al.* (1987) over 24 hours was only 73 pg/ml, which is negligible compared with the concentration in tears. Hence, we can effectively neglect this process.

Another possible source is a 'diffusion-limited process' as suggested by Dunn & Ireland (1984) and Barradon & Green (1987), where a source of growth factor at the centre of the wound is "mopped up" by cells at the edge which then migrate inwards, the source being the underlying tissue that has been wounded. In this scheme, it is the rapidity of the degradation that results in the experimentally observed increase in cell proliferation and motility in a band of cells at the wound edge; the cells in this band degrade the additional chemical, preventing it reaching cells further from the wound edge. In Barradon & Green's experiments, the growth of large keratinocyte colonies was observed, *in vitro*, and was found to be dependent on the outward migration of the rapidly proliferating cells located in a thin rim close to

the colony perimeter. Dunn & Ireland examined the growth of 3T3 cells in rotating culture dishes, and showed that in the presence of serum, new growth occurs at the margin of a wound in a cell sheet. They found that increased cell division occurs only downstream from the wound. Since a serum-derived factor would be carried with the flow, this supports the possibility that a serum-derived factor is responsible for the elevated rate of cell division after wounding.

Experimental evidence suggests that EGF is the main agent promoting corneal epithelial wound healing. We thus develop a mathematical model which captures the essential biological processes described above, and focus on the source of EGF as the main area of controversy to be investigated. We first derive a model in which the tear film is the only source of EGF and then extend the model to consider the possibility that the exposed underlying tissue within the wound releases EGF, which is rapidly degraded by cells at the wound edge.

1.2 Mathematical Model

As a first step in investigating the role of EGF in corneal epithelial repair, we construct a simple mathematical model which proposes that the tear film is the main source of EGF and which focuses on the regulation by EGF of cell migration and cell proliferation. The governing equations are conservation equations for cell density and chemical concentration, with the form:

$$\begin{array}{rclclcl}
 \text{Rate of Increase} & = & \text{Cell} & + & \text{Mitotic} & - & \text{Natural} \\
 \text{of Cell Density} & & \text{Migration} & & \text{Generation} & & \text{Loss} \\
 \\
 \text{Rate of Increase} & = & \text{Diffusion} & + & \text{Production} & - & \text{Decay of} \\
 \text{of EGF Conc.} & & & & \text{by Cells} & & \text{Active EGF}
 \end{array}$$

We incorporate the following assumptions:

1. For simplicity, we model cell movement by Fickian diffusion to capture cells moving down gradients in cell density due to contact inhibition, and we assume

that the cell diffusion coefficient increases linearly with the EGF concentration (Nishida *et al.*, 1990).

2. Following a number of previous authors, we use a logistic growth form for the cell mitotic term (Maini *et al.*, 1991; Murray, 1989). However, we consider the control of mitosis to be dependent on the chemical concentration and represent it by an increasing function $s(c)$, where c is the EGF concentration.
3. Sloughing of the outermost epidermal cells is responsible for natural cell loss, and we take this to be a first order process. The cells that are lost are replaced by frequent mitosis in the basal cell layer.
4. The EGF diffusion coefficient is taken to be a positive constant, D_c .
5. Decay of active EGF is due to a combination of natural decay and cellular degradation. We assume the former to be first order in c and model the latter by a saturating term denoting the rate of internal degradation of bound EGF receptors.
6. The production of chemical by the cells is represented by the function $f(n)$, where n is the cell density.

Denoting by $n(\underline{r}, t)$ and $c(\underline{r}, t)$ the cell density and chemical concentration, respectively, at position $\underline{r}$ and time t , the above assumptions imply the following model equations:

$$\frac{\partial n}{\partial t} = \nabla \cdot (D_n(c) \nabla n) + s(c)n \left(\nu - \frac{n}{n_0} \right) - kn \quad (1.1a)$$

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c + f(n) - h(c)n - \delta c \quad (1.1b)$$

where D_c , ν , n_0 , k and δ are all positive constants; the functional forms of $D_n(c)$, $s(c)$, $f(n)$ and $h(c)$ are as discussed below.

The thickness of the epithelium is much smaller than the wound length and hence we can treat the epithelium as two-dimensional. Furthermore, by considering

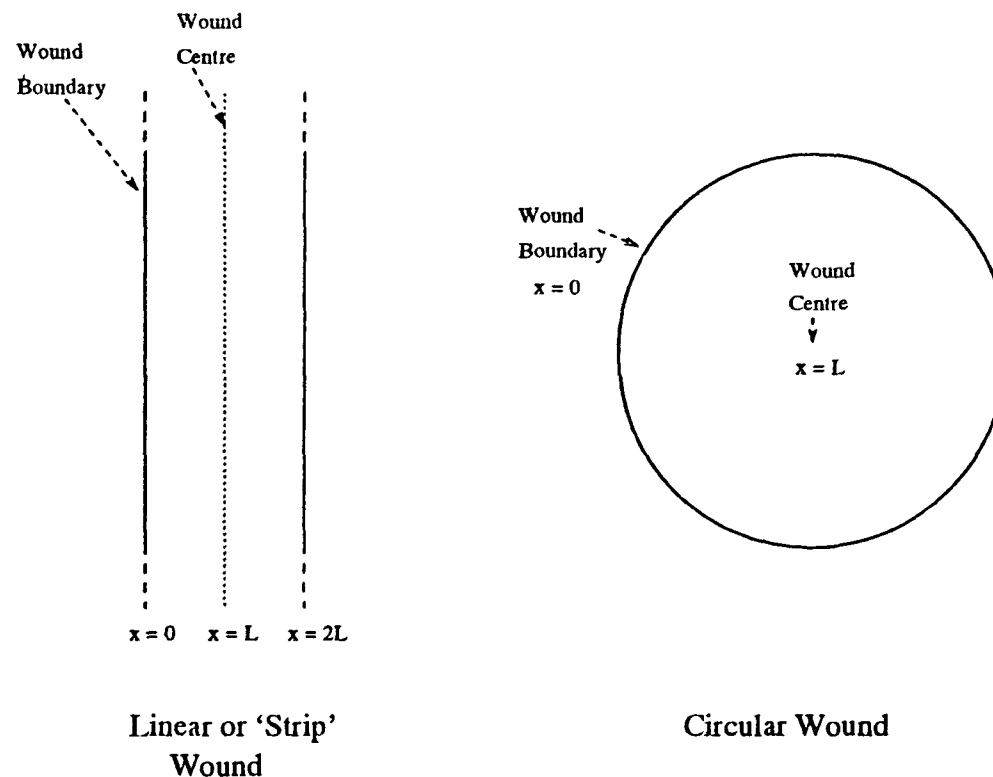


Figure 1.3: Pictorial representation of a typical wound, showing the wound centre and boundary for linear 'strip' wounds and circular wounds

a 'linear' wound geometry, which models a long 'strip' wound, we simplify the spatial domain from three dimensions to only one. We solve the equations on a semi-infinite domain $-\infty \leq x < L$, with $0 \leq x < L$ representing half the initial wound domain, where 0 is the boundary of the epithelium and L is the centre of the wound (Figure 1.3). Since the epithelium does not contract, 0 is a fixed boundary.

Biologically relevant initial and boundary conditions are

1. $n(x, 0) = c(x, 0) = 0$ for $0 \leq x < L$
 $n(x, 0) = n_0, \quad c(x, 0) = c_0$ for $-\infty < x < 0$
2. $n(-\infty, t) = n_0, \quad c(-\infty, t) = c_0 \quad \forall t \geq 0$
 $n_x(L, t) = c_x(L, t) = 0$ by symmetry $\forall t \geq 0$

where n_0 and c_0 represent the unwounded levels of cell density and EGF concentration, respectively.

1.2.1 Estimating the Parameters

Estimating the parameter values is vital to the comparison of model predictions with experimental data. Subsequently, we will use the superscript dim to denote a dimensional quantity.

1. The cell cycle time (the time for a basal cell to undergo mitosis and push upwards resulting in desquamation) is approximately 6.6 days (Hanna *et al.*, 1961). Hence

$$k^{dim} = \frac{1}{6.6 \times 24} = 6.31 \times 10^{-3} \text{hr}^{-1}$$

2. The release of EGF in tears has been estimated experimentally by Ohashi *et al.* (1989) who found the concentration in reflex tears to be 4 ng ml⁻¹. Hence the unwounded level of chemical concentration, c_0 , is 4×10^{-6} g l⁻¹. The molecular weight of EGF is 6145 (Carpenter & Cohen, 1976), so that 1 mole of EGF corresponds to 6145 g. Hence

$$c_0^{dim} = \frac{4 \times 10^{-6}}{6145} \text{mol l}^{-1} = 6.6 \times 10^{-10} \text{M}$$

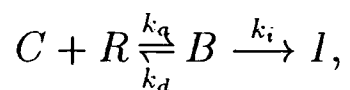
3. The corneal epithelial cell length is approximately 10 μm (Klyce *et al.*, 1988). Hence the unwounded level of cell density, n_0 , is

$$n_0^{dim} = \left(\frac{1}{10^{-5}} \right)^3 = 10^{15} \text{m}^{-3} = 10^{12} \text{l}^{-1}$$

4. In the absence of cellular degradation, EGF has a half-life of approximately 1 hour *in vivo*, (Chan *et al.*, 1991) so we can estimate $\delta^{dim} = \log 2 \text{hr}^{-1}$.
5. To estimate ν , we follow Sherratt & Murray (1990) and assume that when the chemical concentration, c , is at its unwounded level c_0 , the net reaction term in the cell conservation equation is of logistic growth form $kn(1 - n/n_0)$. Therefore $\nu = 2$ and $s(c_0^{dim}) = k^{dim}$.
6. To estimate the cell diffusion coefficient we use experimental data for *in vitro* and *in vivo* situations and, in the simplest case, assume a linear relationship

of the form $D_n(c) = \alpha c + \beta$ where α and β are positive constants. Previous modelling experience suggests that the order of magnitude of a dimensional cell diffusion coefficient, *in vivo*, is $10^{-9} \text{ cm}^2 \text{ s}^{-1}$ (Murray, 1989; Sherratt & Murray, 1992). We estimate the values of α and β by matching model solutions with experimental data, within the constraint that $\alpha c_0 + \beta \approx 10^{-9} \text{ cm}^2 \text{ s}^{-1}$.

7. There is no experimental data to enable us to estimate D_c^{dim} but, knowing the molecular weight of EGF, the dimensional diffusion coefficient in aqueous solution can be estimated theoretically as $9.75 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ (Barrow, 1981; Berg & Von Hippel, 1985). We expect this to be a reasonable order of magnitude estimate for the diffusion coefficient *in vivo*.
8. To determine the functional form for the cellular degradation term $h(c)$, we begin by considering the interaction between the chemical (C), the free receptors on the cell surface (R), the bound receptors (B) and the internalized receptor-chemical complex (I), which can be represented by



where k_a , k_d and k_i are positive rate constants. Using the Law of Mass Action we derive the equations

$$\frac{dc}{dt} = -k_a cr + k_d b \quad (1.2a)$$

$$\frac{dr}{dt} = -k_a cr + k_d b \quad (1.2b)$$

$$\frac{db}{dt} = k_a cr - k_d b - k_i b \quad (1.2c)$$

where c , r and b are the concentrations of C, R and B respectively. Several experimental groups have made direct measurements of the rate of internal degradation of bound EGF receptors, which are in agreement. Typically, cells are labelled with S-methionine and then the medium is replaced by nonradioactive methionine. The receptors are analysed and the amount of radiolabeled

receptor present is quantified by scanning. In the presence of EGF, but with no other growth factors, Soderquist *et al.* (1986) predicted that the EGF receptor is degraded with a half-life of about 1 hour. We consider the equation for the internal degradation of bound receptors

$$\frac{db}{dt} = -k_i b$$

and obtain the solution

$$b = \alpha e^{-k_i t}$$

where $\alpha = b(0)$. Using $t_{1/2} = 1$ hr, we estimate $k_i = \log 2 \approx 0.693$ hr⁻¹. The processes represented in equations (1.2) occur on a considerably faster time scale than either cell movement or chemical diffusion. Sherratt (1992) has shown that with this difference in time scales, equation (1.2c) is approximately at a steady state at all points, so that

$$b = k_a c r / (k_i + k_d) \quad (1.3)$$

To circumvent the complexities of receptor recycling, we follow Sherratt *et al.*, (1993) and assume that the total number of receptors is a function of the number of bound receptors, specifically $R + B = \rho + \gamma B$, where ρ and γ are positive constants to be determined. Hence $b(1 - \gamma) = \rho - r$, and substituting this and (1.3) into (1.2a) we find

$$\frac{dc}{dt} = -\frac{k_i \rho}{(1 - \gamma)} \cdot \frac{c}{\frac{k_i + k_d}{k_a(1 - \gamma)} + c} \quad (1.4)$$

This leads us to look for a function of the form $h(c) = \mu c / (\hat{c} + c)$ in (1.1b), where μ and $\hat{c}$ are positive constants. Sunada *et al.* (1991) investigated the number of EGF receptors expressed by the cell line A431 by Scatchard analysis of EGF binding to the cell surface. From their plots of bound/free receptors against bound receptors we can estimate γ to be 2.80665×10^{-6} . Now the quotient $K_D = \frac{k_a}{k_i + k_d}$ is about 0.5 nM⁻¹ (Harris and Nicholson, 1988; Sunada *et al.*,

1990) and Soderquist *et al.* (1986) showed the existence of 50,000 receptors per cell. To convert to mol cell^{-1} , we use a conversion factor of 6.02×10^{23} and hence estimate ρ to be

$$\rho = \frac{5 \times 10^4}{6.02 \times 10^{23}} = 8.3 \times 10^{-20} \text{mol cell}^{-1}$$

Using (1.4) we find

$$\hat{c}^{dim} \approx K_D^{-1} = 2 \times 10^{-9} M$$

$$\mu^{dim} = k_i \rho = 5.75 \times 10^{-20} \text{mol cell}^{-1} \text{hr}^{-1}$$

9. Any functional form for $s(c)$ is subject to the nondimensional constraint $s(c_0) = 1$. As a first approximation, we assume $s(c)$ is linear and we use data for the mitotic rate in the absence of any additional EGF, obtained by Chan *et al.* (1989) using a time-lapse videomicroscopic study of *in vitro* wound closure in rabbit corneal cells, to estimate $s(0) = 6 \times 10^{-4}$. Later we will show that $s(c)$ must saturate for large concentrations of EGF to agree with other experimental data.
10. The overlying tear film supplies a constant source of EGF, independent of cell number, and in the simplest model we take $f(n) = A$, where A is a constant, evaluated using the condition that $n = n_0, c = c_0$ is a steady state. Therefore

$$A = \frac{\mu n_0 c_0}{(\hat{c} + c_0)} + \delta c_0.$$

We consider a more complex form for $f(n)$ in a later refinement of the model.

Having established these parameter values, we non-dimensionalise the model using a typical initial wound length, L , and introducing the typical time scale, $1/k^{dim}$, which is of the order of the cell cycle time. We define the following dimensionless quantities

$$\begin{aligned} n^* &= n/n_0 & c^* &= c/c_0 & x^* &= x/L \\ t^* &= kt & \hat{c}^* &= \hat{c}/c_0 & s^*(c_0 c^*) &= s(c)/k \\ D_c^* &= D_c/kL^2 & f^*(n_0 n^*) &= f(n)/kc_0 & \mu^* &= \mu n_0/kc_0 \\ \delta^* &= \delta/k & D_n^*(c_0 c^*) &= D_n(c)/kL^2. \end{aligned}$$

Dropping the asterisks for algebraic convenience, we obtain the dimensionless model

$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial x} \left((\alpha c + \beta) \frac{\partial n}{\partial x} \right) + (\alpha_1 c + \beta_1) n(2 - n) - n \quad (1.5a)$$

$$\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} + f(n) - \frac{\mu n c}{(\hat{c} + c)} - \delta c \quad (1.5b)$$

with conditions

1. $n(x, 0) = c(x, 0) = 0$ for $0 \leq x < 1$
 $n(x, 0) = c(x, 0) = 1$ for $-\infty < x < 0$
2. $n(-\infty, t) = c(-\infty, t) = 1 \ \forall t \geq 0$
 $n_x(1, t) = c_x(1, t) = 0$ by symmetry $\forall t \geq 0$.

By considering a wound length, L , to be 2 mm (Crosson *et al.*, 1986), we determine the dimensionless parameters shown in Table 1.1.

In the rest of this chapter, we solve the system of reaction diffusion equations numerically, first assuming that the tear film is the only source of EGF and then amending the model to include a source from the underlying tissue. By considering the speed of the travelling wave solutions, we are able to comment on the different possible source terms. Furthermore, we investigate the relative importance of mitosis and migration in the healing process and, finally, we consider the experimental problem of determining at which point the wound is said to be healed.

$D_n(c) = \alpha c + \beta = 0.01c + 0.05$
$D_c = 25$
$\mu = 1.37 \times 10^4$
$\hat{c} = 3$
$\delta = 110$
$s(c) = \alpha_1 c + \beta_1 = 0.915c + 0.0851$
$A = \frac{\mu}{(\hat{c}+1)} + \delta = 3556$

Table 1.1: Typical parameter values for a corneal wound, 2mm in radius.

1.3 Tear Film Source of EGF

In this section, we propose that the tear film, overlying the corneal surface, provides a constant source, A , of EGF. We consider numerical solutions of the model for different geometries and different domain sizes.

1.3.1 Linear Stability Analysis

We begin by investigating the linear stability of the steady states $(0, A/\delta)$ and $(1, 1)$ of the nondimensional equations (1.5), corresponding to the wounded and unwounded levels respectively, on an infinite domain. We require the unwounded steady state $(1, 1)$ to be stable to small perturbations in cell density and EGF concentration, while requiring the steady state $(0, A/\delta)$ to be unstable.

We linearize about the positive steady state by setting $n = 1 + n_1$ and $c = 1 + c_1$, where $|n_1|, |c_1| \ll 1$. Expanding in a Taylor series and neglecting quadratic and higher order terms in the usual way, we obtain the linearized system

$$\frac{\partial n_1}{\partial t} = D_n(1) \frac{\partial^2 n_1}{\partial x^2} + s'(1)c_1 - n_1 \quad (1.6a)$$

$$\frac{\partial c_1}{\partial t} = D_c \frac{\partial^2 c_1}{\partial x^2} - \frac{\mu \hat{c} c_1}{(\hat{c} + 1)^2} - \frac{\mu n_1}{\hat{c} + 1} - \delta c_1. \quad (1.6b)$$

On the infinite domain we look for solutions of the form

$$n_1 = u e^{i(kx - \omega t)} \quad c_1 = v e^{i(kx - \omega t)}.$$

Since the equations (1.6) are linear, any solution can be written as a sum of such plane wave solutions. Substituting in the above equations we find

$$(1 + D_n(1)k^2 - iw)u = s'(1)v$$

$$\frac{\mu u}{\hat{c} + 1} = \left(\frac{-\mu \hat{c}}{(\hat{c} + 1)^2} - \delta - D_c k^2 + iw \right) v.$$

Hence for nontrivial solutions we require

$$\frac{\mu s'(1)}{\hat{c} + 1} = \left(iw - D_c k^2 - \frac{\mu \hat{c}}{(\hat{c} + 1)^2} - \delta \right) (1 + D_n(1)k^2 - iw).$$

This gives the quadratic dispersion relation for $w(k^2)$

$$w^2 + ipw - q = 0 \tag{1.7}$$

where

$$p = \left(1 + (D_n(1) + D_c)k^2 + \frac{\mu \hat{c}}{(\hat{c} + 1)^2} + \delta \right) > 0 \tag{1.8a}$$

$$q = (1 + D_n(1)k^2) \left(D_c k^2 + \delta + \frac{\mu \hat{c}}{(\hat{c} + 1)^2} \right) + \frac{\mu s'(1)}{(\hat{c} + 1)} > 0. \tag{1.8b}$$

For linear stability of (1,1), we require $\text{Im } w(k^2) < 0 \quad \forall k$. If $p^2 < 4q$ then $2w = -ip \pm r$ where r is real, whereas if $p^2 > 4q$ then $2w = -p(1 \pm \eta)i$ where $\eta < 1$. Hence $\text{Im } w(k^2) < 0$ and the steady state (1,1) is linearly stable for all parameter values.

Linearising about the wounded steady state $(0, A/\delta)$ and carrying out the analysis as above, we obtain the dispersion relation

$$(1 - 2s(A/\delta) + D_n(0)k^2 - iw)(iw - D_c k^2 - \delta) = 0. \tag{1.9}$$

For linear instability, one of the roots must have positive imaginary part. Hence, for some k we need

$$D_n(0)k^2 - 2s(A/\delta) + 1 < 0.$$

But for the parameter values derived from experimental data in the previous chapter, we know that $s(A/\delta) > 1/2$ and therefore the wounded steady state is unstable to small perturbations in cell density and EGF concentration, of sufficiently small wave number.

1.3.2 Numerical Simulation

We use the NAG routine D03PGF to solve the pair of partial differential equations (1.5) using linear geometry, with the only source of EGF being from the tear film. The routine uses the method of lines to convert a system of non-linear parabolic partial differential equations in one space variable to a system of ordinary differential equations, and then integrates using Gear's method.

The biological observation of a front of cells moving into the wound at constant speed suggests that the model solutions should have a travelling wave form. However, numerical solutions of the model equations with $f(n) = A$, over the realistic semi-infinite domain, corresponding to a finite wound size and infinite unwounded epithelium (hereafter we shall refer to this as the “finite domain” case), indicate that the ‘model wound’ is far from healed in the experimentally observed time, which is 37 hours for a corneal wound 2mm in radius (Crosson *et al.*, 1986) (Figure 1.4a). A plot of the mitotic generation term against space ($x = 0$ and $x = 1$ representing the edge and centre of the wound respectively) also indicates a maximum which is much less than the experimentally observed nondimensional value of approximately 13 (Danjo *et al.*, 1989). Solving the equations for longer times reveals a “fill up” rather than a “travelling wave” mechanism (Figure 1.4b). This is inconsistent with experimental results since it implies that the wound heals by a uniform increase of cells in each epithelial layer rather than by the inward migration of cells at the wound edge. Qualitatively similar results are obtained for a range of parameter values.

We suspected that the absence of wave fronts was due to a long transient time for the evolution of a wave profile. To give a clearer perspective on the model solutions, we thus solved the equations on $-\infty < x < \infty$, subject to boundary conditions $n(-\infty, t) = c(-\infty, t) = 1$ and $n_x = c_x = 0$ as $x \rightarrow \infty$. These new end conditions are not directly relevant to the biological problem; rather, they enable us to obtain a clearer understanding of mathematical aspects of our model. Numerical solutions

show that with these amended end conditions, the system evolves to travelling waves of constant speed and shape in n and c (Figure 1.5), but the speed of these travelling waves ($\approx 40\mu\text{m h}^{-1}$) is slower than the healing rate of actual corneal wounds ($\approx 60\mu\text{m h}^{-1}$). When the simulations are repeated using circular geometry, rather than linear geometry, qualitatively similar results are obtained.

Table 1.2 shows the final values of cell density and chemical concentration predicted after 37 hours for a set of different diffusion coefficients. To compare our results with experimental observations, we need to carefully define the level at which a wound is said to be healed, as discussed later. Using experimental data to estimate values for D_c and $D_n(c)$ we can compare the healing time predicted by the model with the known values, for different ‘healed levels’, as shown in Table 1.3. If we take a wound to be healed when the cell density reaches 80% of its unwounded level then the cell diffusion coefficient has to be several orders of magnitude higher than expected from previous modelling experience for the wound to heal in the required time. Furthermore the mechanism of healing is still inconsistent with experimental results.

1.3.3 Conclusion

Numerical simulations of the simple model, based on the assumption that the tear film is the only source of EGF, show that there is insufficient EGF concentration to heal the wound in the experimentally observed time. With biologically realistic parameters, the healing time for this process is up to 1.5 times longer than observed in experiments. Simulations over a finite domain indicate that the wounds heal via a “fill up” mechanism, which is inconsistent with the observed inward migration of cells, and these results are robust to small changes in parameters values. It is this discrepancy in the healing mechanism and speed that suggests that there is an additional source of mitotic stimulation in the normal healing process. In the remainder of the chapter we focus on one possible additional source, the underlying stroma releasing EGF in response to injury.

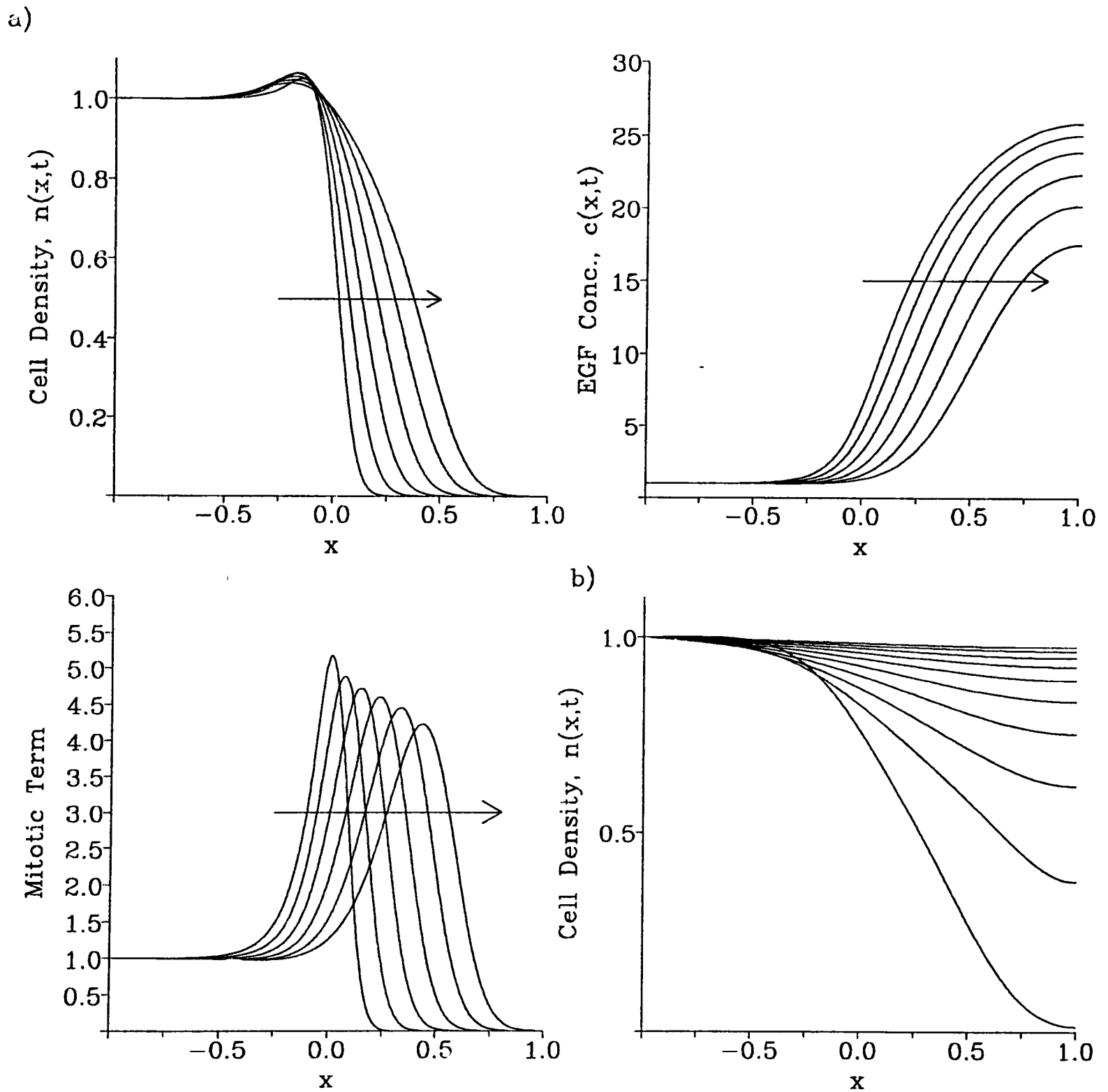


Figure 1.4: Numerical solution of (1.5) showing changes in cell density, EGF concentration and mitotic activity at equal time intervals, a) up to 37 hours and b) up to 25 days. The parameter values are as in Table 1.1.

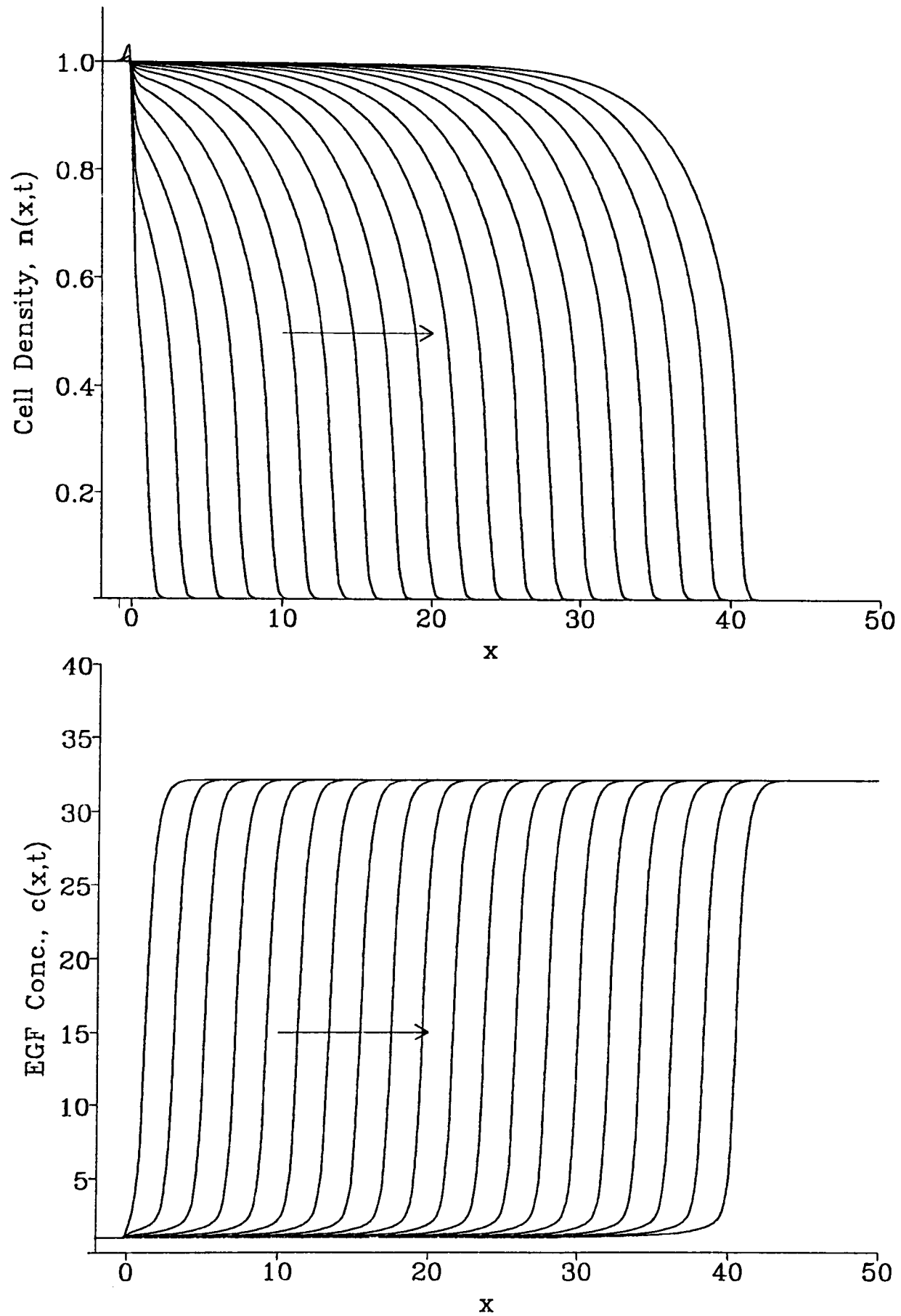


Figure 1.5: Numerical solution of (1.5), over an infinite domain, showing changes in cell density and EGF concentration, at equal time intervals, of 50 hours. The parameter values are as in Table 1.1. The solutions evolve to travelling waves of constant speed and shape.

D_c	D_n	n	c
50	$50c + 50$	1.00	1.00
25	$25c + 25$	0.99	1.00
25	$c + 1$	0.69	1.64
25	$0.5c + 0.5$	0.58	2.82
25	$0.1c + 0.5$	0.44	6.21
25	$0.01c + 0.1$	0.10	12.10
25	$0.01c + 0.05$	0.01	17.47
1	$(c + 1)$	0.69	1.63
1	$0.5c + 0.5$	0.64	1.83
1	$0.1c + 0.5$	0.58	2.13
1	$0.01c + 0.5$	0.52	2.51
1	$0.01c + 0.1$	0.43	3.45
1	$0.01c + 0.05$	0.41	3.83

Table 1.2: Values of n and c at the centre of the wound, after 37 hours, for different diffusion coefficients. Note that the wounded steady state is $n = 0$, $c = A/\delta = 33.2$.

1.4 Improved Model

The results discussed in the previous section suggest that other sources of EGF are required to account for the normal healing of corneal epithelial wounds, in addition to the tear film. We thus consider the possibility that the exposed underlying tissue within the wound releases EGF, which is rapidly degraded by cells at the wound edge. The possibility of the underlying tissue acting as a source of EGF is suggested by the experiments performed on other cell types by Dunn & Ireland (1984) and Barrandon & Green (1987), as discussed in Section 1.1.

The amended nondimensional model, in one space dimension, is therefore

$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial x} \left((\alpha c + \beta) \frac{\partial n}{\partial x} \right) + s(c) \cdot n \cdot (2 - n) - n \quad (1.10a)$$

$$\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} + A + B(n) - \frac{\mu n c}{(\hat{c} + c)} - \delta c. \quad (1.10b)$$

For $B(n)$, we look for a function which is constant for low cell densities and decreases linearly to zero for larger cell densities, motivated by the above experimental results.

Wound Length (mm)	Expected Time (hours)	Predicted Time (hrs) for 25% Level	Predicted Time (hrs) for 80% Level
1	21	44	72
2	37	52	86
4	57	75	125

Table 1.3: Comparison of predicted and expected healing times for different ‘healed’ levels. The parameters are as shown in Table 1.1.

We thus take $f(n) = A + B(n)$, where $B(n)$ has the form

$$B(n) = \begin{cases} \sigma & \text{if } n < n_1 \\ \sigma(p - qn) & \text{if } n_1 \leq n \leq n_2 \\ 0 & \text{if } n > n_2 \end{cases}$$

where σ , p and q are positive constants and n_1 and n_2 are chosen cell densities. Our numerical simulations (see Section 1.4.1) show that consistent results are obtained provided $B(n)$ is non-zero only for n_2 less than about 0.5. To be specific, we choose $n_1 = 0.2$ and $n_2 = 0.4$ (Figure 1.6), but the results are insensitive to any small change in these threshold values.

Using the same parameters as before, our simulations show that we need the source coefficient, σ to be greater than about 1000 to obtain travelling waves, and to match the experimentally observed healing rate we must take $\sigma = 4000$. This is the same order of magnitude as the tear film source A and is hence biologically realistic. Smaller values of σ give reduced mitotic activity, reduced wave speed and loss of ‘travelling wave’ form. The unwounded and wounded steady states of (1.10) are $(1, 1)$ and $(0, \frac{A+B(0)}{\delta})$ respectively. As before, we first use numerical techniques to analyse the stability of these steady states.

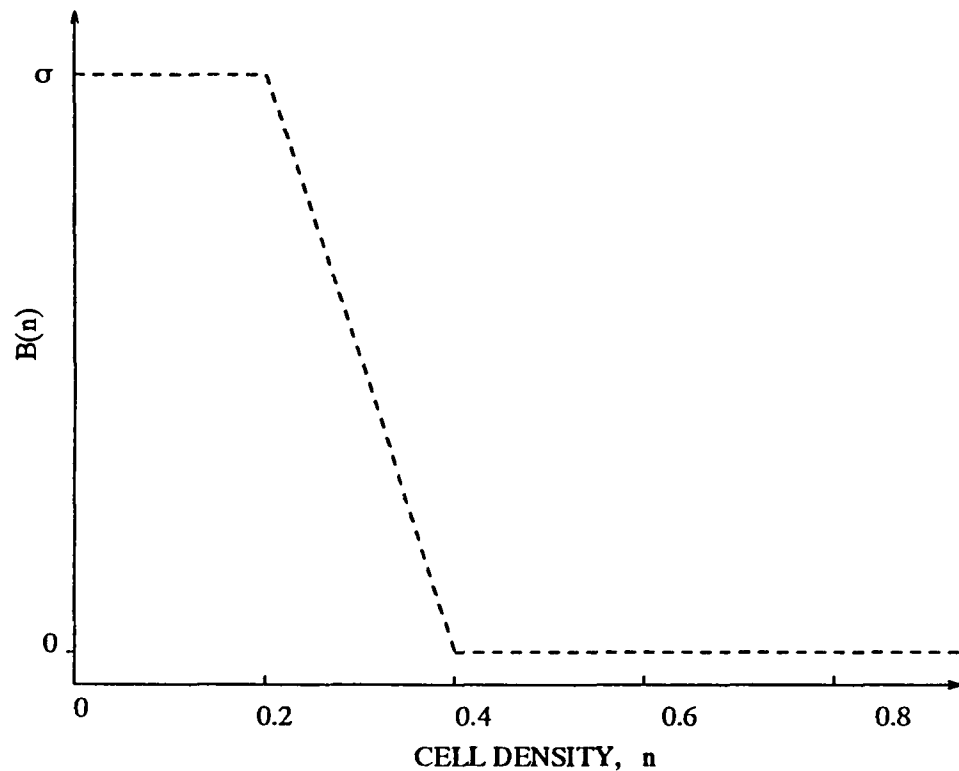


Figure 1.6: The functional form for the additional source term $B(n)$, showing high EGF concentration for small cell density and zero concentration for high cell densities.

1.4.1 Numerical Analysis

We use the NAG routine D03PGF to solve the pair of partial differential equations (1.5) using the same step-function initial condition, $n = c = 1$ outside and $n = c = 0$ inside the wound, for different geometries. The solutions of this amended model have the experimentally expected form of a front of cells moving into the wound, with an associated wave of EGF tending towards the unwounded steady state (1, 1) (Figure 1.7). Moreover, a plot of the mitotic generation term $s(c)n(2 - n)$ shows a wave moving with the cell front and peaking at approximately 14 times the unwounded level, which agrees favourably with the experimentally observed increase in mitotic rate (Danjo *et al.*, 1989) (Figure 1.7). The results are also qualitatively very similar to those obtained by Winter (1972) for the mitotic rate as a function of distance from the wound edge in the healing of epidermal wounds in domestic pigs. Figure 1.7 shows that the wound length is reduced significantly within the experimentally observed healing time, but that healing is still incomplete. However, by extending

our domain size, as discussed previously, long term simulations evolve to fronts of constant shape, moving with a speed ($\approx 64\mu\text{m h}^{-1}$) that compares favourably with the experimentally predicted healing rate (Figure 1.8). It should be noted that the small variation in shape at the wound edge can be explained by excess EGF “seeping into” the wound during the initial stages of healing, promoting cell proliferation and cell migration. Qualitatively similar solutions are obtained when radially symmetric circular geometry is used rather than linear geometry. The radially symmetric case is of course more relevant to applications, but the advantage of the linear geometry is that the solutions can be studied analytically as travelling waves (see Section 2.2).

In Figure 1.7, the wound length has been reduced significantly after 37 hours but we notice that the cell density only reaches 30% of its unwounded level. When the numerical simulations are run for longer time periods, we still observe incomplete healing. This discrepancy between model solutions and experiments may be explained by noting that experimentalists only monitor the healing time until the wound radius is reduced by about 75% and then extrapolate to obtain the ‘total’ healing time. However, our model is invalid for the final stages of healing, since the processes involved become more complex, with the re-establishment of cell-cell contacts and other remodelling processes as the opposing wound fronts meet (Clark, 1989). Therefore, it is the rate of healing, rather than the exact healing time, that we require our model solutions to capture. A detailed study of the final stages of the healing process could be a valuable area for both experimental and theoretical research.

1.5 The Relative Importance of Migration and Mitosis

An important biological question is the relative importance of mitosis versus migration in the healing process. The equations in (1.10) are strongly coupled by

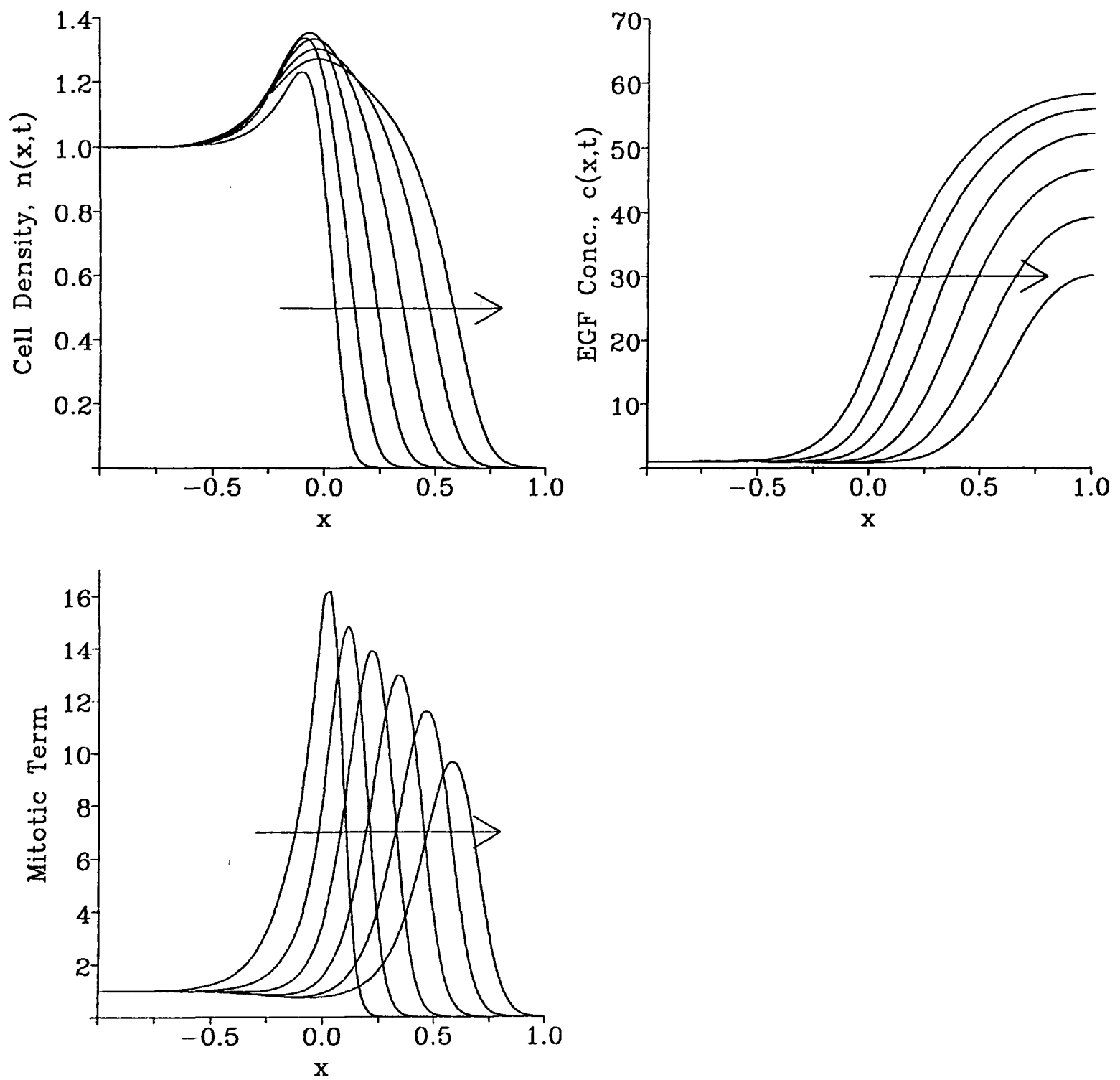


Figure 1.7: Numerical solutions of (1.10), over a finite domain, showing cell density, EGF concentration and mitotic activity as functions of space at equal time intervals, up to 37 hours, using linear geometry. The parameters are as shown in Table 1.1.

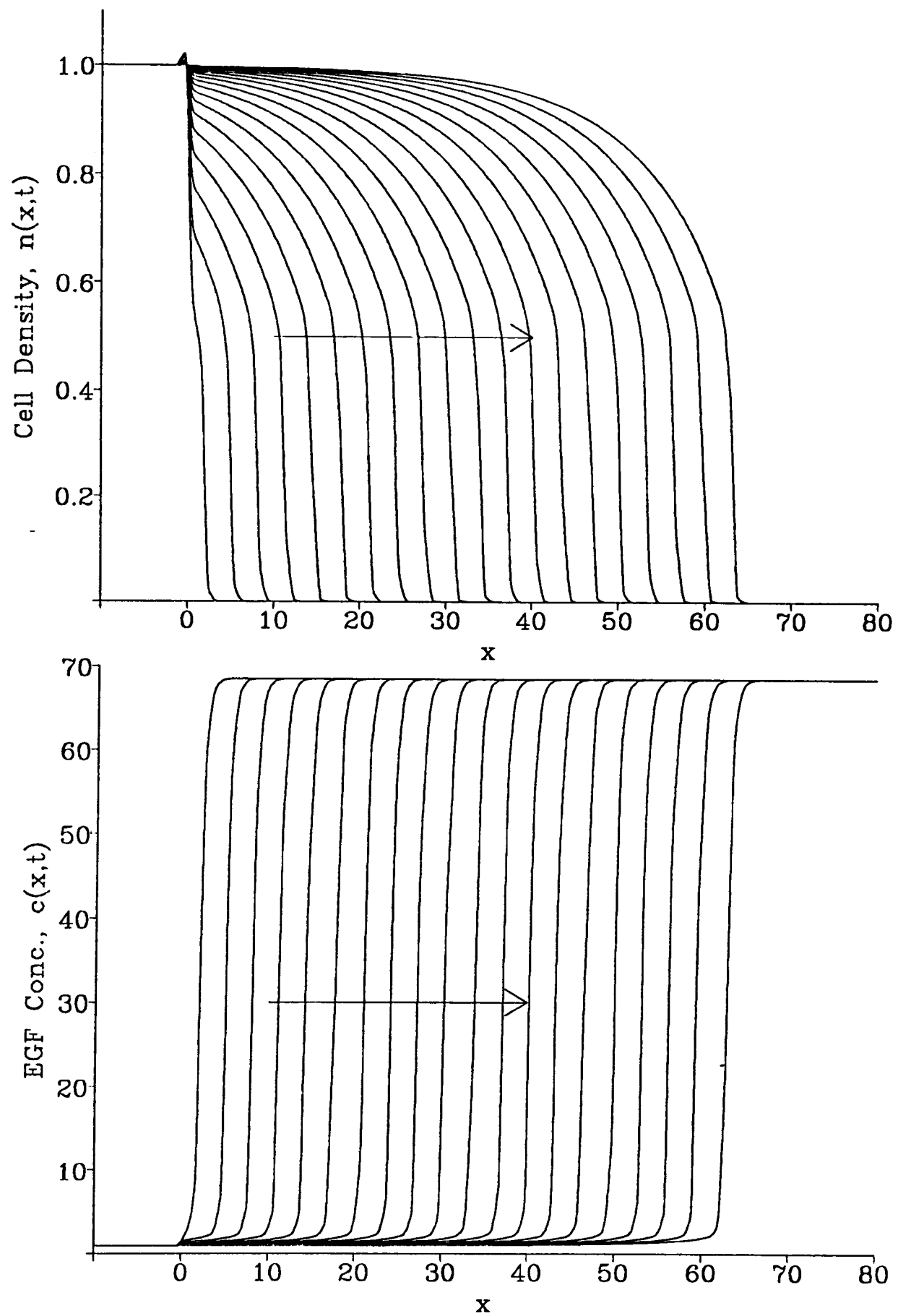


Figure 1.8: Numerical solutions of (1.10), over an infinite domain, showing cell density and EGF concentration as functions of space at equal time intervals of 50 hours, using linear geometry. The parameters are as shown in Table 1.1.

terms accounting for these processes. We investigate this by first reducing α and β in (1.10a) by two orders of magnitude, which corresponds to a healing process dominated by mitosis. In this case, the solutions evolve to travelling waves (Figure 1.9a), of greater slope, moving across the wound at a greatly reduced speed of about $8.8 \mu\text{mh}^{-1}$ for the given parameter values. Again, the peak at the wound edge over the first few time intervals can be explained by excess growth factor seeping into the wound milieu. Similarly, when the mitotic generation term is reduced by one order of magnitude, healing occurs at a slower rate of about $8.2 \mu\text{mh}^{-1}$ (Figure 1.9b). Hence, although our model predicts that wounds can heal in the virtual absence of either mitosis or migration, both processes are important for effective wound healing.

A separate but equally important question concerns the contribution of EGF to migration and mitosis. In the derivation of the functional form $B(n)$, we evaluated σ by matching the increase in mitotic rate with experimental data. Hence, EGF has been shown to have an enhancing effect on mitosis. The effect on migration can be investigated by varying the parameters α and β , with the sum $\alpha c_0 + \beta$ maintained at a biologically realistic value of about $10^{-9} \text{cm}^2 \text{s}^{-1}$. Numerical solutions indicate loss of travelling wave fronts if β is very small, resulting in a ‘fill up’ mechanism. However, if α is very small, fronts of the same constant speed and shape are still observed. This suggests that the role of EGF as a regulator of cell mobility is relatively unimportant; however, EGF is crucial to healing as a mitotic regulator. Hence, to simplify the analysis, we shall subsequently take $\alpha = 0$, corresponding to constant cellular diffusion.

In Figure 1.10 we compare the model predictions of the change in wound radius during healing with the experimental results of Crosson *et al.* (1986), using a radially symmetric circular geometry in the numerical solutions. Here we assume the wound to be “healed” when the cell density is 25% of its unwounded level. The overall process agrees moderately well with the experimental observations, although the model fails to capture the lag phase exhibited in the early stages of healing. By contrast, if we plot wound radius against time for a critical healing density level

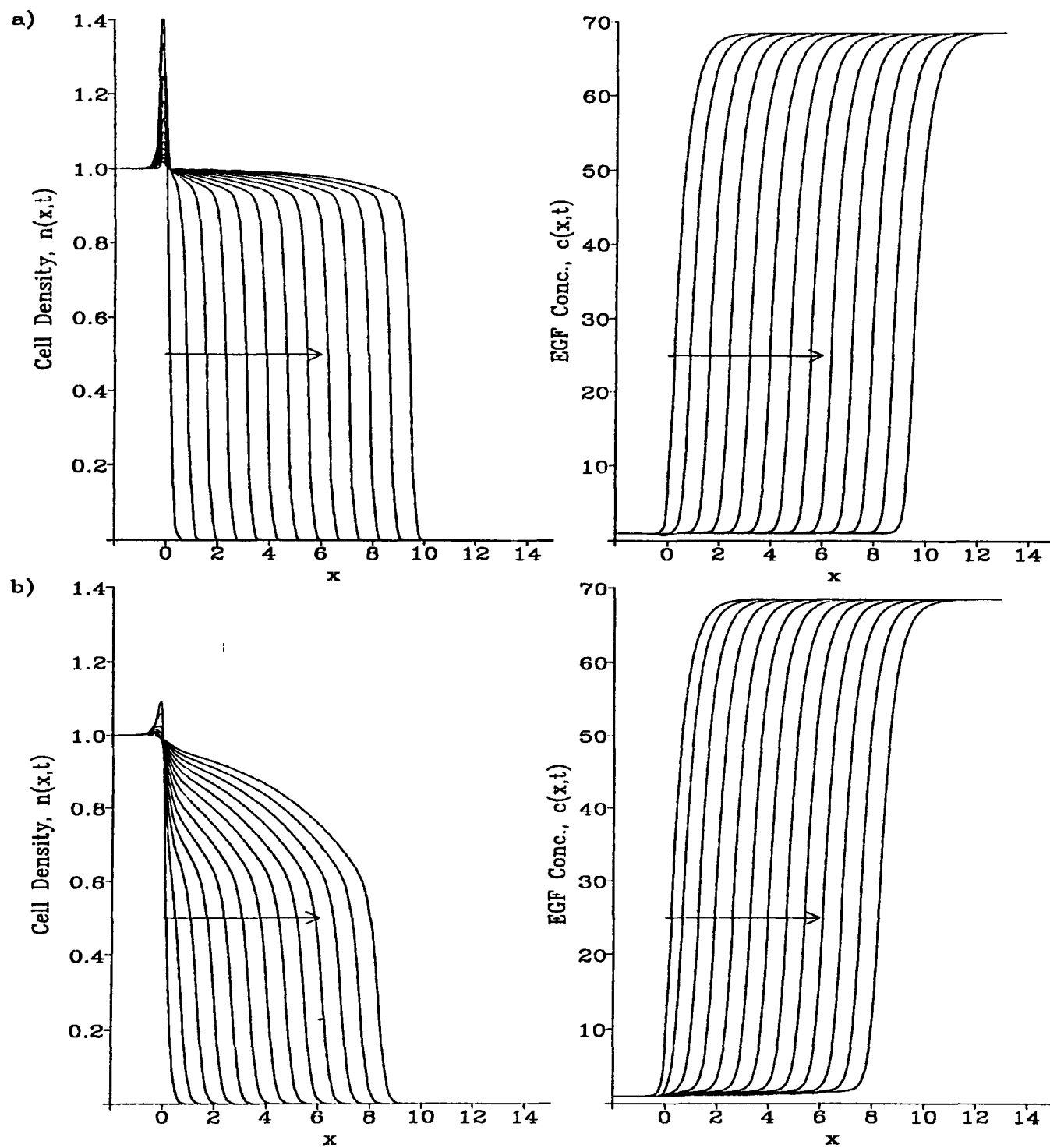


Figure 1.9: Numerical solutions of the model equations (1.10), over an infinite domain, showing cell density and EGF concentration as functions of space at equal time intervals of 80 hours. In (a) we take $\alpha = \beta = 0.001$, which corresponds to healing dominated by mitosis and in (b) we take we take $s(c) = 0.01c + 0.99$ which corresponds to healing dominated by migration. In both cases, the other parameters are as in Table 1.1. Wave fronts are still observed but the rate of healing in greatly reduced.

of 80% (Figure 1.10b), there is strikingly good correspondence with experimental data for the first 20 hours of healing; in particular, we can now obtain a lag phase. However, in the latter stages of healing, the model results deviate markedly from the experimental observations, but, as discussed in Section 1.4, the model is not relevant for these stages.

1.6 Summary

In this chapter we have developed a mathematical model for corneal epithelial wound healing, and used experimental data to estimate parameter values. The model has been used to investigate possible sources of the growth factor EGF. We first considered the tear film as the only source of EGF, and numerical simulations showed that the speed of healing is much slower than experimentally predicted and that the healing occurs via a fill up rather than a travelling wave mechanism. This result strongly suggests that there is an additional source of EGF in the normal healing process, in addition to the tear film. We thus chose to focus on one possible source of growth factor and have developed Dunn & Ireland's (1984) idea of a serum derived growth factor inducing growth at the wound margin. We amended our model to include this additional source term, released within the wound, which is constant for small densities and zero for large cell densities. This corresponds to the underlying stroma releasing EGF in the absence of cells but, as the wound heals, the tear film becomes the sole source of growth factor. Numerical simulations show a definite front of cells and EGF moving into the wound domain, with a speed that compares favourably with experimental data. Hence our modelling suggests that serum-derived factors can alone account for the overall features of the healing process. Furthermore, by reducing the parameters representing the cell diffusion and chemical control of mitosis, we have shown that wounds can heal in the absence of either migration or mitosis, but that both processes are important for fast, effective wound healing. During the

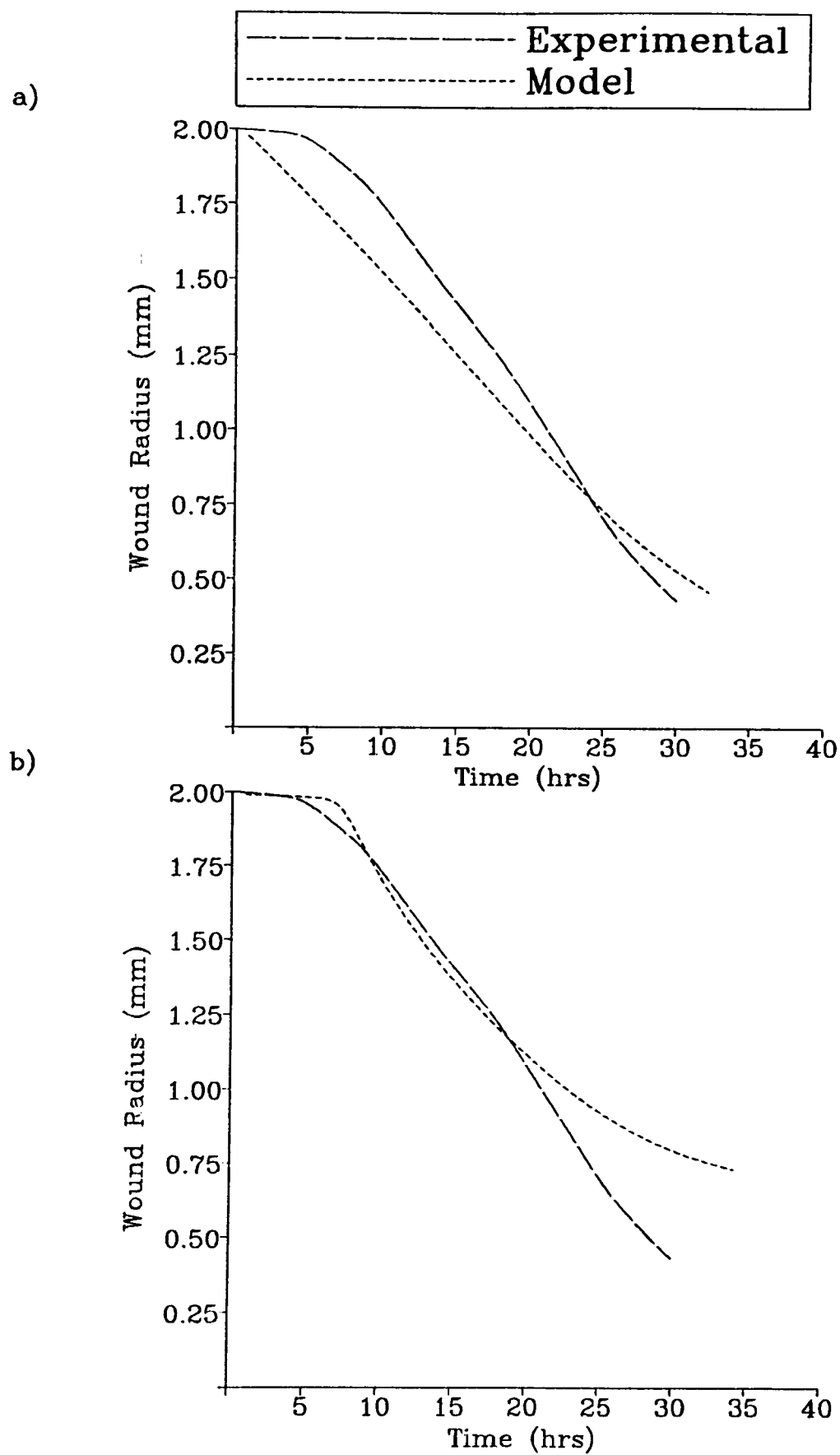


Figure 1.10: Comparison of model predictions of change in wound radius during healing with experimental results. In a) we assume the wound to be healed when the cell density is 25% of its unwounded level. In b) the critical density for healing is taken to be 0.8. Extrapolation of the experimentally determined data line shows the healing time of 37 hours.

final stages of healing, more complex processes are involved, which our mathematical model does not capture. By considering model predictions for the change of wound radius during healing, we have raised an important biological question as to when the wound is said to be healed. The details of the way in which the tears release EGF, and in particular whether the release is constant, and when the wound is said to be healed are possible areas of further experimental study.

Chapter 2

The Speed of Healing

2.1 Introduction

In the previous chapter we constructed a mathematical model for corneal epithelial wound healing. The parameter values are based as far as possible on experimental data, and the numerical solutions suggest that the exposed underlying tissue within the wound acts as a source of EGF, in addition to the tear film. Kolmogorov *et al.* (1937) determined bounds on the wave speed for a single reaction-diffusion equation by considering travelling wave coordinates and studying the resulting ordinary differential equation. In this chapter, we first explore this approach for our system of reaction-diffusion equations, and solve the resulting eigenvalue problem using numerical techniques. We then derive an analytical approximation for the wave speed in terms of the model parameters, and verify the numerical speeds simulated in Chapter 1. The topical application of EGF to the wound increases the speed of healing, and in Section 2.4 we show that our model does indeed capture this effect. The cornea is, of course, a curved surface, whereas we have thus far only considered linear wounds. In Section 2.5, the effect of curvature on the speed of healing is investigated and, in fact, is shown to be negligible for wounds of typical size.

2.2 Phase Plane Analysis

We have shown that when equations (1.5) are solved on an infinite space domain, the solutions evolve to travelling waves. In this section, we consider the possibility of determining the speed of the waves analytically, as a function of the model parameter values.

2.2.1 Transition Wave Speeds in Single Reaction Diffusion Equations

One of the most important properties of nonlinear parabolic systems is their ability to support travelling wave solutions. By far the best studied example of this is for the equation

$$\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} + f(u) \quad (2.1)$$

where f is continuously differentiable on $[u_1, u_2]$, with

$$f(u_1) = f(u_2) = 0$$

$$f(u) > 0, \quad u_1 < u < u_2$$

$$f'(u_1) = \alpha > 0; \quad f'(u) < \alpha, \quad (u_1 < u \leq u_2).$$

We now summarize some of the standard results concerning travelling wave solutions of (2.1), that is solutions of the form

$$u(x, t) = U(z), \quad z = x - at, \quad a > 0, \quad a \text{ a constant.}$$

This is a wave with profile U , moving with speed a in the positive x -direction. In the moving coordinate frame, $U(z)$ satisfies

$$U'' + aU' + f(U) = 0, \quad (2.2)$$

where prime denotes d/dz . This equation has two steady states, $U = U_1 \equiv u_1$ and $U = U_2 \equiv u_2$. We look for solutions which are fixed at one steady state as $z \rightarrow -\infty$,

and fixed at the other as $z \rightarrow \infty$. Thus we have a boundary value problem and must determine if there exist values of a such that (2.1) has a non-negative solution U satisfying

$$\lim_{z \rightarrow \infty} U(z) = U_1 \quad \lim_{z \rightarrow -\infty} U(z) = U_2.$$

In the (U, V) phase plane, we obtain the system

$$\begin{aligned} U' &= V \\ V' &= -aV - f(U) \end{aligned}$$

which has steady states $(U_1, 0)$ and $(U_2, 0)$. It can be easily shown that $(U_2, 0)$ is a saddle point and $(U_1, 0)$ is either a stable spiral or a stable node depending on the wave speed. A stable spiral would result in negative values, which is biologically implausible for most systems and hence we determine the minimum wave speed by seeking a change in the nature of the solution. Straightforward phase plane analysis shows that there is a trajectory connecting $(U_2, 0)$ and $(U_1, 0)$, lying entirely in the region $U \geq U_1$, $V \leq 0$, $U_1 \leq U \leq U_2$, for all wave speeds $a \geq 2[f'(u_1)]^{1/2}$. This trajectory corresponds to a travelling wave solution (Grindrod, 1991). For any initial conditions having compact support, solutions of the partial differential equation (2.1) evolve towards the travelling wave solution with the minimum wave speed $a = 2[f'(u_1)]^{1/2}$ (Kolmogorov *et al.*, 1937). A number of authors have derived bounds on possible wave speeds (Gibbs, 1980; Dunbar, 1984; Sherratt & Murray, 1991) and, moreover, Dunbar (1984) has also investigated connections in four dimensional space for predator-prey systems. For our model, we have a fourth order system, and we do not attempt to consider the existence of a heteroclinic connection in the four dimensional phase space. Instead, we find bounds on the wave speed such that the wounded steady state is unstable whilst the unwounded steady state is stable and then, by analogy with the above analysis, we will conjecture the existence of travelling waves.

2.2.2 Travelling Waves

Travelling wave solutions of (1.5) have the form $n(x, t) = N(z)$, $c(x, t) = C(z)$, where $z = x - at$ and a is the wave speed in the positive x -direction. These solutions must satisfy

$$\frac{d}{dz} \left[(\alpha C + \beta) \frac{dN}{dz} \right] + a \frac{dN}{dz} + s(C)N(2 - N) - N = 0 \quad (2.3a)$$

$$D_c \frac{d^2 C}{dz^2} + a \frac{dC}{dz} + A + B(N) - \frac{\mu NC}{\hat{C} + C} - \delta C = 0 \quad (2.3b)$$

Equations (1.5) hold on a finite space domain and a semi-infinite time domain. During the majority of the ‘linear phase’ of healing, the numerical solutions have $n = c = 1$ near the wound edge and $n = 0$, $c = \frac{A+B(0)}{\delta}$ near the centre of the wound, and they differ from these steady state values only in a localized region. Hence the wave form can be reasonably represented by solving (2.3) on $-\infty < z < \infty$. As a first approximation, we consider the cell diffusion to be independent of the EGF concentration and set $\alpha = 0$ (as discussed in Section 1.5). We let $dN/dz = V$ and $dC/dz = W$, hence the above pair of ordinary differential equations can be written as four first order equations:

$$\frac{dN}{dz} = V \quad (2.4a)$$

$$\frac{dC}{dz} = W \quad (2.4b)$$

$$\frac{dV}{dz} = \frac{1}{\beta} (-aV - s(C)N(2 - N) + N) \quad (2.4c)$$

$$\frac{dW}{dz} = \frac{1}{D_c} \left(\frac{\mu NC}{\hat{C} + C} + \delta C - A - B(N) - aW \right). \quad (2.4d)$$

This system of ordinary differential equations is difficult to analyse globally. We therefore attempt only a linear analysis about the steady states $(0, (A+B(0))/\delta, 0, 0)$ and $(1, 1, 0, 0)$. We examine stability of each steady state by considering the eigenvalues of the Jacobian matrix, J , at each steady state in turn. The Jacobian matrix

for this system is

$$J = \begin{pmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ \frac{1}{\beta}(-2s(C)(1-N) + 1) & \frac{-s'(C)}{\beta}N(2-N) & \frac{-a}{\beta} & 0 \\ \frac{\mu C}{D_c(\hat{C}+C)} & \frac{1}{D_c} \left(\frac{\mu N \hat{C}}{(\hat{C}+C)^2} + \delta + B'(N) \right) & 0 & -\frac{a}{D_c} \end{pmatrix}$$

We let $\underline{u} = (N, C, V, W)$ and rewrite (2.4) as $\frac{d\underline{u}}{dz} = J\underline{u}$. Looking for solutions of the form $\underline{u} = e^{\lambda z}$, we determine that if there are no eigenvalues of the Jacobian matrix with positive real part then the steady state is stable. We will investigate the stability of each steady state by determining the number of positive real roots using *Descarte's Rule of Signs*, which states that

“Given an n th order polynomial, suppose N is the number of sign changes in the sequence of coefficients $\{a_n, a_{n-1}, \dots, a_0\}$, ignoring any which are zero. Then there are at most N roots which are real and positive, and further, there are either N or $N - 2$ or $N - 4, \dots$ real positive roots.”

We consider this analytical approach before resorting to numerical techniques.

Unwounded steady state

At the unwounded steady state $N = C = 1, V = W = 0$, the eigenvalues satisfy the quartic equation

$$\lambda^4 + p_u \lambda^3 + q_u \lambda^2 + r_u \lambda + s_u = 0 \quad (2.5)$$

where

$$p_u = a \left(\frac{1}{\beta} + \frac{1}{D_c} \right) > 0 \quad (2.6a)$$

$$q_u = \frac{1}{\beta D_c} \left(a^2 - D_c - \delta \beta - \frac{\mu \hat{C}}{(\hat{C} + 1)^2} \right) \quad (2.6b)$$

$$r_u = -\frac{a}{\beta D_c} \left(1 + \delta + \frac{\mu \hat{C}}{(\hat{C} + 1)^2} \right) < 0 \quad (2.6c)$$

$$s_u = \frac{1}{\beta D_c} \left(\frac{\mu \hat{C}}{(\hat{C} + 1)^2} + \frac{s'(1)\mu}{(\hat{C} + 1)} + \delta \right) > 0. \quad (2.6d)$$

Using Descartes's Rule of Signs, it is clear in both the tear film and improved models, that the dispersion relation at the unwounded steady state has either 2 or 0 real positive roots and hence 2 or 0 positive eigenvalues. We require the unwounded steady state to have an unstable direction in phase space and thus have 2 positive eigenvalues. This is verified numerically.

Wounded steady state

Solving for the eigenvalues of this matrix at the wounded steady state $N = V = W = 0$, $C = \frac{(A+B(0))}{\delta}$, we obtain the quartic equation

$$\lambda^4 + p_w \lambda^3 + q_w \lambda^2 + r_w \lambda + s_w = 0 \quad (2.7)$$

where

$$p_w = a \left(\frac{1}{D_c} + \frac{1}{\beta} \right) > 0 \quad (2.8a)$$

$$q_w = \frac{1}{\beta D_c} \left(a^2 - \beta \delta - D_c + 2D_c s\left(\frac{A+B(0)}{\delta}\right) \right) \quad (2.8b)$$

$$r_w = \frac{a}{\beta D_c} \left(2s\left(\frac{A+B(0)}{\delta}\right) - 1 - \delta \right) \quad (2.8c)$$

$$s_w = \frac{\delta}{\beta D_c} \left(1 - 2s\left(\frac{A+B(0)}{\delta}\right) \right). \quad (2.8d)$$

Using our expression for $s(c)$ and the parameter values obtained from experimental data, we know that $s(A/\delta) > 1/2$ and $s((A+B(0))/\delta) > 1/2$. Thus $s_w < 0$ in both the tear film and the improved model. There are 4 possible cases:

1. $q_w < 0, r_w < 0 \Rightarrow 1$ sign change
2. $q_w < 0, r_w > 0 \Rightarrow 3$ sign changes
3. $q_w > 0, r_w < 0 \Rightarrow 1$ sign change
4. $q_w > 0, r_w > 0 \Rightarrow 1$ sign change

Wave Speed, a	Eigenvalues	Eigenvectors
3	-30 + 16.157i -30 - 16.157i 2.038 -2.158	(-0.023-0.013i,-0.001-0.012i,0.906,0.231+0.355i) (-0.023+0.013i,-0.001+0.012i,0.906,0.231-0.355i) (0,0.44,0,0.898) (0,-0.42,0,0.907)
3.4	-34 + 2.245i -34 - 2.245i 2.031 -2.167	(-0.027-0.002i,-0.012-0.002i,0.905,0.42+0.056i) (-0.027+0.002i,-0.012+0.002i,0.905,0.42-0.056i) (0,0.442,0,0.897) (0,-0.419,0,0.908)
3.41	-35.431 -32.769 2.031 -2.179	(-0.026,-0.011,0.917,0.397) (-0.029,-0.044,0.5499,0.834) (0,0.442,0,0.897) (0,-0.417,0,0.908)
3.5	-60.951 -19.049 2.019 -2.179	(-0.016,-0.002,0.989,0.144) (-0.029,-0.044,0.549,0.834) (0,0.444,0,0.896) (0,-0.417,0,0.909)

Table 2.1: Eigenvalues and corresponding eigenvectors at the wounded steady state $(0, A/\delta, 0, 0)$ for different nondimensional wave speeds, a , in the tear film model. There is a local bifurcation at $a = 3.41$. The parameter values are as in Table 1.1.

Hence, regardless of the signs of q_w and r_w , there is always at least one real, negative root and we conclude that the wounded steady state always has one stable direction in phase space.

2.2.3 Numerical Evaluation of Eigenvalues

Tear Film Model

For general parameter values, it is unfeasible to determine the eigenvalues (2.4) analytically. However, it is straightforward to calculate the eigenvalues and eigenvectors of the Jacobian matrix numerically. In the single reaction-diffusion equation (2.1), it is the change from complex eigenvalues corresponding to a stable spiral to real eigenvalues corresponding to a stable node which determines the speed of the travelling waves (see Section 2.2.1). It therefore seems natural to look for similar changes

in eigenvalues for the system of equations (1.5). For the unwounded steady state, numerical calculation suggests that there are always two eigenvalues with positive real part, independent of the source term, Λ . Hence, we look for any change in the eigenvalues of the Jacobian matrix at the wounded steady state as we increase the wave speed. Table 2.1 shows the eigenvalues and corresponding eigenvectors for a range of wave speeds. At $a = 3.41$ there is a change from a complex conjugate pair with negative real part, a positive and a negative real root to one positive and three negative real roots, corresponding to a change in the nature of the unwounded steady state. This value of a is very close to the observed wave speed in numerical solutions of the partial differential equations, suggesting that this bifurcation may determine the observed wave speed.

To confirm this, we use the partial differential equation solutions to investigate the form of the conjectured heteroclinic connection near the wounded steady state. The eigenvector along which the heteroclinic connection approaches the steady state is evaluated numerically by running long term simulations and using the fact that we are looking for solutions of the form $n \sim n_0 e^{\lambda x}$. Hence, by estimating $\frac{d(\log n)}{dx}$ numerically for large x , we can verify that the trajectory corresponding to the travelling wave solution will approach the equilibrium point along the eigenvector corresponding to the eigenvalue with the most negative real part. Figure 2.1 shows the dominant eigenvalue tending towards -34 as x increases, which is the value of the real part of the eigenvalue of the Jacobian, J , when the wounded steady state changes from a spiral to a node.

The above results indicate that there is a change in solution behaviour as the wave speed increases beyond the critical value, $a_{crit} = 3.41$. Moreover, consistent results are obtained for changes in mesh and domain size and we confirm the robustness for a range of parameter sets. This analysis of the eigenvalues and corresponding eigenvectors determines a dimensionalized speed of

$$a^{dim} = 43 \mu\text{m hr}^{-1},$$

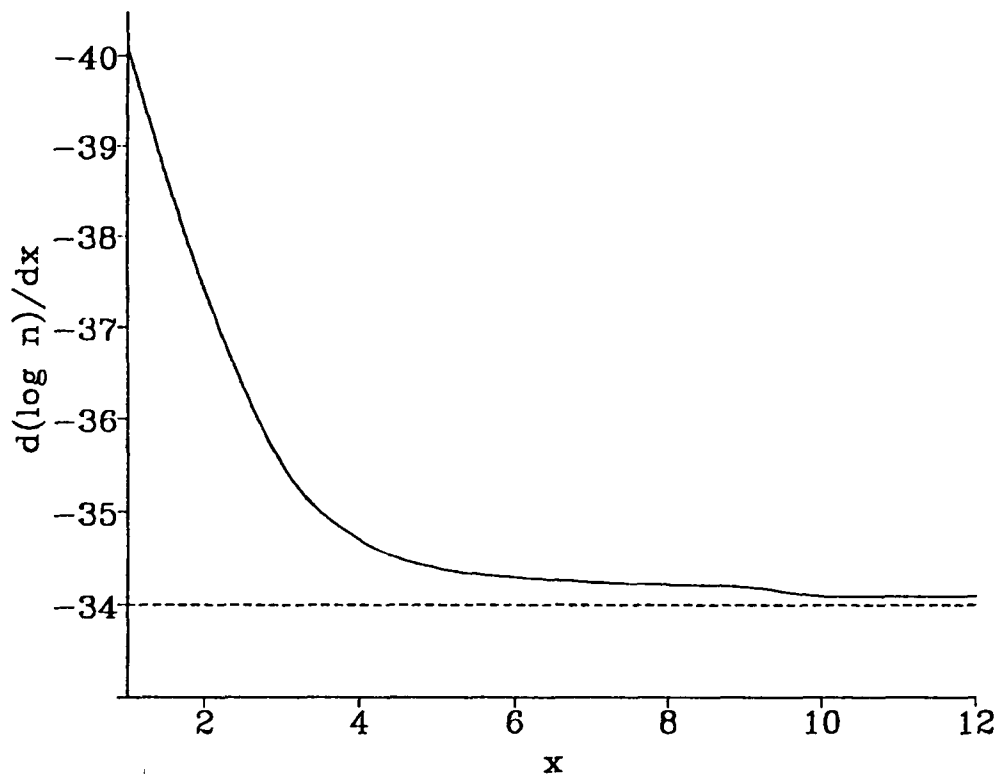


Figure 2.1: The change in $\frac{d(\log n)}{dx}$ with x for the waves illustrated in Figure 1.5. Under the assumption that $n \sim n_0 e^{\lambda x}$ as $x \rightarrow \infty$, this function should tend to λ . Here $\lambda = -34$, which is the real part of the eigenvalue that changes character at the critical wave speed, $a = 3.41$, corresponding to a dimensional speed of $43 \mu\text{m h}^{-1}$.

using the parameter values estimated in Table 1.1, which agree well with the results obtained from our numerical simulations. A comparison with the rates of wound healing in Table 2.4 show that this maximum speed is at least 1.5 times slower than experimentally predicted.

Improved Model

By calculating the eigenvalues and corresponding eigenvectors at the wounded steady state, for a range of wave speeds, a , we observe a local bifurcation at $a = 5$, corresponding to a change from a complex conjugate pair with negative real part, a positive and a negative real root to one positive and three negative real roots (Table 2.2). This is to within 2% of wave speed in numerical solutions of the equations, thus we focus on the form of the conjectured heteroclinic connection to confirm that this bifurcation determines the observed wave speed. By estimating $\frac{d(\log n)}{dx}$ for large

Wave speed a	Eigenvalues	Corresponding Eigenvectors
4.5	-45+21.609i -45-21.609i 2.010 -2.19	(-0.018-0.008i,-0.001-0.004i, 0.976, 0.134+0.168i) (-0.018+0.008i,-0.001+0.004i, 0.976, 0.134-0.168i) (0,0.446,0,0.895) (0,-0.415,0,0.91)
4.99	-49.9+1.392i -49.9 1.392i 2.00 -2.20	(-0.02 -0.001i,-0.004,0.976,0.215+0.012i) (-0.02 +0.001i,-0.004,0.976,0.215-0.012i) (0,0.447,0,0.894) (0,-0.414,0,0.91)
5	-52.838 -47.162 2.00 -2.20	(-0.019,-0.004,0.981,0.193) (-0.021,-0.005,0.917,0.24) (0,0.447,0,0.894) (0,-0.414,0,0.91)
5.5	-78.088 -31.912 1.99 -2.21	(-0.013,-0.001,0.996,0.089) (-0.028,-0.015,0.879,0.476) (0,0.449,0,0.894) (0,-0.4112,0,0.911)

Table 2.2: Eigenvalues and corresponding eigenvectors of the Jacobian matrix at the wounded steady state of the improved model, for different wave speeds.

x , we verify once more that the trajectory along which we approach the equilibrium point is asymptotic to the eigenvector corresponding to the eigenvalue with the most negative real part. Figure 2.2 shows the dominant eigenvalue tending towards -50 , which agrees well with the real part of the eigenvalue at the bifurcation point.

The numerical results are not sensitive to changes in mesh size or domain size, and similar results are obtained for a wide range of other parameters. Table 2.3 compares wave speeds and eigenvalues for different parameter domains. It should be noted that the wave speed appears to depend crucially on the cell diffusion coefficient, β , with large values of β resulting in loss of travelling wave form and a “fill up” mechanism. In dimensional form the wave speed is

$$a^{dim} = 63 \mu\text{m hr}^{-1}$$

which compares well with the numerically simulated wave speed of $64 \mu\text{m hr}^{-1}$.

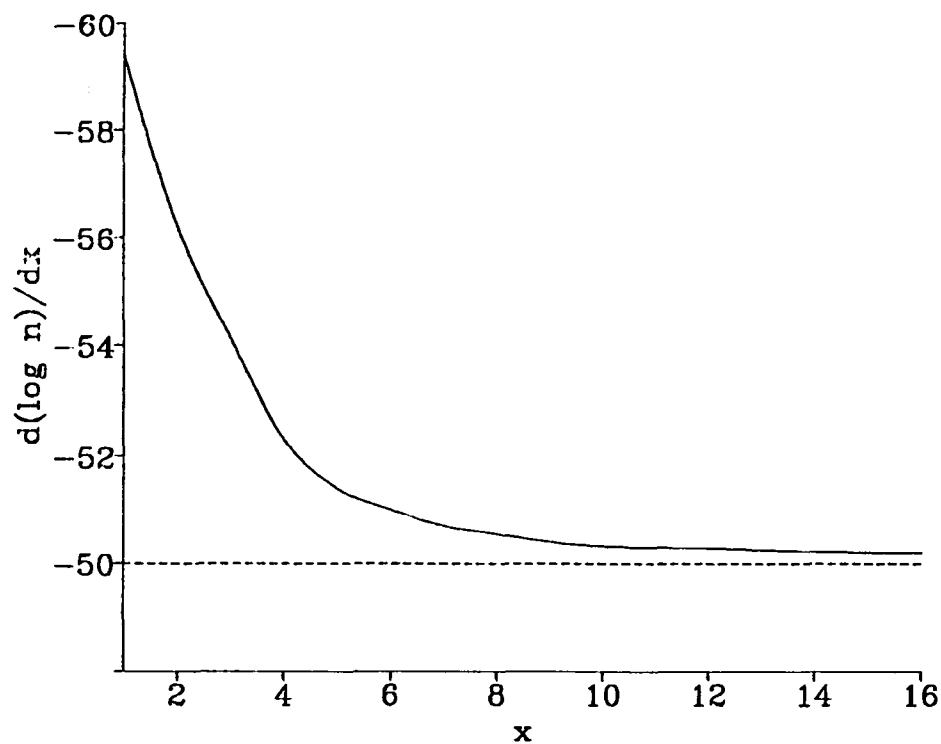


Figure 2.2: Numerically determined dominant eigenvalue for increasing domain size. The eigenvalues tend towards -50 which agrees reasonably well with the real part of the eigenvalue evaluated numerically at the bifurcation point

Cell diffusion coefficient β	Chemical diffusion coefficient D_c	Numerically simulated speed $\mu\text{m hr}^{-1}$	Numerically evaluated speed $\mu\text{m hr}^{-1}$	Eigenvalue	Dominant eigenvalue
0.01	25	28	28.5	$-112 \pm 2.8i$	-114
0.05	25	64	63	$-50 \pm 1.4i$	-50.5
0.1	25	88	89	$-35 \pm 0.97i$	-37.5
0.5	25	192	198	$-16 \pm 0.7i$	-17
1.0	25	270	281	$-11 \pm 0.5i$	-11.5
0.1	1	61	63	$-50 \pm 1.4i$	-51.5
0.1	100	62	63	$-50 \pm 1.4i$	-51

Table 2.3: Comparison of the numerically simulated and evaluated wave speeds, together with the numerically determined eigenvalue and the dominant eigenvalue predicted by long time simulations of the improved model, for different parameter domains.

Author	Wound Radius (mm)	EGF Conc. (ng/ml)	Rate ($\mu\text{m/hr}$)
Crosson, 1986	2	0	64
Pfeister, 1975	6	0	55
Ho & Elliot, 1975	7	0	60
Khododast <i>et al.</i> , 1968	3	0	54
Harris <i>et al.</i> , 1970	2	0	68
Thoft & Friend, 1977	3	0	66
Buck, 1979	1.4	0	45
Brazzell <i>et al.</i> , 1991	5	3.63	86
Brazzell <i>et al.</i> , 1991	5	9.09	91
Brazzell <i>et al.</i> , 1991	5	16.67	92

Table 2.4: Experimentally determined *in vivo* rates of wound healing of corneal wound healing in rabbits and mice.

Comparing this result with the *in vivo* data for the rate of healing (Table 2.4), we observe that wounds of similar size heal at similar rates to the result obtained above.

2.3 Analytical Expression for the Wave Speed

In this section, we derive an analytical expression for the wave speed, in terms of the model parameters, by determining an approximate solution of the quartic equation (2.7) for the eigenvalues of the Jacobian matrix at the wounded steady state, namely

$$\lambda^4 + p\lambda^3 + q\lambda^2 + r\lambda + s = 0 \quad (2.9)$$

where

$$p = a \left(\frac{1}{\beta} + \frac{1}{D_c} \right) \quad (2.10a)$$

$$q = \frac{1}{\beta D_c} (a^2 - \delta\beta - D_c + 2D_c s(A/\delta)) \quad (2.10b)$$

$$r = -\frac{a}{\beta D_c} (\delta + 1 - 2s(A/\delta)) \quad (2.10c)$$

$$s = -\frac{\delta}{\beta D_c} (2s(A/\delta) - 1). \quad (2.10d)$$

The parameter values in Table 1.1 imply that the coefficients q and s dominate p and r , and we will use this to find an approximate solution. In order to make this mathematically rigorous, we introduce a small parameter, ϵ , and consider

$$\delta = \epsilon^{-2}, \quad \beta = \beta_0 \epsilon^{\alpha_1}, \quad D_c = D_0 \epsilon^{-\alpha_2}, \quad \& \quad A = A_0 \epsilon^{-\alpha_3}$$

where α_1 , α_2 and α_3 are parameters to be determined and β_0 , D_0 and A_0 are all first order constants. Our coefficients thus become

$$p = \frac{a(\beta_0 \epsilon^{\alpha_1} + D_0 \epsilon^{-\alpha_2})}{\beta_0 D_0 \epsilon^{\alpha_1 - \alpha_2}} \quad (2.11a)$$

$$q = \frac{1}{\beta_0 D_0 \epsilon^{\alpha_1 - \alpha_2}} \left(a^2 - \beta_0 \epsilon^{\alpha_1 - 2} - D_0 \epsilon^{-\alpha_2} + 2A_0 D_0 \epsilon^{2 - \alpha_2 - \alpha_3} \right) \quad (2.11b)$$

$$r = -\frac{a}{\beta_0 D_0 \epsilon^{\alpha_1 - \alpha_2}} (1 + \epsilon^{-2} - 2A_0 \epsilon^{2 - \alpha_3}) \quad (2.11c)$$

$$s = \frac{1}{\beta_0 D_0 \epsilon^{\alpha_1 - \alpha_2 + 2}} (1 - 2A_0 \epsilon^{2 - \alpha_3}). \quad (2.11d)$$

Focussing on the expression for q , comparison of the relative size of each term shows that $q \gg p, r$ provided that $0 \leq \alpha_2 \leq 2$ and $\alpha_3 \geq 3$ and an inspection of the terms in s reveals that $0 \leq \alpha_1 < 3$. Hence our assumption that p and r are small compared to q and s is valid provided

$$\delta = \epsilon^{-2} \quad (2.12a)$$

$$\beta_0 \epsilon^3 < \beta \leq \beta_0 \quad (2.12b)$$

$$D_0 \leq D_c \leq D_0 \epsilon^{-2} \quad (2.12c)$$

$$A \geq A_0 \epsilon^{-3}. \quad (2.12d)$$

We now choose $\epsilon = 0.1$ so that δ agrees well with the experimentally determined value and hence find that the nondimensional parameters must satisfy

$$1 \leq D_c \leq 100 \quad A \geq 1000 \quad \& \quad 0.001 < \beta \leq 1 \quad (2.13)$$

These inequalities hold for the parameter values we have derived from experimental data. For the subsequent analysis, we fix $\alpha_1 = 1$, $\alpha_2 = 1$ and $\alpha_3 = 4$, in agreement

with the model parameters. The quartic equation for the eigenvalues can thus be written as

$$\lambda^4 + ap_0\epsilon^{-1}\lambda^3 + q_0\epsilon^{-3}\lambda^2 + ar_0\epsilon^{-2}\lambda + s_0\epsilon^{-4} = 0, \quad (2.14)$$

where $p_0 = \frac{\beta + D_c}{\beta D_c}$, $q_0 = \frac{2}{\beta}s\left(\frac{A}{\delta}\right)$, $r_0 = \frac{1}{\beta D_c}(\delta - 2s\left(\frac{A}{\delta}\right))$ and $s_0 = \frac{2\delta}{\beta D_c}s\left(\frac{A}{\delta}\right)$. This equation has 2 roots that are $O(1)$ and two that are $O(\epsilon^{-3/2})$. Previous numerical results imply that it is the larger two roots which change character and hence we consider an asymptotic expansion of the form

$$\lambda = \epsilon^{-3/2}\lambda_0 + \epsilon^{-1}\lambda_1 + \epsilon^{-1/2}\lambda_2 + \dots$$

By comparing coefficients, we find that the linear and constant terms can be neglected. To attain the “richest solution” (Kevorkian & Cole, 1981) we must retain the cubic term, and hence we consider the expansion

$$a = \epsilon^{-1/2}a_0 + a_1 + \dots,$$

which agrees with the numerically simulated wave speeds. The resulting equation for λ_0 is

$$\lambda_0^4 + a_0p_0\lambda_0^3 + q_0\lambda_0^2 = 0, \quad (2.15)$$

from which we determine

$$2\lambda_0 = -a_0p_0 \pm \sqrt{a_0^2p_0^2 - 4q_0}. \quad (2.16)$$

Based on the numerical results in the previous section, we are looking for the critical wave speed, a_{crit} , for which the discriminant is zero. The critical wave speed is thus found to be

$$a_{crit} = \pm \frac{2}{p_0}\sqrt{q_0}\epsilon^{-1/2}. \quad (2.17)$$

Substituting for p_0 and q_0 and noting a is positive, we obtain an analytical expression for the critical wave speed in terms of our model parameters

$$a_{crit} \approx \frac{2}{\beta + D_c}\sqrt{2\beta D_c^2 s\left(\frac{A + \sigma}{\delta}\right)}. \quad (2.18)$$

The analytically determined wave speeds for the tear film and improved models are $22.4\mu\text{m hr}^{-1}$ and $63.3\mu\text{m hr}^{-1}$ respectively, which compare well with the numerically determined wave speeds of $23\mu\text{m hr}^{-1}$ and $63\mu\text{m hr}^{-1}$ respectively.

As $D_c \gg \beta$, (2.18) can be approximated by

$$a_{crit} = \sqrt{8\beta s\left(\frac{A + \sigma}{\delta}\right)}$$

and hence we derive the relationship $a_{crit} \propto \sqrt{\beta}$, which is similar to the relationship between the speed and diffusion coefficient in (2.1) (Grindrod, 1991). In Table 2.5 and Figure 2.3 we compare the analytically and numerically determined wave speeds for different cell diffusion coefficients for the improved model and note that there is a good relationship between numerical and analytical solutions for all values of β less than 1, with the error at this value being 6%. Larger values of β give rise to large discrepancies between numerical and analytical speed due to the loss of travelling wave form. This is exactly as predicted in the above analysis.

An important biological implication of this result is that the rate of healing of corneal epithelial wounds can be increased by increasing the cell diffusion coefficient, β , or the amount of EGF, $A + \sigma$. However, increasing the chemical diffusion coefficient, D_c , does not have a significant effect.

2.4 Topical Application of EGF

In this section, we examine the effects of topical application of EGF to the wound and determine an expression for the rate of healing. Brazzell *et al.* (1991) and Nishida *et al.* (1990) undertook *in vivo* and *in vitro* experiments in which EGF was applied externally to a corneal surface wound and the change in wound radius with time was noted for different concentrations of EGF. Brazzell *et al.* (1991) recorded an increase in the healing rate when EGF was added, the rate saturating at about 45% greater than normal, at an experimentally determined EGF concentration of $16.67\mu\text{g ml}^{-1}$. The mitotic generation function, $s(c)$, determined in Chapter 1 is an

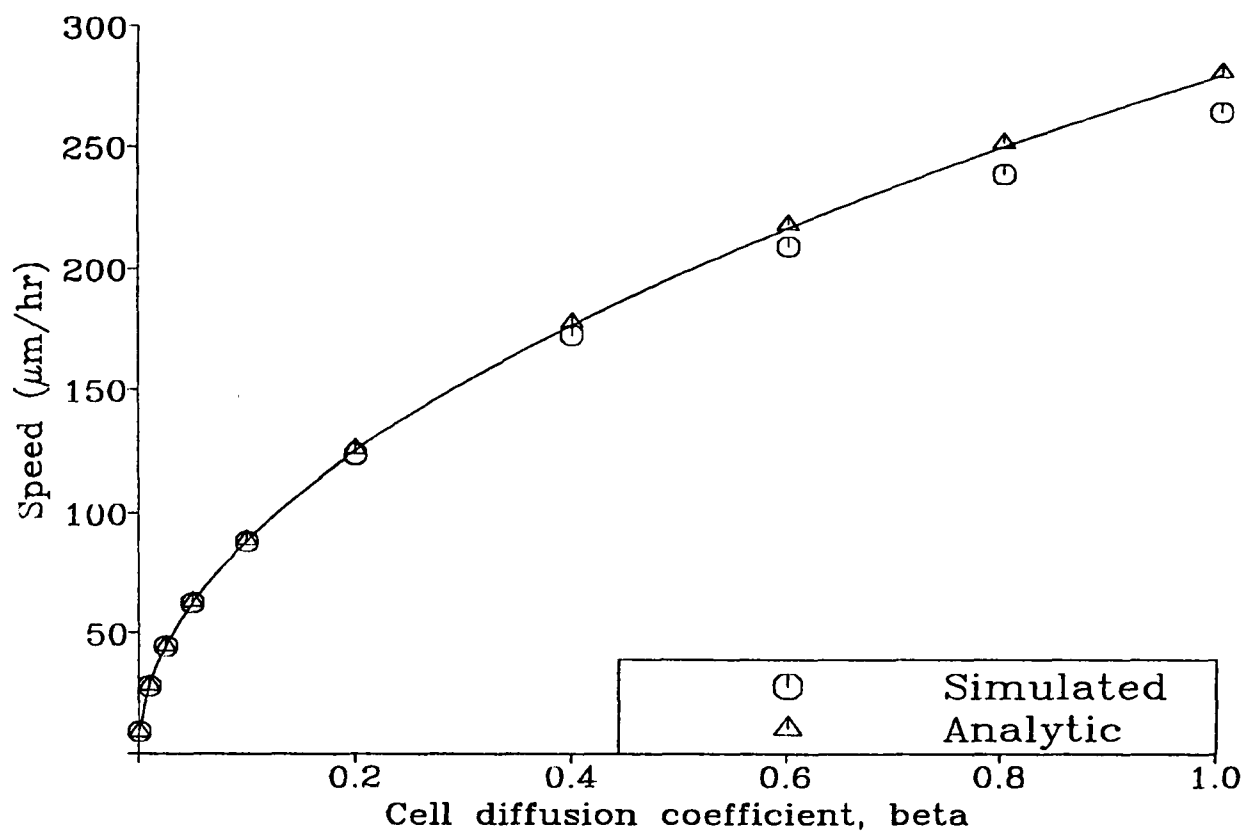


Figure 2.3: Comparison of numerically and analytically determined wave speeds for different cell diffusion coefficients in the improved model (1.5). The full curve represents $a = \sqrt{8\beta s(\frac{A+\sigma}{\delta})}$.

Cell Diffusion Coefficient, β	Numerical Wave Speed, μmhr^{-1}	Analytical Wave Speed, μmhr^{-1}
0.001	8.9	8.85
0.01	28.5	27.96
0.025	44.6	44.2
0.05	63.0	62.4
0.1	89.1	88.0
0.2	126.0	123.7
0.4	178.1	173.0
0.6	218.3	209.33
0.8	252.0	239.0
1.0	281.1	264.1
2.0	398.5	353.0
10.0	890.3	506.25

Table 2.5: Comparison of numerically and analytically determined wave speeds for different cell diffusion coefficients in the improved model.

unbounded, linearly increasing function of the EGF concentration, which contradicts these experimental results. We now derive a more realistic form for $s(c)$, using the experimentally determined results that as higher and higher concentrations of EGF are added to a healing wound, the rate of healing saturates, the speed being approximately $92 \mu\text{m hr}^{-1}$ for wounds 2 mm in radius. Our expression (2.18) for the speed of healing implies that the chemical control of mitosis term, $s(c)$, must also saturate to a nondimensional level of 135. We thus seek a function which is approximately linear for small c and saturates to a level of about 135 for large c . One simple function of the form is $s(c) = \frac{a_1 c}{a_2 + c} + a_3$; we evaluate a_1 , a_2 and a_3 using our fixed data points. As discussed in Chapter 1, $s(c)$ must satisfy the conditions $s(1) = 1$ and $s(0) = 0.1$, which implies that the function is

$$s(c) = \frac{135c}{149 + c} + 0.1. \quad (2.19)$$

Our nondimensional model equations become

$$\frac{\partial n}{\partial t} = \beta \frac{\partial^2 n}{\partial x^2} + s(c)n(2 - n) - n \quad (2.20a)$$

$$\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} + A + B(n) + q - \frac{\mu n c}{(\hat{c} + c)} - \delta c \quad (2.20b)$$

where $s(c)$ is defined in (2.19) and q is an external source term representing the topical application of EGF. Numerical simulations of (2.20) over an infinite domain evolve to travelling waves, the speed of which increases as we increase the external source term. The topical application of EGF simply alters the source term in equation (2.20b) and hence an analytical expression for the wave speed is

$$a_{crit} = \frac{2}{(\beta + D_c)} \sqrt{2\beta D_c^2 s \left(\frac{A + \sigma + q}{\delta} \right)} \quad (2.21)$$

where $s(c)$ is the saturating function defined in (2.19).

In Table 2.6 we compare the analytical speed calculated from (2.21) with the numerically simulated wave speed for different concentrations of EGF, corresponding to the topical application of EGF, and observe a strikingly good agreement, the

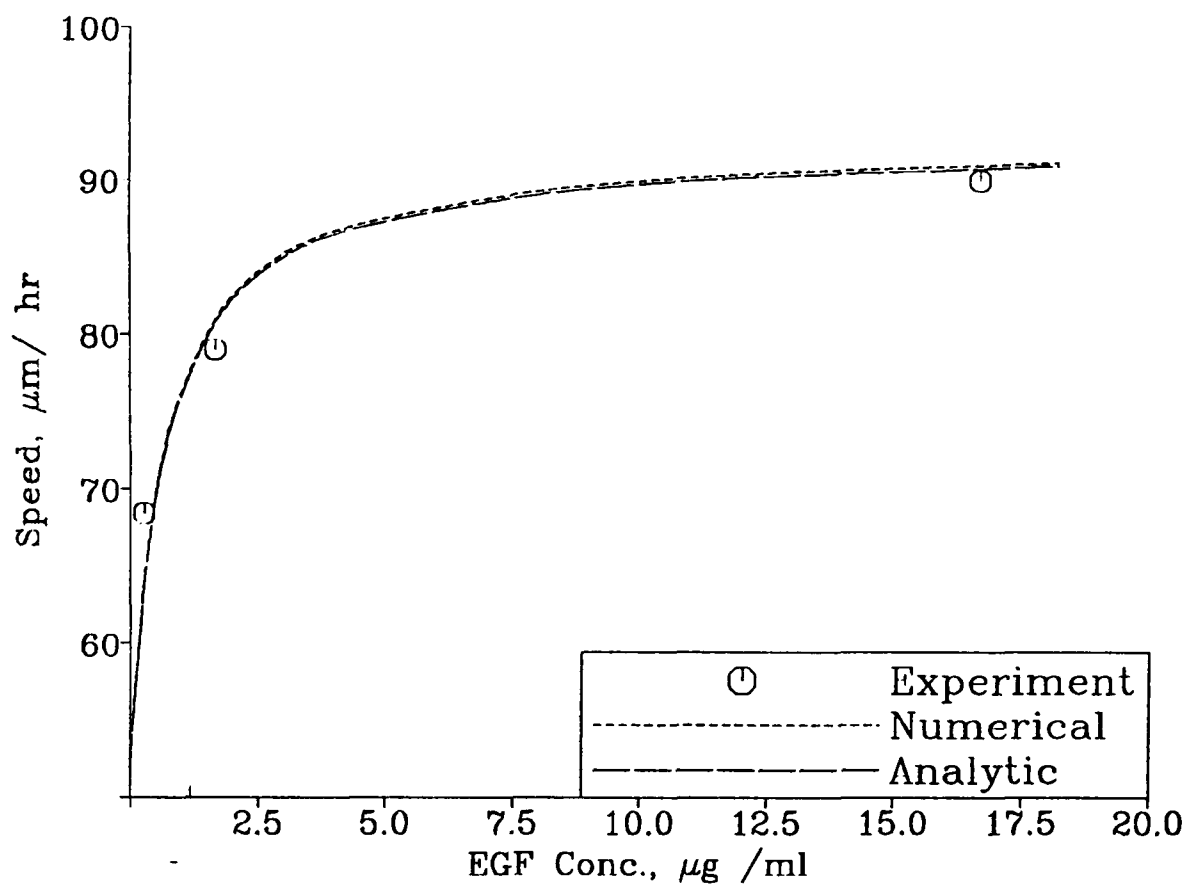


Figure 2.4: The change in the rate of healing of 2 mm corneal surface wounds with topical application of EGF. The rate saturates as we increase the concentration of EGF, in agreement with experimental results. Numerically simulated and analytically derived wave speeds compare favourably (see Table 2.6).

EGF conc., $\mu\text{g ml}^{-1}$	Numerically simulated speed, $\mu\text{m hr}^{-1}$	Analytical speed, $\mu\text{m hr}^{-1}$
0.36	66.31	66.5
0.73	73.01	73.26
1.81	81.45	81.65
2.73	84.35	84.55
3.63	86.03	86.23
9.09	89.56	89.78
18.18	90.92	91.14
27.27	91.39	91.61
36.36	91.63	91.84

Table 2.6: Numerically simulated and analytically calculated rates of healing for a 2mm corneal surface for topical application of different concentrations of EGF

error being less than 0.5%. Figure 2.4 illustrates these results and indicates the good correspondence with the experimentally determined rates of healing. Hence, using (2.21), we can predict the speed of healing of corneal surface wounds with the topical application of any concentration of EGF. This simple result could easily be tested by further experiments similar to those of Brazell *et al.* (1991) and Nishida *et al.* (1990).

2.5 The Effect of Curvature

It has been suggested that current analysis of corneal epithelial wound closure is misleading and inaccurate due to the simplicity of the models, particularly with respect to the validity of using planar kinematics to model the curved surface of the cornea (Kwok, 1991). In the model thus far we have only considered one-dimensional wounds and assumed that the corneal surface is flat. In this section, we take into account the curved surface of the cornea, formulate the model in spherical coordinates and analyse the resulting system to determine the effect of curvature on the speed of healing.

2.5.1 Formulation in Spherical Coordinates

We suppose, for simplicity, that the eyeball is a sphere and introduce coordinates (r, θ, ϕ) for radius r , polar angle measured from the top of the sphere θ , and azimuthal angle ϕ . Since the thickness of the epithelium is very small compared to the radius of the eyeball, we consider solutions independent of r and ϕ . This is equivalent to considering the movement of cells and chemical which are confined to the surface of a rigid sphere, with nondimensional radius R (Figure 2.5). We therefore study a problem where the model variables are solutions of θ and t only. In order to obtain the diffusion terms in (1.5), we require the Laplacian in spherical polars, namely

$$\nabla^2 u = \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial u}{\partial r} \right) + \frac{1}{r^2 \sin \theta} \frac{\partial}{\partial \theta} \left(\sin \theta \frac{\partial u}{\partial \theta} \right) + \frac{1}{r^2 \sin^2 \theta} \frac{\partial^2 u}{\partial \phi^2}.$$

Since there are no r and ϕ variations, the model equations in spherical coordinates are

$$\frac{\partial n}{\partial t} = \frac{\beta}{R^2} \left(\frac{\partial^2 n}{\partial \theta^2} + \cot \theta \frac{\partial n}{\partial \theta} \right) + s(c)n(2-n) - n \quad (2.22a)$$

$$\frac{\partial c}{\partial t} = \frac{D_c}{R^2} \left(\frac{\partial^2 c}{\partial \theta^2} + \cot \theta \frac{\partial c}{\partial \theta} \right) + f(n) - \frac{\mu n c}{\hat{c} + c} - \delta c. \quad (2.22b)$$

We impose zero flux boundary conditions at the wound edge and centre, consider half the wound domain, the polar angle measured from the top of the sphere thus being defined for $0 < \theta \leq \pi/2$. A key feature of these equations is the presence of the polar angle θ via the $\cot \theta$ terms. We note that near the equator, $\theta = \pi/2$, the $\cot \theta$ terms disappear, and our system becomes similar to the linear case, indicating that the assumption that the eyeball is spherical breaks down near this point. Furthermore, the $\cot \theta$ term tends to infinity as θ tends to 0, although this is compensated for by the conditions $n_\theta = c_\theta = 0$ at $\theta = 0$. Note, however, that our model is actually invalid for the final stages of healing, as discussed previously.

2.5.2 Numerical Simulations

We solve the system (2.22) numerically using the NAG routine D03PGF. To implement this scheme, we expand the $\cot \theta$ term in the form

$$\cot \theta = \frac{1}{\theta} - \frac{\theta}{3} - \frac{\theta^3}{45} - \dots - \frac{2^{2n} B_n \theta^{2n-1}}{(2n)!} - \dots, \quad 0 < \theta < \pi$$

where B_n is the Bernoulli number. The system of equations can thus be approximated by

$$\frac{\partial n}{\partial t} = \frac{\beta}{R^2} \left(\frac{\partial^2 n}{\partial \theta^2} + \frac{1}{\theta} \frac{\partial n}{\partial \theta} \right) + s(c)n(2-n) - n - \frac{\beta}{R^2} \left(\frac{\theta}{3} + \frac{\theta^3}{45} \right) \frac{\partial n}{\partial \theta} \quad (2.23a)$$

$$\frac{\partial c}{\partial t} = \frac{D_c}{R^2} \left(\frac{\partial^2 c}{\partial \theta^2} + \frac{1}{\theta} \frac{\partial c}{\partial \theta} \right) + f(n) - \frac{\mu n c}{\hat{c} + c} - \delta c - \frac{D_c}{R^2} \left(\frac{\theta}{3} + \frac{\theta^3}{45} \right) \frac{\partial c}{\partial \theta} \quad (2.23b)$$

valid for $0 < \theta < \pi/2$. For the purpose of numerical simulations, we assume that the wound edge occurs at $\pi/2$ and the wound centre at $\theta = 0$ and solve at equal

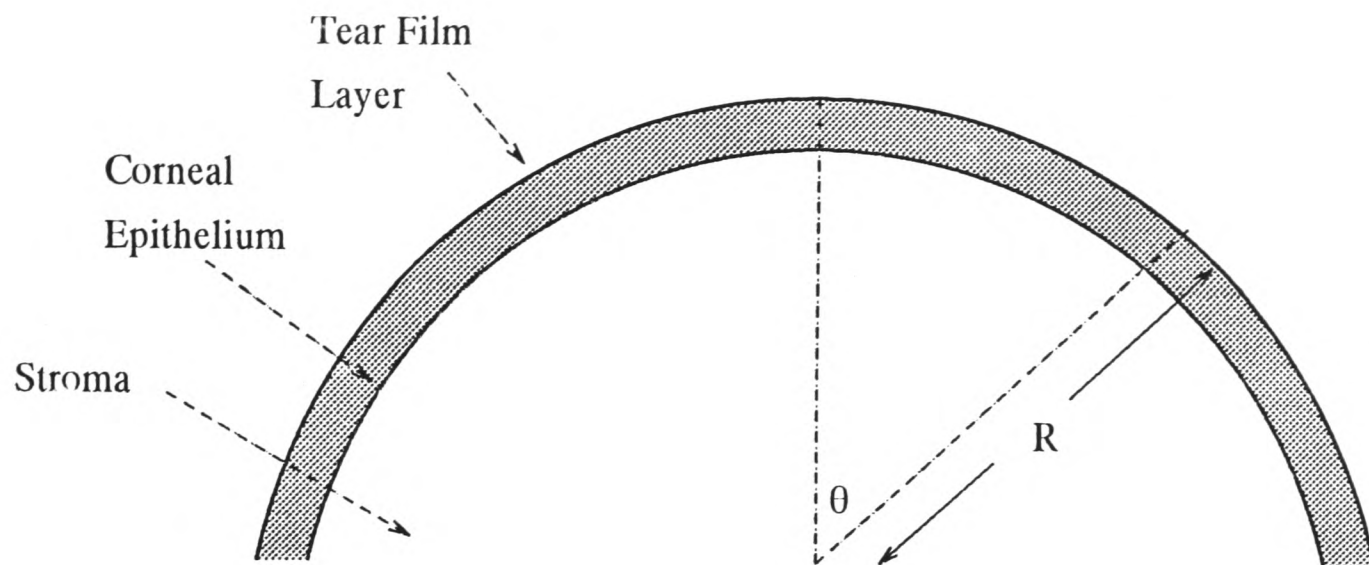


Figure 2.5: Pictorial representation of the eyeball as a sphere., showing the epithelium of negligible thickness in comparison with the eyeball radius.

time intervals. For the model parameters we are using (Table 1.1), we observe that there is a long transient time for evolution to a wave profile, but the restriction on the polar angle θ prevents us from extending to an infinite domain. To obtain a qualitative understanding of the effects of curvature on the wave speed, we thus reduce the time for transition to wave profiles by decreasing the diffusion coefficients. This is equivalent to extending our domain for the linear case. Taking $\beta = 0.002$ and $D_c = 1$, the solutions consist of a front of cells and an associated wave of EGF propagating towards the wound centre (Figure 2.6). Since the curves are plotted at equal time intervals, it is clear that, after an initial rapid increase, the speed of the wave increases monotonically as θ decreases but this increase is very small. The numerical simulations thus imply that the speed of healing increases slightly as the wound closes. We will now derive an analytical expression for the wave speed in terms of the model parameters and θ to investigate exact relationship between the speed and the polar angle.

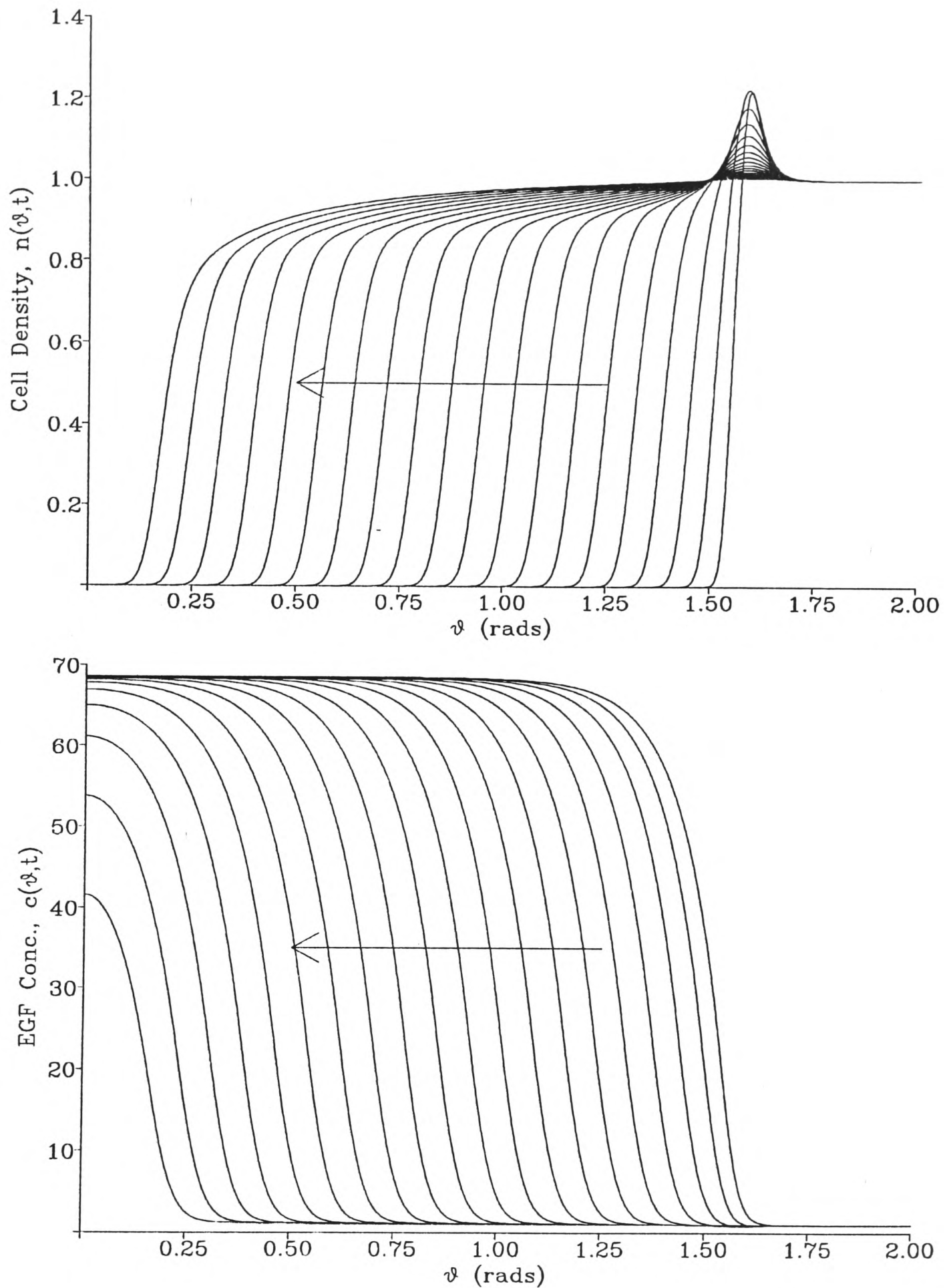


Figure 2.6: Numerical solution of (2.23) over a finite domain, with $\pi/2$ representing the wave edge and 0 the wound centre. The solutions evolve to travelling waves whose speed increases with decreasing values of θ . We take $\beta = 0.002$ and $D_c = 1$ to overcome the restrictions of the wound domain (see text for details).

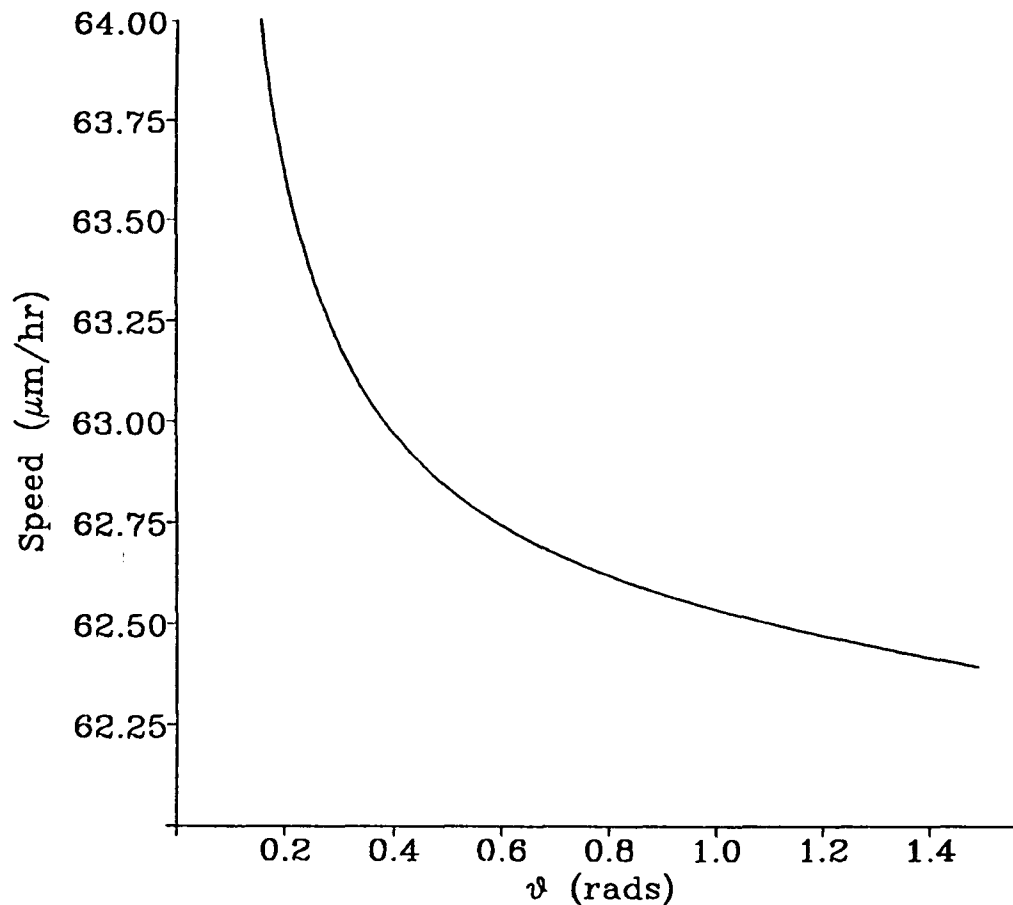


Figure 2.8: The analytically determined rate of healing for a 2mm wound over the curved corneal surface. The speed increases as the wound closes but for $\theta > 20^\circ$, the change is less than 1%.

space and find a bound on the wave speed by considering the change in the nature of the steady state from a spiral to prevent unrealistic, negative values. Numerical evaluation of the eigenvalues suggests a wave speed which compares favourably with the numerically simulated speed. Furthermore, we have derived an analytical approximation for the wave speed in terms of the model parameters and hence determined the key parameters to be the cell diffusion coefficient, β and the chemical source term $f(n)$. In Section 2.4, we have amended the model to include the saturating effect of the chemical control of mitosis and thus determined the rates of healing for the topical application of EGF. Finally, we have taken into account the curved surface of the cornea and, by solving in spherical co-ordinates, have shown that the increase in wave speed with polar angle is small, thus indicating that the linear model is sufficient for experimentally testable predictions for rates of healing.

Chapter 3

Analytical Solutions of the Reduced System

3.1 Introduction

In this chapter, we look at methods of determining analytical solutions to a single reaction diffusion equation for cell density (3.1), with the chemical concentration taken to be in equilibrium. Perturbation theory yields an ordinary differential equation for the first order term, which we are unable to solve due to the complicated nature of the kinetic term. We can, however, use this asymptotic solution to determine the slope of the wave front (3.8). It is the form of the kinetic term which causes problems for analytical work, and since some work has been done previously on single reaction diffusion with piecewise linear kinetics (Crank, 1956; Ockendon and Taylor, 1985; Grindrod, 1991), we consider a piecewise linear approximation. We first use travelling wave coordinates and determine a smooth, continuous solution with general kinetics (3.13), before considering the improved model kinetics (3.12). We then look at techniques for solving the partial differential equation explicitly, using Laplace and Fourier transform methods. The solutions obtained are in terms of inversion formulae and are thus of little practical value. However, in the Laplace transform case we are able to show the consistency between these solutions and those derived in travelling wave form (3.35).

3.2 Model Equation and Numerical Simulations

In travelling wave coordinates, equation (2.4) is transformed to a fourth order system of coupled, nonlinear ordinary differential equations and is thus difficult to analyse globally. Hence we consider an approximation to (1.5) which reduces the order of the system. The production and decay of EGF occur on a considerably faster time scale than diffusion and we thus assume that the EGF concentration is in equilibrium. The single partial differential equation resulting from this approximation is

$$\frac{\partial n}{\partial t} = \beta \frac{\partial^2 n}{\partial x^2} + g(n) \quad (3.1)$$

where

$$\begin{aligned} g(n) &= \frac{1}{2\delta} ((1-b)m(n) + b)n(2-n) - n, \\ m(n) &= (A + B(n) - \mu n - \delta\hat{c}) + \sqrt{(\mu n + \delta\hat{c} - A - B(n))^2 + 4(A + B(n))\delta\hat{c}} \end{aligned} \quad (3.2)$$

Hence we have reduced our two partial differential equations to a single equation of the form (2.1). Kolmogorov *et al.* (1937) showed that for a system of the form (3.1), initial conditions having compact support evolve to the travelling wave solution with wavespeed $a = 2\sqrt{\beta g'(0)}$. Using our model parameters, this wave speed is approximately 5.17, which corresponds to a dimensional wave speed of $65\mu\text{m hr}^{-1}$. This compares very well with the wave speed $64\mu\text{m hr}^{-1}$ found in the numerical solutions of the partial differential equation system (1.10). Numerical simulations for the reduced system over an infinite domain (Figure 3.1) show evolution to travelling wave solutions with a speed which is approximately equal to $2\sqrt{\beta g'(0)}$. The wave profiles have a constant shape, which differs from that obtained when the chemical is not in equilibrium.

3.2.1 First Approximation

We now use perturbation theory to derive an analytical approximation to the travelling wave solution of the reduced model (3.1). The analysis is analogous to that

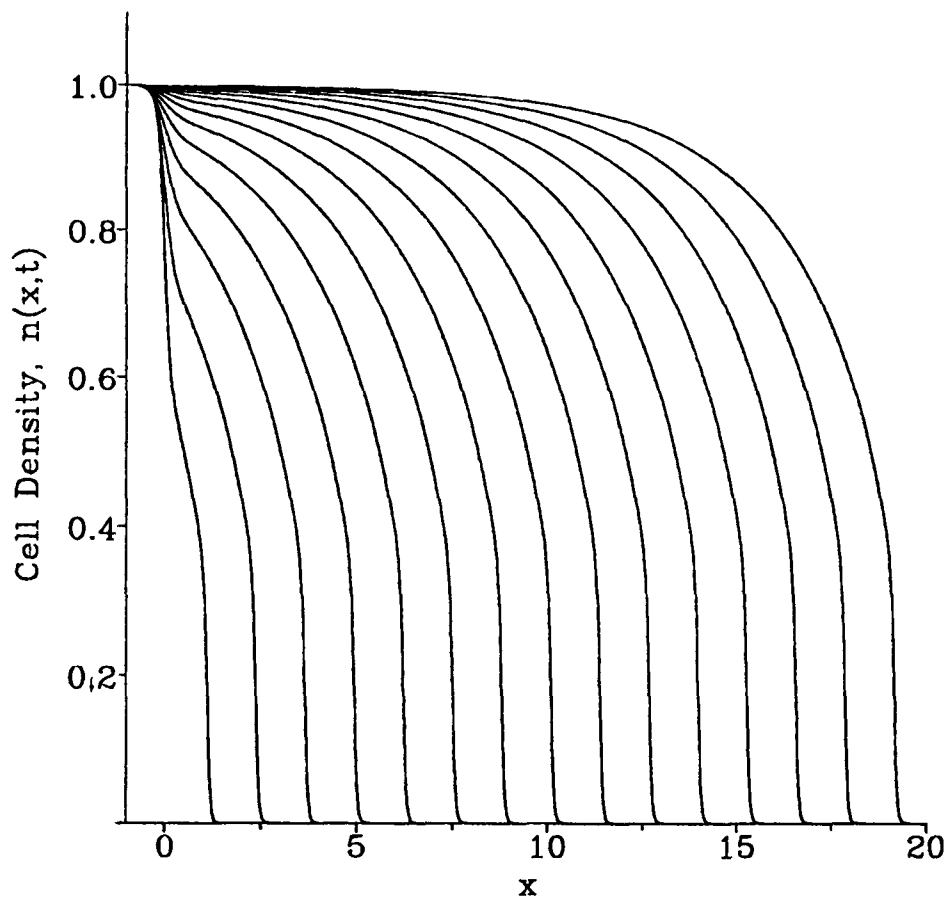


Figure 3.1: Travelling wave solutions for the simplified system (3.1), showing the evolution of cell density at equal time intervals of 31.6 hours, over an infinite domain.

for the Fisher equation (Canosa, 1973). The travelling wave satisfies the boundary value problem

$$N'' + \frac{a}{\beta}N' + \frac{1}{\beta}g(N) = 0 \quad (3.3)$$

subject to $N(-\infty) = 1$ and $N(\infty) = 0$; $g(N)$ is defined by (3.2). The system (3.3) has singular points $N = N' = 0$ and $N = 1, N' = 0$. The eigenvalues are $(-a \pm (a^2 - 4\beta g'(N))^{1/2})/2\beta$ for $N = 0$ and $N = 1$ and, as discussed previously, we obtain the critical wave speed, $a_{crit} = 2\sqrt{\beta g'(0)}$. Rescaling the independent variable, $\zeta = z/a$, in (3.3) gives

$$\epsilon N'' + N' + g(N) = 0 \quad (3.4)$$

where the prime denotes $d/d\zeta$ and $\epsilon = \beta/a^2 = 1.87 \times 10^{-3}$ for our model parameters. Since ϵ multiplies the highest derivative, equation (3.4) looks like the standard singular perturbation problem. However, the nonlinear term $g(N)$ vanishes at $N = 0$ and $N = 1$ and the reduced equation gives a uniformly valid first order approxi-

mation. We can thus consider (3.4) as a regular perturbation problem in the small parameter ϵ by looking for a solution of the form

$$N(\zeta; \epsilon) = N_0(\zeta) + \epsilon N_1(\zeta) + \epsilon^2 N_2(\zeta) + \dots$$

Substituting this into (3.4) and equating coefficients of powers of ϵ gives

$$N'_0 = -g(N_0) \quad (3.5a)$$

$$N'_1 = -N_1 \frac{dg(N_0)}{dN_0} - N''_0 \quad (3.5b)$$

$$\cdot = \cdot$$

$$\cdot = \cdot$$

subject to $N_0(-\infty) = -1$, $N_0(0) = k$, $N_0(\infty) = 0$, and $N_i(-\infty) = N_i(0) = N_i(\infty) = 0$ for all $i \geq 1$. We must specify k to give a unique solution; we choose $k = 1/2$.

Integrating (3.5a) gives

$$\zeta = \int_k^{N_0} -\frac{1}{g(\xi)} d\xi = \int_k^{N_0} \frac{1}{\xi(1 - (2 - \xi)s(\xi))} d\xi. \quad (3.6)$$

where

$$s(N) = \frac{m(N)}{2\delta}$$

The square root in the denominator prevents an analytical evaluation so we attempt to approximate this term by writing

$$s(N) = \frac{1}{2\delta} [(A + B(N) - \mu N - \delta\hat{c}) + (\mu N + \delta\hat{c} - A - B(N)) \sqrt{1 + \frac{4(A + B(N))\delta\hat{c}}{(\mu N + \delta\hat{c} - A - B(N))^2}}].$$

The square root term can be expanded binomially, resulting in the approximation

$$g(N) = \frac{N(A + B(N) + 2(A + B(N))\hat{c} - \delta\hat{c} - N((A + B(N))\hat{c} + \mu))}{(\mu N + \delta\hat{c} - A - B(N))} \quad (3.7)$$

which agrees well with (3.2) provided N is greater than about 0.4 (Figure 3.2). This can be integrated using partial fractions and the first order approximation for cell

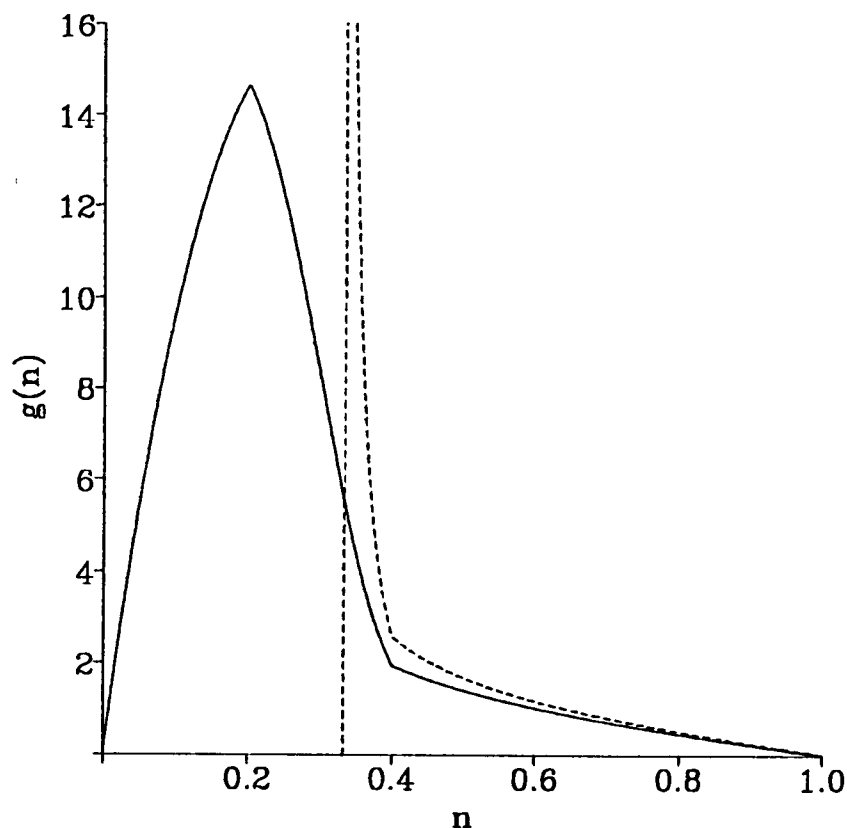


Figure 3.2: The kinetic term, $g(n)$ (solid line) and the approximation (dotted line), obtained a binomial expansion of the square root term (3.7). We note the good agreement for $N > 0.4$. The solid line is not smooth because of the piecewise linear form of $B(n)$.

densities greater than 0.4 is determined to be

$$\zeta = \frac{(A - \delta\hat{c})}{(A + 2A\hat{c} - \delta\hat{c})} \log\left(\frac{N_0}{k}\right) + \frac{A\hat{c}(2\mu - A + \delta\hat{c})}{(A\hat{c} + \mu)(2A\hat{c} + A - \delta\hat{c})} \log\left[\frac{N_0 - \frac{(A+2A\hat{c}-\delta\hat{c})}{(A\hat{c}+\mu)}}{k - \frac{(A+2A\hat{c}-\delta\hat{c})}{(A\hat{c}+\mu)}}\right].$$

The discontinuity in $s(N)$ prevents us from using another binomial expansion to approximate the function for small cell densities and we thus resort to piecewise linear approximations, as discussed in Section 3.3.

The first approximation to the solution can be used to investigate the dependence of the slope of the wave front on the model parameter values. Since the gradient is negative everywhere, a measure of the steepness, $\hat{s}$, is the magnitude of the maximum of the gradient $N'(z)$ at the point of inflexion of the wave front. Using (3.5), the

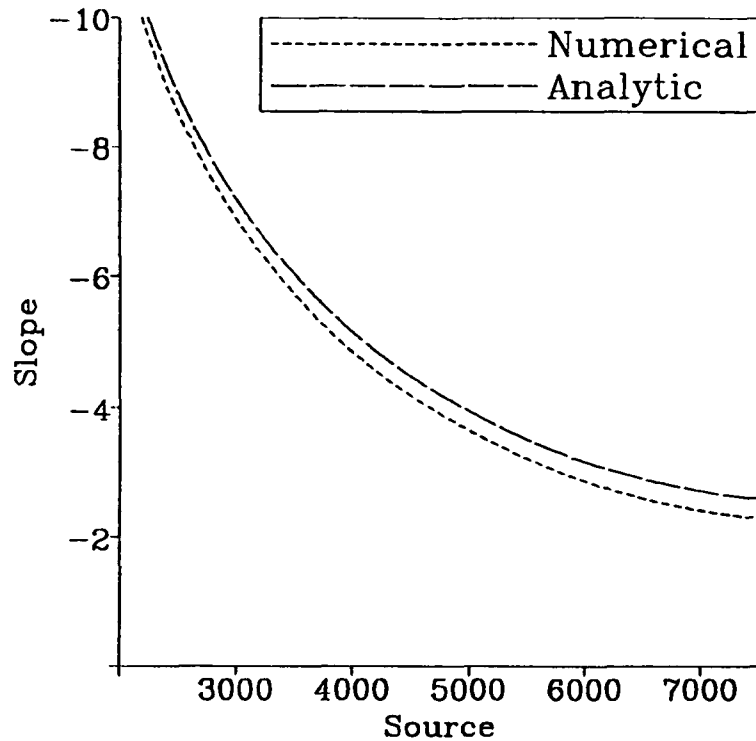


Figure 3.3: The change in steepness of slope as we increase the source term, σ . The wave front becomes less steep with increasing EGF concentration (corresponding to increasing wave speed). The discrepancy between the analytically determined wave slope and the steepness of the numerically simulated waves is due to our approximation that the derivative terms in the EGF equations are negligible compared to the reaction terms.

gradient at $N = 0.2$ gives

$$\hat{s} = -\frac{g(0.2)}{a}, \quad (3.8)$$

which implies that faster waves have a less steep wavefront. Using (3.2), we find that the steepness depends crucially on the source term. In Figure 3.3 we plot the change in slope with increasing concentration of EGF; there is a good correspondence between the steepness of the numerically simulated wave front and the analytically determined slope.

3.2.2 Width of the Wave in Spherical Coordinates

In Section 2.5.3, we derived an analytical expression (2.27) for the speed of healing, in terms of the model parameters. The expression suggests that the wave speed increases slightly with decreasing polar angle θ and it applies while θ is sufficiently large that the wavefront is a distance greater than the width of the wave away from $\theta = 0$, the wound centre. A practical measure of the width of the wave, W , is simply the inverse of the steepness, $\hat{s}$. We thus determine the width of the wave in order to examine the minimum value of θ beyond which the analytical solution breaks down. We have derived an expression for the steepness in Cartesian coordinates, and this result can easily be extended to polars. In travelling wave form, the second order differential equation for the cell density is given by

$$\beta N'' + \left(a + \frac{\beta}{R} \cot \theta \right) N' + g(N) = 0 \quad (3.9)$$

where prime denotes d/dz and $g(N)$ is as shown in (3.2). We note that this equation is the same as (3.3) with the wave speed a replaced by $a + \frac{\beta}{R} \cot \theta$, for each fixed θ . By rescaling the independent variable, $\zeta = z/a$, and solving the equations as a regular perturbation problem as discussed in the previous section, we obtain the leading order term

$$N'_0 = -\frac{g(N_0)}{(1 + \frac{\beta}{aR} \cot \theta)}.$$

The width of the wave is thus given by

$$W = \frac{a(1 + \frac{\beta}{aR} \cot \theta)}{g(k)} \quad (3.10)$$

where the wave speed a is defined by (2.27) and k is taken to be 0.2. By substituting in the model parameters, we find that the width of the wave is

$$W = 0.002 \cot \theta + 0.426.$$

Hence, the analytical expression is valid provided

$$\theta > 5^\circ \quad (3.11)$$

and the maximum speed is thus $a_{max} = 65.2 \mu\text{mhr}^{-1}$.

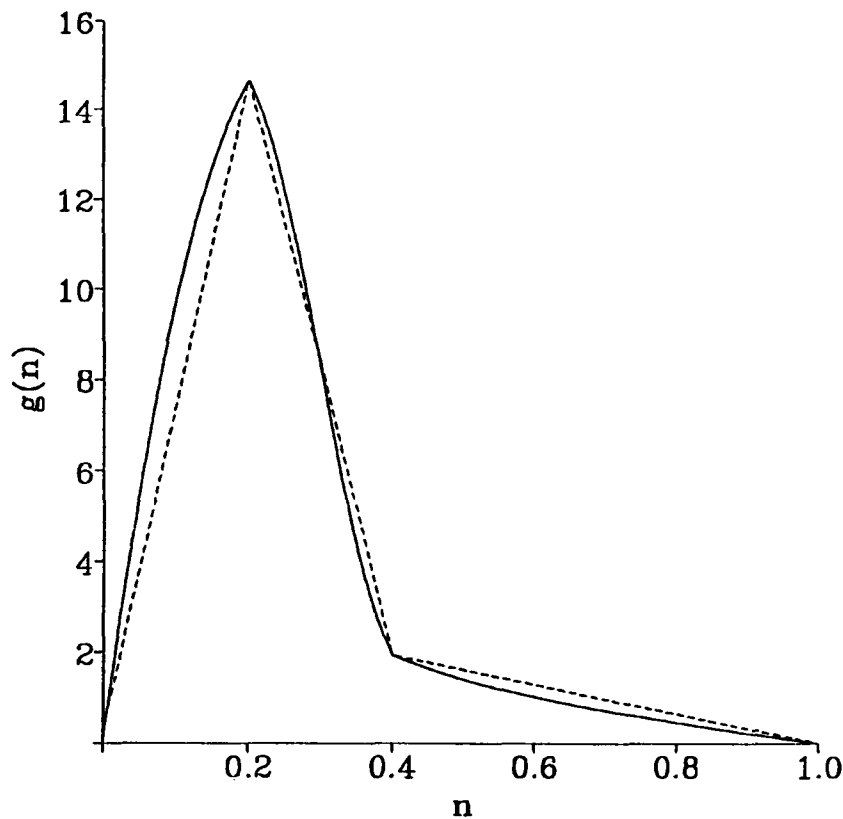


Figure 3.4: The kinetic term $g(n)$ (solid line), and the piecewise linear approximation (dotted line).

3.3 Piecewise Linear Approximation in Travelling Wave Coordinates

In this section, we find a solution for the cell density in travelling wave coordinates, by considering a piecewise linear approximation to the kinetic term $g(n)$. By plotting $g(n)$ against n (Figure 3.4), we simply consider the gradients of straight line approximations to the curves, for given intervals of n , to derive linear approximations of the following form

$$g(n) = \begin{cases} 133n & \text{if } n < 0.005 \\ 71.95n + 0.25 & \text{if } 0.005 \leq n \leq 0.2 \\ 27.33 - 63.47n & \text{if } 0.2 \leq n \leq 0.4 \\ 3.24(1 - n) & \text{if } n > 0.4 \end{cases} \quad (3.12)$$

making sure that the gradients match near the origin, which is crucial for correct wave speeds. Numerical simulations of the reduced system with our piecewise linear approximation, over an infinite domain, evolve to travelling waves whose constant speed and shape agree well with the solutions for the actual function $g(n)$ given in

(3.2). We thus take advantage of the linear kinetics and look for analytical solutions in travelling wave coordinates.

3.3.1 General Solutions in Travelling Wave Coordinates

We consider the general piecewise linear approximation

$$g(n) = \begin{cases} \gamma_1 n & \text{if } 0 < n < q_1 \\ \gamma_2 n + \gamma_3 & \text{if } q_1 \leq n \leq q_2 \\ \gamma_4 - \gamma_5 n & \text{if } q_2 \leq n \leq q_3 \\ \gamma_6(1 - n) & \text{if } q_3 < n < 1 \end{cases} \quad (3.13)$$

with travelling wave solution as shown in Figure 3.5. In travelling wave coordinates, we have

$$\beta N'' + aN' + g(N) = 0. \quad (3.14)$$

Solving (3.14) in the four separate regions, we determine solutions of the form

$$n(z) = \begin{cases} n_1(z) = \alpha_1 e^{\lambda_1 z} + \alpha_2 e^{\lambda_2 z} & \text{if } z > z_3 \\ n_2(z) = \alpha_3 e^{\lambda_3 z} + \alpha_4 e^{\lambda_4 z} - \frac{\gamma_3}{\gamma_2} & \text{if } z_2 < z < z_3 \\ n_3(z) = \alpha_5 e^{\lambda_5 z} + \alpha_6 e^{\lambda_6 z} - \frac{\gamma_4}{\gamma_5} & \text{if } z_1 < z < z_2 \\ n_4(z) = \alpha_7 e^{\lambda_7 z} + \alpha_8 e^{\lambda_8 z} + 1 & \text{if } z < z_1 \end{cases} \quad (3.15)$$

where

$$\begin{aligned} \lambda_{1,2} &= \frac{1}{2\beta}(-a \pm \sqrt{a^2 - 4\gamma_1\beta}) & \lambda_{3,4} &= \frac{1}{2\beta}(-a \pm \sqrt{a^2 - 4\gamma_2\beta}) \\ \lambda_{5,6} &= \frac{1}{2\beta}(-a \pm \sqrt{a^2 + 4\gamma_5\beta}) & \lambda_{7,8} &= \frac{1}{2\beta}(-a \pm \sqrt{a^2 + 4\gamma_6\beta}). \end{aligned}$$

Without loss of generality, we can assume that $z_1 = 0$, corresponding to a simple translation in z . Since we require a smooth, continuous solution, we have the conditions

$$n_1 = n_2 = q_1, \quad \frac{dn_1}{dz} = \frac{dn_2}{dz} \quad \text{at } z = z_3$$

with similar conditions at z_2 and 0. We also require $n_4(z) \rightarrow 1$ as $z \rightarrow -\infty$. After much algebraic manipulation, we can derive the following expressions for the coefficients α_i in terms of q_i and the eigenvalues λ_i

$$\alpha_8 = q_3 - 1 \quad (3.16a)$$

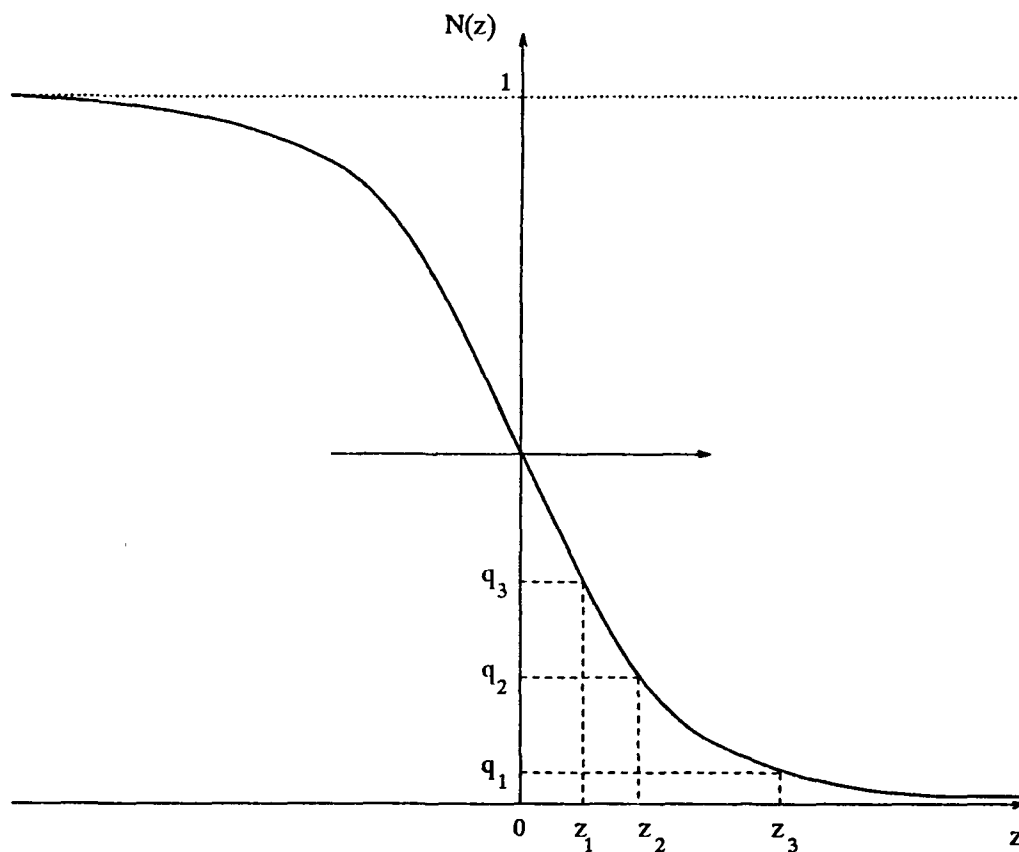


Figure 3.5: Travelling wave form for a general piecewise linear approximation (3.13) to the kinetic term (see text for details).

$$\alpha_7 = 0 \quad (3.16b)$$

$$\alpha_6 = \frac{q_3(\lambda_8 - \lambda_5) + \frac{\lambda_5 \gamma_4}{\gamma_5} - \lambda_8}{\lambda_6 - \lambda_5} \quad (3.16c)$$

$$\alpha_5 = \frac{q_3(\lambda_8 - \lambda_6) + \frac{\lambda_6 \gamma_4}{\gamma_5} - \lambda_8}{\lambda_5 - \lambda_6} \quad (3.16d)$$

$$\alpha_4 = \frac{e^{-\lambda_4 z_2}}{\lambda_4 - \lambda_3} \left(\lambda_5 \alpha_5 e^{\lambda_5 z_2} + \lambda_6 \alpha_6 e^{\lambda_6 z_2} - \lambda_3 (q_2 + \gamma_3/\gamma_2) \right) \quad (3.16e)$$

$$\alpha_3 = \frac{e^{-\lambda_3 z_2}}{\lambda_3 - \lambda_4} \left(\lambda_5 \alpha_5 e^{\lambda_5 z_2} + \lambda_6 \alpha_6 e^{\lambda_6 z_2} - \lambda_4 (q_2 + \gamma_3/\gamma_2) \right) \quad (3.16f)$$

$$\alpha_2 = \frac{e^{-\lambda_2 z_3}}{\lambda_2 - \lambda_1} \left(\lambda_3 \alpha_3 e^{\lambda_3 z_3} + \lambda_4 \alpha_4 e^{\lambda_4 z_3} - \lambda_1 q_1 \right) \quad (3.16g)$$

$$\alpha_1 = \frac{e^{-\lambda_1 z_3}}{\lambda_1 - \lambda_2} \left(\lambda_3 \alpha_3 e^{\lambda_3 z_3} + \lambda_4 \alpha_4 e^{\lambda_4 z_3} - \lambda_2 q_1 \right) \quad (3.16h)$$

where z_2 and z_3 satisfy the transcendental equations

$$\alpha_5 e^{\lambda_5 z_2} + \alpha_6 e^{\lambda_6 z_2} = (q_2 - \gamma_4/\gamma_5) \quad (3.17a)$$

$$\alpha_3 e^{\lambda_3 z_3} + \alpha_4 e^{\lambda_4 z_3} = (q_1 + \gamma_3/\gamma_2), \quad (3.17b)$$

which may need to be solved numerically. Hence we have a smooth, continuous solution for $n(z)$ in terms of the wave speed and the slope of the linear approximations.

Solution for the improved model

We now use the above formulae to derive an analytical expression for the wave form of the improved model with the particular kinetic term (3.12). This yields the eigenvalues

$$\lambda_1 = -55.29 \quad \lambda_2 = -48.10 \quad \lambda_3 = -86.8 \quad \lambda_4 = -16.57$$

$$\lambda_5 = -114.49 \quad \lambda_6 = 11.09 \quad \lambda_7 = -104 \quad \lambda_8 = 0.62 \quad .$$

After much algebraic manipulation, the solutions are found to be

$$n(z) = \begin{cases} n_1(z) = -14786337e^{-55.29z} + 1346947e^{-48.10z} & \text{if } z > 0.3759 \\ n_2(z) = 4.2966e^{-16.57z} - 75811.15e^{-86.8z} - 0.00347 & \text{if } 0.1807 < z < 0.3759 \\ n_3(z) = -0.031e^{11.09z} + 0.00026e^{-114.49z} + 0.43 & \text{if } 0 < z < 0.1807 \\ n_4(z) = 1 - 0.6e^{0.62z} & \text{if } z < 0 \end{cases} \quad (3.18)$$

Comparison of this solution with the exact solution (Figure 3.6) indicates a very close agreement for $n < 0.4$ but a small error for $n > 0.4$. This can be explained by considering the approximation (Figure 3.4), and noting that in deriving the solutions, we divide any error in our approximation by the slope of the line. The maximum error occurs for $0 < n < 0.4$, but a large slope results in negligible error in the solution whereas, for $0.4 < n < 1$ the slope is less steep and the error is more marked. In general, piecewise linear approximations give rise to solutions which compare favourably with the true solutions provided the error in approximation is small or the line gradients are steep.

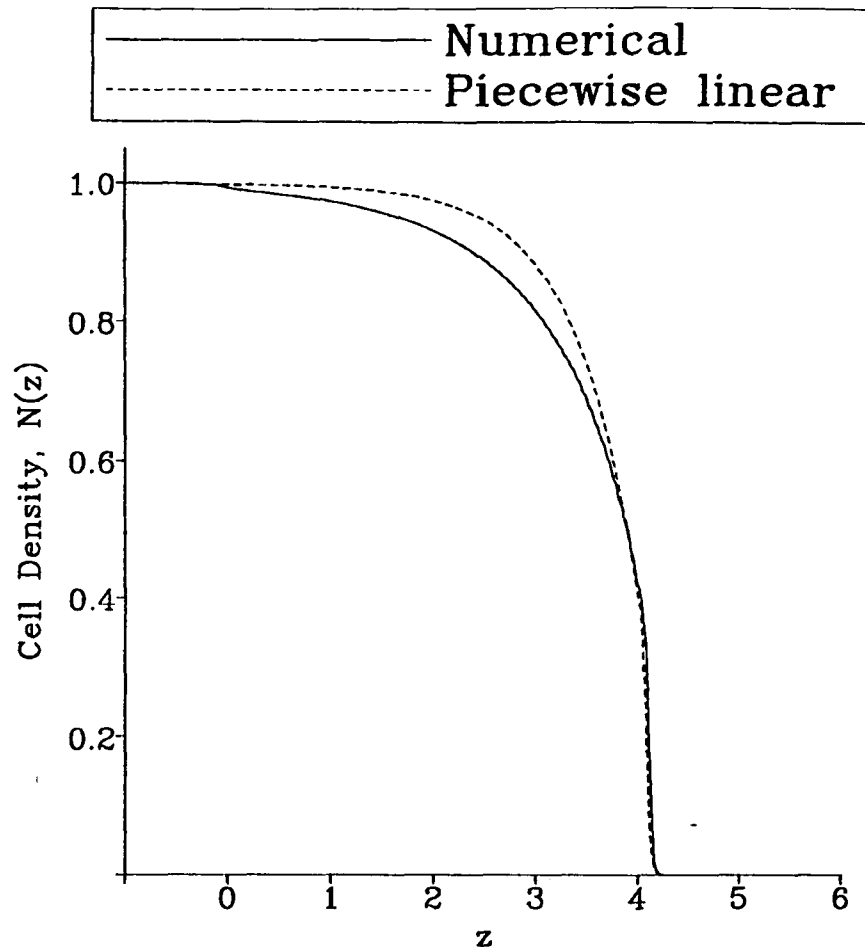


Figure 3.6: Comparison of numerically simulated solution and the travelling wave solution derived from the piecewise linear approximation (3.12) to the kinetic term (3.2).

3.4 Analytical Solutions - Piecewise Linear Approximations

We have shown that travelling wave solutions to equation (3.1) with piecewise linear approximations can be found analytically. In this section, we exploit the linearity of the kinetic terms and attempt to solve the partial differential equation to obtain an expression for the cell density as a function of space and time. We consider a simplified kinetic term of the form

$$g(n) = \begin{cases} \frac{\alpha n}{\gamma} & \text{if } 0 < n < \gamma \\ \frac{\alpha}{1-\gamma}(1-n) & \text{if } \gamma \leq n \leq 1 \end{cases}, \quad (3.19)$$

illustrated in Figure (3.7), but the analysis could easily be extended to the term shown in equation (3.12).

Suppose that at time t the discontinuity in kinetics occurring at $n = \gamma$ is at

$x = X(t)$, a function of t which needs to be determined. Without loss of generality, we choose $X(0) = 0$. At time t , we let the cell density in the region $x > X(t)$ be denoted by $n_1(x, t)$, and in the region $x < X(t)$ by $n_2(x, t)$. At the discontinuity, both the cell densities n_1 , n_2 and the spatial gradients ($f(t)$, to be determined), must match, so we have

$$n_1 = n_2 = \gamma, \quad x = X(t) \quad (3.20)$$

$$\frac{\partial n_1}{\partial x} = \frac{\partial n_2}{\partial x} = f(t), \quad x = X(t). \quad (3.21)$$

In the region $0 < n < \gamma$, we have to satisfy

$$\frac{\partial n_1}{\partial t} = \beta \frac{\partial^2 n_1}{\partial x^2} + \frac{\alpha n_1}{\gamma}, \quad x > X(t), \quad (3.22)$$

with initial conditions

$$n_1(x, 0) = g_1(x),$$

whereas for $\gamma < n < 1$ we have

$$\frac{\partial n_2}{\partial t} = \beta \frac{\partial^2 n_2}{\partial x^2} + \frac{\alpha}{1 - \gamma}(1 - n_2), \quad x < X(t), \quad (3.23)$$

with initial conditions

$$n_2(x, 0) = g_2(x).$$

Both (3.22) and (3.23) are linear partial differential equations, which can thus be solved using standard methods. Specifically, we look for solutions of the form $n_i(x, t) = e^{\lambda_i t} \phi_i(x, t)$, $i = 1, 2$ and substitute into the above equations. By choosing λ_i appropriately, we can reduce equations (3.22) and (3.23) to diffusion equations for ϕ_i , with coefficient β . Many different methods for solving the diffusion equation have been investigated, including separation of variables, Green functions and transform methods. In his book, “Mathematics of Diffusion”, Crank (1956) deals with moving boundary problems in which the diffusion coefficients have a discontinuity at one density. However, the method employed is invalid for discontinuities in kinetics due to the presence of temporal exponential terms.

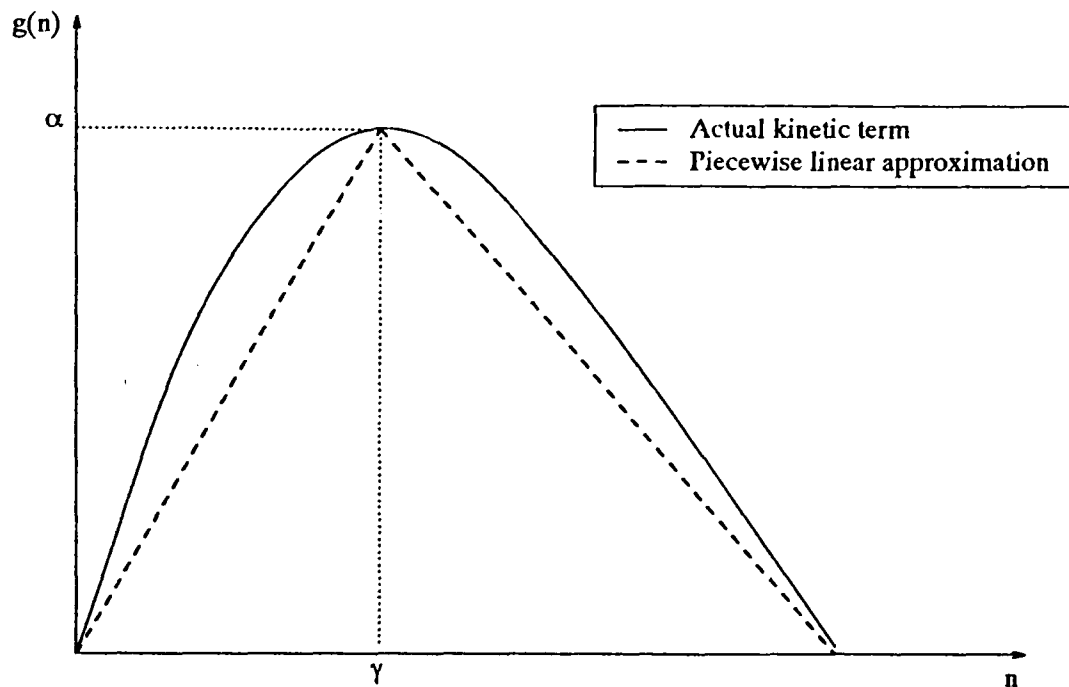


Figure 3.7: Piecewise linear approximation to a simplified kinetic term.

To solve equations (3.22) and (3.23), we first consider a Fourier transform and impose the initial conditions

$$n_1(x, 0) = \begin{cases} 1 + f_1(x) & \text{if } x < 0 \\ 0 & \text{if } x > 0 \end{cases} \quad n_2(x, 0) = \begin{cases} 1 & \text{if } x < 0 \\ f_2(x) & \text{if } x > 0 \end{cases} \quad (3.24)$$

since n_1 and n_2 are initially defined for $x > 0$ and $x < 0$, respectively. Using standard techniques in Fourier transform theory and the convolution theorem we determine the solution

$$n_1(x, t) = \frac{1}{2} e^{\frac{\alpha t}{\gamma}} \operatorname{erfc} \left(\frac{x}{2\sqrt{\beta t}} \right) + \frac{e^{\frac{\alpha t}{\gamma}}}{\sqrt{\pi}} \int_{-\infty}^{\frac{-x}{2\sqrt{\beta t}}} f_1(x + 2\theta\sqrt{\beta t}) e^{-\theta^2} d\theta \quad (3.25a)$$

$$n_2(x, t) = 1 - \frac{1}{2} e^{\frac{-\alpha t}{1-\gamma}} \operatorname{erfc} \left(\frac{-x}{2\sqrt{\beta t}} \right) + \frac{e^{\frac{-\alpha t}{1-\gamma}}}{\sqrt{\pi}} \int_{\frac{-x}{2\sqrt{\beta t}}}^{\infty} f_2(x + 2\theta\sqrt{\beta t}) e^{-\theta^2} d\theta, \quad (3.25b)$$

where $\operatorname{erfc}(x)$ is defined to be $\frac{2}{\sqrt{\pi}} \int_x^{\infty} e^{-u^2} du$. The functional forms of $f_1(x)$ and $f_2(x)$ are arbitrary and any further progress with the Fourier transform method requires knowledge of these forms. Even for a simple case, such as step function initial

conditions, $f_i = 0$, $i = 1, 2$, we find that a smooth, continuous solution cannot be attained using matching techniques. We thus consider a Laplace transform method.

3.4.1 Laplace Transform Method

Suppose $h(x)$ is a known function of space for positive values of x . Then the Laplace transform $\bar{h}(p)$ of $h(x)$ is defined as

$$\bar{h}(p) = \int_0^\infty e^{-px} h(x) dx.$$

We first apply this method to solve (3.22) for n_1 (i.e. in the region $x > X(t)$). By considering $n_1(x, t) = e^{\lambda_1 t} \phi_1(x, t)$ with $\lambda_1 = \alpha/\gamma$ and introducing a change of variables

$$y = x - X(t), \quad \tau = t$$

so that the discontinuity occurs at $y = 0$, the equation becomes

$$\frac{\partial \phi_1}{\partial \tau} - \dot{X} \frac{\partial \phi_1}{\partial y} = \beta \frac{\partial^2 \phi_1}{\partial y^2} \quad \text{valid for } y > 0, \quad (3.26)$$

where the dot represents $\partial/\partial \tau$. The boundary conditions are

$$\phi_1(0, \tau) = \gamma e^{-\lambda_1 \tau} \quad (3.27)$$

$$\frac{\partial \phi_1}{\partial y}(0, \tau) = f(\tau) e^{-\lambda_1 \tau} \quad (3.28)$$

with initial conditions

$$\phi_1(y, 0) = g_1(y). \quad (3.29)$$

We take the Laplace transform of the equation with respect to y , giving

$$\int_0^\infty \left(\frac{\partial \phi_1}{\partial \tau} - \dot{X} \frac{\partial \phi_1}{\partial y} \right) e^{-py} dy = \int_0^\infty \beta \frac{\partial^2 \phi_1}{\partial y^2} e^{-py} dy.$$

Using standard results for Laplace transforms, we obtain the following differential equation for $\bar{\phi}_1(p, \tau)$

$$\frac{\partial \bar{\phi}_1}{\partial \tau} - (\beta p^2 + p \dot{X}) \bar{\phi}_1 = -\dot{X} \phi_1(0, \tau) - \beta p \phi_1(0, \tau) - \beta \frac{\partial \phi_1}{\partial y}(0, \tau)$$

which can be solved using integrating factors and the transform of the initial condition, giving

$$\begin{aligned}\bar{\phi}_1(p, \tau) &= e^{-(\beta p^2 \tau + pX(\tau))} (\bar{g}_1(p) \\ &\quad - \int_0^\tau (p\beta\gamma + \beta f(\tau_1) + \gamma \dot{X}) e^{-(\beta p^2 \tau_1 + pX(\tau_1) + \lambda_1 \tau_1)} d\tau_1) \\ &= \frac{\gamma}{p} e^{-\lambda_1 \tau} + \left(\bar{g}_1(p) - \frac{\gamma}{p} \right) e^{(\beta p^2 \tau + pX(\tau))} \\ &\quad + e^{(\beta p^2 \tau + pX(\tau))} \int_0^\tau \left(\frac{\gamma \lambda_1}{p} - \beta f(\tau_1) \right) e^{-(\beta p^2 \tau_1 + pX(\tau_1) + \lambda_1 \tau_1)} d\tau_1.\end{aligned}$$

Hence we derive the solution for the transform $\bar{n}_1(p, \tau)$

$$\begin{aligned}\bar{n}_1(p, \tau) &= \frac{\gamma}{p} + \left(\bar{g}_1(p) - \frac{\gamma}{p} \right) e^{(\beta p^2 \tau + pX(\tau) + \lambda_1 \tau)} \\ &\quad + e^{(\beta p^2 \tau + pX(\tau) + \lambda_1 \tau)} \int_0^\tau \left(\frac{\gamma \lambda_1}{p} - \beta f(\tau_1) \right) e^{-(\beta p^2 \tau_1 + pX(\tau_1) + \lambda_1 \tau_1)} d\tau_1.\end{aligned}\tag{3.30}$$

To solve the equation (3.23) for $n_2(x, t)$, we write $n_2(x, t) = 1 - e^{\lambda_2 t} \phi_2(x, t)$, and, by choosing $\lambda_2 = \frac{-\alpha}{1-\gamma}$, we again find that ϕ_2 satisfies the diffusion equation. We introduce the change of variables

$$z = X(t) - x, \quad \tau = t$$

so that the boundary conditions hold at $z = 0$ and the equation is valid for $z > 0$.

The Laplace transform method yields the solution

$$\begin{aligned}\bar{\phi}_2(p, \tau) &= \frac{(1-\gamma)}{p} e^{-\lambda_2 \tau} + \left(\frac{1}{p} + \bar{g}_2(p) - \frac{(1-\gamma)}{p} \right) e^{(\beta p^2 \tau - pX(\tau))} \\ &\quad + e^{(\beta p^2 \tau - pX(\tau))} \int_0^\tau \left(\frac{(1-\gamma)\lambda_2}{p} - \beta f(\tau_1) \right) e^{-(\beta p^2 \tau_1 - pX(\tau_1) + \lambda_2 \tau_1)} d\tau_1\end{aligned}$$

and hence the solution for the transform $\bar{n}_2(p, \tau)$ is

$$\begin{aligned}\bar{n}_2(p, \tau) &= \frac{\gamma}{p} + \left(\bar{g}_2(p) - \frac{\gamma}{p} \right) e^{(\beta p^2 \tau - pX(\tau) + \lambda_2 \tau)} \\ &\quad - e^{(\beta p^2 \tau - pX(\tau) + \lambda_2 \tau)} \int_0^\tau \left(\frac{(1-\gamma)\lambda_2}{p} - \beta f(\tau_1) \right) e^{-(\beta p^2 \tau_1 - pX(\tau_1) + \lambda_2 \tau_1)} d\tau_1.\end{aligned}\tag{3.31}$$

From this, it is clear that analytical solutions for $n_1(x, t)$ and $n_2(x, t)$ can be determined by using the Inversion theorem for Laplace transforms and changing the variables back to x and t , giving

$$n_1(x, t) = \gamma + e^{\frac{\alpha t}{\gamma}} \frac{1}{2\pi i} \lim_{R \rightarrow \infty} \int_{\varrho - iR}^{\varrho + iR} e^{(\beta p^2 t + px)} \left(\bar{g}_1(p) - \frac{\gamma}{p} \right) dp \quad (3.32a)$$

$$+ e^{\frac{\alpha t}{\gamma}} \frac{1}{2\pi i} \lim_{R \rightarrow \infty} \int_{\varrho - iR}^{\varrho + iR} e^{(\beta p^2 t + px)} \left(\int_0^\tau \left(\frac{\alpha}{p} - \beta f(\tau_1) \right) e^{-(\beta p^2 \tau_1 + pX(\tau_1) + \lambda_1 \tau_1)} d\tau_1 \right) dp$$

$$n_2(x, t) = \gamma + e^{\frac{-\alpha t}{1-\gamma}} \frac{1}{2\pi i} \lim_{R \rightarrow \infty} \int_{\varrho - iR}^{\varrho + iR} e^{(\beta p^2 t - px)} \left(\bar{g}_2(p) - \frac{\gamma}{p} \right) dp \quad (3.32b)$$

$$+ e^{\frac{-\alpha t}{1-\gamma}} \frac{1}{2\pi i} \lim_{R \rightarrow \infty} \int_{\varrho - iR}^{\varrho + iR} e^{(\beta p^2 t - px)} \left(\int_0^\tau \left(\frac{\alpha}{p} + \beta f(\tau_1) \right) e^{-(\beta p^2 \tau_1 - pX(\tau_1) + \lambda_1 \tau_1)} d\tau_1 \right) dp,$$

where ϱ is an arbitrary real constant. We can thus, in theory, determine $X(t)$ and $f(t)$ by matching these solutions and the gradients at $x = X(t)$. However, since the form of the solution does not lend itself to further algebraic manipulation, we resort to simply showing that these solutions are consistent with those obtained by using travelling wave coordinates, as discussed in Section 3.3.

Consistency with travelling wave solutions

The analytical solutions (3.32) to the reaction-diffusion equation with piecewise linear approximations to the kinetics are in terms of the inversion of Laplace transforms. We now show that these solutions agree with the solutions determined using travelling wave analysis.

Consider equation (3.30) for the transformed solution $\bar{n}_1(p, \tau)$. Suppose we take a travelling wave solution as our initial condition, so that

$$X(t) = at \quad f(t) = A$$

where a is the constant wave speed and A is the constant slope. Then the exponential term in the integral is a linear function of t and the coefficient is constant. Hence the transformed solution is

$$\begin{aligned}\bar{n}_1(p, \tau) = & \frac{\gamma}{p} - \frac{\left(\frac{\alpha}{p} - \beta A\right)}{(\beta p^2 + ap + \frac{\alpha}{\gamma})} + \\ & \left[\bar{g}_1(p) - \frac{\gamma}{p} + \frac{\left(\frac{\alpha}{p} - \beta A\right)}{(\beta p^2 + ap + \frac{\alpha}{\gamma})} \right] e^{-(\beta p^2 + ap + \frac{\alpha}{\gamma})\tau}.\end{aligned}\quad (3.33)$$

Now if we impose a travelling wave as the initial condition, the solution will remain in travelling wave form and the solution for $\bar{n}_1$ will be independent of time. Thus we have $\bar{n}_1(p) = \bar{g}_1(p)$, and by looking at time independent and dependent terms we find

$$\bar{g}_1(p) = \frac{\gamma}{p} - \frac{\left(\frac{\alpha}{p} - \beta A\right)}{(\beta p^2 + ap + \frac{\alpha}{\gamma})}.$$

Hence

$$\bar{n}_1(p) = \frac{\gamma\beta p + \gamma a + \beta A}{\beta p^2 + ap + \frac{\alpha}{\gamma}} \quad (3.34)$$

By factorizing the denominator and using partial fractions, we can rewrite the transformed solution as

$$\bar{n}_1(p) = \frac{C}{p - \zeta_1} + \frac{D}{p - \zeta_2}$$

where

$$\zeta_1 = \frac{1}{2\beta} \left(-a - \sqrt{a^2 - \frac{4\beta\alpha}{\gamma}} \right) \quad \zeta_2 = \frac{1}{2\beta} \left(-a + \sqrt{a^2 - \frac{4\beta\alpha}{\gamma}} \right)$$

and

$$C = \frac{A - \gamma\zeta_2}{\zeta_1 - \zeta_2} \quad D = \frac{A - \gamma\zeta_1}{\zeta_2 - \zeta_1}.$$

Functions of the form $h(x) = qe^{rx}$ have the Laplace transform $\bar{h}(p) = \frac{q}{p-r}$ and we can therefore invert the above expression to obtain

$$n_1(x - at) = \frac{A - \gamma\zeta_2}{\zeta_1 - \zeta_2} e^{\zeta_1(x-at)} + \frac{A - \gamma\zeta_1}{\zeta_2 - \zeta_1} e^{\zeta_2(x-at)}. \quad (3.35)$$

We note that this solution is a function of $x - at$, which motivates us to consider travelling wave coordinates. In the region $x > X(t)$ we solve the ordinary differential equation

$$\beta N_{zz} + aN_z + \frac{\alpha}{\gamma}N = 0$$

subject to

$$N = \gamma \quad \text{and} \quad \frac{dN}{dz} = A \quad \text{at} \quad z = z_1.$$

Seeking solutions of the form $N(z) = c_1 e^{\eta_1 z} + c_2 e^{\eta_2 z}$, where $z = x - at$, and choosing $z_1 = 0$, we find that $\zeta_1 = \eta_1$ and $\zeta_2 = \eta_2$, $c_1 = C$ and $c_2 = D$. Hence the solution derived using the transform method is identical to the travelling wave solution. Furthermore, the minimum wave speed, a_{min} , is $2\sqrt{\alpha\beta/\gamma}$, in agreement with the speed predicted by Kolmogorov *et al.* (1937).

The form of $n_2(x, t)$, the solution in the region $x < X(t)$, is determined in a similar fashion and found to be

$$n_2(x - at) = 1 + \frac{(1 - \gamma)\zeta_2 + A}{\zeta_1 - \zeta_2} e^{\zeta_1(x-at)} + \frac{(1 - \gamma) + A\zeta_1}{\zeta_2 - \zeta_1} e^{\zeta_2(x-at)}, \quad (3.36)$$

which is identical to the solution derived using travelling wave analysis. This clearly demonstrates that the solutions given by equations (3.32), with travelling wave initial conditions, are consistent with solutions determined using travelling wave analysis.

In principle we can extend the above analysis to our equation for the cell density with the kinetics given in (3.19), and determine analytical solutions in terms of space and time. However, due to the complicated nature of our kinetic term, this leads to a very cumbersome solution which is not shown here. The method of Laplace transform is a useful technique for solving reaction-diffusion equations with piecewise linear approximations to the kinetic term but, in general, the complicated form of the solutions means they are of little use. However, by considering exact forms for the speed, $X(t)$, and the slope, $f(t)$, we are able to use standard inversion techniques to determine explicit solutions.

3.5 Summary

In this chapter, we have focused on a reduced model, in which the EGF concentration is taken to be in equilibrium. By considering travelling wave coordinates, we solved the resulting boundary value problem as a regular perturbation problem and derived expressions for the slope and width of the waves. We then considered a piecewise linear approximation to the kinetic term and derived analytical solutions. Diffusion equations with a discontinuity in the diffusion coefficients at one concentration have been studied in some depth (Crank, 1956) and analytical solution have been found using transform methods and matching techniques. To our knowledge, no work has been done on discontinuities in kinetics. The Fourier transform method yielded solutions which depend on the initial conditions, with restrictions imposed by smoothness and continuity conditions. A more fruitful technique was found to be the Laplace transform, where, by considering the discontinuity as a moving boundary, we were able to derive solutions in terms of the speed and slope. However, complicated algebra prevented further analysis and we resorted to solving with constant speed and slope, assuming a travelling wave form initially. We thus showed the consistency with the travelling wave solutions and highlighted the advantages of working in travelling wave coordinates.

Chapter 4

Discussion on Corneal Epithelial Wound Healing

Despite much experimental investigation, a full understanding of the complex interaction of processes involved in epidermal wound healing still poses a major challenge to biologists. Previous models have been proposed for general epidermal wound healing, both deterministic (Sherratt *et al.*, 1994) and stochastic (Boyarsky, 1986). Here, we have presented a model for the specific case of corneal epithelial repair, focusing on the effect of EGF on mitotic and migratory activity. Numerical simulation of the model equation has enabled us to compare the model with experimental data, and analytical investigation has provided an understanding of the roles of the various parameters. The parameters were estimated, as far as possible, from the large quantity of experimental data available for corneal epithelial wound healing.

Specifically, we have concentrated on the role of epidermal growth factor (EGF). We first assumed that the tear film is the only source of EGF and that the rate of supply was constant. Numerical simulations of the resulting model showed that there is insufficient EGF concentration to heal the wound in the experimentally observed time. Moreover, since a wound front is observed experimentally (Buck, 1979), we expect travelling waves of cells and chemical whereas in the model solutions we observed a “fill-up” mechanism. The details of the way in which the tears release EGF, and in particular whether the release is constant, are possible areas of further

experimental study. However, the results of this basic model strongly suggested that there is an additional source of mitotic stimulation in the normal healing process.

We amended our model to include an additional source of chemical, released at the centre of the wound, in response to injury. This source term decays as the cell density increases in the wound and hence further amendments are necessary to model the final stages of healing. Numerical simulations of the amended model evolved to travelling waves, with constant shape and speed. Based on experience with scalar reaction diffusion equations (Kolmogorov *et al.*, 1937), we derived an analytical approximation to the healing rate by looking for a change in the type of eigenvalue at the leading steady state in the ordinary differential equations governing travelling wave solutions. The predicted wave speeds compare favourably with experimental data. An important biological implication of this result is that the rate of healing of corneal epithelial wounds can be increased by increasing the cell diffusion coefficient or the secretion rate of the EGF but the chemical diffusion coefficient does not have a significant effect. The results of our simple model thus suggest that serum-derived factors can alone account for the overall features of the healing process and that both migration and mitosis are necessary for effective healing. Furthermore, modification of the analytical expression for the wave speed, to include a saturating mitotic generation term, has enabled us to make clinically testable predictions of the healing rate when EGF is applied topically to the wound.

There has been some debate as to the validity of current models of corneal wound closure, due to the curvature of the surface of the eye being neglected. We have assumed that the eye can be modelled as a sphere and that the thickness of the epithelial layer is negligible compared with the radius of the eyeball. By writing our model in spherical coordinates, both numerical and analytical solutions suggested that the rate of healing increases as the wound closes. However, for typical parameter values and wound sizes, the increase is very small for the majority of healing, and the speed can thus be taken to be constant. We also considered a reduced system in which the EGF concentration was in equilibrium, thus resulting

in a single reaction diffusion equation for cell density, and used regular perturbation techniques to derive an expression for the slope and width of the wave. This expression suggested that the analytical wave speed in spherical coordinates is valid except for the final stages of healing, as the wave fronts meet at the wound centre. Furthermore, by considering piecewise linear approximations to the kinetic term for this reduced system, we have determined analytical solutions for the cell density, in terms of inverse Laplace transforms and showed the consistency with travelling wave solutions. These methods highlight the difficulties in analysing systems with discontinuities in the kinetic term.

An important question arising from the model is “When is the wound healed?”. Sherratt & Murray (1990) modelled the epidermal wound healing process using a source term linearly dependent on the cell density, reflecting auto-induction of mitosis. The travelling waves observed for their model had sharp fronts of constant shape and hence the critical cell density at which the wound was said to be healed was unimportant. In our model, numerical solutions give rise to waves with constant speed but differing shape at the wound edge. Simulations on a larger domain show that these solutions do evolve to travelling waves, so any variation can be explained by the finiteness of the wound domain. The wave fronts are more shallow, so that the results on healing time depend crucially on this critical cell density. For example, plots of change in wound length with time show that to model the lag phase of the healing process we require a high critical cell density, approximately 80% of the unwounded level. However, we are then unable to model the final stages of healing effectively. Conversely, if we take a low critical density, approximately 25% of the unwounded level, then the overall process agrees moderately well with experimental observations but the model fails to capture the lag phase. This illustrates one drawback of our model.

A further question which is highlighted is “What is the healing time?”. Conventionally, experimentalists determine the time for which the wound is reduced by about 75% and extrapolate the results to obtain the total healing time. In our

model solutions, the final stages of healing are much more time consuming since the additional source of growth factor disappears as the wound heals. In reality, additional factors that are not reflected in either our model or the experimentalists extrapolation, enter the healing process near closure, as the epithelial sheet returns to confluence (Clark, 1989). The importance of these additional factors is suggested by the invalidity of the wave speed expression as the wave fronts meet and the polar angle decreases. A detailed study of the final stages of the healing process could be a valuable area for both experimental and theoretical research.

In this thesis, we have concentrated on the chemical EGF, which has been established as an important regulator of epithelial repair. We now briefly discuss a number of the other growth factors which play overlapping and synergetic roles during the general wound healing process. Type α transforming growth factor (TGF- α) is a very similar protein to EGF, which binds and activates the EGF receptor but, although topical application of TGF- α has been shown to increase healing rates in burn wounds (Schultz *et al.*, 1987), its presence in the corneal epithelium is biologically unproven. Fibroblast growth factor (FGF) has both basic and acidic forms and topical application of a cocktail of FGFs has been shown to increase mitotic rates and hence speed up healing of corneal epithelial lesions (Fredj - Reygrobellet *et al.*, 1986). However, there is again no evidence for its presence in the tear film or its secretion by epithelial cells in response to injury. The effects of platelet derived growth factor and transforming growth factor- β are restricted to the healing of full depth wounds (reviewed in Martin *et al.*, 1992).

In the presence of serum-derived growth factors, rapid cell division occurs at the margin of a wound in an epithelial cell sheet. Numerous attempts have been made to explain this band of increased proliferation. Coffey *et al.*, (1987) suggested an auto-induction process, for a general epithelial wound, showing that wounding by abrasion increases the detectable quantities of TGF- α by freeing bound TGF- α from the epidermis of the normal adult breast skin and activating a process which releases bound TGF- α from the cells. These EGF-like peptides bind to EGF receptors and

induce an enhanced TGF- α synthesis in epithelial cells to increase their rate of proliferation. This autocrine feedback process is the basis of numerous models for epithelial repair (Sherratt, 1992), and, although there is evidence to support it in some epithelium, the process remains biologically unproven. Ohashi *et al.*, (1989) determined the average concentration of EGF released by damaged cells and found it to be negligible compared with the tear film concentration. Hence we have chosen to neglect any autocrine mechanism in the specific case of the cornea. Absence of contact inhibition and change in cell shape also increase the proliferation of the basal cells at the wound edge. Our model does reflect these effects, although it does not focus on them. However, a very simple model of these processes involving a single reaction diffusion equation for the cell density has been studied previously and predicts greatly reduced speeds of healing (Sherratt, 1992).

In this thesis, we have considered a quite different explanation for the elevated mitotic rate at the wound edge, based upon the previously discussed ideas of Barandon & Green (1987) and Dunn & Ireland (1984). Although these experiments were performed on different cell types, we speculate similar results for EGF and assume that damage to the underlying layers of the cornea cause these to release EGF, near the centre of the wound. The EGF released by this additional source is then “mopped up” by the leading row of cells at the wound edge, because of their very high affinity for EGF, resulting in a preferential increase in the mitotic rate and an inwards migration to heal the wound. This hypothesis and our other quantitative analytical results are highly amenable to further experimental verification.

Part 2

Dermal Wound Healing

“Now to him who is able to do immeasurably more than all we ask or imagine, according to his power that is at work within us to him be the glory in the church and in Christ Jesus throughout all generations, for ever and ever! Amen.”

Ephesians 3: 20-21

Chapter 5

Scarring in Foetal and Adult Dermal Tissue

5.1 Biological Background

5.1.1 Overview of Dermal Wound Healing

Mammalian skin forms the external covering of the body and consists of three parts; the epidermis, the dermis and the subcutis (Figure 5.1). The epidermis has an average thickness of 0.1 mm and is continually renewed with new cells being produced through mitotic division and the outermost cells being “sloughed off” (Odland, 1983). The subcutis lies beneath the dermis and consists of firm fibrous bands which anchor the skin to the underlying structures (Mast, 1992). The dermis contains blood vessels, nerves, inelastic collagen fibres, elastin fibres, pigment cells, fat cells and fibroblasts, all of which are embedded in a “ground substance”, and is composed of two structurally distinct layers; the papillary layer and the reticular layer. The papillary layer consists of free connective tissue cells whereas in the reticular layer there are densely interwoven collagen fibres (Asmussen & Söllner, 1993). The basal lamina separates the epidermis and the dermis, with numerous finger-like connective tissue projections ‘anchoring’ the two layers (Furthmayr, 1988).

In the epithelial repair discussed in the previous chapters, wound healing results in complete regeneration of lost tissue. In contrast, in the dermis the damaged tissue is repaired by non-specific connective tissue which then forms a scar. The process of

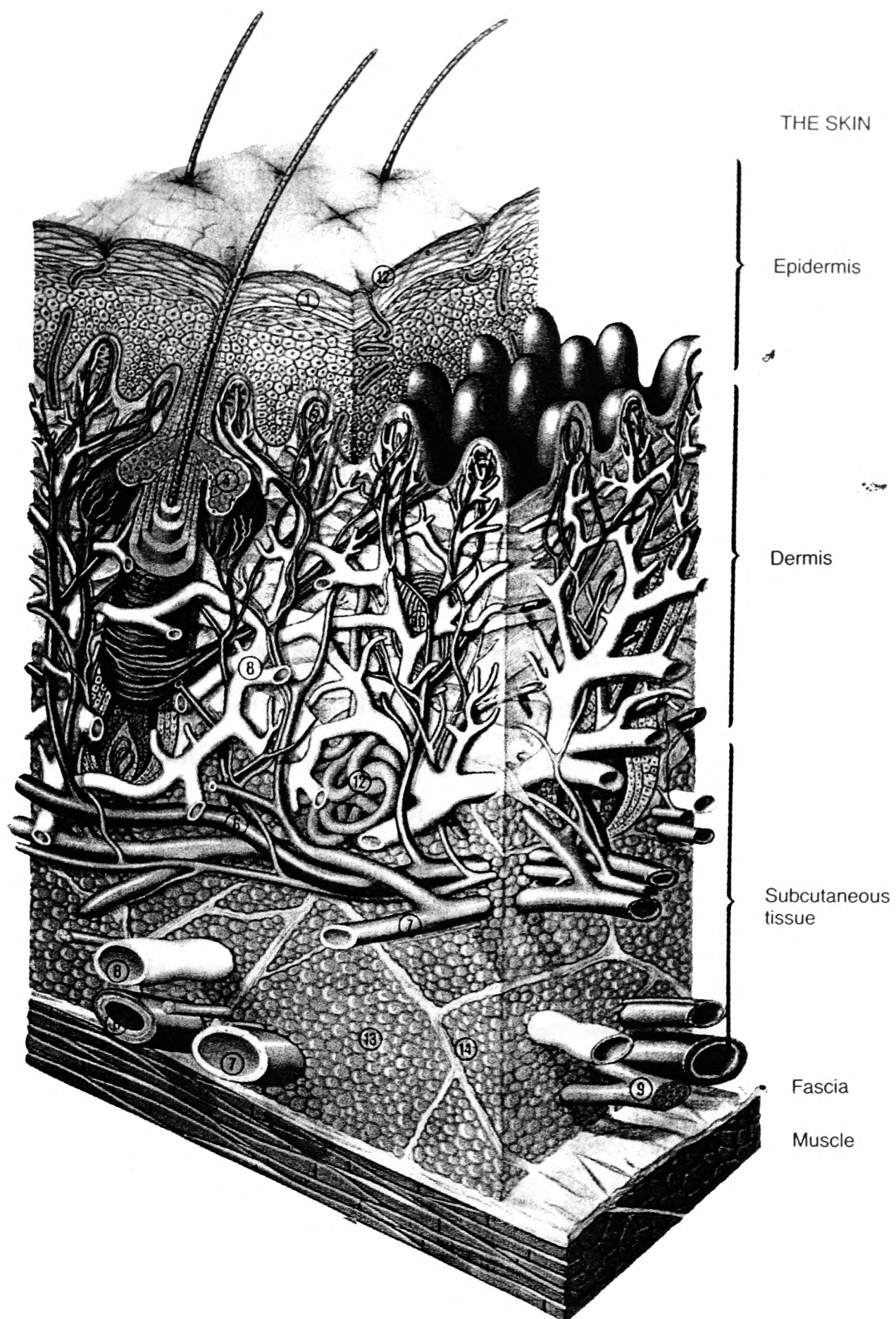


Figure 5.1: Anatomical features of the skin, showing the three distinct layers: epidermis, dermis and subcutaneous tissue (taken from Mast, 1992).

dermal wound healing therefore involves the complex interaction of many cell types and occurs as a sequential cascade of overlapping processes as shown in Figure 5.2 (Jennings & Hunt, 1992).

Immediately after injury, there is heavy bleeding which has a cleansing effect but the constriction of blood vessels prevents further blood loss. The process of coagulation releases biologically active substances that begin the early and late inflammatory phase of tissue repair and culminates in the formation of a stable, crosslinked fibrin clot (Dvorak *et al.*, 1988). Within 24 hours of wounding, the inflammation stage begins with the infiltration of the wound by granulocytes (white blood cells) in response to chemotactic factors (Clark, 1989). The primary role of the cells is to fight infection by consuming contaminating bacteria and debris. The granulocytes are gradually replaced by macrophages, which release a range of growth factors such as $TGF\alpha$ and $TGF\beta$ (Transforming Growth Factor α and β) which stimulate proliferation and matrix production by fibroblasts.

The defect, cleansed by inflammatory processes, can now be covered in new tissue. The upper portion of the blood clot gradually forms a scab which sloughs during the healing process (Dvorak *et al.*, 1988), whilst the lower portion becomes 'granulation tissue'. This is composed of fibroblasts, macrophages and neovasculature embedded in a loose matrix of types I and III collagen, fibronectin and hyaluronic acid (Clark, 1988). Angiogenesis, the process of formation of new blood vessels by directed endothelial cell migration and growth, gives rise to the deep red colour of the granulation tissue (Whalen & Zetter, 1992; Wahl & Wahl, 1992). Parallel to vascularization, fibroblasts migrate along the fibrin network, divide at a rapid rate and produce the connective tissue ground substance consisting of proteoglycans and the water-insoluble collagen fibres essential for tissue stability (McDonald, 1988).

With the formation of granulation tissue, epidermal migration and wound contraction begins. Epidermal migration involves the spreading of epidermal cells across the wound between the scab and the granulation tissue whereas wound contraction involves the contraction of granulation tissue and the inward movement of the wound

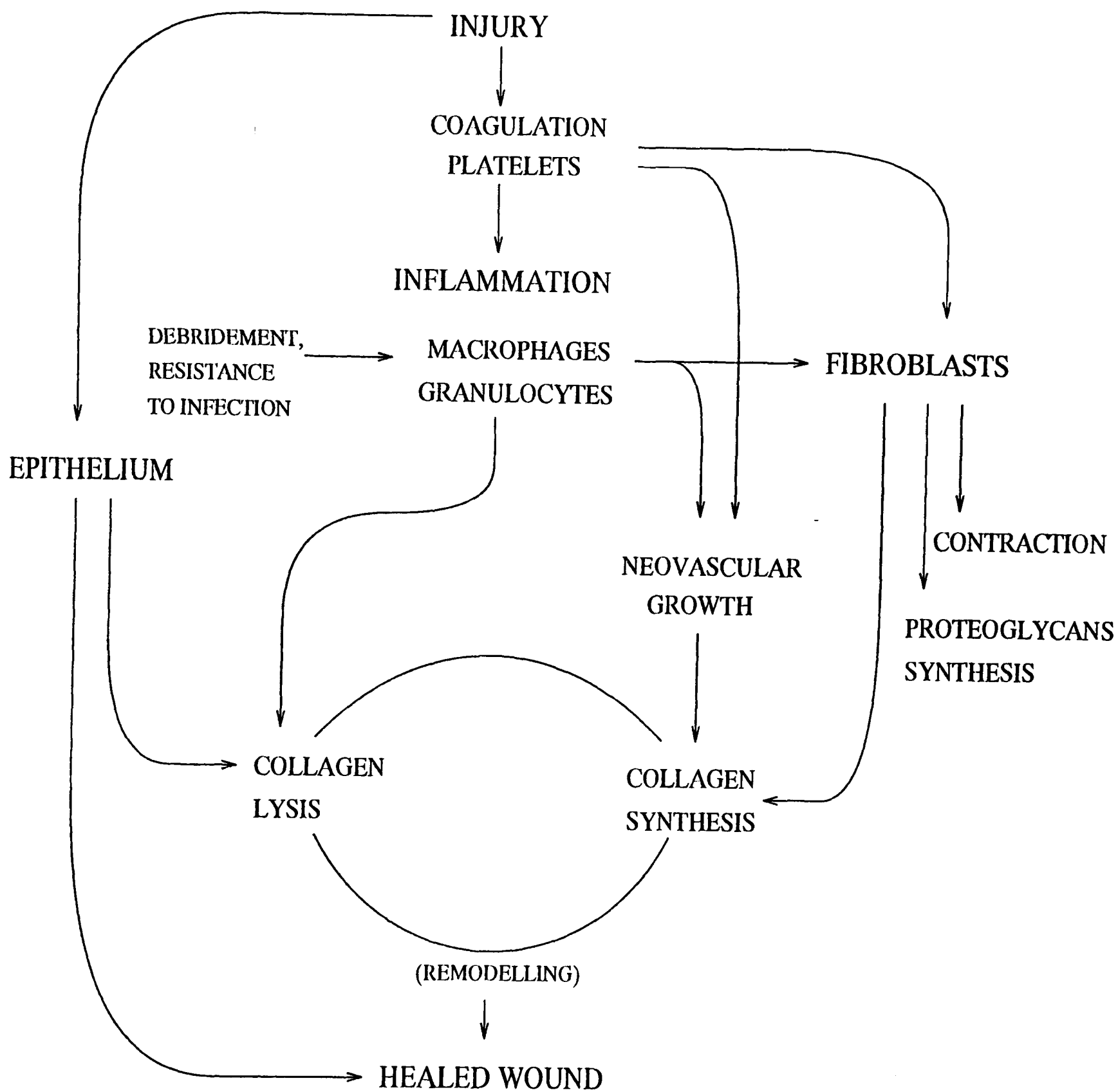


Figure 5.2: The complex interaction of the many cell types to achieve repair after an injury.

edges. During the contraction stage, fibroblasts are transformed into myofibroblasts, which contain contractile elements allowing them to draw together. The collagen fibres align, the scar tissue shrinks and the wound margin contracts leaving only a small defect (Rudolph *et al.*, 1992).

Once the wound has closed, the slow process of matrix remodelling can begin. This process can take up to 20 years after an injury and is concerned with the removal of loose extracellular matrix materials and the slow restructuring of type I and III collagen fibres which are critical for the return of tissue integrity, flexibility and strength (Jennings & Hunt, 1992). In postnatal mammalian wound repair, in contrast to foetal healing, the end result is scar formation. This initially reddish, slightly elevated scar gradually turns pale and becomes slightly recessed. It is less functional than the surrounding uninjured tissue due to the lack of some components of the normal tissue (Rudolph *et al.*, 1992). Figure 5.3 shows the overlapping processes involved in wound healing from the onset of injury to full scar maturation.

5.1.2 Foetal and Adult Healing

Experimental understanding of foetal wounds has increased over the past 15 years and this has led to the possibility of improved adult healing. Foetal and postnatal mammalian wounds have been shown to heal differently, a comparison being illustrated in Table 5.1. The major difference is that foetal wounds heal without scar formation; during the late foetal stages and early childhood, there is a gradual transition to the adult response to injury, repair by scar (Adzick & Longaker, 1992). Scar tissue results from the remodelling of the extracellular matrix of the granulation tissue and involves the removal of fibronectin and hyaluronic acid by enzymatic action, the increase in size of collagen bundles and the deposition of proteoglycans (Clark, 1989). The tensile strength of the scar tissue depends on the collagen fibrils and the degree of fibril cross-linking (Cohen & McCoy, 1983). In scar tissue, the fibres are longer and thinner than in normal tissue, with the orientation being in the

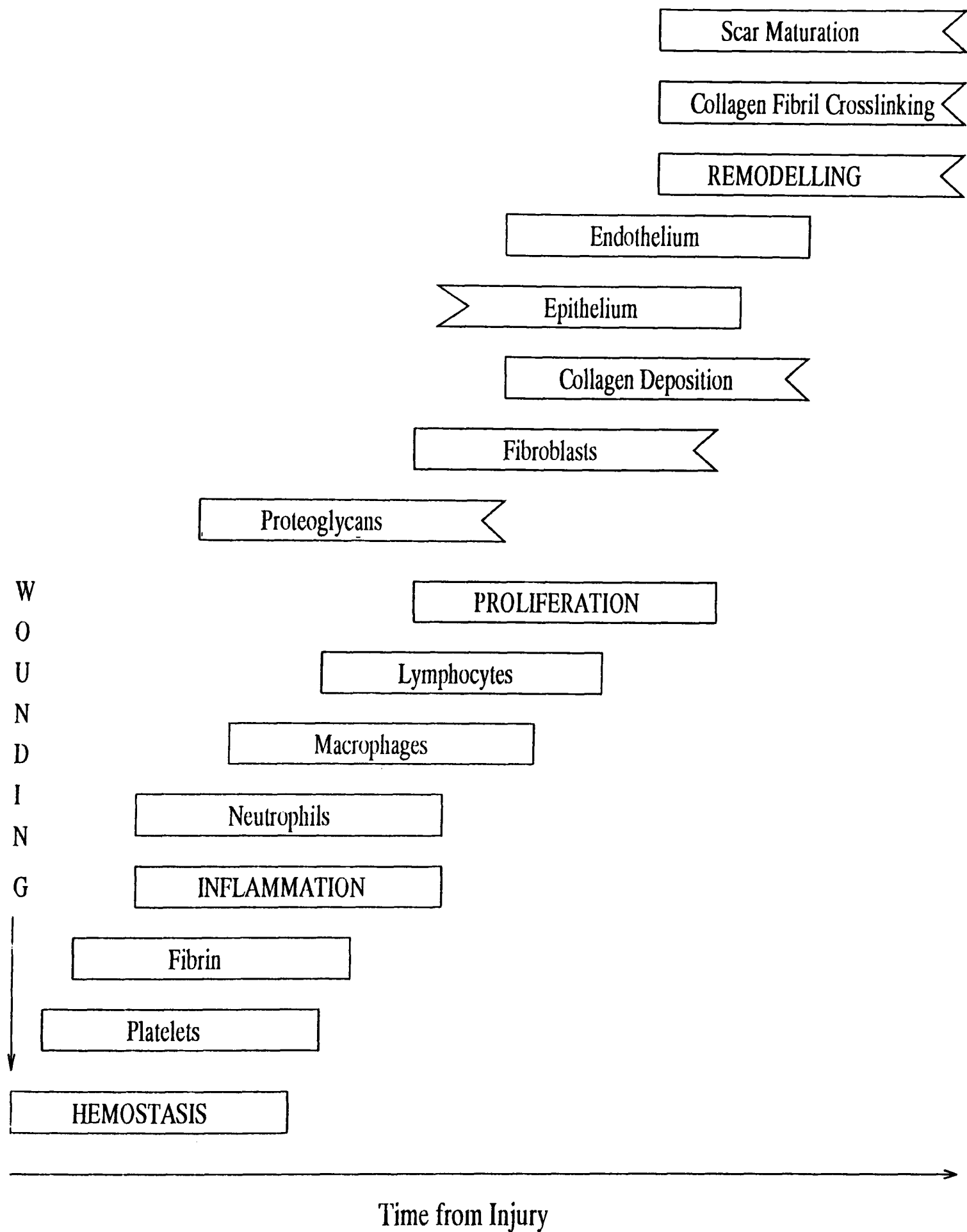


Figure 5.3: Temporal relationship between the multiple processes occurring in dermal wound healing.

- | |
|---|
| 1. No scar formation |
| 2. Fast |
| 3. No scab |
| 4. Poor inflammatory response |
| 5. Lower growth factor profile |
| 6. Rapid epithelialization |
| 7. Reduced angiogenesis |
| 8. Higher rates of cell division and migration |
| 9. Different wound metabolism, pH and pO ₂ |
| 10. Quantitative and qualitative differences in the ECM molecules |
| 11. Reduced wound contraction and tension |
| 12. Enhanced regeneration of epithelial and mesenchymal tissues |

Table 5.1: Properties of foetal wound healing compared with the adult case.

direction of tension. The reduction in fibril diameters and cross-linking results in weaker tissue. Although the strength increases with tissue maturity, it never attains that of the original dermis. In particular, the lack of elastin fibres and the presence of proteoglycans causes scar tissue to be inelastic.

The fibroblasts migrate extensively into the wounded area, the process being critically dependent on fibroblast contractility and the activity of degrading enzymes such as plasmin at the leading edge of the fibroblast (Clark, 1988). It has been suggested that in foetal wounds, fibroblasts migrate into the wounded area very rapidly and easily due to minimal clotting and increased motility or higher levels of tissue degrading enzymes (Siebert *et al.*, 1990). In adults, however, the fibroblasts proliferate in the sub-cutaneous compartment, and migrate preferentially between the margins of the clot and the healthy tissue. There may also be major differences in the integrin receptor profile on the foetal and adult fibroblasts, and thus how they interact and organise the matrix (Mast *et al.*, 1992). Furthermore, TGF β is known to influence the integrin profile of fibroblasts.

The intensity and orientation of the collagen fibres is crucial in scar formation. A few days after wounding, adult scar tissue is characterized by an accumulation of

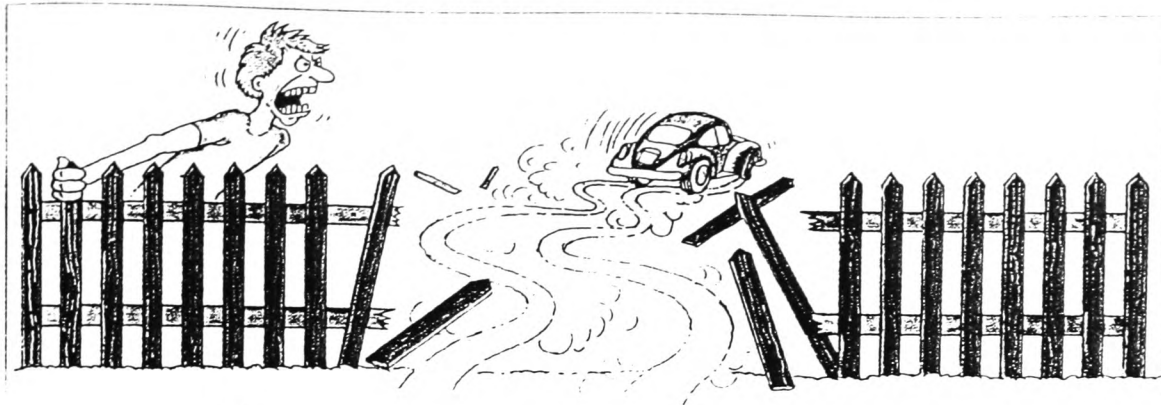
fibroblasts, blood vessels and a dense collagenous connective tissue, together with the absence of sweat glands and hair follicles (Peacock, 1984). With the extracellular matrix remodelling, the fibroblasts and blood vessels return to a similar density to those of the surrounding skin, and some hair follicle regeneration occurs. However, the density of collagen fibrils remains much higher than that of normal skin, especially in the subcutaneous region of the wound (Clore *et al.*, 1979). In normal skin, the collagen fibres in the dermis exhibit a basketweave-like arrangement. After injury, long, thinner collagen fibres are present throughout the wound but are more prominent at the wound edges (Whitby & Ferguson, 1991). Also, the fibres are orientated perpendicular to the basement membrane (i.e. in the direction of the tension) (Peacock, 1984). We focus on the density and diameter of collagen fibres as a measure of scar tissue.

Other forms of wound healing include incomplete repair, when there is a deficiency of connective tissue, and, at the other extreme, hypertrophic scar formation due to excessive connective tissue (Figure 5.4).

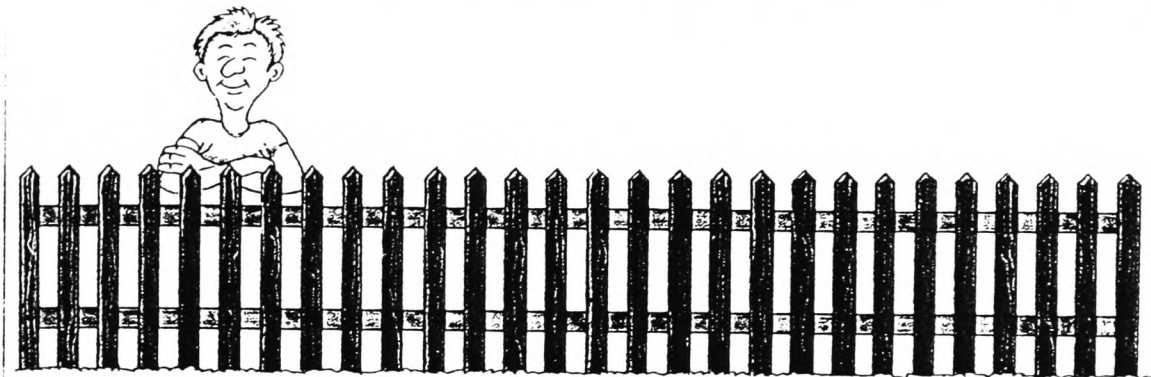
5.1.3 Scar Formation

The process of wound healing and scar tissue formation is complicated and in this section, we introduce the basic elements of the model. We aim to produce a model which explains the differences between foetal and adult healing in terms of the density and the diameter of collagen fibres. Accumulation of collagen in the wound is dependent on the amount of collagen synthesized compared to collagen degraded by local enzymes. The control of collagen synthesis, secretion, cross-linking and degradation is incompletely understood but collagen is obviously critical for the return of tissue integrity and strength (Jeffrey, 1992).

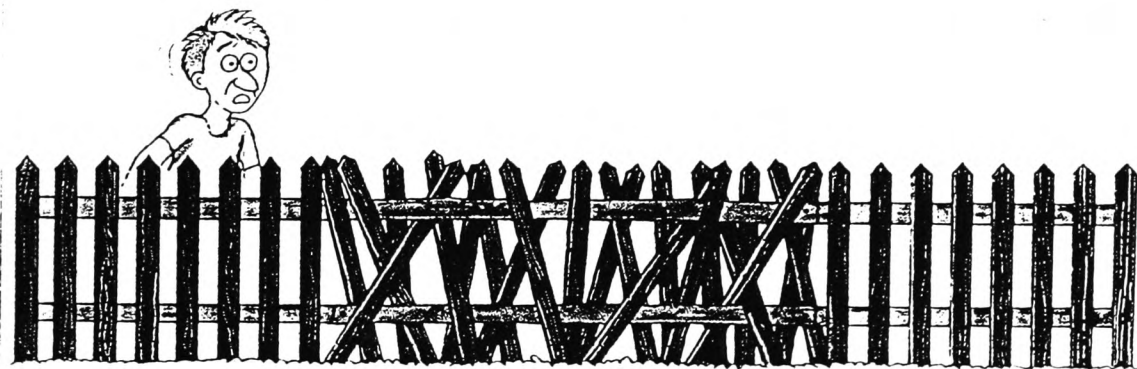
During the healing process, mobile fibroblasts synthesize much of the intercellular substance, including various growth factors (including Transforming Growth Factor β) and different collagens and collagenases. TGF β is autoinductive and experiments



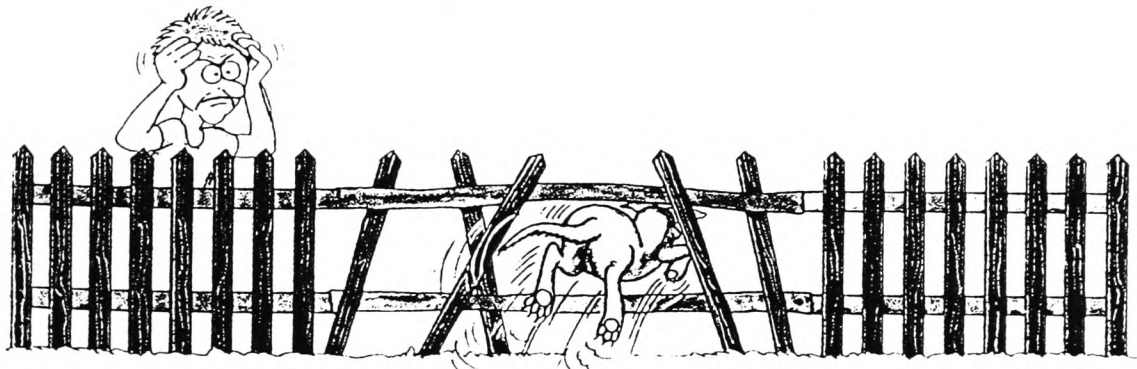
a) The tissue is destroyed by an injury and substance is lost.



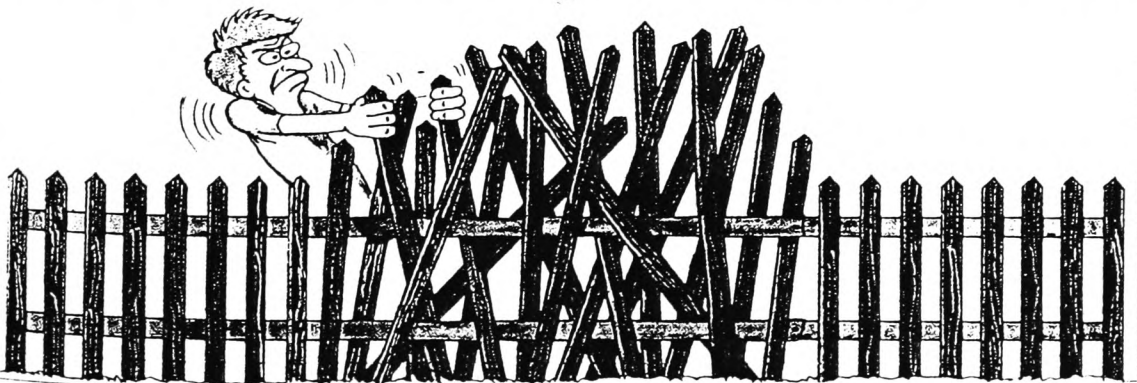
b) In regeneration the original state is exactly restored. This is the case in foetal wound healing.



c) In the repair process, the gap is closed by unspecific elements. The structure of the scar tissue differs from the healthy tissue. This is the case in adult wound healing.



d) In disturbances of wound healing, repair may be incomplete, for example when there is a deficiency of connective tissue.



e) Excess formation of connective tissue can lead to hypertrophic scars.

Figure 5.4: Pictorial representation of possible forms of wound healing (taken from Asmussen & Söllner, 1993).

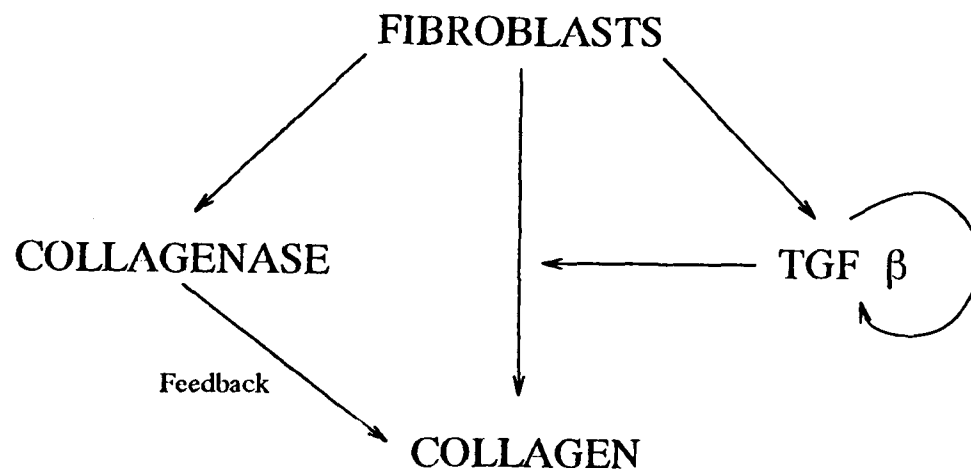


Figure 5.5: The basic interaction of fibroblasts, growth factor, collagen and collagenase during the healing process.

indicate that the growth factor stimulates the secretion of collagen by fibroblasts. Collagenase is responsible for the downregulation of collagen. The collagen level in a healed wound is thus dependent on fibroblasts, $\text{TGF}\beta$ and collagenases, as shown in Figure 5.5. In order to capture the essential features of the healing process, we extend this basic framework and look at each element in more detail.

Collagen

The collagens are a group of structural matrix proteins (up to type XIII) which provide connective tissues with their strength and are a key component of all phases of wound repair (Miller and Gay, 1992). Collagen becomes the foundation of the new extracellular matrix, the normal adult dermis containing 85% type I and 15% type III collagen (Wenstrup *et al.*, 1983; Lapiere *et al.*, 1988). The fibroblasts synthesize procollagen, chains of amino acids, in response to injury (McDonald, 1988). The synthesis is activated by growth factors such as $\text{TGF}\beta$ (Appling *et al.*, 1989) and in the absence of specific enzymes, the procollagens undergo natural decay. Biological experiments show that the wound site contains enzymes which

initiate the stabilization of the precursor collagens to collagen (Miller & Gay, 1992). In our model, we consider a generic enzyme for the activation of the procollagens. The chains of amino acids are twisted in threes in helical formation and polymerize to produce microfibrils, which in turn assemble to form a collagen fibril. Several of these fibrils arrange themselves into bundles, called collagen fibres, with diameter 1000-1200 nm (Asmussen & Söllner, 1993). In healthy tissue the collagen fibres are aligned in certain patterns following the main contours of tension of the skin. This organized structure is not achieved in wound healing, which explains the disorganized appearance of the fibres in scar tissue (Peacock, 1984). The fibres in the wound are also much thinner and are much more densely packed, with fewer spaces between them (Whitby & Ferguson, 1991).

Collagenase

Recent research has shown that collagen degradation is a two stage process (Jeffrey, 1992). Specific collagenases are secreted by dermal fibroblasts, and these break down the fibres into smaller protein units, which are then degraded by a variety of less specific proteases. In human skin collagenase is synthesized and secreted by fibroblasts as a 'zymogen', a proenzyme with molecular weight of 52kDa (Stricklin *et al.*, 1978). The zymogen is incapable of binding to the collagen, and degradation cannot occur until activated by a variety of reagents. *In vitro* experiments activate the procollagenase by using trypsin to remove a "pro-" piece of protein approximately 10kDa in mass. Once activated, collagenases bind avidly to collagen. A similar process may take place *in vivo*, although this is an area of much biological controversy. We hypothesize that zymogens are activated by specific enzymes within the wound site, and for the purpose of our model, we consider a generic enzyme.

Transforming Growth Factor β (TGF β)

The TGF β family consists of three different isoforms which bind with a high affinity to a wide variety of cell types (Wakefield *et al.*, 1987). TGF β is released by platelet degranulation and is secreted at high levels by macrophages, monocytes, fibroblasts and keratinocytes adjacent to the wounded area (Assoian *et al.*, 1983; Wakefield *et al.*, 1988). Biologically inactive (latent) forms of the TGF β s associated with a binding protein are secreted by many cells (Martin *et al.*, 1992; Streuli *et al.*, 1993). The latent form of the growth factor has a high molecular weight (220 kDa) and a considerably longer half-life than the active forms (Roberts & Sporn, 1990). Latent TGF β is also autoinductive, which accounts for its prolonged activity after a single exogenous administration. The latent growth factors are stored until activated by macrophages and enzymatic activity. We represent the activation chemical by a single generic enzyme. Active TGF β has been shown to increase fibroblast proliferation and addition of TGF β results in fibroblasts secreting more procollagen, elastin and fibronectin, hence inducing extracellular matrix synthesis (Krummel *et al.*, 1988; Appling *et al.*, 1989).

Platelet Derived Growth Factor (PDGF)

PDGF is a glycoprotein, consisting of two chains, which is contained in the α -granules of the platelets and is released in abundance at the time of wounding by degranulating platelets (reviewed in Martin *et al.*, 1992). However, research has shown that the effect of PDGF on collagen deposition is small compared to the effect of TGF β (Pierce *et al.*, 1991). We have thus chosen to neglect PDGF and concentrate on the role of TGF β .

Hyaluronic Acid

Hyaluronic acid (HA) is a high molecular weight glycosaminoglycan, which plays a role in the structure and organisation of the extracellular matrix during wound repair

(Clark, 1988). HA inhibits cell differentiation, creating an environment to promote cell proliferation and a burst of IIA synthesis just prior to mitosis allows dissociation from neighbouring cells (Toole, 1981; Wiegel *et al.*, 1986). The regulation of HA deposition is not fully understood but PDGF and TGF β have both been shown to stimulate HA in fibroblasts through HA-stimulating activity (HASA) (Stern *et al.*, 1992). In adult wound healing, there is an early deposition of HA which peaks after a few days and then falls rapidly. In contrast, IIA in fetal wound healing maintains its elevated level (Stern *et al.*, 1992). In adults, a substance may be produced to inhibit biosynthesis and interact with hyaluronidase to decrease the level of HA or elevated levels of HA may themselves inhibit synthesis. The effect of HA on reducing scar formation is an important area of current research but preliminary results show that there is very little effect when exogenous hyaluronic acid is added to adult wounds. There are effects, but if a neutralising antibody to TGF β 1 has an anti-scarring activity of 10 (on the score of 1-10), exogenous hyaluronic acid has an anti-scarring score of 0.5 (Ferguson, personal communication). Hence we choose not to focus on HA in our basic model.

5.2 Mathematical Modelling

In this section, we derive a detailed mathematical model for foetal dermal wound healing which explains the lack of scar tissue formation, in terms of the effect of TGF β on fibroblast synthesis of collagens and collagenases. Dermal tissue contains two main types of collagen, types I and III. Type III collagen decorates the surface of the type I collagen fibril so that a higher ratio of type III to type I results in thinner fibres (Flint *et al.*, 1984; Whitby & Ferguson, 1991). We note that scar tissue in adults has higher levels of type III collagen than most normal tissues whereas foetal scar tissue retains the normal levels of types I and III collagen (Mast *et al.*, 1992; Merkel *et al.*, 1988). Therefore we concentrate on the fibre density and the ratio of collagen types I to III as an indication of the level of scarring. Figure 5.6 shows the complex interactions of the variables included in our model.

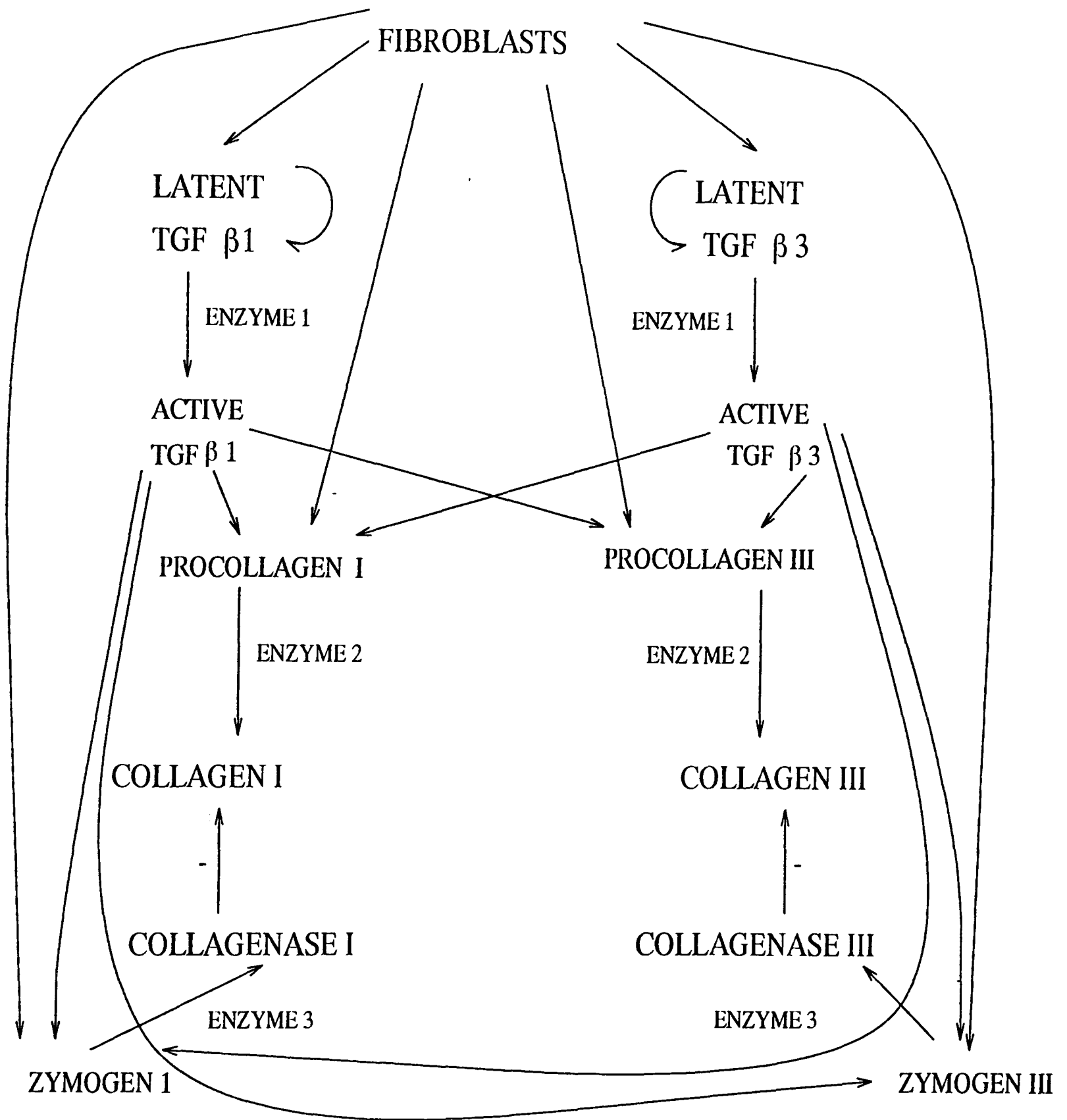


Figure 5.6: Schematic representation of the interactions between growth factors, proteins, fibroblasts and enzymes during the wound healing process.

5.2.1 Derivation of Model Equations

We introduce the following model variables, all at position $\underline{r}$ and at time t :

$f(\underline{r}, t)$	=	density of fibroblasts,
$l_1(\underline{r}, t)$	=	concentration of latent TGF β 1,
$l_3(\underline{r}, t)$	=	concentration of latent TGF β 3,
$\beta_1(\underline{r}, t)$	=	concentration of active TGF β 1,
$\beta_3(\underline{r}, t)$	=	concentration of active TGF β 3,
$e_1(\underline{r}, t)$	=	generic enzyme 1, activating latent TGF β ,
$e_2(\underline{r}, t)$	=	generic enzyme 2, activating procollagens,
$e_3(\underline{r}, t)$	=	generic enzyme 3, activating zymogens,
$p_1(\underline{r}, t)$	=	procollagen I,
$p_3(\underline{r}, t)$	=	procollagen III,
$c_1(\underline{r}, t)$	=	collagen I,
$c_3(\underline{r}, t)$	=	collagen III,
$z_1(\underline{r}, t)$	=	zymogen I,
$z_3(\underline{r}, t)$	=	zymogen III,
$s_1(\underline{r}, t)$	=	collagenase I,
$s_3(\underline{r}, t)$	=	collagenase III.

Fibroblasts

Fibroblasts are the main cell type in the dermis, and in normal, uninjured tissue, they are sparsely distributed throughout the connective tissue matrix. After injury, the fibroblasts migrate into the wound site, where they proliferate and produce numerous substances (Morgan & Pledger, 1992).

Rate of Increase of Fibroblast Density	=	Fibroblast Migration	+	Mitotic Generation	-	Natural Loss
---	---	-------------------------	---	-----------------------	---	-----------------

$$\frac{\partial f}{\partial t} = D_1 \nabla^2 f + (A_1 + A_2 \beta_1 + A_3 \beta_3) f \left(1 - \frac{f}{k_1} \right) - A_4 f \quad (5.1)$$

We model the movement of fibroblasts by Fickian diffusion to capture cells moving down gradients in cell density. The rate of diffusion may be dependent on the TGF β concentration and the amount of collagen but, to avoid further complexity, we consider a constant diffusion coefficient, D_1 . In the absence of TGF β , the cell population increases exponentially at low densities but saturates for high cell densities and we thus model fibroblast proliferation by a chemically enhanced logistic growth

term, $s(\beta_1, \beta_3)f(1 - f/k_1)$, where $s(\beta_1, \beta_3)$ represents the chemical control of mitosis and k_1 is the carrying capacity. The effect of $TGF\beta$ is dependent on the number of $TGF\beta$ -bound cell surface receptors per cell (Morgan & Pledger, 1992) and, for simplicity, we consider the linear functional form $s(\beta_1, \beta_3) = A_1 + A_2\beta_1 + A_3\beta_3$. We assume that normal dermal fibroblasts die at a constant rate, A_4 .

Latent $TGF\beta$

Recent biological research has shown that the ratio of $TGF\beta$ isoforms in the wound is very important (Chen *et al.*, 1993; Shah *et al.*, 1992). Essentially, $TGF\beta_3$ causes the dermis to reform in the wound area, with thick, less densely packed collagen fibres. Conversely, $TGF\beta_1$ and $TGF\beta_2$ tend to produce thinner collagen fibres, which are much more densely packed. It is still not fully understood how the $TGF\beta$ isoforms differentially regulate the collagen. In our model we consider only two isoforms, $TGF\beta_1$ (representing both 1 and 2) and $TGF\beta_3$.

$$\begin{array}{ccccccc} \text{Rate of} & = & \text{Chemical} & + & \text{Production by} & - & \text{Natural} \\ \text{Increase of} & & \text{Diffusion} & & \text{Fibroblasts} & & \text{Decay} \\ \text{Latent } TGF\beta & & & & & - & \text{Activation} \\ \text{Concentration} & & & & & & \text{by Enzyme} \end{array}$$

$$\frac{\partial l_1}{\partial t} = D_2 \nabla^2 l_1 + \frac{A_5 f l_1}{1 + A_6 l_3 + A_7 l_1} - A_8 l_1 - A_{16} e_1 l_1 \quad (5.2a)$$

$$\frac{\partial l_3}{\partial t} = D_3 \nabla^2 l_3 + \frac{A_9 f l_3}{1 + A_{10} l_3} - A_{11} l_3 - A_{17} e_1 l_3 \quad (5.2b)$$

Latent $TGF\beta$ diffuses freely and we assume constant diffusion coefficients D_2 and D_3 for latent $TGF\beta_1$ and $TGF\beta_3$ respectively. The fibroblasts are stimulated by the growth factors and secrete latent forms of $TGF\beta$ (Wakefield *et al.*, 1988) but the production of growth factors does not increase unboundedly, hence the saturating functional form in the above equations. Latent $TGF\beta$ also undergoes an autocrine mechanism, whereby $TGF\beta$ induces self-secretion, thus explaining the prolonged presence of chemical after a single application (Roberts & Sporn, 1990). An important experimental fact represented in this term is the modulation of the secretion

of latent TGF β 1 by latent TGF β 3, which is not mimicked in the corresponding latent TGF β 3 equation (Shah, personal communication). Latent TGF β has a short half-life and we model natural decay as a first order process (Wakefield, 1990). The concentration of latent growth factor is also decreased due to activation by specific enzymes, and we use the law of mass action to derive the functional forms $-A_{16}e_1l_1$ and $-A_{17}e_1l_3$.

Active TGF β

We consider two isoforms of active TGF β , which have been shown to increase fibroblast proliferation and collagen synthesis (Krummel *et al.*, 1988).

$$\begin{array}{ccccccc} \text{Rate of Increase of} & = & \text{Chemical} & + & \text{Activation of} & - & \text{Natural} \\ \text{Active TGF}\beta & & \text{Diffusion} & & \text{Latent Form} & & \text{Decay} \\ \text{Concentration} & & & & \text{by Enzyme} & & \end{array}$$

$$\frac{\partial \beta_1}{\partial t} = D_4 \nabla^2 \beta_1 + A_{12}e_1l_1 - A_{13}\beta_1 \quad (5.3a)$$

$$\frac{\partial \beta_3}{\partial t} = D_5 \nabla^2 \beta_3 + A_{14}e_1l_3 - A_{15}\beta_3 \quad (5.3b)$$

We again model chemical movement by simple Fickian diffusion, where D_4 and D_5 are constant. Latent forms of TGF β 1 & 3 are activated by specific enzymes and experiments have shown active TGF β to undergo rapid decay, which we model as a first order process for both isoforms (Roberts & Sporn, 1990).

Enzymes

Biological research indicates that, during the inflammation stage, white blood cells release specific enzymes, which activate growth factors, procollagens and zymogens (Sinclair and Ryan, 1994). These pools of enzyme are rapidly degraded during the healing process. Due to lack of experimental data, and for simplicity, we consider generic enzymes acting on the latent TGF β , procollagen and zymogen.

$$\begin{array}{ccc} \text{Rate of Increase} & = & - \text{Activation of} \\ \text{of Enzyme} & & \text{Latent Forms} \end{array}$$

$$\frac{de_1}{dt} = -e_1(A_{16}l_1 + A_{17}l_3) \quad (5.4a)$$

$$\frac{de_2}{dt} = -e_2(A_{18}p_1 + A_{19}p_3) \quad (5.4b)$$

$$\frac{de_3}{dt} = -e_3(A_{40}z_1 + A_{41}z_3) \quad (5.4c)$$

The increase of enzyme concentration is taken to be spatially independent, since the kinetic time scale is known to be much faster than any diffusion. We use the law of mass action to model the activation of latent TGF β 1 & 3 in (5.4a), and the activation of type I and type III collagen and collagenases in (5.4b) and (5.4c) respectively.

Procollagens

Procollagen is synthesized by fibroblasts, in response to injury (McDonald, 1988), the synthesis being stimulated by TGF β . We focus on two types of procollagens, types I and III.

$$\begin{array}{ccccccc} \text{Rate of Increase} & = & \text{Secretion by} & - & \text{Natural} & - & \text{Conversion by} \\ \text{of Procollagen} & & \text{Fibroblasts} & & \text{Decay} & & \text{Enzyme} \end{array}$$

$$\frac{dp_1}{dt} = (A_{20} + A_{21}\beta_1 + A_{22}\beta_3)f - A_{23}p_1 - A_{18}e_2p_1 \quad (5.5a)$$

$$\frac{dp_3}{dt} = (A_{24} + A_{25}\beta_1 + A_{26}\beta_3)f - A_{27}p_3 - A_{19}e_2p_3 \quad (5.5b)$$

The procollagen fibres do not diffuse within the wound milieu but are synthesized by the migrating fibroblasts (Miller & Gay, 1992). In the absence of chemicals, we assume constant secretion rate but experiments show upregulation of procollagen synthesis by active TGF β (Appling *et al.*, 1989), hence the inclusion of a linear function of the active chemical concentrations. We assume procollagen fibres have a constant life-span and model natural decay as a first order process. Procollagen fibres are activated by specific enzymes, denoted by the generic enzyme e_2 , to form the triple helical collagen fibrils, procollagen I being activated to form collagen I, and procollagen III forms collagen III.

Collagens

Collagen I and III are triple helical structural matrix proteins, formed by the activation of procollagens I and III. Type III collagen decorates the surface of the type I fibril, with a higher ratio of III to I producing thinner fibres (Flint *et al.*, 1984).

$$\begin{array}{lcl} \text{Rate of Increase} & = & \text{Activation of Procollagen} - \text{Degradation by} \\ \text{of Collagen} & & \text{by Enzyme} \quad \text{Collagenases} \end{array}$$

$$\frac{dc_1}{dt} = A_{28}p_1e_2 - A_{29}s_1c_1 \quad (5.6a)$$

$$\frac{dc_3}{dt} = A_{30}p_3e_2 - A_{31}s_3c_3 \quad (5.6b)$$

There is no experimental evidence for collagen diffusion and hence we assume no spatial dependence. We use the law of mass action to model the activation of procollagen fibres to collagen fibrils. Collagenases I and III gradually digest collagen I and III, respectively. There is little experimental evidence for the processes involved in the degradation. Therefore, for simplicity we assume a linear form.

Zymogens

Zymogens are proenzymes, the inactive forms of collagenase secreted by fibroblasts, which are incapable of degrading collagen until activated. We consider two specific zymogens, types I and III.

$$\begin{array}{lcl} \text{Rate of Increase} & = & \text{Secretion by} - \text{Natural} - \text{Activation by} \\ \text{of Zymogens} & & \text{Fibroblasts} \quad \text{Decay} \quad \text{Enzyme} \end{array}$$

$$\frac{dz_1}{dt} = \frac{A_{32}}{1 + A_{33}\beta_1 + A_{34}\beta_3} f c_1 - A_{35}z_1 - A_{40}e_3z_1 \quad (5.7a)$$

$$\frac{dz_3}{dt} = \frac{A_{36}}{1 + A_{37}\beta_1 + A_{38}\beta_3} f c_3 - A_{39}z_3 - A_{41}e_3z_3 \quad (5.7b)$$

Zymogen I and III are not known to undergo any diffusion. The zymogens are synthesized and secreted by the migrating fibroblasts but the secretion is inhibited by the presence of active TGF β (Jeffrey, 1992). We assume linear inhibition, as shown in the first terms of the above equations. The natural decay of zymogens is taken to be first order and zymogens are activated by specific enzymes, e_3 , to form collagenases.

Collagenases

Collagenases I and III are the active form of zymogens, which bind avidly to collagen I and III, breaking down the fibres. As discussed previously, collagenases are the subject of much biological debate.

$$\begin{array}{rcl} \text{Rate of Increase} & = & \text{Activation of Zymogens} - \text{Natural} \\ \text{of Collagenase} & & \text{by Enzyme} \quad \text{Degradation} \end{array}$$

$$\frac{ds_1}{dt} = A_{42}z_1e_3 - A_{43}s_1 \quad (5.8a)$$

$$\frac{ds_3}{dt} = A_{44}z_3e_3 - A_{45}s_3 \quad (5.8b)$$

We hypothesize that the only source of active collagenase is the zymogens and hypothesize that the activation by the enzyme is independent of $\text{TGF}\beta$. We model natural decay as a first order process.

Governing Equations

Using the above functional forms, we have the following dimensional equations:

$$\frac{\partial f}{\partial t} = D_1 \nabla^2 f + (A_1 + A_2\beta_1 + A_3\beta_3)f \left(1 - \frac{f}{k_1}\right) - A_4f \quad (5.9a)$$

$$\frac{\partial l_1}{\partial t} = D_2 \nabla^2 l_1 + \frac{A_5 f l_1}{1 + A_6 l_3 + A_7 l_1} - A_8 l_1 - A_{16} e_1 l_1 \quad (5.9b)$$

$$\frac{\partial l_3}{\partial t} = D_3 \nabla^2 l_3 + \frac{A_9 f l_3}{1 + A_{10} l_3} - A_{11} l_3 - A_{17} e_1 l_3 \quad (5.9c)$$

$$\frac{\partial \beta_1}{\partial t} = D_4 \nabla^2 \beta_1 + A_{12} e_1 l_1 - A_{13} \beta_1 \quad (5.9d)$$

$$\frac{\partial \beta_3}{\partial t} = D_5 \nabla^2 \beta_3 + A_{14} e_1 l_3 - A_{15} \beta_3 \quad (5.9e)$$

$$\frac{de_1}{dt} = -e_1(A_{16}l_1 + A_{17}l_3) \quad (5.9f)$$

$$\frac{de_2}{dt} = -e_2(A_{18}p_1 + A_{19}p_3) \quad (5.9g)$$

$$\frac{de_3}{dt} = -e_3(A_{40}z_1 + A_{41}z_3) \quad (5.9h)$$

$$\frac{dp_1}{dt} = (A_{20} + A_{21}\beta_1 + A_{22}\beta_3)f - A_{23}p_1 - A_{18}e_2p_1 \quad (5.9i)$$

$$\frac{dp_3}{dt} = (A_{24} + A_{25}\beta_1 + A_{26}\beta_3)f - A_{27}p_3 - A_{19}e_2p_3 \quad (5.9j)$$

$$\frac{dc_1}{dt} = A_{28}p_1e_2 - A_{29}s_1c_1 \quad (5.9k)$$

$$\frac{dc_3}{dt} = A_{30}p_3e_2 - A_{31}s_3c_3 \quad (5.9l)$$

$$\frac{dz_1}{dt} = \frac{A_{32}}{1 + A_{33}\beta_1 + A_{34}\beta_3}f c_1 - A_{35}z_1 - A_{40}e_3z_1 \quad (5.9m)$$

$$\frac{dz_3}{dt} = \frac{A_{36}}{1 + A_{37}\beta_1 + A_{38}\beta_3}f c_3 - A_{39}z_3 - A_{41}e_3z_3 \quad (5.9n)$$

$$\frac{ds_1}{dt} = A_{42}z_1e_3 - A_{43}s_1 \quad (5.9o)$$

$$\frac{ds_3}{dt} = A_{44}z_3e_3 - A_{45}s_3. \quad (5.9p)$$

5.3 Estimating the Parameters

The estimation of dimensional parameter values is essential for biologically realistic model predictions. The governing equations contain a large number of parameters whereas there is a limited source of experimental data. In the following section, we present detailed accounts for the accurate estimation of a few parameters but we have to resort to order of magnitude estimates for a large number of the unknown quantities. Table 5.2 shows the biological significance of each parameter and whether or not the parameter is known. In Section 6.5, we investigate the sensitivity of the model to changes in these parameter values.

- f^0 : The foetal dermis contains approximately 100 cells per cubic millimetre (Morgan & Pledger, 1992) and each cell occupies a volume of the order of $1 \times 10^{-13}\text{m}^3$. Hence we estimate $f^0 = 10^5\text{ml}^{-1}$.
- l_1^0 & l_3^0 : Latent $\text{TGF}\beta$ is released by platelets and experimental results suggest unwounded levels of approximately 2 ng ml^{-1} (Streuli *et al.*, 1993).
- c_1^0 & c_3^0 : The total collagen content in normal foetal dermal tissue is $17\mu\text{g mg}^{-1}$ dry weight (Merkel *et al.*, 1988). Normal dermal tissue consists of 26% type III collagen yielding the values $c_1^0 = 12.5\mu\text{g}$ per mg dry weight and $c_3^0 = 4.5\mu\text{g}$ per mg dry weight.

Parameter	Biological Interpretation	Known/Unknown
D_1	Fibroblast diffusion coefficient	Known
D_2	Latent TGF β 1 diffusion coefficient	Known
D_3	Latent TGF β 3 diffusion coefficient	Known
D_4	Active TGF β 1 diffusion coefficient	Known
D_5	Active TGF β 3 diffusion coefficient	Known
k_1	Fibroblast carrying capacity	Known
A_1	Fibroblast proliferation rate	Calculated
A_2	TGF β 1 stimulated rate of proliferation	Unknown
A_3	TGF β 3 stimulated rate of proliferation	Unknown
A_4	Fibroblast rate of mortality	Known
A_5	Rate of latent TGF β 1 synthesis by fibroblasts	Calculated
A_6	Modulation of latent TGF β 1 by TGF β 3	Calculated
A_7	Latent TGF β 1 saturation coefficient	Calculated
A_8	Natural decay rate of latent TGF β 1	Known
A_9	Rate of latent TGF β 3 synthesis by fibroblasts	Calculated
A_{10}	Latent TGF β 3 saturation coefficient	Calculated
A_{11}	Natural decay rate of latent TGF β 3	Known
A_{12}	Rate of activation of latent TGF β 1	Unknown
A_{13}	Natural decay rate of active TGF β 1	Known
A_{14}	Rate of activation of latent TGF β 3	Unknown
A_{15}	Natural decay rate of active TGF β 3	Known
A_{16}	Latent TGF β 1 stimulated enzyme 1 decay rate	Unknown
A_{17}	Latent TGF β 3 stimulated enzyme 1 decay rate	Unknown
A_{18}	Procollagen I stimulated enzyme 2 decay rate	Unknown
A_{19}	Procollagen III stimulated enzyme 2 decay rate	Unknown
A_{20}	Natural procollagen I secretion rate	Unknown
A_{21}	TGF β 1 stimulated procollagen I secretion rate	Unknown
A_{22}	TGF β 3 stimulated procollagen I secretion rate	Unknown
A_{23}	Natural rate of decay of procollagen I	Unknown
A_{24}	Natural procollagen III secretion rate	Unknown
A_{25}	TGF β 1 stimulated procollagen III secretion rate	Unknown
A_{26}	TGF β 3 stimulated procollagen III secretion rate	Unknown
A_{27}	Natural rate of decay of procollagen III	Unknown
A_{28}	Rate of activation of procollagen I	Unknown
A_{29}	Rate of degradation of collagen I by collagenase I	Unknown
A_{30}	Rate of activation of procollagen III	Unknown
A_{31}	Rate of degradation of collagen III by collagenase III	Unknown
A_{32}	Rate of zymogen I secretion	Unknown
A_{33}	Modulation of zymogen I by TGF β 1	Unknown
A_{34}	Modulation of zymogen I by TGF β 3	Unknown
A_{35}	Natural rate of decay of zymogen III	Unknown
A_{36}	Rate of zymogen III secretion	Unknown

Parameter	Biological Interpretation	Known/Unknown
A_{37}	Modulation of zymogen III by TGF β 1	Unknown
A_{38}	Modulation of zymogen III by TGF β 3	Unknown
A_{39}	Natural rate of decay of zymogen III	Unknown
A_{40}	Zymogen I stimulated enzyme 3 decay rate	Unknown
A_{41}	Zymogen III stimulated enzyme 3 decay rate	Unknown
A_{42}	Rate of activation of zymogen I	Unknown
A_{43}	Natural rate of degradation of collagenase I	Unknown
A_{44}	Rate of activation of zymogen III	Unknown
A_{45}	Natural rate of degradation of collagenase III	Unknown

Table 5.2: Biological interpretation of model parameters

- D_1 : The maximum rate of human adult fibroblast migration is $1\mu\text{m min}^{-1}$ (Bard & Hay, 1975). Foetal fibroblasts migrate about ten times more rapidly, and we thus derive a diffusion coefficient of $1.7 \times 10^{-9}\text{cm}^2\text{s}^{-1}$.
- k_1 : Experiments using high-density fibroblast cultures show that mitosis is totally inhibited at a value of 2×10^6 cells per millilitre (Morgan & Pledger, 1992). Thus, $k_1 = 2 \times 10^6\text{ml}^{-1}$.
- A_4 : The half-life of foetal fibroblasts is approximately 1 day (Ferguson & Howarth, 1992) and hence we calculate the coefficient of natural loss by $A_4 = \frac{\log 2}{1} = 0.7 \text{ day}^{-1}$.
- A_1 : In the absence of active TGF β , the fibroblast density is at a steady state level. This imposes a constraint on A_1 and substituting into equation (5.9a), we find that $A_1(1 - f^0/k_1) = A_4$. We thus calculate A_1 to be 1.4 day^{-1} .
- D_2 & D_3 : The diffusivity of latent TGF β has not been measured directly so we calculate these parameters using the Stokes-Einstein formula, namely *Diffusion Coefficient* $\propto (\text{Molecular Weight})^{-1/3}$ for large proteins in aqueous solution. We showed in Chapter 1 that epidermal growth factor has a diffusion coefficient of $9.75 \times 10^{-7}\text{cm}^2\text{s}^{-1}$ and a molecular weight of 6145, and we know that the molecular weight of latent TGF β is approximately 220kDa (Wakefield *et al.*,

1988). Hence we can use this data to calculate the constant of proportionality in the formula and obtain the values $D_2 = D_3 = 2.94 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$.

- A_8 & A_{11} : The half-life of latent TGF β in adult plasma is around 100 mins (Wakefield, 1990). This yields parameter values of 9.98 day^{-1} which is much faster than the decay of fibroblasts.
- A_5 , A_6 & A_7 : There is no experimental data from which these values can be estimated. We know, however, that there is an unwounded steady state level, from which we derive a relationship between these parameters.
- D_4 & D_5 : The molecular weight of active TGF β is 25kDa (Roberts & Sporn, 1990) and we again use the Stokes-Einstein formula to calculate the diffusion coefficients $D_4 = D_5 = 6.074 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$.
- A_{13} & A_{15} : Experimental data is only available for the half-life of active TGF β in adult plasma (Roberts & Sporn, 1990). This value of 2 mins yields parameters $A_{13} = A_{15} = 400 \text{ day}^{-1}$.

There is little experimental data available for the enzymes, collagens and collagenases and hence we are unable to estimate, with any accuracy, any of the parameters in the equations corresponding to these variables. We do know, however, the time scale of the healing process and can hence obtain order of magnitude estimates for some of the remaining parameters.

5.4 Nondimensionalization

The model equations are simplified by reducing the spatial domain from three dimensions to one. The wound depth is much smaller than the wound length and hence we treat the dermis as two dimensional. By considering a ‘linear’ wound geometry, which models a long ‘strip’ wound, we reduce the dimensions to one. In this case, the wound domain, Ω , is defined to be $0 \leq x \leq 2M$ and the zero flux

boundary conditions are thus imposed at the centre of the wound, $x = M$. A typical length scale, M , for foetal dermal wounds is 0.05 mm and, for algebraic simplicity we introduce a typical time scale, k , of 1 day⁻¹.

We define the following dimensionless quantities

$$\begin{array}{llll}
\tilde{x} & = & \frac{x}{M} & \tilde{t} & = & kt & \tilde{f} & = & \frac{f}{f^0} \\
\tilde{l}_1 & = & \frac{l_1}{l_1^0} & \tilde{l}_3 & = & \frac{l_3}{l_3^0} & \tilde{\beta}_1 & = & \frac{\beta_1}{\beta_1^0} \\
\tilde{\beta}_3 & = & \frac{\beta_3}{\beta_3^0} & \tilde{e}_1 & = & \frac{e_1}{e_1^0} & \tilde{e}_2 & = & \frac{e_2}{e_2^0} \\
\tilde{e}_3 & = & \frac{e_3}{e_3^0} & \tilde{p}_1 & = & \frac{p_1}{p_1^0} & \tilde{p}_3 & = & \frac{p_3}{p_3^0} \\
\tilde{c}_1 & = & \frac{c_1}{c_1^0} & \tilde{c}_3 & = & \frac{c_3}{c_3^0} & \tilde{z}_1 & = & \frac{z_1}{z_1^0} \\
\tilde{z}_3 & = & \frac{z_3}{z_3^0} & \tilde{s}_1 & = & \frac{s_1}{s_1^0} & \tilde{s}_3 & = & \frac{s_3}{s_3^0} \\
\tilde{D}_1 & = & \frac{D_1}{kM^2} & \tilde{A}_1 & = & \frac{A_1}{k} & \tilde{A}_2 & = & \frac{A_2\beta_1^0}{k} \\
\tilde{A}_3 & = & \frac{A_3\beta_3^0}{k} & \tilde{A}_4 & = & \frac{A_4}{k} & \tilde{k}_1 & = & \frac{k_1}{f^0} \\
\tilde{D}_2 & = & \frac{D_2}{kM^2} & \tilde{A}_5 & = & \frac{A_5f^0}{k} & \tilde{A}_6 & = & A_6l_3^0 \\
\tilde{A}_7 & = & \frac{A_7l_1^0}{k} & \tilde{A}_8 & = & \frac{A_8}{k} & \tilde{D}_3 & = & \frac{D_3}{kM^2} \\
\tilde{A}_9 & = & \frac{A_9f^0}{k} & \tilde{A}_{10} & = & \frac{A_{10}l_3^0}{k} & \tilde{A}_{11} & = & \frac{A_{11}}{k} \\
\tilde{D}_4 & = & \frac{D_4}{kM^2} & \tilde{A}_{12} & = & \frac{A_{12}e_1^0l_1^0}{k\beta_1^0} & \tilde{A}_{13} & = & \frac{A_{13}}{k} \\
\tilde{D}_5 & = & \frac{D_5}{kM^2} & \tilde{A}_{14} & = & \frac{A_{14}e_1^0l_3^0}{k\beta_3^0} & \tilde{A}_{15} & = & \frac{A_{15}}{k} \\
\tilde{A}_{16} & = & \frac{A_{16}l_1^0}{k} & \tilde{A}_{17} & = & \frac{A_{17}l_3^0}{k} & \tilde{A}_{18} & = & \frac{A_{18}p_1^0}{k} \\
\tilde{A}_{19} & = & \frac{A_{19}p_3^0}{k} & \tilde{A}_{20} & = & \frac{A_{20}f^0}{kp_1^0} & \tilde{A}_{21} & = & \frac{A_{21}f^0\beta_1^0}{kp_1^0} \\
\tilde{A}_{22} & = & \frac{A_{22}f^0\beta_3^0}{kp_1^0} & \tilde{A}_{23} & = & \frac{A_{23}}{k} & \tilde{A}_{24} & = & \frac{A_{24}f^0}{kp_3^0} \\
\tilde{A}_{25} & = & \frac{A_{25}\beta_1^0f^0}{kp_3^0} & \tilde{A}_{26} & = & \frac{A_{26}\beta_3^0f^0}{kp_3^0} & \tilde{A}_{27} & = & \frac{A_{27}}{k} \\
\tilde{A}_{28} & = & \frac{A_{28}p_1^0e_2^0}{kc_1^0} & \tilde{A}_{29} & = & \frac{A_{29}s_1^0}{k} & \tilde{A}_{30} & = & \frac{A_{30}p_3^0e_2^0}{kc_1^0} \\
\tilde{A}_{31} & = & \frac{A_{31}s_1^0}{k} & \tilde{A}_{32} & = & \frac{A_{32}f^0c_1^0}{kz_1^0} & \tilde{A}_{33} & = & A_{33}\beta_1^0 \\
\tilde{A}_{34} & = & A_{34}\beta_3^0 & \tilde{A}_{35} & = & \frac{A_{35}}{k} & \tilde{A}_{36} & = & \frac{A_{36}f^0c_1^0}{kz_3^0} \\
\tilde{A}_{37} & = & A_{37}\beta_1^0 & \tilde{A}_{38} & = & A_{38}\beta_3^0 & \tilde{A}_{39} & = & \frac{A_{39}}{k} \\
\tilde{A}_{40} & = & \frac{A_{40}z_1^0}{k} & \tilde{A}_{41} & = & \frac{A_{41}z_3^0}{k} & \tilde{A}_{42} & = & \frac{A_{42}z_1^0e_3^0}{ks_1^0} \\
\tilde{A}_{43} & = & \frac{A_{43}}{k} & \tilde{A}_{44} & = & \frac{A_{44}z_3^0e_3^0}{ks_1^0} & \tilde{A}_{45} & = & \frac{A_{45}}{k} \\
\tilde{\mu}_1 & = & \frac{A_{17}}{A_{16}l_1^0} & \tilde{\mu}_2 & = & \frac{A_{19}}{A_{18}l_3^0} & \tilde{\mu}_3 & = & \frac{A_{41}}{A_{40}} \\
\tilde{\beta}_1^0 & = & \frac{A_{12}l_1^0}{A_{16}} & \tilde{\beta}_3^0 & = & \frac{A_{14}l_3^0}{A_{16}} & \tilde{e}_1^0 & = & \frac{k}{A_{16}} \\
\tilde{e}_2^0 & = & \frac{k}{A_{18}} & \tilde{e}_3^0 & = & \frac{k}{A_{40}} & \tilde{s}_1^0 & = & \frac{k}{A_{29}} \\
\tilde{s}_3^0 & = & \frac{k}{A_{31}} & & & & & &
\end{array}$$

Using these parameter groupings, we can nondimensionalize the parameters estimated above. Table (5.3) shows typical parameter values for a 0.1 mm foetal dermal

$D_1 = 0.015$	$D_2 = 0.25$	$D_3 = 0.25$	$A_1 = 1.4$	$A_2 = 200$
$A_3 = 200$	$A_4 = 0.7$	$A_5 = 100$	$A_6 = 5$	$A_7 = 4$
$A_8 = 10$	$A_9 = 100$	$A_{10} = 9$	$A_{11} = 10$	$A_{12} = 1$
$A_{13} = 400$	$A_{14} = 1$	$A_{15} = 400$	$A_{16} = 0.1$	$A_{17} = 0.1$
$A_{18} = 1$	$A_{19} = 1$	$A_{20} = 3$	$A_{21} = 600$	$A_{22} = 1400$
$A_{23} = 1$	$A_{24} = 3$	$A_{25} = 600$	$A_{26} = 800$	$A_{27} = 1$
$A_{28} = 1.8$	$A_{29} = 1$	$A_{30} = 0.6$	$A_{31} = 1$	$A_{32} = 1$
$A_{33} = 400$	$A_{34} = 1200$	$A_{35} = 10$	$A_{36} = 1$	$A_{37} = 1200$
$A_{38} = 400$	$A_{39} = 10$	$A_{40} = 50$	$A_{41} = 50$	$A_{42} = 1.5$
$A_{43} = 1$	$A_{44} = 4.5$	$A_{45} = 1$	$k_1 = 2$	$\mu_1 = 1$
$\mu_2 = 1$	$\mu_3 = 1$			

Table 5.3: Typical parameter values for a 0.1 mm foetal dermal wound

wound, including the order of magnitude estimates which will be used for the numerical work discussed later.

Reduced model

The complex system of equations defined in (5.9) can be somewhat simplified, and we discuss this before considering our simulations. Experimental data suggest that the degradation of active TGF β occurs on a much faster time scale than that of latent TGF β (Roberts & Sporn, 1990), and thus we assume that the concentrations of active TGF β 1 & 3 are always in equilibrium. Therefore equations (5.9d) and (5.9e) yield the equilibrium values

$$\beta_1 = \frac{A_{12}e_1l_1}{A_{13}} \quad \beta_3 = \frac{A_{14}e_1l_3}{A_{15}}.$$

Using a similar argument, we assume that the procollagens and zymogens are both in equilibrium and hence derive from the model equations the following pseudo-steady state values

$$p_1 = \frac{(A_{20} + A_{21}\beta_1 + A_{22}\beta_3)}{A_{23} + A_{18}e_2}$$

$$p_3 = \frac{(A_{24} + A_{25}\beta_1 + A_{26}\beta_3)}{A_{25} + A_{19}e_2}$$

$$z_1 = \frac{f c_1}{(A_{35} + A_{40} e_3)(A_{32} + A_{33} \beta_1 + A_{34} \beta_3)}$$

$$z_3 = \frac{f c_3}{(A_{39} + A_{41} e_3)(A_{36} + A_{37} \beta_1 + A_{38} \beta_3)}.$$

Dropping tilde's for notational simplicity, we derive the nondimensional model:

$$\frac{\partial f}{\partial t} = D_1 \frac{\partial^2 f}{\partial x^2} + (A_1 + \frac{A_2 A_{12} e_1 l_1}{A_{13}} + \frac{A_3 A_{14} e_1 l_3}{A_{15}}) f \left(1 - \frac{f}{k_1}\right) - A_4 f \quad (5.10a)$$

$$\frac{\partial l_1}{\partial t} = D_2 \frac{\partial^2 l_1}{\partial x^2} + \frac{A_5 f l_1}{1 + A_6 l_3 + A_7 l_1} - A_8 l_1 - e_1 l_1 \quad (5.10b)$$

$$\frac{\partial l_3}{\partial t} = D_3 \frac{\partial^2 l_3}{\partial x^2} + \frac{A_9 f l_3}{1 + A_{10} l_3} - A_{11} l_3 - \mu_1 e_1 l_3 \quad (5.10c)$$

$$\frac{de_1}{dt} = -e_1 (A_{16} l_1 + A_{17} l_3) \quad (5.10d)$$

$$\begin{aligned} \frac{de_2}{dt} = & -e_2 f \left[\frac{A_{18}}{A_{23} + e_2} (A_{20} + \frac{A_{21} A_{12} e_1 l_1}{A_{13}} + \frac{A_{22} A_{14} e_1 l_3}{A_{15}}) \right. \\ & \left. + \frac{A_{19}}{A_{27} + \mu_2 e_2} (A_{24} + \frac{A_{25} A_{12} e_1 l_1}{A_{13}} + \frac{A_{26} A_{14} e_1 l_3}{A_{15}}) \right] \end{aligned} \quad (5.10e)$$

$$\begin{aligned} \frac{de_3}{dt} = & -e_3 f \left[\frac{A_{40} c_1}{A_{35} + e_3} \left(\frac{A_{32}}{1 + \frac{A_{33} A_{12} e_1 l_1}{A_{13}} + \frac{A_{34} A_{14} e_1 l_3}{A_{15}}} \right) \right. \\ & \left. + \frac{A_{41} c_3}{A_{37} + \mu_3 e_3} \left(\frac{A_{36}}{1 + \frac{A_{37} A_{12} e_1 l_1}{A_{13}} + \frac{A_{38} A_{14} e_1 l_3}{A_{15}}} \right) \right] \end{aligned} \quad (5.10f)$$

$$\frac{dc_1}{dt} = \frac{A_{28} e_2 f}{A_{23} + e_2} \left(A_{20} + \frac{A_{21} A_{12} e_1 l_1}{A_{13}} + \frac{A_{22} A_{14} e_1 l_3}{A_{15}} \right) - s_1 c_1 \quad (5.10g)$$

$$\frac{dc_3}{dt} = \frac{A_{30} e_2 f}{A_{27} + \mu_2 e_2} \left(A_{24} + \frac{A_{25} A_{12} e_1 l_1}{A_{13}} + \frac{A_{26} A_{14} e_1 l_3}{A_{15}} \right) - s_3 c_3 \quad (5.10h)$$

$$\frac{ds_1}{dt} = \frac{A_{42} e_3 f c_1}{A_{35} + e_3} \left(\frac{A_{32}}{1 + \frac{A_{33} A_{12} e_1 l_1}{A_{13}} + \frac{A_{34} A_{14} e_1 l_3}{A_{15}}} \right) - A_{43} s_1 \quad (5.10i)$$

$$\frac{ds_3}{dt} = \frac{A_{44} e_3 f c_3}{A_{39} + \mu_3 e_3} \left(\frac{A_{36}}{1 + \frac{A_{37} A_{12} e_1 l_1}{A_{13}} + \frac{A_{38} A_{14} e_1 l_3}{A_{15}}} \right) - A_{45} s_1. \quad (5.10j)$$

Hence the number of equations is reduced from 16 to 10, and numerical solutions of the full system verify that this reduction does not affect the results. Moreover, the first four equations decouple from the other six and thus can be solved separately. The e_2 equation is only dependent on the first four variables and thus can be solved independently. We can then solve the remaining collagen and collagenase equations.

5.5 Summary

In this chapter we have discussed, in detail, the biological process of dermal wound healing and highlighted the main differences in foetal and adult wounds. We have determined the key variables for scar tissue models and developed a detailed mathematical model. The model contains a number of parameters, only a few of which can be estimated from the data available. However, we are able to make order of magnitude estimates for many of the parameters. Using a pseudo-steady state hypothesis, we have reduced the number of equations to ten and specified relevant initial and boundary conditions. In the following chapters, we first look at a temporal model and consider the effect of exogenous application of $TGF\beta$. We then consider a reaction-diffusion model to consider the role of fibroblast and growth factor diffusion.

Chapter 6

Temporal Model

6.1 Introduction

In the previous chapter, we developed a mathematical model based on the complex interactions of cells, growth factors, collagens and collagenases during dermal wound healing. There has been little experimental work on the role of diffusion of growth factors and migration of fibroblasts into the wound milieu. In this absence of experimental detail, there is a controversy concerning whether fibroblasts move into the wound from the underlying subcutaneous tissue or from the neighbouring dermis. In the latter case, a spatially dependent model is essential, but in the former case spatial variations within the wound will be quite small and we can reasonably consider a purely temporal model. We first consider this spatially homogeneous model equations for foetal wounds and investigate the temporal changes in the wound milieu. We determine the steady states of the system and carry out linear stability analysis, and then solve the system numerically. We investigate the effect of exogenous application of various factors and consider the effect of later addition of growth factors. We observe a good agreement with the experimental data for the foetal model. We then consider the changes in parameters that are required to model adult wound healing. By changing initial conditions to model the addition of $\text{TGF}\beta$, we find that the model results compare favourably with experimental data. Furthermore, the model enables us to make experimentally testable predictions as to the effect of $\text{TGF}\beta$ application on collagen fibre diameters and densities.

6.2 Linear Stability Analysis

6.2.1 Steady States

We consider the spatially homogeneous model equations and determine the steady states of the resulting tenth order system of ordinary differential equations by solving the algebraic system

$$0 = (A_1 + \frac{A_2 A_{12} e_1 l_1}{A_{13}} + \frac{A_3 A_{14} e_1 l_3}{A_{15}}) f \left(1 - \frac{f}{k_1}\right) - A_4 f \quad (6.1a)$$

$$0 = \frac{A_5 f l_1}{1 + A_6 l_3 + A_7 l_1} - A_8 l_1 - e_1 l_1 \quad (6.1b)$$

$$0 = \frac{A_9 f l_3}{1 + A_{10} l_3} - A_{11} l_3 - \mu_1 e_1 l_3 \quad (6.1c)$$

$$0 = -e_1 (A_{16} l_1 + A_{17} l_3) \quad (6.1d)$$

$$0 = -e_2 f \left[\frac{A_{18}}{A_{23} + e_2} \left(A_{20} + \frac{A_{21} A_{12} e_1 l_1}{A_{13}} + \frac{A_{22} A_{14} e_1 l_3}{A_{15}} \right) + \frac{A_{19}}{A_{27} + \mu_2 e_2} \left(A_{24} + \frac{A_{25} A_{12} e_1 l_1}{A_{13}} + \frac{A_{26} A_{14} e_1 l_3}{A_{15}} \right) \right] \quad (6.1e)$$

$$0 = -e_3 f \left[\frac{A_{40} c_1}{A_{35} + e_3} \left(\frac{A_{32}}{1 + \frac{A_{33} A_{12} e_1 l_1}{A_{13}} + \frac{A_{34} A_{14} e_1 l_3}{A_{15}}} \right) + \frac{A_{41} c_3}{A_{37} + \mu_3 e_3} \left(\frac{A_{36}}{1 + \frac{A_{37} A_{12} e_1 l_1}{A_{13}} + \frac{A_{38} A_{14} e_1 l_3}{A_{15}}} \right) \right] \quad (6.1f)$$

$$0 = \frac{A_{28} e_2 f}{A_{23} + e_2} \left(A_{20} + \frac{A_{21} A_{12} e_1 l_1}{A_{13}} + \frac{A_{22} A_{14} e_1 l_3}{A_{15}} \right) - s_1 c_1 \quad (6.1g)$$

$$0 = \frac{A_{30} e_2 f}{A_{27} + \mu_2 e_2} \left(A_{24} + \frac{A_{25} A_{12} e_1 l_1}{A_{13}} + \frac{A_{26} A_{14} e_1 l_3}{A_{15}} \right) - s_3 c_3 \quad (6.1h)$$

$$0 = \frac{A_{42} e_3 f c_1}{A_{35} + e_3} \left(\frac{A_{32}}{1 + \frac{A_{33} A_{12} e_1 l_1}{A_{13}} + \frac{A_{34} A_{14} e_1 l_3}{A_{15}}} \right) - A_{43} s_1 \quad (6.1i)$$

$$0 = \frac{A_{44} e_3 f c_3}{A_{39} + \mu_3 e_3} \left(\frac{A_{36}}{1 + \frac{A_{37} A_{12} e_1 l_1}{A_{13}} + \frac{A_{38} A_{14} e_1 l_3}{A_{15}}} \right) - A_{45} s_1. \quad (6.1j)$$

As discussed in the previous chapter, the first four equations decouple from the other six and hence we focus on these initially. Equation (6.1d) implies that at a steady state either $e_1 = 0$ or $l_1 = l_3 = 0$. In either case, it follows that $e_1 l_1$ and $e_1 l_3$ are zero. Substituting this into the fibroblast equation, (6.1a), we deduce that

either

$$f = f^w \equiv 0, \text{ or } f = f^u \equiv \frac{k_1}{A_1}(A_1 - A_4),$$

corresponding to the wounded and unwounded steady states, respectively. We can thus solve the latent TGF β equations (6.1b) & (6.1c) to obtain

$$l_1^w = 0 \text{ and } l_1^u = \frac{1}{A_7} \left(\frac{A_5 k_1}{A_8 A_1} (A_1 - A_4) - 1 - \frac{A_6}{A_{10}} \left(\frac{A_9 k_1}{A_{11} A_1} (A_1 - A_4) - 1 \right) \right)$$

$$l_3^w = 0 \text{ and } l_3^u = \frac{1}{A_{10}} \left(\frac{A_9 k_1}{A_{11} A_1} (A_1 - A_4) - 1 \right).$$

These equilibrium values are in fact the normal dermal levels of foetal fibroblasts and TGF β (i.e. $f^u = 1$, $l_1^u = 1$ and $l_3^u = 1$). We now substitute these expressions into the remaining six equations, noting that all the terms inside the brackets in equations (6.1e) and (6.1f) are positive, and deduce that only the trivial steady state exists for both the enzymes e_2 and e_3 . We can thus deduce from equations (6.1i) and (6.1j) that $s_1 = 0$ and $s_3 = 0$ are the only equilibrium values for the collagenases. Hence, by solving equations (6.1g) and (6.1h) we find a continuum of possible non-trivial collagen I and III steady states, c_1^* and c_3^* respectively. This is a crucial property of the model, since experimental results suggest that the final steady levels of collagen I and III are altered by exogenous application of TGF β . Since the collagen steady states differ from the normal dermal levels, we call them the ‘healed’ levels.

In summary, we find there is a ‘trivial’ or ‘wounded’ steady state and an infinite number of non-zero steady states, which we can write as

$$\begin{aligned} \text{Wounded : } & \underline{q}_s^w = (f = l_1 = l_3 = e_1 = e_2 = e_3 = c_1 = c_3 = s_1 = s_3 = 0) \\ \text{Healed : } & \underline{q}_s^u = (f = l_1 = l_3 = 1, e_1 = e_2 = e_3 = 0, c_1 = c_1^*, c_3 = c_3^*, s_1 = s_3 = 0) \end{aligned}$$

6.2.2 Linear Stability

We investigate the linear stability of $\underline{q}_s^w$ and $\underline{q}_s^u$ by linearizing about the steady state using $\underline{W} = \underline{q}_s + \delta\underline{W}$ where $\underline{W}$ is the set of model variables and $||\delta\underline{W}|| \ll 1$ is the perturbation of $\underline{W}$ from it's uniform steady state value. We neglect quadratic and higher order terms and hence infer linear stability from calculating the eigenvalues of the Jacobian

$$J = \begin{bmatrix} B & 0 \\ 0 & C \end{bmatrix}$$

where B is a 4×4 matrix, C is a 6×6 matrix, given by

$$B = \begin{pmatrix} A_1(1 - \frac{2f}{k_1}) - A_4 & 0 & 0 & (\frac{A_2 l_1}{A_{13}} + \frac{A_3 l_3}{A_{15}})f(1 - \frac{f}{k_1}) \\ \frac{A_5 l_1}{1 + A_6 l_3 + A_7 l_1} & \frac{A_5 f(1 + A_6 l_3)}{(1 + A_6 l_3 + A_7 l_1)^2} & -\frac{A_5 A_6 f l_1}{(1 + A_6 l_3 + A_7 l_1)^2} & -l_1 \\ \frac{A_9 l_3}{1 + A_{10} l_3} & 0 & \frac{A_9 f}{(1 + A_{10} l_3)^2} - A_{11} & -\mu_1 l_3 \\ 0 & 0 & 0 & -(A_{16} l_1 + A_{17} l_3) \end{pmatrix}$$

and

$$C = \begin{pmatrix} -f(\frac{A_{18} A_{20}}{A_{23}} + \frac{A_{19} A_{24}}{A_{27}}) & 0 & 0 & 0 & 0 & 0 \\ 0 & -f(\frac{A_{40} A_{32} c_1^*}{A_{35}} + \frac{A_{41} A_{36} c_3^*}{A_{39}}) & 0 & 0 & 0 & 0 \\ \frac{A_{28} A_{20} f}{A_{23}} & 0 & -s_1 & 0 & -c_1 & 0 \\ \frac{A_{30} A_{24} f}{A_{27}} & 0 & 0 & -s_3 & 0 & -c_3 \\ 0 & \frac{A_{32} A_{42} f c_1}{A_{35}} & 0 & 0 & -A_{43} & 0 \\ 0 & \frac{A_{36} A_{44} f c_3}{A_{39}} & 0 & 0 & 0 & -A_{44} \end{pmatrix}.$$

with f , l_1 , l_3 , c_1 , c_3 , s_1 and s_3 all evaluated at the appropriate steady state. Note that the block structure of this matrix is due to the fact that the steady state values of the enzymes, $e_j, j = 1, 2, 3$, are all zero.

Wounded Steady State

At the trivial steady state, the eigenvalues of the linear stability matrix are

$$\lambda = A_1 - A_4, -A_8, -A_{10}, 0, 0, 0, 0, 0, -A_{43}, -A_{45}$$

where, for all biologically realistic parameters, $A_1 > A_4$. We therefore have one positive, four negative and five zero eigenvalues. This, together with numerical evidence, suggests that the trivial steady state is linearly unstable.

Healed Steady State

As already discussed, there is a spectrum of healed steady states, depending on the equilibrium values of the collagens. The eigenvalues corresponding to the healed steady state are given by

$$\begin{aligned} \lambda_1 &= A_1(1 - 2/k_1) - A_4 & \lambda_2 &= \frac{A_5(1+A_6)}{(1+A_6+A_7)^2} - A_8 \\ \lambda_3 &= \frac{A_9}{(1+A_{10})^2} - A_{11} & \lambda_4 &= -(A_{16} + A_{17}) \\ \lambda_5 &= -\left(\frac{A_{18}A_{20}}{A_{23}} + \frac{A_{19}A_{24}}{A_{27}}\right) & \lambda_6 &= -\left(\frac{A_{40}A_{32}c_1^*}{A_{35}} + \frac{A_{41}A_{36}c_3^*}{A_{39}}\right) \\ \lambda_7 &= 0 & \lambda_8 &= 0 \\ \lambda_9 &= -A_{43} & \lambda_{10} &= -A_{45} \end{aligned}$$

Now, in the absence of TGF β , the fibroblasts are at their normal dermal level, which implies that $A_1(1-1/k_1) = A_4$. Hence $\lambda_1 = -A_1/k_1 < 0$. The steady state condition for latent TGF β 1 gives us the parameter relation $A_5 = A_8(1 + A_6 + A_7)$, and after some simple algebraic manipulation we find that $\lambda_2 < 0$ provided $A_8(1 + A_6) < A_5$. Now it is not biologically realistic for the chemical concentration to become negative, therefore this condition holds for all parameter values and hence λ_2 is always negative. A similar argument for latent TGF β 3 reveals that $\lambda_3 < 0$. The collagen levels c_1^* and c_3^* are always positive and thus λ_6 is always negative, only the magnitude being dependent on the equilibrium values. Hence we find that all the eigenvalues are non-positive. The two zero eigenvalues suggest neutral stability, but from numerical evidence we can deduce that the unwounded steady state is always linearly stable, for all possible levels of collagen I and III.

6.3 Numerical Solution of the Foetal Temporal Model

Experimentalists have shown that foetal wounds heal without scarring, and that a key aspect of this perfect repair is that the overall density of collagen in the healed wound remains close to the unwounded levels and the ratio of collagen types I to III is maintained at approximately 3:1 (Whitby & Ferguson, 1992). We first consider our spatially homogeneous model for foetal wounds and look for this type of behaviour. The model is a tenth order system of ordinary differential equations ((5.10) without the diffusion terms), which we solve numerically using a NAG-routine (D02BBF) based on the Runge-Kutta-Merson method. Now, in the absence of diffusion, we require a small number of fibroblasts and a low concentration of growth factors to initiate the healing process and we thus impose the initial conditions

$$\begin{array}{ll} f(x, 0) &= 0.1 & l_1(x, 0) &= 0.1 \\ l_3(x, 0) &= 0.1 & e_1(x, 0) &= 1 \\ e_2(x, 0) &= 1 & e_3(x, 0) &= 1 \\ c_1(x, 0) &= 0 & c_3(x, 0) &= 0 \\ s_1(x, 0) &= 0 & s_3(x, 0) &= 0, \end{array}$$

for $x \in [0, 1]$. This corresponds to a few fibroblasts and growth factors scattered uniformly throughout the wound, with the generic enzymes at the nondimensionalised level of 1. Numerical solutions suggest robustness for initial amounts of fibroblasts and growth factors less than 0.1. Different initial conditions will be investigated later. Figure 6.1 shows the time evolution of the model variables to their steady states. There is a rapid initial increase of fibroblasts and TGF β before they settle down to the normal dermal levels. This is in agreement with experimental observations (Ferguson & Howarth, 1992). The initial decrease in growth factor illustrates exponential decay at low cell densities. Enzyme 1 gradually decreases with time whereas enzymes 2 and 3 undergo a much more rapid decay. We use these solutions to fix the degradation parameters A_{18} , A_{19} , A_{40} and A_{41} and use experimental observations to choose A_{21} , A_{22} , A_{25} and A_{26} . The parameters A_{28} and A_{30} are fixed by comparing model solutions with experimental results, to obtain the required colla-

gen ratio. The new levels of collagen I and III are quickly attained and these levels, 0.89 and 0.295 respectively, are maintained throughout the healing process. Thus both the density of collagen and the ratio of the two types after healing agrees with the data.

6.3.1 Addition of $\text{TGF}\beta$

Much experimental work has been carried out on the effects of the addition of various growth factors on wounds, mainly adult wounds. Dermal adult rat wounds injected at the margin with neutralizing antibody (NA) to $\text{TGF}\beta 1$ have been shown to heal without scarring and with fewer macrophages and blood vessels, lower collagen and fibronectin content, but identical tensile strength and more normal dermal architecture than other wounds. The advantageous effects on scarring in the NA treated wounds were not accompanied by a delay in wound healing or a reduction in wound strength (Shah *et al.*, 1992). Research has also shown that when exogenous $\text{TGF}\beta$ is added to foetal wounds in rabbits (Krummel *et al.*, 1988), the final ratio of the collagens changes. In particular, it has been shown that topical application of $\text{TGF}\beta 1$ increases the amount of collagen III relative to collagen I.

To model the addition of $\text{TGF}\beta$ in our system, we simply alter the initial conditions so that $l_i = b$, where b is a positive constant, for $i = 1$ or 3 depending on which isoform of the growth factor is added. By varying the initial concentration of the $\text{TGF}\beta$'s, numerical simulations indicate that the ratio of the collagen I to III is changed. Furthermore, we find that there is a saturating level of $l_i = 200$, corresponding to a dimensional concentration of $0.4 \mu\text{g ml}^{-1}$, beyond which addition of more chemical has no effect on the ratio of collagens (Figure 6.2). This is due to the saturating term representing the secretion of growth factor by the fibroblasts. The concentration is in agreement with the order of magnitude of the concentrations of chemical applied topically in the experiments (Krummel *et al.*, 1988). Figure 6.3 shows the effect of changing the initial concentration of $\text{TGF}\beta 1$ so that $l_1 = 1000$, one thousand times the normal dermal concentration. The graphs show the new

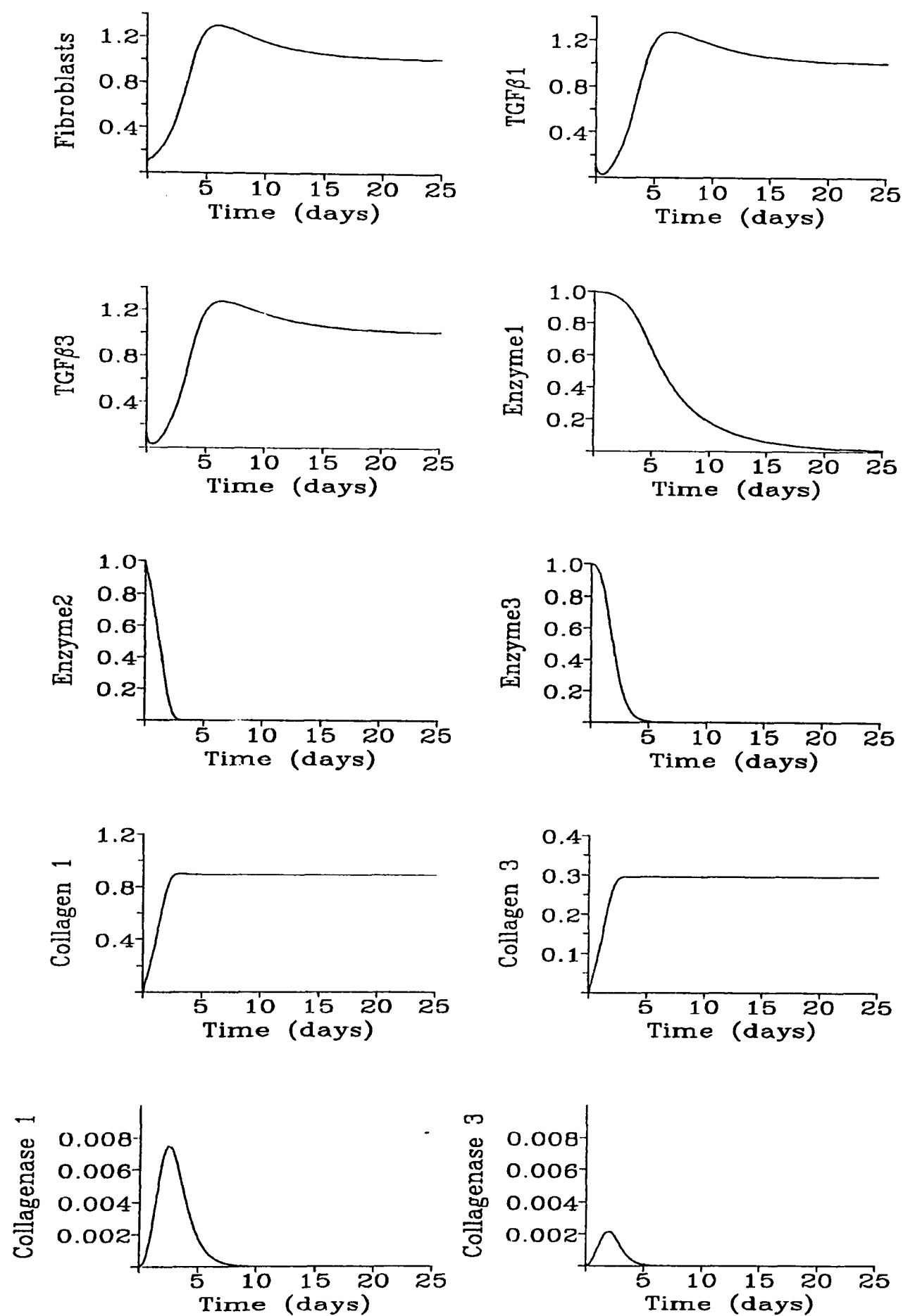


Figure 6.1: Numerical solution of the system of first order ordinary differential equations with spatially homogeneous initial conditions, up to 20 days. The parameters as shown in Table 5.3. The field variables rapidly attain their steady state values and the ratio of collagen types I to III is 3:1.

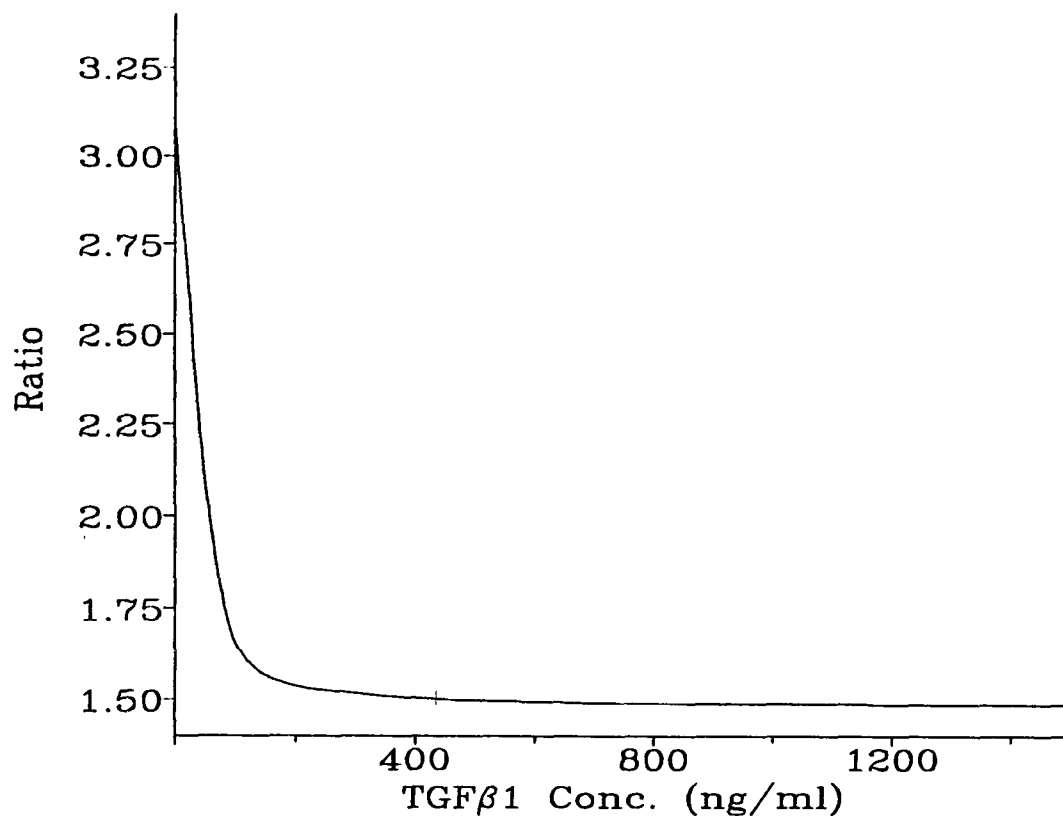


Figure 6.2: The change in the ratio of collagen I and III with the addition of increasing quantities of TGF β 1. There is a saturating level at a concentration of $0.4 \mu\text{g ml}^{-1}$.

steady state levels of collagen I and III, at 0.59 and 0.4 respectively. This enhanced ratio of 3:2 and the reduction in the total amount of collagen implies that the collagen fibrils in the healed wound are much thinner in diameter than in normal foetal wounds, which, in turn, implies scarring. Similarly, by changing the initial conditions to model the addition of TGF β 3, we find the ratio of collagen I to III increases, corresponding to thicker fibres. Since we have not considered the orientation of the fibres, we do not know whether this corresponds to really good healing or abnormal healing (such as hypertrophic scarring). The addition of TGF β 3 to foetal wounds is thus an essential area of research.

We can similarly investigate the effect on the collagen ratio of altering the initial conditions of the other variables. In Table 6.1, we alter one or more of the initial conditions and record the final levels of collagen I and III, together with their ratio. We note that the other 8 variables all attain their normal dermal values $f = l_1 = l_3 = 1$ and $e_1 = e_2 = e_3 = s_1 = s_3 = 0$. By analysing these results, we conclude that

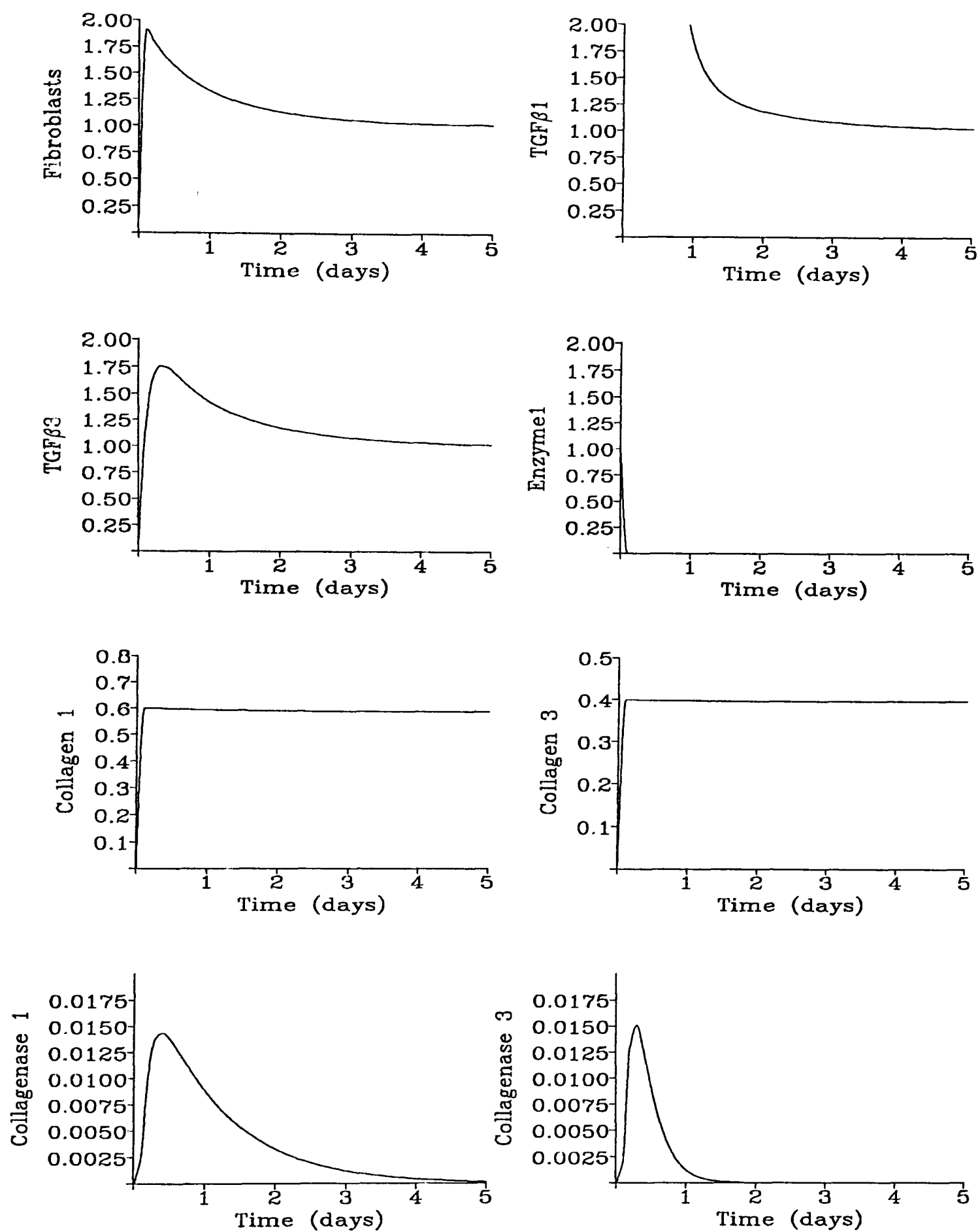


Figure 6.3: Numerical solution of the system of first order ordinary differential equations with spatially homogeneous initial conditions, with $b = 1000$, showing the effect of exogenous application of TGF β 1. The parameters as shown in Table 5.3. The field variables rapidly attain their steady state values and the ratio of collagen types I and III is 3:2, indicating thinner fibres and enforced scarring.

1. The final steady state levels of collagen I and III are not changed if fibroblasts or enzymes are topically applied to the wound.
2. Addition of $\text{TGF}\beta 1$ decreases the level of collagen I down to a minimum level of 0.59 but increases the level of collagen III to 0.4, thus changing the ratio to 3:2. Normal foetal dermal tissue contains $12.5\mu\text{g}$ per mg dry weight of collagen I and $4.5\mu\text{g}$ per mg dry weight of type III. Hence, using our nondimensionalisation, addition of $\text{TGF}\beta 1$ yields final amounts of 7.375 and $5\mu\text{g}$ per mg dry weight for collagen I and III, respectively.
3. Addition of $\text{TGF}\beta 3$ increases the level of collagen I up to a saturating level of 1.11 but decreases the final level of collagen III to 0.218. This corresponds to dimensional amounts 13.875 and $2.725\mu\text{g}$ per mg dry weight, respectively, thus changing the ratio to a maximum of 5 : 1.
4. Addition of collagen I increases the final level of collagen I but has no effect on the level of collagen III, thus increasing the ratio.
5. Addition of collagen III increases the final level of collagen III but has no effect on the level of collagen I, thus decreasing the ratio.
6. Adding collagenase I only decreases the final amount of collagen I, thus decreasing the ratio.
7. Adding collagenase III only decreases the final amount of collagen III, thus increasing the ratio.

We also investigate the importance of the time at which $\text{TGF}\beta$ is added to wounds. By changing the initial conditions to represent addition of $0.4\mu\text{g ml}^{-1}$ of $\text{TGF}\beta 3$ at later stages of the healing process, numerical simulations indicate that early addition is essential. In fact, addition of growth factor after day 2 of healing has little effect on either the density of collagen or the ratio of types I to III (Figure 6.4).

Change in Initial Condition	Final level of Collagen I	Final level of Collagen III	Ratio of Collagen I to Collagen III	Change in Fibre Diameters
$f = 1$	0.89	0.295	3	No change
$l_1 = 10$	0.84	0.31	2.7	Thinner
$l_1 = 1000$	0.59	0.4	1.475	Thinner
$l_3 = 10$	0.95	0.275	3.45	Thicker
$l_3 = 1000$	1.11	0.218	5.1	Thicker
$c_1 = 1$	1.865	0.295	6.3	Thicker
$c_3 = 0.36$	0.89	0.65	1.37	Thinner
$s_1 = 1$	0.638	0.295	2.16	Thinner
$s_3 = 1$	0.89	0.28	3.18	Thicker

Table 6.1: The final levels of collagens type I and III, together with the ratio of types, when the initial conditions are changed to model the exogenous application of growth factors and collagens in foetal dermal wound healing.

One biological feature which is included in our model is the modulation of latent $TGF\beta 1$ by latent $TGF\beta 3$ (Shah, personal communication), a phenomenon which is not mimicked in the corresponding $TGF\beta 3$ equation. This is represented by an asymmetry in the latent growth factor equations. We investigate the importance of this term by numerically solving the system of ordinary differential equations with $A_6 = 0$, corresponding to no modulation. Neither the final levels of collagen I and III nor the ratio are significantly altered by the removal of this term, indicating that the modulation of $TGF\beta 1$ is not crucial for foetal dermal wound healing.

We can conclude from this section that the temporal model for foetal wound healing predicts levels of collagen I and III which compare favourably with experimental data, both in normal healing and when $TGF\beta 1$ is applied topically to the wound. The model also predicts that early addition of $TGF\beta 3$ results in thicker fibres, a result which could be tested experimentally. We now look at a temporal model for adult wound healing, which involves altering the model parameters in accordance with experimental data.

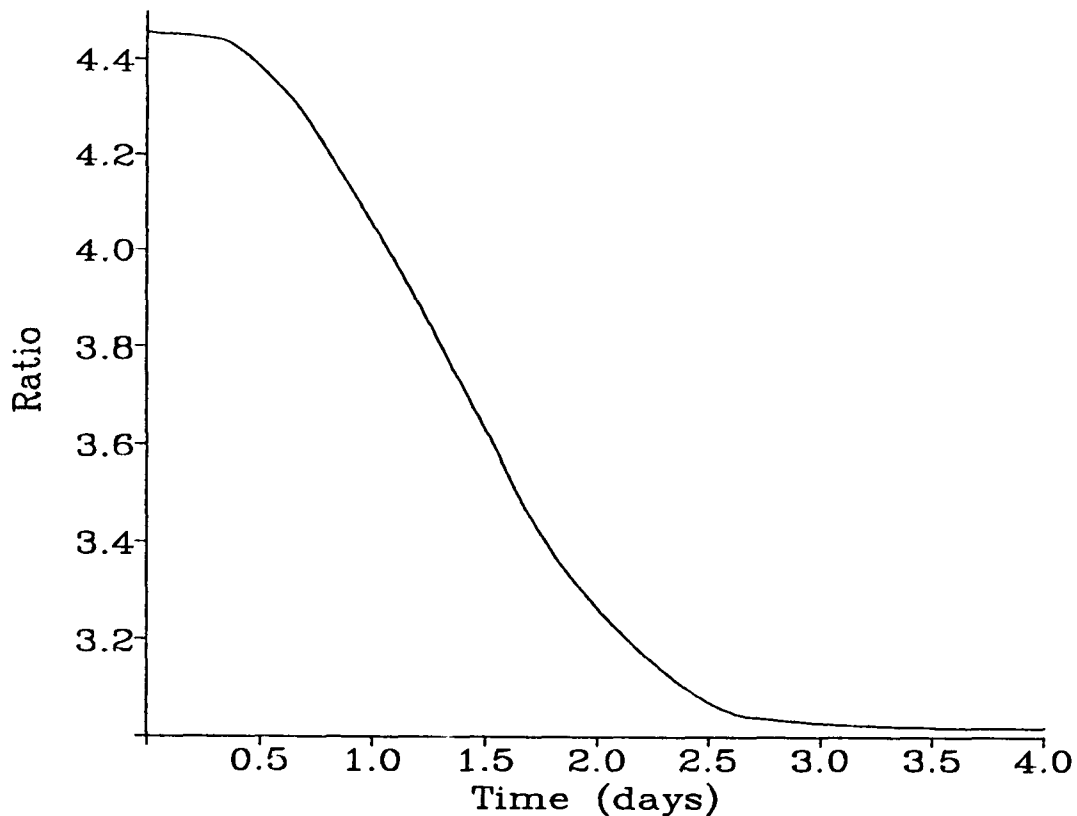


Figure 6.4: The change in the ratio of collagen I and III with the addition of TGF β 1 at later stages in the healing process. After day 2, addition of growth factor has little effect on the ratio.

6.4 Adult Wound Healing

Foetal dermal wounds heal rapidly, with minimal scar formation and a lack of inflammatory response. There is a gradual transition in the rate and quality of wound healing as the foetus becomes a neonate (at approximately two thirds of the gestation period), then a young child and eventually an adult. Adult wound healing is much slower and results in scar tissue formation, which consists of thinner collagen fibrils, more densely packed and orientated perpendicular to the basement membrane (Whitby & Ferguson, 1992). In this section, we model the time evolution of adult scar tissue formation and look at possible ways of reducing scarring and enabling the adult wound to heal in a similar fashion to the foetus.

6.4.1 Estimating the Parameters

We adapt our model for foetal healing by changing some of the parameters based on experimental evidence. The biological interpretation of each model parameter is shown in Table 5.2.

Fibroblasts

1. The cell cycle time for adult fibroblasts is much slower than in the foetal case. Experiments have shown the half-life of adult fibroblasts to be 100 hours (Morgan & Pledger, 1992) and thus we find the fibroblast rate of mortality, A_4 , to be $\frac{\log 2}{100} \text{hr}^{-1} = 0.166 \text{ day}^{-1}$.
2. We can use the new value of A_4 and the constraint of the steady state fibroblast level to calculate the linear birth rate A_1 to be 0.332 day^{-1} .

TGF β

1. The concentration of TGF β in adult dermal tissue is much higher than in the foetal case. The unwounded concentrations are 80 and 40 ng ml⁻¹ for TGF β 1 and 3, respectively (Roberts & Sporn, 1990), thus resulting in new nondimensionalised steady state levels of 40 and 20, respectively.
2. The new values of l_1^0 and l_3^0 effect the nondimensionalisation of various other parameters, namely $A_6 = 0.25$, $A_7 = 0.1$, $A_{10} = 0.45$, $A_{16} = 0.0025$ and $A_{17} = 0.005$.

Collagen

1. The total collagen content in normal adult dermal tissue is 331 μg per mg dry weight (Merkel *et al.*, 1988). Normal dermal tissue consists of 15% type III collagen, yielding the new values $c_1^0 = 281.35$ and $c_3^0 = 49.65 \mu\text{g}$ per mg dry weight. The new nondimensional steady state levels of collagen I and III are thus 22.5 and 3.97 respectively.

$D_1 = 0.015$	$D_2 = 0.25$	$D_3 = 0.25$	$A_1 = 0.332$	$A_2 = 200$
$A_3 = 200$	$A_4 = 0.166$	$A_5 = 100$	$A_6 = 0.255$	$A_7 = 0.1$
$A_8 = 10$	$A_9 = 100$	$A_{10} = 0.45$	$A_{11} = 10$	$A_{12} = 1$
$A_{13} = 400$	$A_{14} = 1$	$A_{15} = 400$	$A_{16} = 0.1$	$A_{17} = 0.1$
$A_{18} = 1$	$A_{19} = 1$	$A_{20} = 3$	$A_{21} = 600$	$A_{22} = 1400$
$A_{23} = 1$	$A_{24} = 3$	$A_{25} = 600$	$A_{26} = 800$	$A_{27} = 1$
$A_{28} = 67.5$	$A_{29} = 1$	$A_{30} = 22.5$	$A_{31} = 1$	$A_{32} = 1$
$A_{33} = 400$	$A_{34} = 1200$	$A_{35} = 10$	$A_{36} = 1$	$A_{37} = 1200$
$A_{38} = 400$	$A_{39} = 10$	$A_{40} = 50$	$A_{41} = 50$	$A_{42} = 1.5$
$A_{43} = 1$	$A_{44} = 4.5$	$A_{45} = 1$	$k_1 = 2$	$\mu_1 = 1$
$\mu_2 = 1$	$\mu_3 = 1$			

Table 6.2: Typical parameter values for a 0.1 mm adult dermal wound

2. The new levels of collagen I and III effect various other parameters, namely $A_{28} = 67.5$ and $A_{30} = 22.5$.

All the other parameters remain the same as in the foetal model, and are shown in Table 6.2.

6.4.2 Numerical Solution of the Model

We solve the system of ordinary differential equations using the same numerical routine, D02BBF, with the same initial conditions of a few fibroblasts and growth factors uniformly scattered throughout the wound. Figure 6.5 shows the time evolution of the field variables to their steady state values. The level of fibroblasts, TGF β 1 and 3 all increase initially but settle down, after approximately 60 days, to the healed levels 1, 40 and 20 respectively, which correspond to the normal dermal levels. The collagenases are gradually degraded to zero whilst the collagens rapidly attain their healed levels of 29.6 for type I and 8.28 for type III. Our model thus predicts more collagen and thinner fibres, the ratio 3.57 : 1 being in fair agreement with the experimentally determined ratio of 4 : 1.

Experimental evidence suggests that the addition of neutralising antibodies to TGF β 1 reduces scarring in adult wounds by producing thicker fibres whose orien-

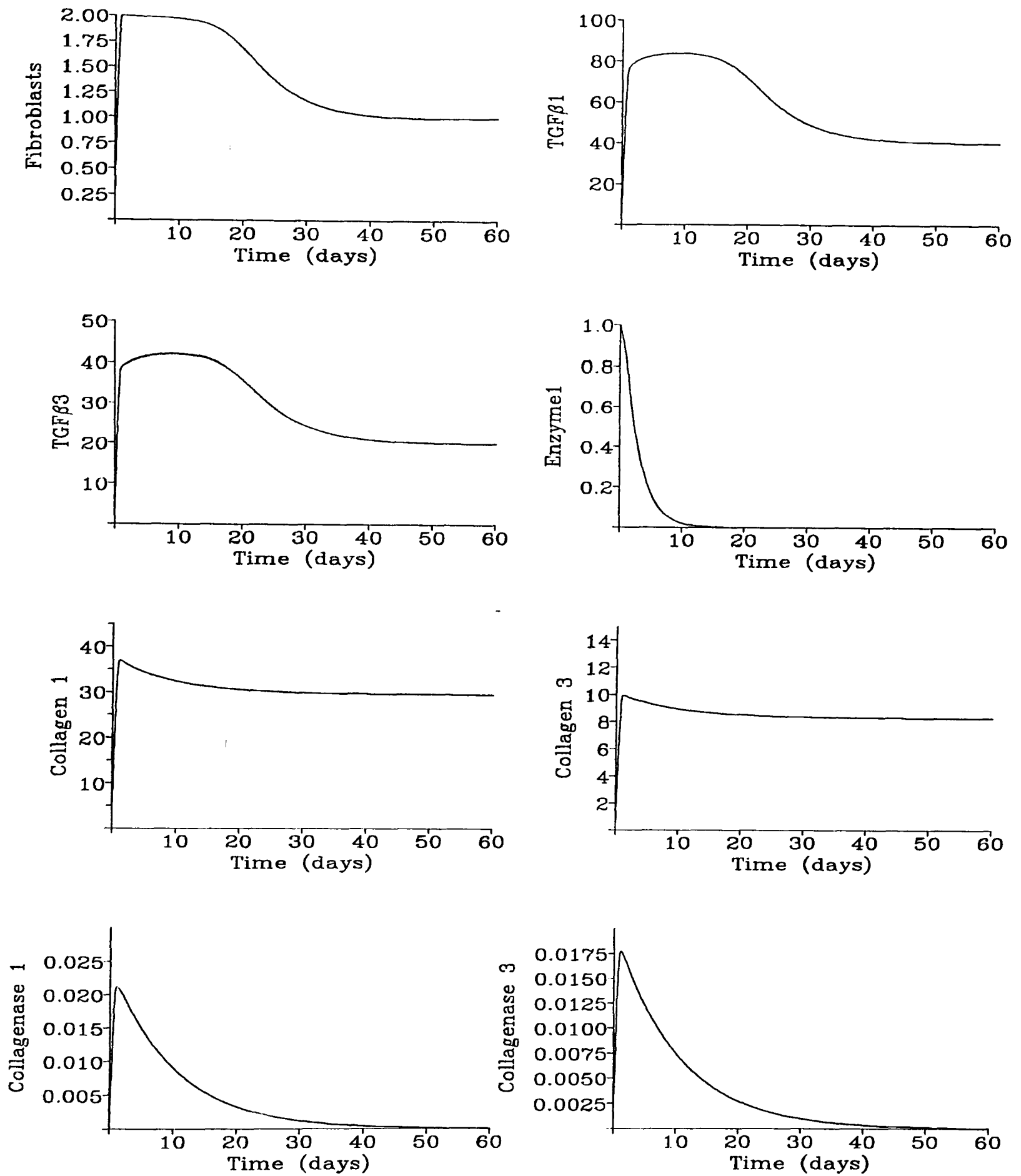


Figure 6.5: Numerical solution of the system of first order ordinary differential equations corresponding to the model for adult dermal wound healing, up to 100 days. We impose spatially homogeneous initial conditions and use the parameters shown in Table 6.2. The ratio of collagen types I and III is 3.57:1, implying thinner, more densely packed fibres as seen in scar tissue.

tation are similar to those of normal dermal tissue (Shah *et al.*, 1992). Addition of $\text{TGF}\beta 1$ causes the fibres to be much thinner and hence induces more scarring. We once again investigate these results by altering our initial conditions, such that $l_i = b$ for $i = 1$ or 3 , for increasing b , as discussed previously. Once again we observe a saturating level for the amount of $\text{TGF}\beta$ added (Figure 6.6). Figure 6.7 shows the effect of adding $\text{TGF}\beta 1$ and we note that the final levels of collagen I and III are 25.93 and 10.079 respectively, resulting in a ratio of 2.57 : 1 and implying that the fibrils are much thinner. Shah *et al.* (1992) showed that when $\text{TGF}\beta 1$ is applied topically to an adult wound at day 0, there is a transient increase in the total collagen level but after 14 days it is of a similar magnitude to the healed level. The phenomenon of an initial increase in collagen is observed in our numerical solutions and the final collagen levels indicate that diameters of the fibrils agree well with the experimental results. In Figure 6.8 we have added $\text{TGF}\beta 3$ and observe collagen levels of 34.9 and 6.17 for types I and III, respectively, the new ratio of 5.65 : 1 corresponding to 15% type III collagen. Again it should be noted that early addition of $\text{TGF}\beta$ is essential, with little effect for application after day 15. We thus predict that addition of $\text{TGF}\beta 3$ reduces scarring, with the healed tissue having a similar percentage of type III collagen as normal dermal tissue.

We again investigate the role of the modulation of $\text{TGF}\beta 1$ by $\text{TGF}\beta 3$ by setting A_6 to zero. Numerical solutions indicate that the final ratio of collagen I to III is decreased by the removal of this term. This is as expected intuitively since the steady state level of $\text{TGF}\beta 1$ is increased and this has already been shown to produce thinner fibres. Further simulations reveal that for addition of $\text{TGF}\beta 3$, the saturating levels for collagen I and III and the final ratio is also decreased. Hence, we conjecture that the modulation of $\text{TGF}\beta 1$ by $\text{TGF}\beta 3$ is more important in adult wound healing than in the foetal case because the intrinsic growth factor levels are higher. More experimental work is necessary to investigate possible ways of increasing the modulation term. This may be a viable alternative to the addition of growth factors to reduce scar tissue formation in adult dermal wounds.

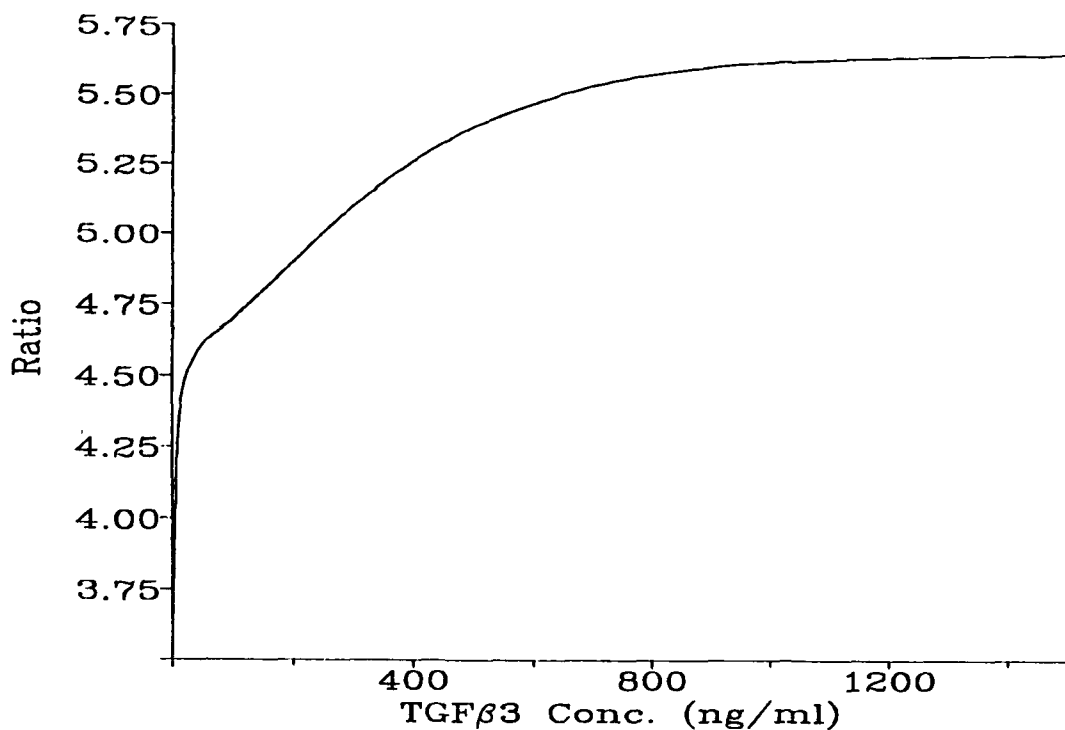


Figure 6.6: The change in the ratio of collagen I and III with the addition of increasing quantities of TGFβ3. There is a saturating level at a concentration of $1 \mu\text{g ml}^{-1}$.

6.5 Parameter Sensitivity Analysis

Numerical solutions of the temporal model suggest healed levels of collagen which are in agreement with experimental results. However, a large number of the model parameters are either unknown or fixed by comparing numerical solutions with experimental data. In this section we consider the robustness of the model to changes in parameter space. This enables us to determine the key parameters which influence the final levels of collagen, and we thus hypothesize which parameters account for the differences between adult and foetal wound healing.

We consider model variables $\underline{u} \in \mathbb{R}^n$ and parameter space $\underline{p} \in \mathbb{R}^m$. Suppose we are solving the ordinary differential equation system

$$\frac{d\underline{u}}{dt} = f(\underline{u}; \underline{p}) \quad (6.2)$$

subject to initial conditions $\underline{u}(0) = \underline{u}_0$. To determine the parameter sensitivity we perturb the parameters such that

$$\underline{p} \longrightarrow \underline{p}(1 + \Delta p), \quad \Delta p \in \mathbb{R}.$$

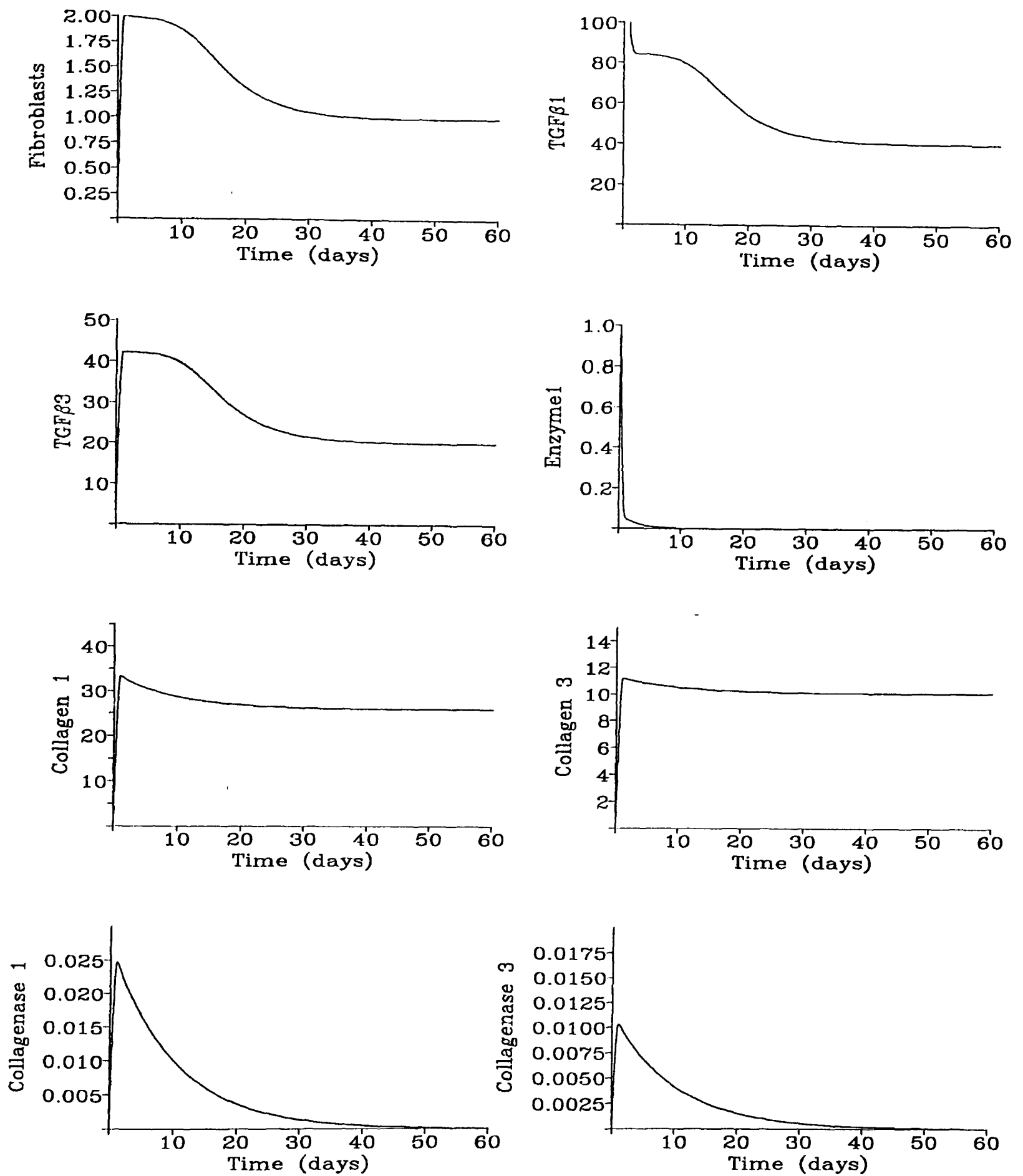


Figure 6.7: Numerical solution of the system of first order ordinary differential equations corresponding to the model for adult dermal wound healing, up to 100 days, showing the effect of exogenous application of TGF β 1. We impose spatially homogeneous initial conditions and use the parameters shown in Table 6.2. The ratio of collagen types I and III is 2.57:1, resulting in even thinner fibres, thus increasing scarring.

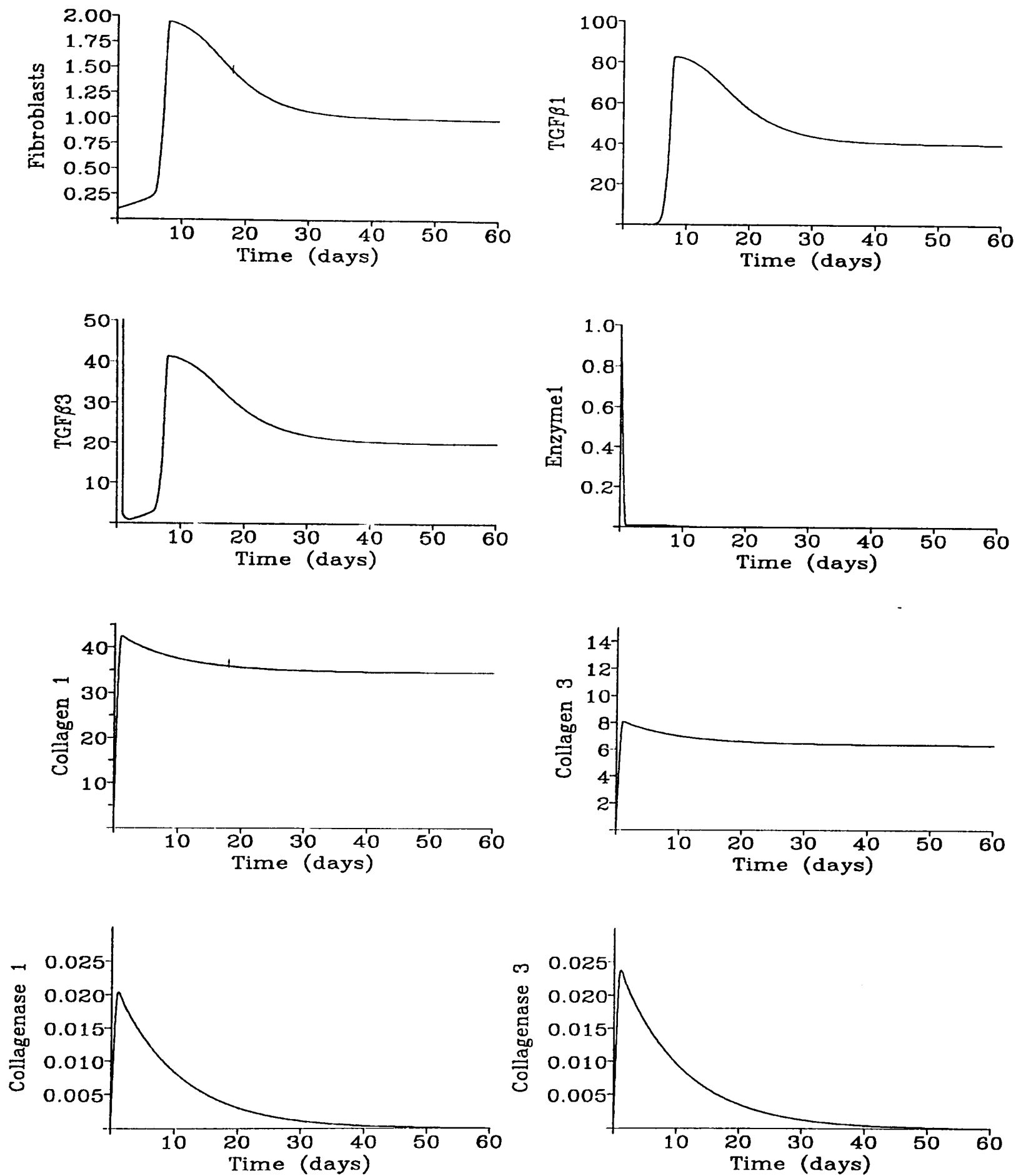


Figure 6.8: Numerical solution of the system of first order ordinary differential equations corresponding to the model for adult dermal wound healing, up to 100 days, showing the effect of exogenous application of TGF β 3. We impose spatially homogeneous initial conditions and use the parameters shown in Table 6.2. The ratio of collagen types I and III is 5.67:1, similar to normal dermal tissue. Addition of TGF β 3 thus reduces scarring.

The corresponding change in the solution, $\underline{\Delta u}$, is given by

$$\underline{\Delta u}(t; \underline{p}, \Delta p) = \underline{u}(t; \underline{p}(1 + \Delta p)) - \underline{u}(t; \underline{p}).$$

We define the sensitivity coefficient S to be

$$S(t; p_i, \Delta p) = \frac{\frac{\|\Delta u\|}{\|u\|}}{\Delta p}, \quad (6.3)$$

where $\|\cdot\|$ is the vector 2-norm.

In practice, we perturb each parameter in turn in the foetal model, by choosing different values Δp , and calculate the sensitivity coefficient when t is 5 days. We arbitrarily fix the critical level at $S = 1$, such that if $S < 1$ the parameter is insensitive whereas if $S > 1$ the parameter is sensitive. We first set $\Delta p = -1/2$, corresponding to halving each parameter in turn. Figure 6.9 shows the sensitivity coefficient for each parameter A_i and indicates that the key parameters are the fibroblast proliferation and mortality rates and the latent TGF β parameters, particularly the decay rates. However, we note that it is unrealistic to perturb a single parameter since this results in the loss of the stable steady state. For example, if we halve the rate of fibroblast proliferation without changing the rate of mortality accordingly, the normal dermal level of fibroblasts is no longer a steady state. By perturbing the parameters in such a way that this criterion is satisfied, we find that the most sensitive parameters are the natural decay rates of latent TGF β 1 and 3. We next increase each parameter 10-fold by setting $\Delta p = 9$ (Figure 6.10). Again, most of the parameters are insensitive to the perturbation, except for A_5 , the rate of latent TGF β 1 synthesis by fibroblasts. However, changing A_8 so that the normal dermal level of TGF β 1 is still a steady state results in a sensitivity coefficient $S < 1$, implying insensitivity.

Parameter sensitivity analysis repeated for different values of Δp (1, 99, -0.9 and -0.99 , corresponding to doubling, increasing 100-fold, decreasing 10-fold and 100-fold, respectively) indicates that the model is in fact very robust to changes in parameter values. The key parameters are A_8 and A_{11} , the natural decay rates of

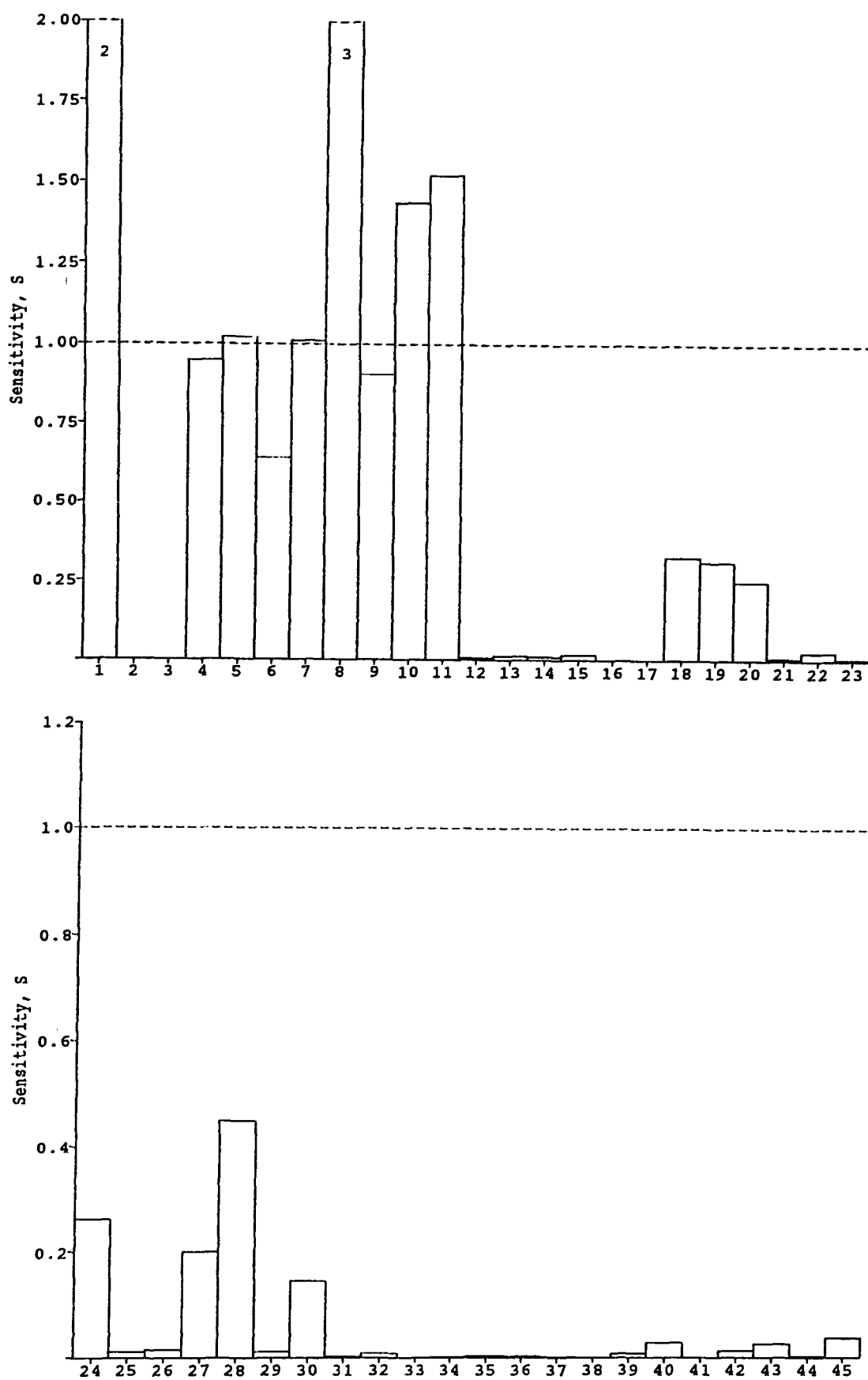


Figure 6.9: A histogram to show the sensitivity, S , of the model parameters, A_i , where i is the value shown on the horizontal axis. We set $\Delta p = -1/2$, corresponding to halving each parameter. The critical level is taken to be $S = 1$. The dotted line represents sensitivity levels beyond the scale of the graph.

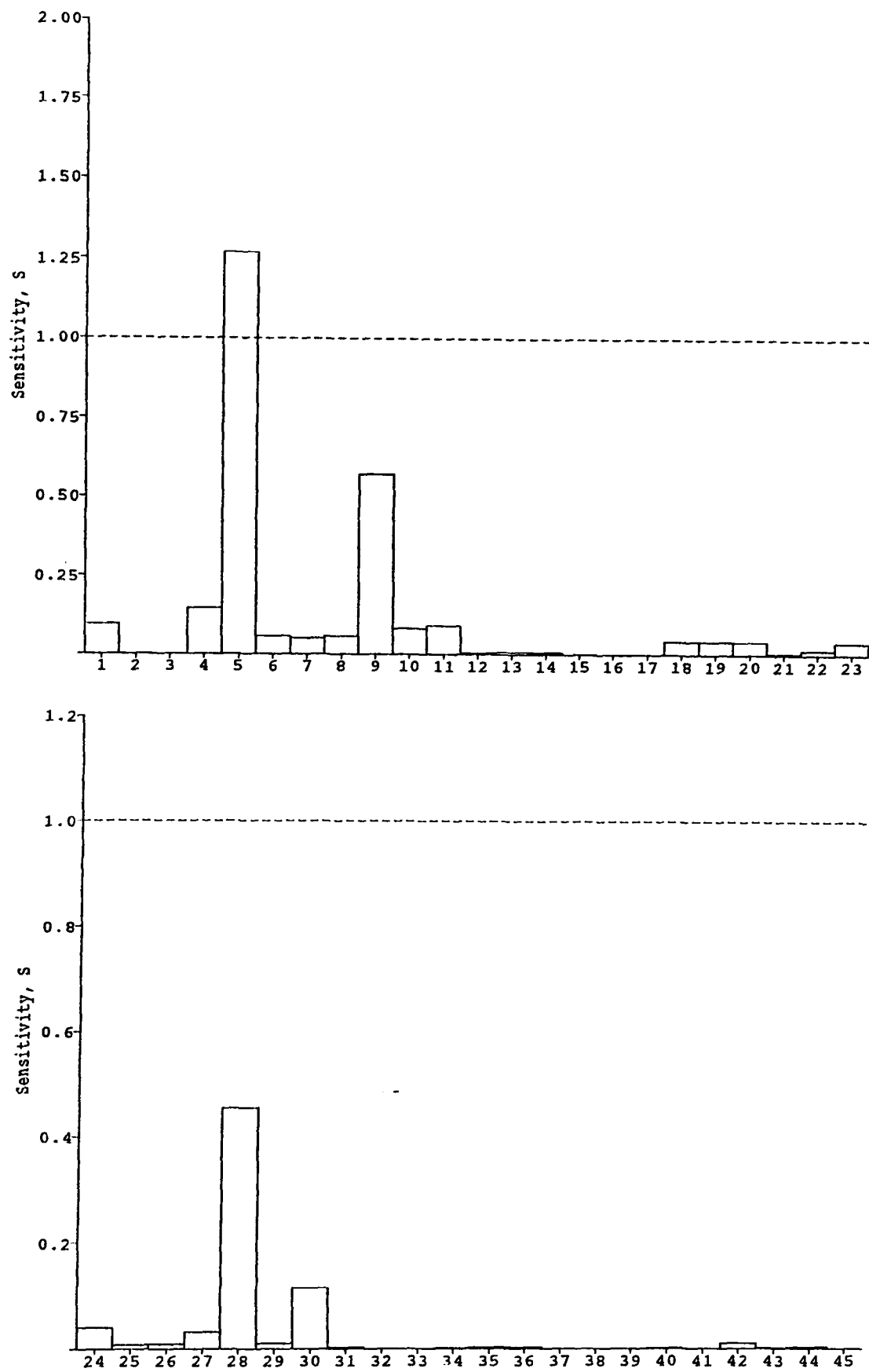


Figure 6.10: A histogram to show the sensitivity, S , of the model parameters, A_i , where i is the value shown on the horizontal axis. We set $\Delta p = 9$, corresponding to increasing each parameter 10-fold. The critical level is taken to be $S = 1$.

latent TGF β 1 and 3 respectively, A_{28} and A_{30} , the rates of activation of procollagen I and III to produce collagen I and III. By varying the parameters A_8 and A_{11} , numerical solutions indicate that there is a less than 2% change in collagen levels, with the ratio being maintained at 3 : 1, but the healing time is altered by up to 20%. Moreover, changes in A_{28} and A_{30} results in new collagen levels for types I and III, and a new ratio. We can thus conclude from numerical solutions that these two parameters crucially affect the solution behaviour. It should be noted that there is little experimental data for these values, and these are thus vital areas of research. Furthermore, parameter sensitivity analysis for the adult model indicates that A_6 , the parameter for the modulation of TGF β 1 by TGF β 3, is also crucial. The above analysis suggests that more experimental work should be carried out to highlight the differences between the rates of activation of procollagen and the modulation of TGF β 1 by TGF β 3 in foetal and adult dermal wounds.

6.6 Summary

In this chapter we have developed a temporal model which captures the essential processes in wound healing. The foetal case heals with no scar formation whereas the adult model predicts more densely packed, thinner collagen fibrils corresponding to scar tissue. Parameter sensitivity analysis reveals that the key parameters which explain this difference are A_{28} and A_{30} , the rates of activation of procollagen I and III to collagen I and III, respectively. Furthermore, the model predicts that addition of TGF β 3 reduces the scarring in adult wounds, resulting in thicker fibres, whereas when TGF β 1 is applied topically to the wound the fibrils are much thinner. Addition of TGF β 3 in foetal wound results in either very good healing or abnormal healing, and since there is a transition phase between foetal and adult healing, research should be carried out on topical application of TGF β 3 in young adults. Moreover, the model highlights that early application of growth factor is essential for effective

control of scar tissue formation. The temporal model assumes that initially there are a few fibroblasts and growth factors uniformly scattered throughout the wound and does not take into account the biologically realistic processes of fibroblast and $\text{TGF}\beta$ migration. Therefore, in the next chapter we look at the reaction diffusion system (5.10).

Chapter 7

Reaction Diffusion Equations for the Foetal Model

7.1 Introduction

We have developed a mathematical model for foetal dermal wound healing which follows the evolution in time of fibroblast density and $\text{TGF}\beta$, collagen and collagenase concentrations. This temporal model assumes no spatial variations within the wound and corresponds to the underlying subcutaneous tissue being the source of fibroblasts. The model predicts collagen fibre diameters and densities which compare favourably with experimental results. There is divided biological opinion as to whether the cells and growth factors enter the wound space predominantly from the subcutaneous tissue or from the unwounded dermis. We now consider the latter case and look at the system of reaction-diffusion equations (5.10). We solve the system numerically and investigate the effect of addition of $\text{TGF}\beta$ on the healed levels of collagen I and III.

7.2 Numerical Solution of the Model

The mathematical model is a system of nonlinear parabolic partial differential equations in one spatial variable (5.10). We solve the system numerically using the method of lines on a uniform space mesh, and integrating the resulting system of ordinary differential equations using Gear's Method (NAG routine D03PGF).

7.2.1 Initial and Boundary Conditions

Initial Conditions

We are considering full thickness, excisional, ‘clean’ wounds, with the cells and chemicals migrating from the unwounded tissue, and we thus assume that no fibroblasts or growth factors are initially present within the wound domain. Experimental evidence suggests that there is no active TGF β or collagenase in normal dermal tissue and, once activated by enzymes on wounding, the concentrations quickly decay to zero (Whitby & Ferguson, 1992). There is little experimental data available for the activating enzymes, although current research implies that, on wounding, a ‘pool’ of enzymes is released, which is rapidly degraded by the latent growth factors, procollagen and zymogen. Hence we make the following assumptions for the initial conditions, based on the biological background to the wounding process.

1. The density of fibroblasts outside the wound domain is at the ‘unwounded’ level, f^0 , whereas no fibroblasts are present inside the wound.
2. The latent isoforms of TGF β are not present in the wound space but the ‘unwounded’ concentrations, l_1^0 and l_3^0 , are maintained outside the wound.
3. The concentration of active TGF β is zero everywhere.
4. The enzymes are released inside the domain on wounding.
5. The amount of procollagen and zymogen is zero inside the wound but the unwounded levels, p_1^0 , p_3^0 , z_1^0 and z_3^0 , are maintained outside the wound domain.
6. The collagens are at their unwounded level, c_1^0 and c_3^0 , respectively, outside the milieu and are zero inside.
7. The collagenase concentration is zero everywhere.

Using the nondimensionalisation discussed previously, we denote the initial wound domain by $0 \leq x \leq 1$ and the above assumptions lead to the following initial

conditions

$$f(x, 0) = \begin{cases} 0 & \text{if } 0 \leq x \leq 1 \\ 1 & \text{otherwise} \end{cases} \quad (7.1a)$$

$$l_1(x, 0) = \begin{cases} 0 & \text{if } 0 \leq x \leq 1 \\ 1 & \text{otherwise} \end{cases} \quad (7.1b)$$

$$l_3(x, 0) = \begin{cases} 0 & \text{if } 0 \leq x \leq 1 \\ 1 & \text{otherwise} \end{cases} \quad (7.1c)$$

$$e_1(x, 0) = \begin{cases} 1 & \text{if } 0 \leq x \leq 1 \\ 0 & \text{otherwise} \end{cases} \quad (7.1d)$$

$$e_2(x, 0) = \begin{cases} 1 & \text{if } 0 \leq x \leq 1 \\ 0 & \text{otherwise} \end{cases} \quad (7.1e)$$

$$e_3(x, 0) = \begin{cases} 1 & \text{if } 0 \leq x \leq 1 \\ 0 & \text{otherwise} \end{cases} \quad (7.1f)$$

$$c_1(x, 0) = \begin{cases} 0 & \text{if } 0 \leq x \leq 1 \\ 1 & \text{otherwise} \end{cases} \quad (7.1g)$$

$$c_3(x, 0) = \begin{cases} 0 & \text{if } 0 \leq x \leq 1 \\ 0.36 & \text{otherwise} \end{cases} \quad (7.1h)$$

$$s_1(x, 0) = 0 \quad \forall x \quad (7.1i)$$

$$s_3(x, 0) = 0 \quad \forall x \quad (7.1j)$$

In Section 7.2.3, we investigate the effect of different initial conditions on the final levels of collagen I and III.

Boundary Conditions

We require that the system remains at the unwounded ‘dermal’ steady state far from the wound and hence fix the variables at their unwounded level. Due to symmetry, we impose ‘zero flux’ boundary conditions at the centre of the wound. The boundary conditions are thus

$$f(-\infty, t) = 1 \quad \& \quad \frac{\partial f}{\partial x}(1, t) = 0 \quad (7.2a)$$

$$l_1(-\infty, t) = 1 \quad \& \quad \frac{\partial l_1}{\partial x}(1, t) = 0 \quad (7.2b)$$

$$l_3(-\infty, t) = 1 \quad \& \quad \frac{\partial l_3}{\partial x}(1, t) = 0 \quad (7.2c)$$

$$e_1(-\infty, t) = 0 \quad \& \quad \frac{\partial e_1}{\partial x}(1, t) = 0 \quad (7.2d)$$

$$e_2(-\infty, t) = 0 \quad \& \quad \frac{\partial e_2}{\partial x}(1, t) = 0 \quad (7.2e)$$

$$e_3(-\infty, t) = 0 \quad \& \quad \frac{\partial e_3}{\partial x}(1, t) = 0 \quad (7.2f)$$

$$c_1(-\infty, t) = 1 \quad \& \quad \frac{\partial c_1}{\partial x}(1, t) = 0 \quad (7.2g)$$

$$c_3(-\infty, t) = 0.36 \quad \& \quad \frac{\partial c_3}{\partial x}(1, t) = 0 \quad (7.2h)$$

$$s_1(-\infty, t) = 0 \quad \& \quad \frac{\partial s_1}{\partial x}(1, t) = 0 \quad (7.2i)$$

$$s_3(-\infty, t) = 0 \quad \& \quad \frac{\partial s_3}{\partial x}(1, t) = 0. \quad (7.2j)$$

7.2.2 Numerical Simulations

We first solve the system for the foetal case over a large spatial domain, as explained in Chapter 2, and use the numerical simulations to determine a number of parameters by comparing model solutions with experimental data from studies in foetal wound healing. Given the uncoupling of the first four equations, we focus on these initially, the numerical solutions being shown in Figure 7.1, with $x = 0$ representing the edge of the wound. We observe a front of fibroblasts moving into the wound domain with constant speed and shape. There is an influx of cells due to the TGF β stimulated proliferation and the system evolves towards the dermal steady state, $f = 1$. Intuitively, we also expect a chemical front travelling into the wound but with the experimentally determined diffusion coefficients, D_2 and D_3 , we observe a ‘fill up’ mechanism as shown in Figure 7.1. Since these coefficients were estimated using data corresponding to adult dermal tissue, we can solve the system numerically for a range of diffusion coefficients. We obtain biologically consistent results when the rate of diffusion is decreased by one order of magnitude and thus conclude that the TGF β must diffuse more slowly in the foetus than in adult dermal tissue. The simulations for the chemical activation enzyme, e_1 , show a definite wound boundary, with the enzyme decaying as the chemical moves into the wound.

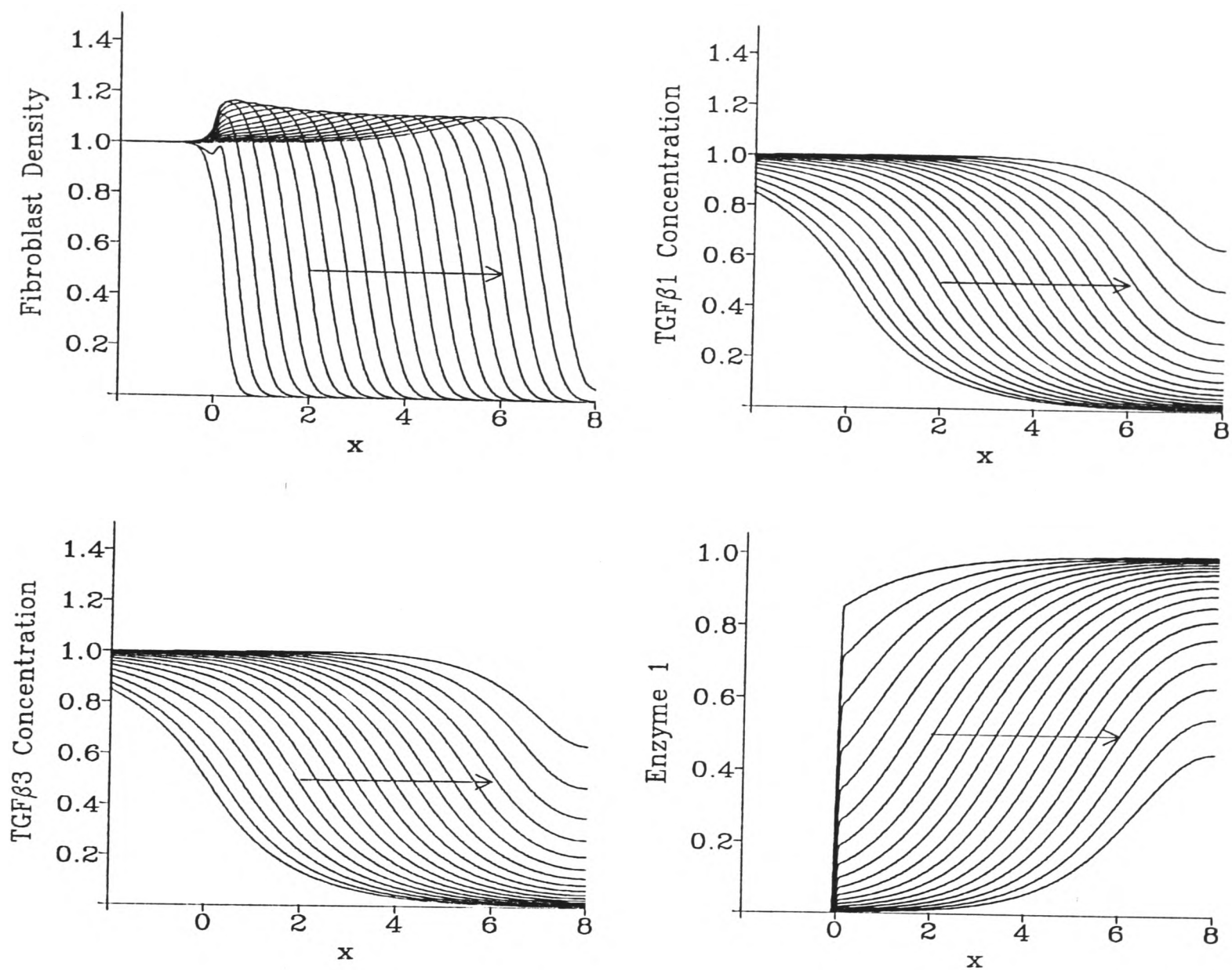


Figure 7.1: Numerical solutions of the four uncoupled model equations (5.10) over a large domain, showing the field variables as functions of space at equal time intervals of 36 hours, using linear geometry. The parameters are as shown in Table 5.3.

Focusing on the remaining six equations, numerical solutions for the enzymes e_2 and e_3 evolve to the zero steady state, as expected intuitively. Since we have chosen to measure the extent of scarring by the final levels of collagen, the numerical solutions for collagen I and III are encouraging (Figure 7.2). The solution profiles show fronts of collagen moving into the wound with constant speed and shape, evolving to new steady state levels for both collagen I and collagen III. The amount of collagen I and III is slightly less than the unwounded level but a 3:1 ratio is obtained by the appropriate choice of the parameters A_{28} and A_{30} , as discussed in Chapter 6. Thus the diameters of the collagen fibrils are unchanged and hence, according to our definition of scar tissue, no scarring occurs. The profiles also show a definite wound edge at $x = 0$, which suggests little intertwining of unwounded dermal collagen fibres and the new fibrils, in agreement with experimental predictions. The collagenase equations show wave pulses moving into the wound domain and both collagenase I and III decay to the zero dermal level. It is the effect of exogenous application of TGF β on the ratio of collagen I to III which is of particular interest and we discuss this in detail in Section 7.4.

Figure 7.2 shows the solution profiles over a large spatial domain, using the model parameters shown in Table 5.3. For a 0.1mm foetal wound, the speed of the fibroblast fronts corresponds to a dimensional wave speed of $0.5\mu\text{m hr}^{-1}$, indicating a healing time of 8 days, which is of the order of magnitude of the experimentally observed time of 5 days for foetal wound healing (Shah *et al.*, 1992). By considering the more biologically realistic semi-finite domain, $x \in [-\infty, 1]$ where 0 is the wound edge and 1 is the centre of the wound, we again observe waves of cells, chemical and collagen (Figure 7.3) but the fronts and the wound boundary are less well defined and the wound fails to heal in the experimentally required time. This may be explained by the fact that experimentalists tend to measure the time for the wound length to be reduced by about 75% and extrapolate to obtain the total healing time. This is discussed in more detail in Chapter 2.

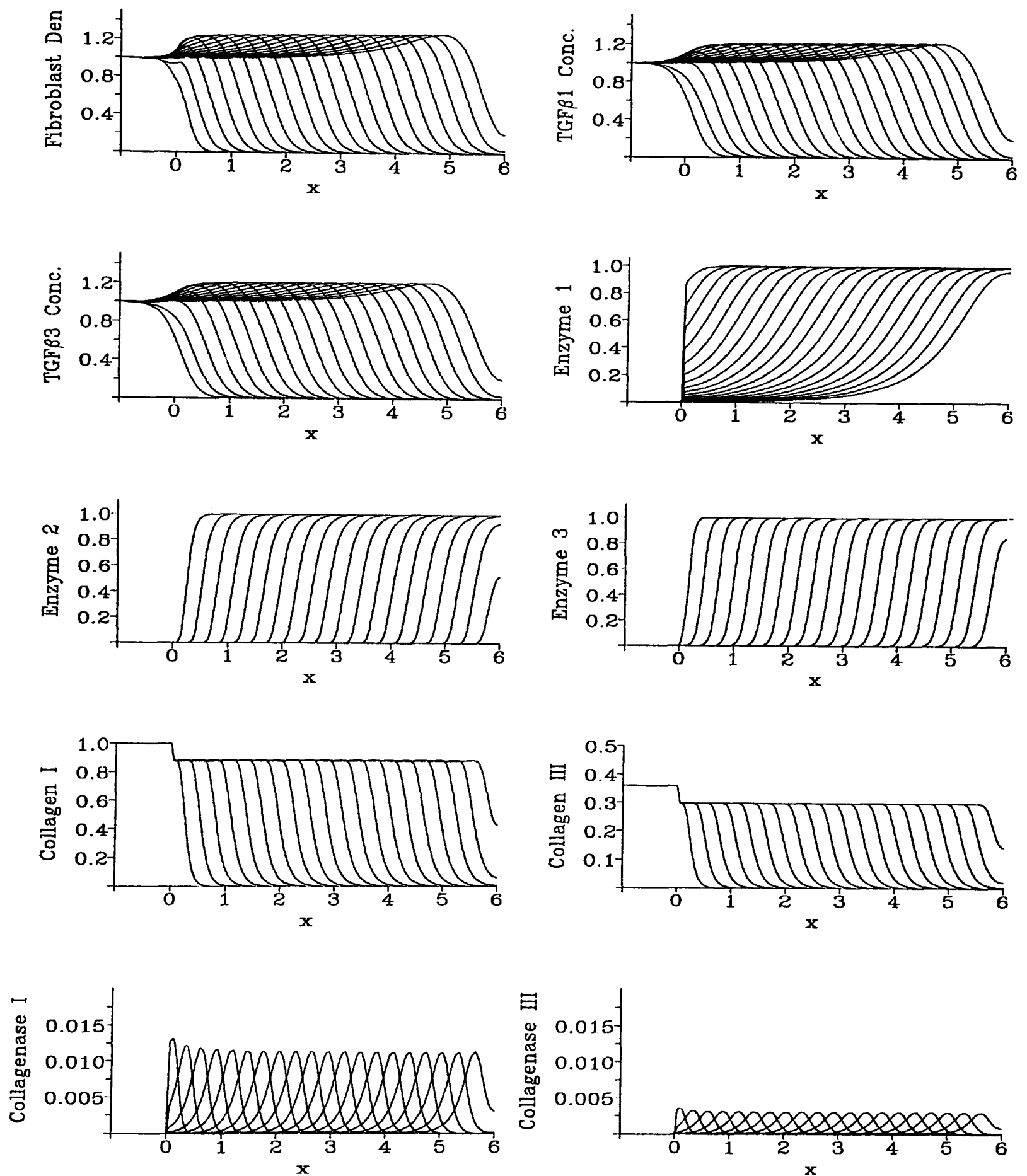


Figure 7.2: Numerical solutions of the model equations (5.10), over an infinite domain, showing the field variables as functions of space at equal time intervals of 36 hours, using linear geometry. The parameters are as shown in Table 5.3.

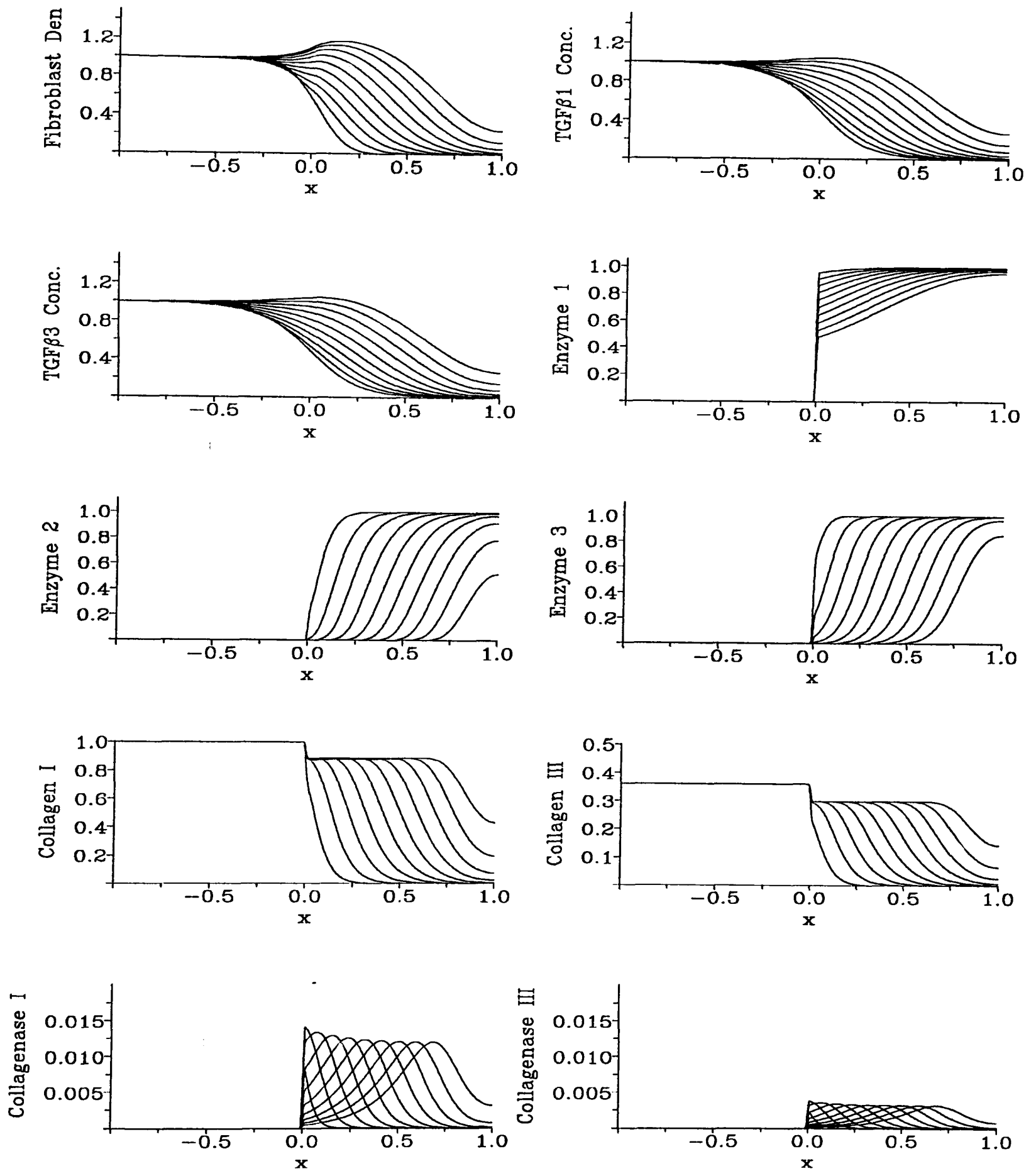


Figure 7.3: Numerical solutions of the model equations (5.10), over a finite domain, showing the field variables as functions of space at equal time intervals of 12 hours, using linear geometry. The parameters are as shown in Table 5.3.

7.2.3 Different Initial Conditions

The important question of what kind of initial conditions will evolve to travelling wave solutions has been widely studied (for discussion see Murray, 1989). In the NAG routine used to solve the system numerically, we have used step function initial conditions, namely, in the notation of Section 6.2, $\underline{q}_s = (1, 1, 1, 0, 0, 0, 1, 0.36, 0, 0)$ for $x < 0$ and $\underline{q}_s = (0, 0, 0, 1, 1, 1, 0, 0, 0, 0)$ for $x > 0$. However, in reality the wound domain is not so well defined and thus we first consider smooth initial conditions and then exponentially decaying initial conditions.

Smooth Initial Conditions

We first smooth out the step function initial conditions by imposing the initial conditions

$$f(x, 0) = l_i(x, 0) = c_i(x, 0) = \begin{cases} 1 & \text{if } x < -1 \\ \frac{(x-1)^p}{1+(x-1)^p} & \text{if } -1 \leq x \leq 1 \\ 0 & \text{if } x > 1, \end{cases} \quad (7.3)$$

for the fibroblasts, TGF β and collagen, where $i = 1$ or 3 and

$$e_j(x, 0) = \begin{cases} 0 & \text{if } x < -1 \\ \frac{1}{1+(x-1)^p} & \text{if } -1 \leq x \leq 1 \\ 1 & \text{if } x > 1 \end{cases} \quad (7.4)$$

for the enzymes, where $j = 1, 2, 3$. Consistent results are obtained provided $p \geq 6$, so that the term $(x - 1)^p$ is approximately zero for all $x > 0.75$. For smaller values of p , the initial conditions indicate that the healing process is already well advanced. In our simulations we thus choose $p = 8$. We may also alter the boundary conditions and impose zero spatial derivatives (zero flux) at both boundaries of the domain. Figure 7.4 shows the numerical solutions with smoothed initial conditions and zero flux boundary conditions. We observe a good correspondence between model solutions and experimental data, with new levels of collagen I and III in a ratio 3 : 1 and a predicted healing time for a 0.1mm foetal dermal wound of 8 days. In particular, we note the smooth transition of the collagen steady state from the

normal dermal level to the new healed level, in contrast to the definite boundary shown in Figure 7.2.

Exponentially Decaying Initial Conditions

We have shown in Section 6.2 that there is a continuum of possible collagen steady states, but found that both the front speed and the steady states to which the system evolves are robust to a variety of changes. We thus try to alter the front speed to see the affect on the collagen steady state by introducing exponentially decaying initial conditions of the form

$$W(x, 0) \sim e^{-Rx} \quad \text{as } x \rightarrow \infty$$

where W is the model variable and R is a positive constant. By looking for the leading edge form of wavefront solutions to a single reaction diffusion equation, with constant diffusion coefficient D_1 , Rothe (1978) showed that

$$\text{if } R \leq \frac{1}{\sqrt{D_1}}, \quad \text{then } b = \frac{1}{R} + D_1 R \quad (7.5a)$$

$$\& \text{ if } R > \frac{1}{\sqrt{D_1}}, \quad \text{then } b = 2\sqrt{D_1}. \quad (7.5b)$$

where b is the wave speed. We solve the system numerically for a range of different values of R and observe that for values of $R > 8$, the solutions evolve to fronts with the same speed, shape and collagen steady states as with compact support initial conditions. However, as R decreases, the speed increases and the collagen steady states are altered, though only slightly, as shown in Figure 7.5. Experimental evidence suggests that the collagen steady state is changed by altering external factors whereas this result implies that although a continuum of steady states exist, the collagen level selected is very insensitive to the details of the initial conditions.

7.3 Travelling Wave Solutions

The qualitative form of the fibroblast, chemical, enzymes, collagens and collagenases solutions is of waves moving into the wound with constant shape and constant speed

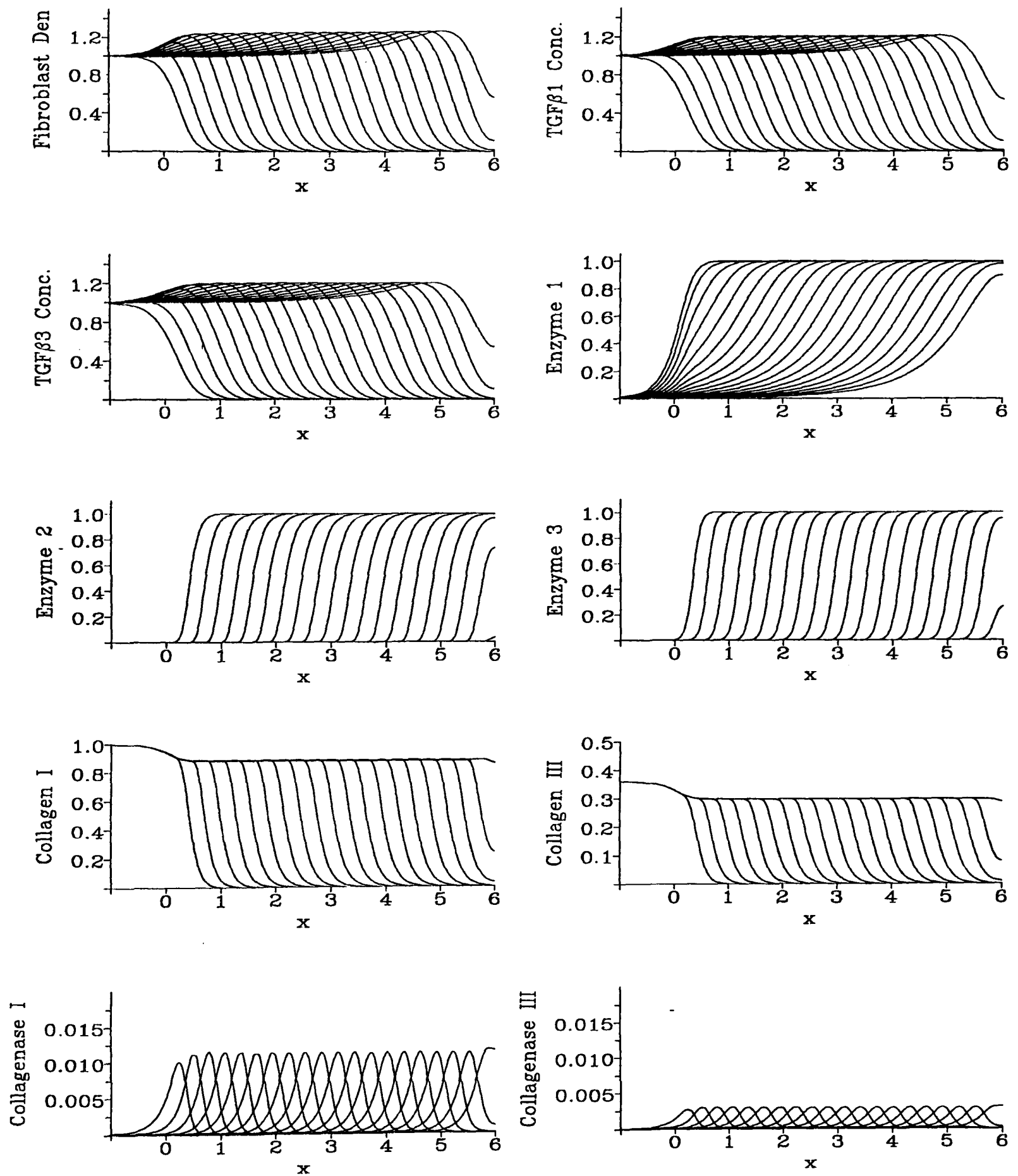


Figure 7.4: Numerical solutions of the model equations (5.10), over an infinite domain, with smoothed initial conditions and zero flux boundary conditions. The plots show the field variables as functions of space at equal time intervals of 36 hours, using linear geometry. We note the smooth transition between the collagen steady states. The parameters are as shown in Table 5.3.

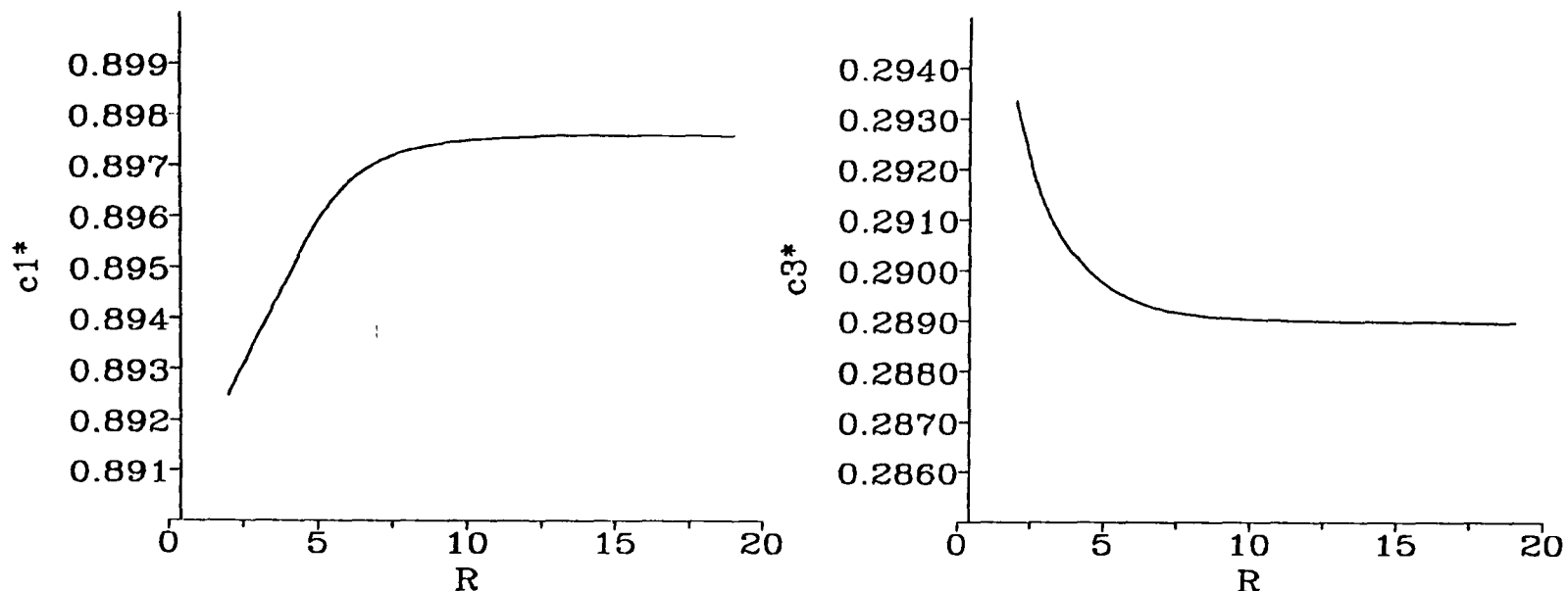


Figure 7.5: The changes in the healed levels of collagen I and III when exponentially decaying initial conditions are imposed.

and, as in Chapter 2, we look for travelling wave solutions of the form $W(x, t) = W(z)$, $z = x - bt$, where W is the model variable, x is the spatial coordinate and b is the wave speed, chosen to be positive since we are considering waves that are moving from left to right. We substitute these forms into the model and obtain the ordinary differential equation system

$$-bf' = D_1 f'' + \left(A_1 + \frac{A_2 A_{12} e_1 l_1}{A_{13}} + \frac{A_3 A_{14} e_1 l_3}{A_{15}} \right) f \left(1 - \frac{f}{k_1} \right) - A_4 f \quad (7.6a)$$

$$-bl'_1 = D_2 l''_1 + \frac{A_5 f l_1}{1 + A_6 l_3 + A_7 l_1} - A_8 l_1 - e_1 l_1 \quad (7.6b)$$

$$-bl'_3 = D_3 l''_3 + \frac{A_9 f l_3}{1 + A_{10} l_3} - A_{11} l_3 - \mu_1 e_1 l_3 \quad (7.6c)$$

$$-be'_1 = -e_1 (A_{16} l_1 + A_{17} l_3) \quad (7.6d)$$

$$-be'_2 = -e_2 f \left[\frac{A_{18}}{A_{23} + e_2} \left(A_{20} + \frac{A_{21} A_{12} e_1 l_1}{A_{13}} + \frac{A_{22} A_{14} e_1 l_3}{A_{15}} \right) + \frac{A_{19}}{A_{27} + \mu_2 e_2} \left(A_{24} + \frac{A_{25} A_{12} e_1 l_1}{A_{13}} + \frac{A_{26} A_{14} e_1 l_3}{A_{15}} \right) \right] \quad (7.6e)$$

$$-be_3 = -e_3f \left[\frac{A_{40}c_1}{A_{35} + e_3} \left(\frac{A_{32}}{1 + \frac{A_{33}A_{12}e_1l_1}{A_{13}} + \frac{A_{34}A_{14}e_1l_3}{A_{15}}} \right) \right. \quad (7.6f)$$

$$\left. + \frac{A_{41}c_3}{A_{37} + \mu_3e_3} \left(\frac{A_{36}}{1 + \frac{A_{37}A_{12}e_1l_1}{A_{13}} + \frac{A_{38}A_{14}e_1l_3}{A_{15}}} \right) \right]$$

$$-bc'_1 = \frac{A_{28}e_2f}{A_{23} + e_2} \left(A_{20} + \frac{A_{21}A_{12}e_1l_1}{A_{13}} + \frac{A_{22}A_{14}e_1l_3}{A_{15}} \right) - s_1c_1 \quad (7.6g)$$

$$-bc'_3 = \frac{A_{30}e_2f}{A_{27} + \mu_2e_2} \left(A_{24} + \frac{A_{25}A_{12}e_1l_1}{A_{13}} + \frac{A_{26}A_{14}e_1l_3}{A_{15}} \right) - s_3c_3 \quad (7.6h)$$

$$-bs'_1 = \frac{A_{42}e_3fc_1}{A_{35} + e_3} \left(\frac{A_{32}}{1 + \frac{A_{33}A_{12}e_1l_1}{A_{13}} + \frac{A_{34}A_{14}e_1l_3}{A_{15}}} \right) - A_{43}s_1 \quad (7.6i)$$

$$-bs'_3 = \frac{A_{44}e_3fc_3}{A_{39} + \mu_3e_3} \left(\frac{A_{36}}{1 + \frac{A_{37}A_{12}e_1l_1}{A_{13}} + \frac{A_{38}A_{14}e_1l_3}{A_{15}}} \right) - A_{45}s_1 \quad (7.6j)$$

where prime denotes d/dz . We consider equations (7.6) on $-\infty < z < \infty$, as discussed in Chapter 2, and letting $df/dz = g$, $dl_1/dz = h_1$ and $dl_3/dz = h_3$ we obtain a system of thirteen first order ordinary differential equations. Any attempt at global analysis would be extremely difficult and we therefore only look at a linear analysis about the steady states. We define $\underline{q} = (f, g, l_1, h_1, l_3, h_3, e_1, e_2, e_3, c_1, c_3, s_1, s_3)$ and rewrite our system of first order ordinary differential equations as $\frac{dq}{dz} = J\underline{q}$, where J is the 13×13 Jacobian matrix evaluated at the steady state. By looking for solutions of the form $\underline{q} = e^{\lambda z}$, we determine a 13^{th} order polynomial for the eigenvalues, λ . In this case analytical investigation is limited and, as discussed in Chapter 2, we resort to numerical techniques to find the eigenvalues. We note that one of the eigenvalues is dependent on the collagen equilibrium level, c_1^* & c_3^* , but since these are always positive, the corresponding eigenvalue is always positive. For the purpose of numerical calculation, we fix c_1^* at 0.89 and c_3^* at 0.295. Table 7.1 shows the eigenvalues and eigenvectors for a range of wave speeds and we see that the eigenvalues λ_1 and λ_2 change from a complex conjugate pair with negative real part to two negative real roots, one of which is the most negative of all the eigenvalues. This change occurs at $b \approx 0.205$, corresponding to a dimensional wave speed of $0.8\mu\text{m hr}^{-1}$, which is of a similar order of magnitude to the numerically simulated and experimentally

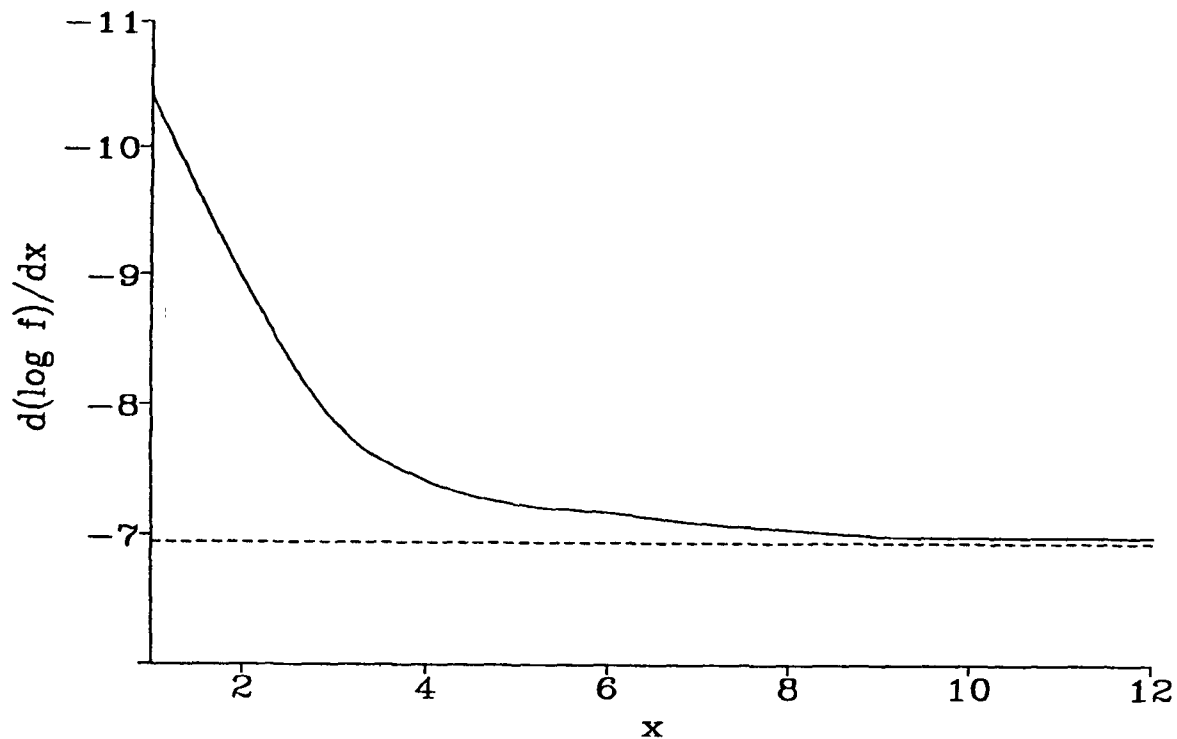


Figure 7.6: The change in $\frac{d(\log f)}{dx}$ with x for the waves illustrated in Figure 7.2. The function tends towards $\lambda = -7$, which is the real part of the eigenvalue that changes character at the critical wave speed, $b = 0.205$, corresponding to a dimensional speed of $0.43 \mu\text{mhr}^{-1}$.

observed speed. We confirm this result by estimating $\frac{d(\log f)}{dz}$ numerically over large x . Figure 7.6 shows the dominant eigenvalue tending towards -6.95 as x increases, which is close to the most negative eigenvalue of the Jacobian evaluated numerically at the bifurcation wave speed. This suggests that there is a change from oscillatory to monotone convergence at the bifurcation point and the good correspondence with experimental and numerical results seems to indicate that this change in behaviour does indeed occur at the critical wave speed. This analysis thus suggests a dimensional wave speed of $0.43 \mu\text{m hr}^{-1}$, predicting that a foetal dermal wound, 0.05mm in radius, heals in 5 days, which is in good agreement with experimental results.

7.4 Addition of $\text{TGF}\beta$

Numerical solutions of the spatially homogeneous system of model equations have been shown to exhibit the phenomenon of a spectrum of collagen I and III steady

states, the values being dependent on the initial amounts of TGF β , collagen I and III and collagenase I and III present within in the wound (Section 6.4). We now look for the same behaviour in the reaction-diffusion model by solving the full system (5.10) with different initial conditions. We first use step function initial conditions, as defined by equations (7.1), except that we model the exogenous application of TGF β 1 by imposing the condition

$$l_1(x, 0) = \begin{cases} 1000 & \text{if } x > 0 \\ 1 & \text{otherwise} \end{cases}$$

Numerical simulations show fronts of fibroblasts and the different isoforms of growth factors moving into the wound with constant speed and shape and we particularly note the more rapid decay of enzyme 1. The profiles for collagen I and III show a definite wound edge and the first wave of collagens attain a steady state value in agreement with the spatially homogeneous solution. However, the equilibrium values increase with time and settle at 0.89 and 0.295 respectively, which correspond to the steady state values in the absence of exogenous application. Figure 7.7 illustrates this result when we impose the smooth initial conditions discussed in Section 7.2.3.

This result suggests that dependence of the final steady state on the transient application of TGF β 's, which is observed in experiment and captured by the temporal model, is lost when the model is extended to include spatial variation. To investigate this further we try to force the collagens to the steady states evaluated in Table 6.1, by selecting specific initial conditions. For example we impose the conditions

$$\begin{aligned} l_1(x, 0) &= \begin{cases} 1000 & \text{if } x > 0 \\ 1 & \text{otherwise} \end{cases} \\ c_1(x, 0) &= \begin{cases} 0 & \text{if } x > 0 \\ 0.59 & \text{otherwise} \end{cases} \\ c_3(x, 0) &= \begin{cases} 0 & \text{if } x > 0 \\ 0.4 & \text{otherwise} \end{cases} \end{aligned}$$

so that the level of collagen outside the wound domain is the same as the level predicted by the ordinary differential equation system. However, the profiles of

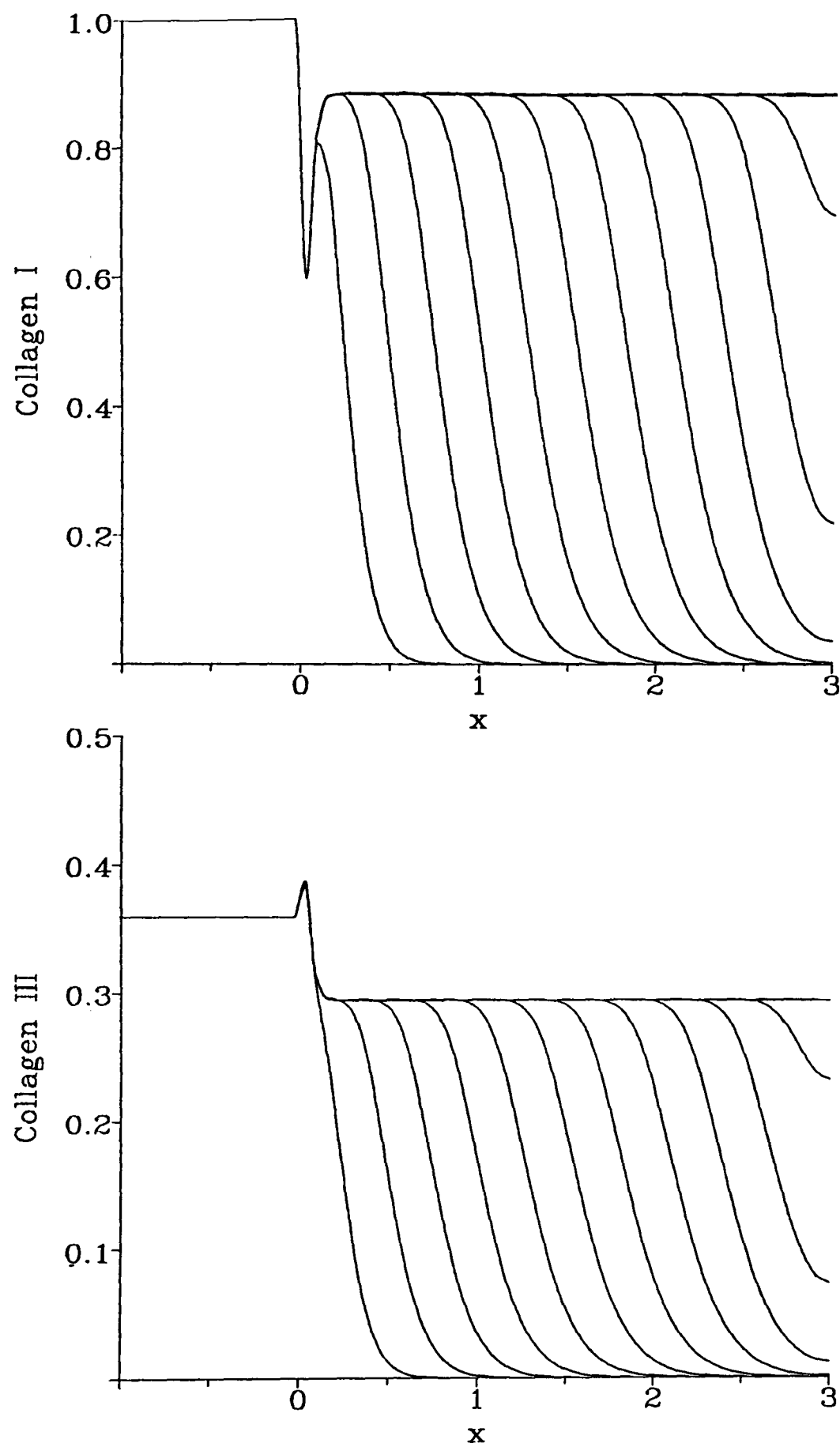


Figure 7.7: Numerical solution of the full system of partial differential equations (5.10) with smooth initial conditions and exogenous application of $TGF\beta 1$, at equal time intervals of 36 hours. The parameters are as shown in Table 5.3. The steady state levels of collagen I and III undergo a transient change but eventually evolve to the same level attained in the absence of excess chemical.

collagen I and III again show a transient behaviour before evolving to the steady states 0.89 and 0.295, respectively. Qualitatively similar results are obtained for the addition of TGF β 3. Now the steady state levels 0.89 and 0.295 depend on the model parameter values but extensive numerical simulations suggest that the insensitivity of the collagen levels to TGF β addition holds for a large range of parameter values. This result suggests that although there is a line of possible wounded collagen steady states in the travelling wave phase space, there is only one trajectory connecting the normal dermal and the wounded steady state. Qualitatively similar results are obtained when all these simulations are performed using the parameters for the adult wound healing model. The system is too complicated for further analysis and we thus develop a caricature model to investigate this behaviour.

7.5 Summary

Numerical solutions of the full system of equations for foetal dermal wound healing (5.10) evolve to fronts of cells, chemicals and collagens moving into the wound domain. The wave speed is determined to be $0.43 \mu\text{m hr}^{-1}$, which compares favourably with experimental data. Furthermore, the collagen profiles indicate new “healed” levels and no intertwining of fibres at the wound edge. The model system has a continuum of possible positive collagen steady states, but numerical solutions for the addition of TGF β suggest that a particular one is picked out. Experiments show that the addition of TGF β alters the collagen density and diameters and hence our model suggests that migration of fibroblasts and TGF β from the unwounded tissue alone cannot account for the essential features of the healing process. We thus hypothesise that fibroblasts and growth factors came mainly from the underlying subcutaneous tissue.

Chapter 8

Caricature Models

8.1 Introduction

Numerical solutions of the reaction diffusion equations (5.10) evolve to waves of fibroblasts, chemicals and collagens moving into the wound domain with constant speed and shape. In particular, there is a spectrum of possible steady state levels of collagen and numerical simulations show a sharp transition between different collagen steady states at the wound edge. Furthermore, addition of $\text{TGF}\beta$ results in only a transient change in the collagen level, and, in contrast to the temporal model, one particular steady state is always selected, independent of the amount of $\text{TGF}\beta$ added. To try to understand this behaviour, we introduce a ‘caricature model’, consisting of a pair of partial differential equations, which mimics the behaviour of the full system.

8.2 Basic Caricature Model

We consider two variables, $n(x, t)$ and $c(x, t)$ at position x and time t , with only n undergoing diffusion. Intuitively one can regard n as mimicking the fibroblast population in the full model, with c mimicking one of the types of collagen. However there is no formal correspondence and the purpose of the caricature model we discuss here is to gain mathematical insight into a system that is analogous to, but simpler than, the full model, in the hope that this insight can be extended to the full model.

The simple system is the following

$$\frac{\partial n}{\partial t} = D_1 \frac{\partial^2 n}{\partial x^2} + n(1 - n) \quad (8.1a)$$

$$\frac{\partial c}{\partial t} = \alpha n(1 - n) - (1 - n)c \quad (8.1b)$$

where D_1 and α are both positive constants. Thus n satisfies the Fisher equation with constant diffusion, and in addition to the trivial steady state $n = c = 0$, there is a spectrum of steady states with $n = 1$ and c taking any value.

8.2.1 Temporal model

In the full model we considered a temporal model with cells undergoing proliferation and death but no migration. Imposing the initial conditions of a few cells scattered uniformly about the wound and no collagen, we obtained final collagen levels which compared favourably with experimental data and, by adding $TGF\beta$, we were able to pick out differing levels of collagen. Analogous to this, we consider the pair of ordinary differential equations

$$\frac{dn}{dt} = n(1 - n) \quad (8.2a)$$

$$\frac{dc}{dt} = \alpha n(1 - n) - (1 - n)c \quad (8.2b)$$

with initial conditions

$$n = n_0 \text{ \& } c = 0 \text{ at } t = 0,$$

where n_0 is an arbitrary constant. We solve equation (8.2a) to obtain

$$n(t) = \frac{1}{1 + Ae^{-t}} \quad (8.3)$$

where A is a constant satisfying $n_0 = 1/(1 + A)$ and $n \rightarrow 1$ as $t \rightarrow \infty$. Substituting (8.3) into (8.2b) we obtain a first order ordinary differential equation for c , with solution

$$c(t) = \frac{\alpha A(A + 2)}{2(A + 1)^2} (1 + Ae^{-t}) - \frac{(\alpha A^2 e^{-t} + 2\alpha A)}{2(A + e^t)} \quad (8.4)$$

This implies that $c^* = \lim_{t \rightarrow \infty} c(t)$ is given by $c^* = \frac{\alpha(1-n_0^2)}{2}$. In practice, $n_0 \ll 1$ and thus $c^* \approx \alpha/2$, as shown in Figure 8.1. Furthermore, this result suggests that the value c^* is bounded above by $\alpha/2$ and decreases as the initial cell density, n_0 increases. Figure 8.2 shows numerical solutions of equations (8.2), with $\alpha = 0.8$, illustrating the dependence of c^* on the initial value n_0 . This differs from the temporal full model, where addition of fibroblasts has no effect on the collagen steady states. Nevertheless, the caricature model does capture the general feature of a continuum of steady states, dependent on the initial conditions.

8.2.2 Numerical Solution of the Caricature Model

Solutions of the full model show that the temporal model yields collagen steady states which can be varied by altering the initial conditions whereas when diffusion is added, the system always evolves to the same collagen steady states. We look for this behaviour in the caricature model by solving the system of partial differential equations (8.1) numerically using the method of lines and Gear's method. We first consider step function initial conditions

$$n(x, 0) = c(x, 0) = 1 \quad \text{if } x < 0 \quad (8.5a)$$

$$n(x, 0) = c(x, 0) = 0 \quad \text{if } x > 0, \quad (8.5b)$$

and impose the boundary conditions

$$n(0, t) = c(0, t) = 1 \quad \forall t \quad (8.5c)$$

$$n_x(\infty, t) = c_x(\infty, t) = 0 \quad \forall t \quad (8.5d)$$

as in the full model. On a large spatial domain, the n profile is a travelling front moving from left to right with constant shape and a speed which compares favourably with the analytically determined wave speed, $2\sqrt{D_1}$. We also observe fronts for the variable c , with a definite wound edge at $x = 0$. This is in agreement with experimental results, which indicate no initial intertwining of collagen fibres at the

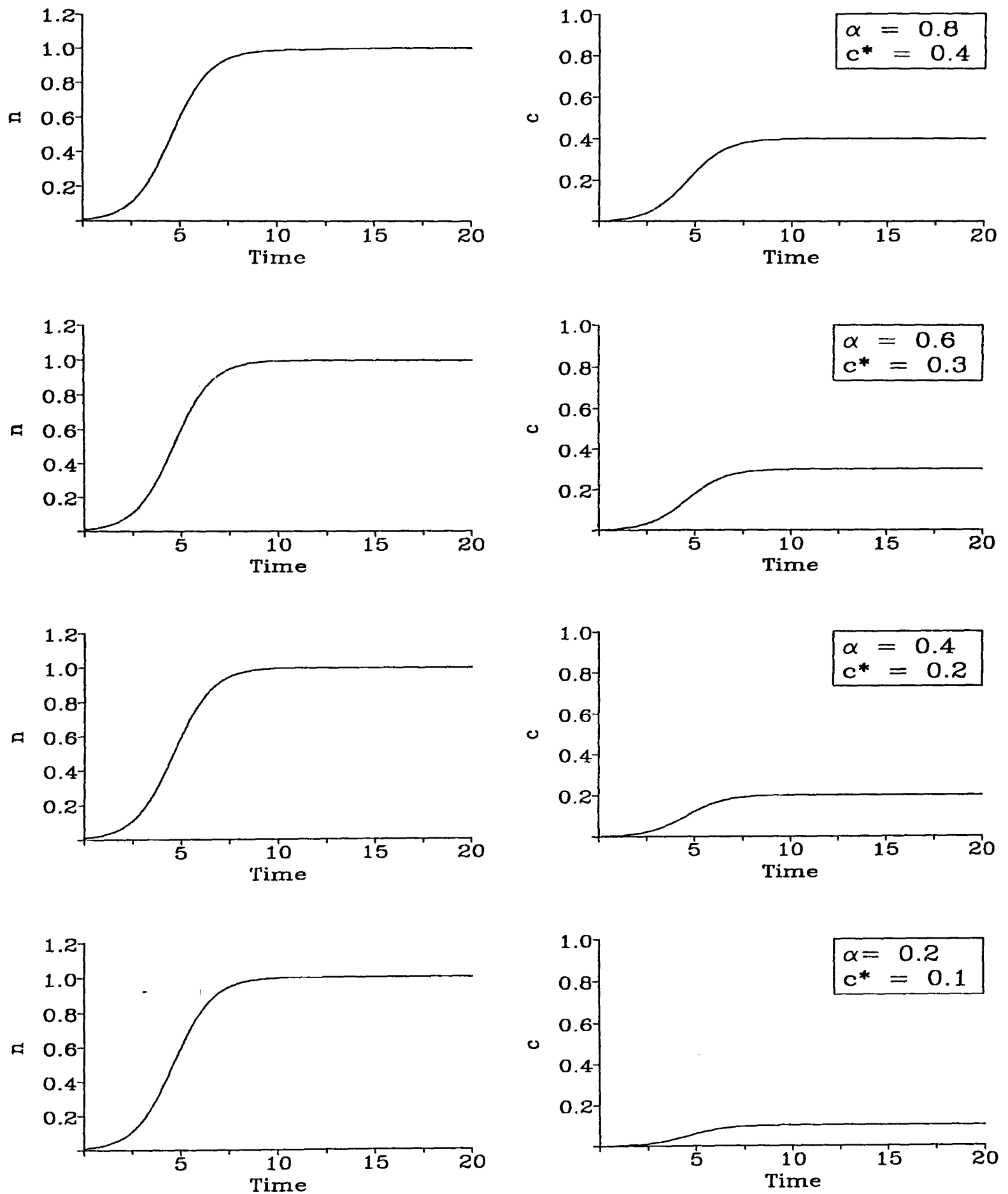


Figure 8.1: Numerical solutions of the caricature temporal model (8.2) showing the time evolution for different values of α , with $n_0 = 0.01$. The steady state c^* is seen to be $\alpha/2$.

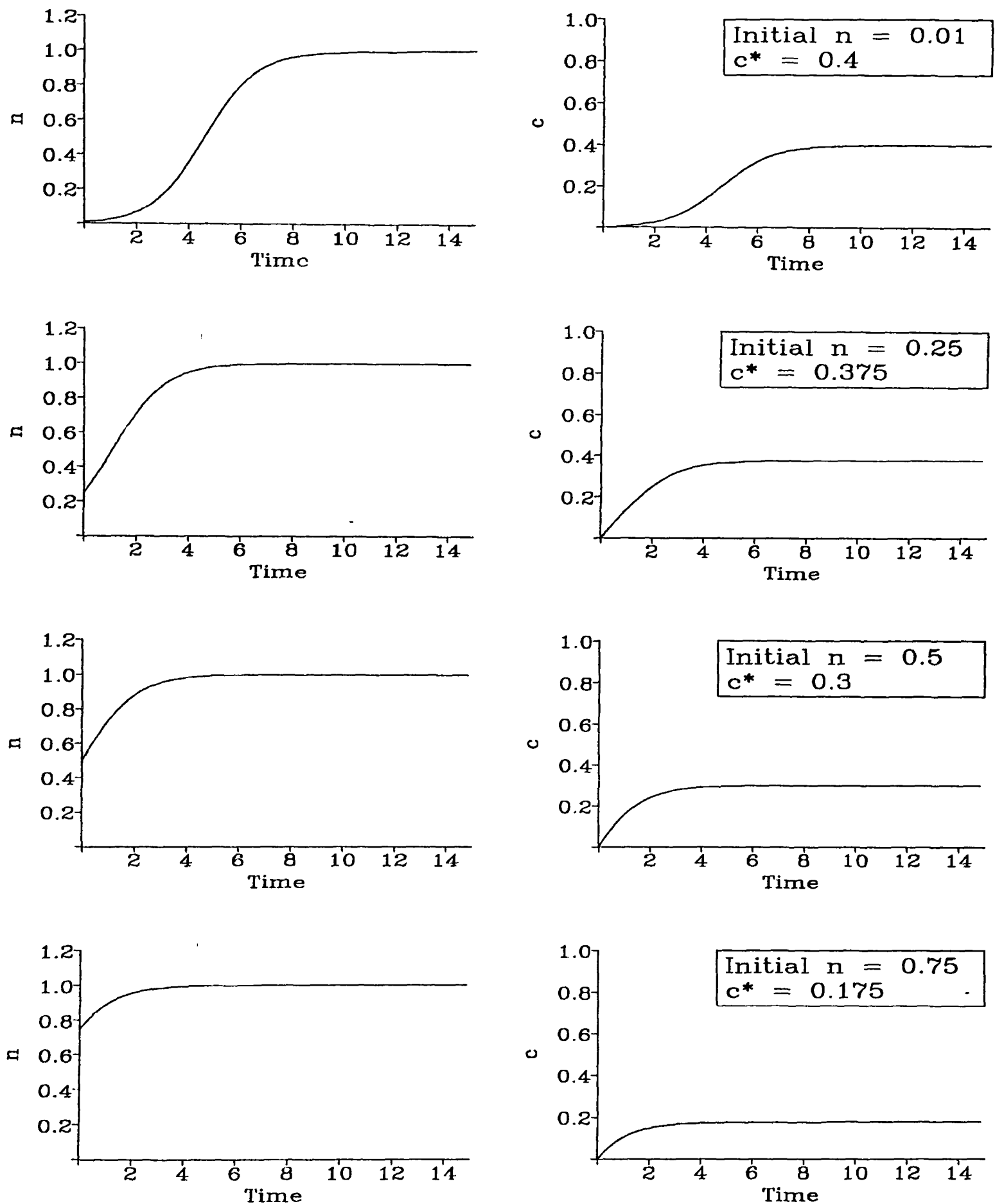


Figure 8.2: Numerical solutions of the caricature temporal model (8.2) showing the time evolution for different initial cell densities, n_0 , with α fixed at 0.8. The steady state c^* is bounded above by $\alpha/2$ and decreases as n_0 increases.

wound edge, with a gradual transition over a number of years. The steady state value depends on α and, by varying α we observe that c^* is approximately $\alpha/2$ (Figure 8.3), in agreement with the temporal model.

In the full model, qualitatively similar results were obtained when the step function initial conditions were smoothed out and also when exponentially decay initial conditions were imposed. By smoothing out the initial conditions in the caricature model, there is a less definite edge at $x = 0$ but both the front speed and the steady states are the same. This motivates us to try to alter the front speed by considering exponentially decaying initial conditions

$$\begin{aligned} n(x, 0) &\sim e^{-Rx} \quad \text{as } x \rightarrow \infty \\ c(x, 0) &\sim e^{-Rx} \quad \text{as } x \rightarrow \infty. \end{aligned}$$

where R is a positive constant (Murray, 1989). Letting $D_1 = 0.05$ and $\alpha = 0.8$, we solve the system numerically for different values of R (Figure 8.4) and find that for values of $R > 5$, the solutions evolve to fronts with the same speed, shape and steady state c^* as with step function initial conditions. However, as R decreases the wave speed increases and the fronts become less steep but the attained value c^* changes by less than 2%, and remains approximately $\alpha/2$ until R becomes very small (Figure 8.5). The numerical scheme breaks down for smaller values of R due to a slow decay rate, but this parameter region is not particularly significant and we do not pursue it here.

The temporal caricature model suggests that the steady state c^* changes when the initial amount of n varies. We investigate the effect of diffusion on this result by altering the step function initial conditions such that

$$\begin{aligned} n(x, 0) &= 1, \quad c(x, 0) = 1 \quad \text{if } x < 0 \\ n(x, 0) &= n_0, \quad c(x, 0) = 0 \quad \text{if } x > 0 \end{aligned}$$

and solve the system numerically for differing n_0 . We observe that the solutions do in fact attain different equilibrium values c^* but a ‘fill up’ mechanism with no

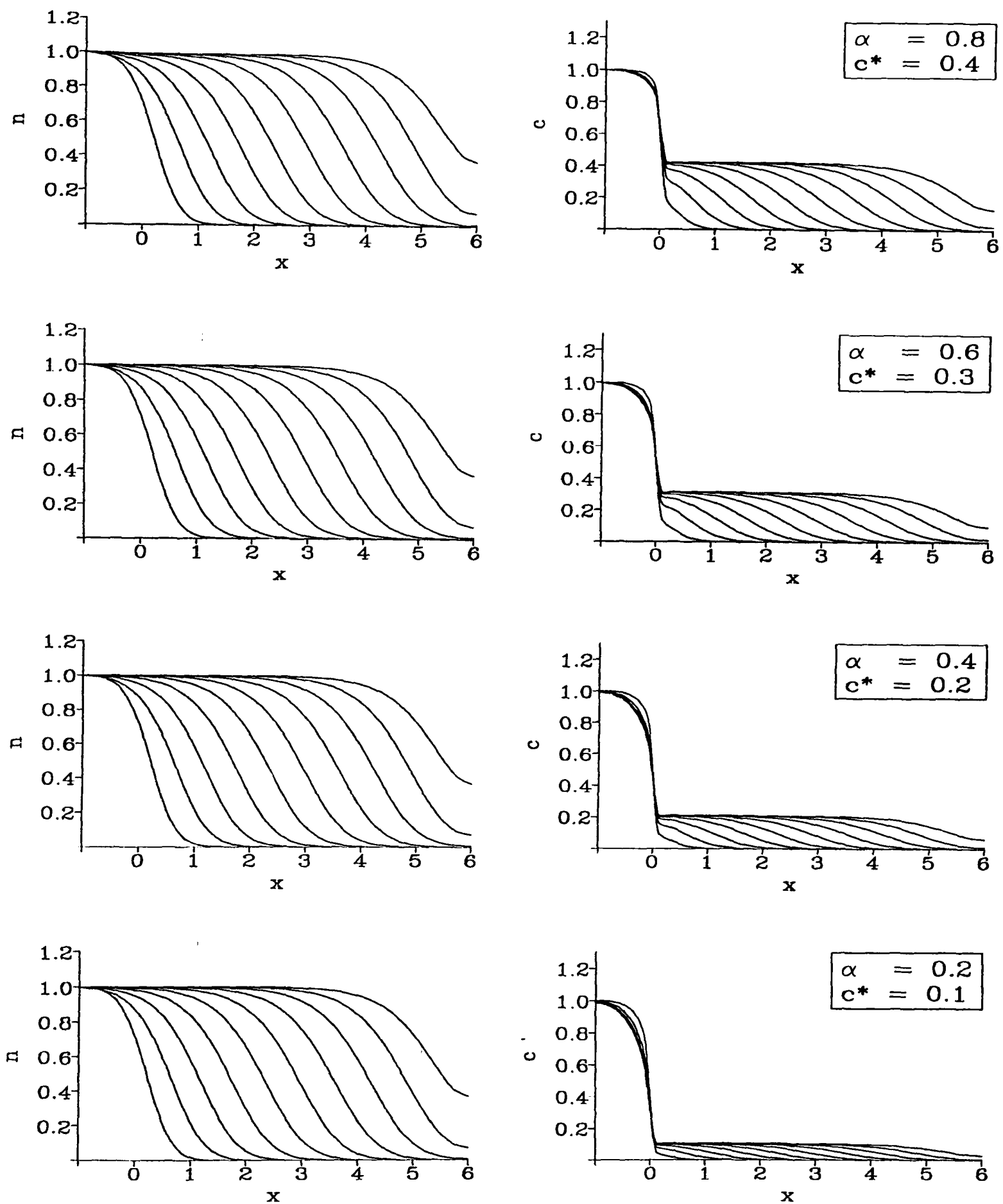


Figure 8.3: Numerical solutions of the nonlinear parabolic partial differential equations (8.1) with step function initial conditions, showing the change in n and c with space, at equal time intervals. The diffusion coefficient D_1 is taken to be 0.05 and we vary α as shown. The steady state c^* is observed to be approximately $\alpha/2$.

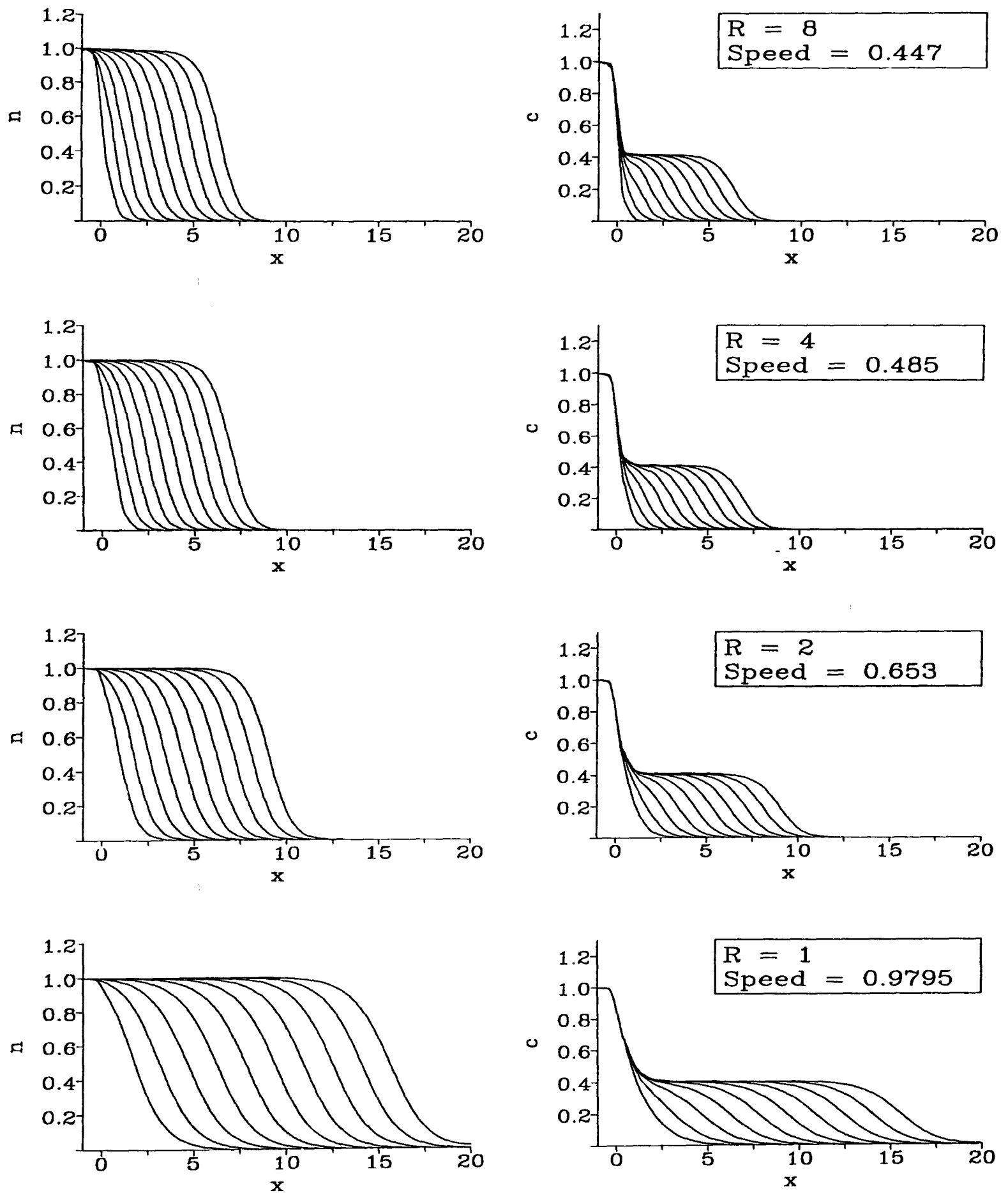


Figure 8.4: Numerical solutions of the nonlinear parabolic partial differential equations (8.1) with exponentially decaying initial conditions, showing the change in n and c with space, at equal time intervals, over a large domain. The diffusion coefficient D_1 is taken to be 0.05 and we let $\alpha = 0.8$. As the parameter R is varied, the speed and slope of the fronts vary but the steady state c^* is unchanged (see Figure 8.5).

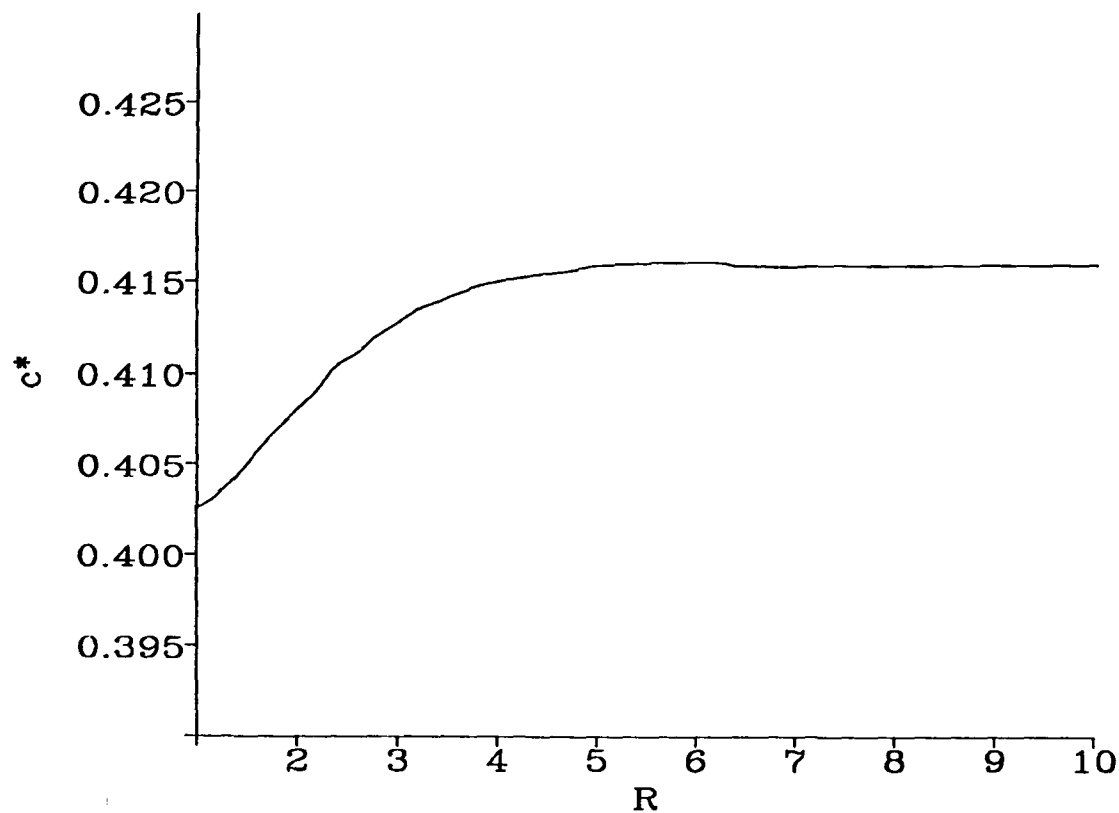


Figure 8.5: The change in the numerically determined steady state c^* as R is varied. The value c^* is unchanged for values of $R > 5$.

wavefronts is observed (Figure 8.6), implying that the kinetics are driving the system, as in the temporal model. Thus the caricature model does not mimic the full model exactly because we cannot look at the effect of additional external factors on c^* . However, we can try to force the variable c to a new equilibrium value by imposing the condition $c(x, 0) = \sigma$ if $x < 0$ and solving the system numerically for a range of values σ . Figure 8.7 shows the transition to the same dominant steady state, independent of the value σ . We will later extend this idea for the caricature model where c diffuses as well as n .

We therefore conjecture from the simulations that although there exists a continuum of positive steady states c^* , a value of approximately $\alpha/2$ is always attained. By changing the initial conditions we can alter the wave speed and steepness of the fronts but the value c^* remains approximately unchanged. This seems to suggest that in the three dimensional phase space of the travelling wave ordinary differential equations, there is only a heteroclinic connection between the ‘trivial’ steady state and one point on the line of the positive steady states. To investigate this further,

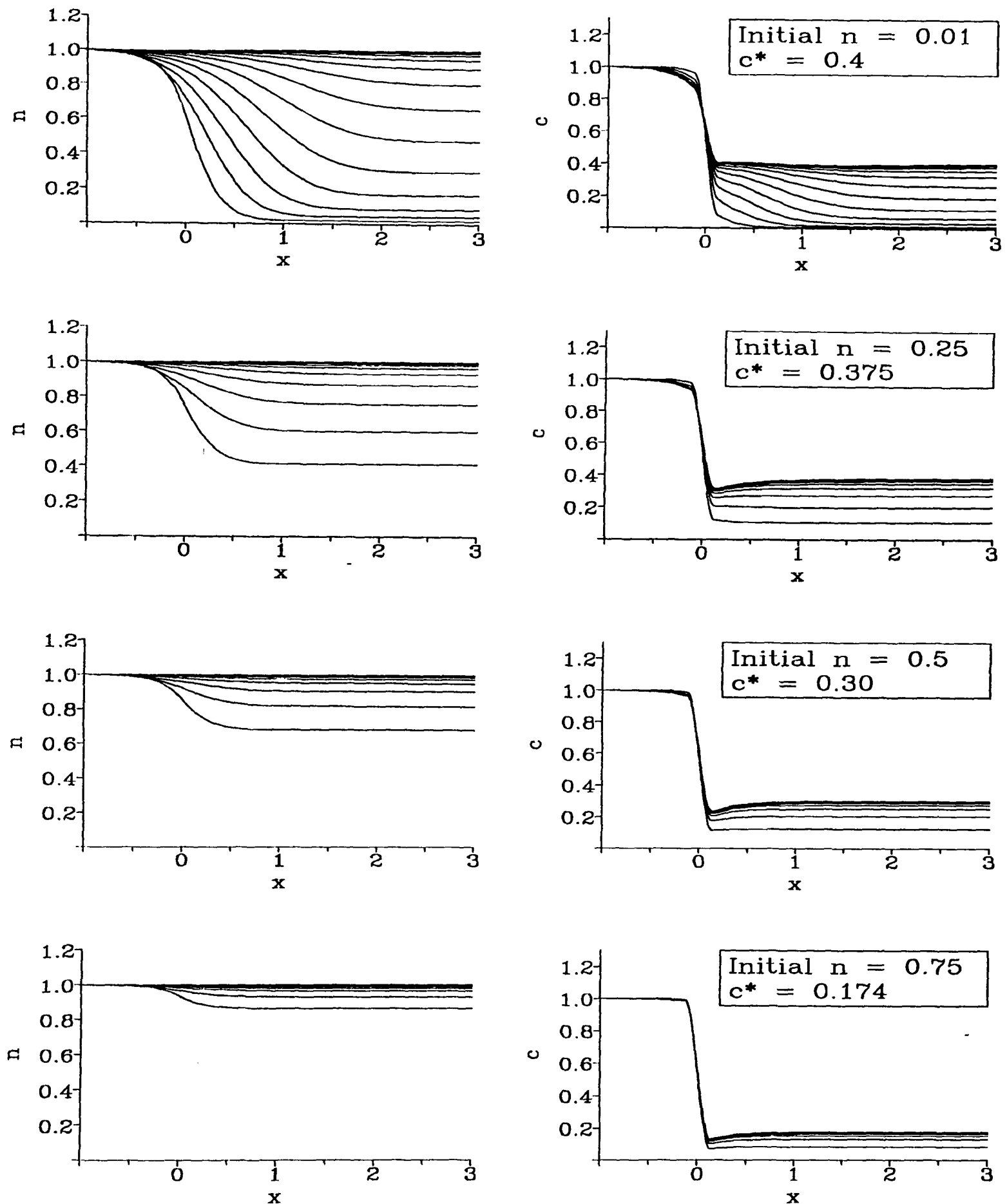


Figure 8.6: Numerical solutions of the nonlinear parabolic partial differential equations (8.1) showing the change in n and c with space, at equal time intervals. $D_1 = 0.05$ and $\alpha = 0.8$ and we vary the parameter n_0 corresponding to the initial amount of n . A ‘fill-up’ mechanism is observed and the steady state c^* varies, satisfying $c^* \approx \frac{\alpha}{2}(1 - n_0^2)$.

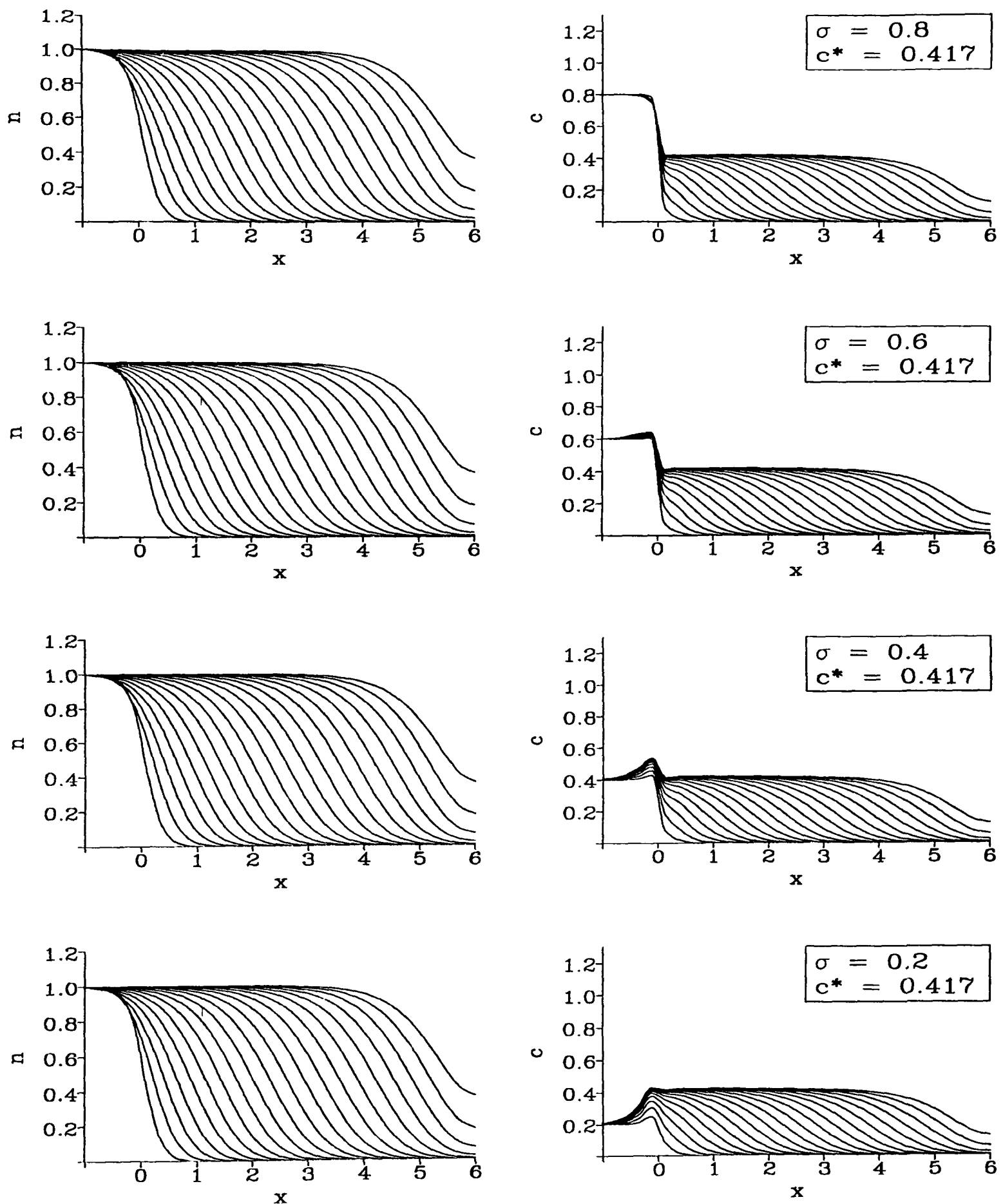


Figure 8.7: Numerical solutions of the nonlinear parabolic partial differential equations (8.1) showing the change in n and c with space, at equal time intervals. $D_1 = 0.05$ and $\alpha = 0.8$ and we try to force the variable c to a different steady state by varying the parameter σ . Travelling wave solutions are observed but the equilibrium value c^* remains unchanged.

we consider the system of equations in travelling wave form.

8.2.3 Travelling Waves

We have shown that in the spatially homogeneous situation the steady states are $n = c = 0$ and $n = 1, c = c^*$ and stability analysis shows that these steady states are, respectively, unstable and stable. This suggests that we look for travelling waves solutions to (8.1) for which $0 \leq n \leq 1$ and $0 \leq c \leq c^*$ and we thus introduce the coordinates $z = x - at$, where a is the wave speed, and let $n(x, t) = N(z)$ and $c(x, t) = C(z)$. N and C thus satisfy

$$D_1 N'' + aN' + N(1 - N) = 0 \quad (8.6a)$$

$$aC' + \alpha N(1 - N) - (1 - N)C = 0 \quad (8.6b)$$

where the prime denotes d/dz and the boundary conditions are

$$\lim_{z \rightarrow \infty} N(z) = 0, \quad \lim_{z \rightarrow -\infty} N(z) = 1 \quad (8.7a)$$

$$\lim_{z \rightarrow \infty} C(z) = 0 \quad \lim_{z \rightarrow -\infty} C(z) = c^*. \quad (8.7b)$$

By letting $dN/dz = V$, the above pair of ordinary differential equations can be written as three first order equations

$$N' = V \quad (8.8a)$$

$$V' = \frac{1}{D_1}(-aV - N(1 - N)) \quad (8.8b)$$

$$C' = \frac{1}{a}((1 - N)C - \alpha N(1 - N)). \quad (8.8c)$$

This system has steady states $N = V = C = 0$ and $N = 1, V = 0, C = C^*$ and we examine the stability of each steady state by considering the eigenvalue of the Jacobian matrix, J , evaluated at the corresponding steady state, where

$$J = \begin{pmatrix} 0 & 1 & 0 \\ -\frac{1}{D_1}(1 - 2N) & -\frac{a}{D_1} & 0 \\ \frac{1}{a}(-C - \alpha(1 - 2N)) & 0 & \frac{1-N}{a} \end{pmatrix}.$$

Solving for the eigenvalues we obtain a cubic equation, the roots of which are

$$\lambda = \frac{1-N}{a}, \quad \lambda = \frac{-a \pm \sqrt{a^2 - 4D_1(1-2N)}}{2D_1}$$

and we note that these are all independent of α and C (because independent of J_{31}) and thus of c^* . At the positive steady state $N = 1$, the roots of the cubic equation are

$$\lambda = 0, \quad \lambda = \frac{-a \pm \sqrt{a^2 + 4D_1}}{2D_1}$$

and thus we have one negative, one positive and one zero root. Similarly, at the trivial steady state $N = 0$ the roots are

$$\lambda = 1/a, \quad \lambda = \frac{-a \pm \sqrt{a^2 - 4D_1}}{2D_1}$$

and we have one positive and two negative roots.

Now a necessary condition for the existence of a travelling wave is that the positive steady state has at least one unstable manifold and the trivial steady state has at least one stable manifold. Looking at the positive steady state we observe that there is only one positive eigenvalue and this is independent of c^* . But there exists a continuum of possible steady states, so the paths out of each must all be parallel, as shown in Figure 8.8, since the eigenvectors corresponding to the positive eigenvalue are independent of c^* . The zero eigenvalue corresponds to neutral stability to a perturbation along the line of steady states. At the trivial steady state, the Jacobian matrix has two negative eigenvalues, corresponding to a stable spiral or stable node, depending on the sign of $a^2 - 4D_1$, from which we can determine the minimum wave speed. Attempts to force the system to a particular value c^* show that the value $\alpha/2$ always is picked out, and we can thus conjecture that there is a unique trajectory connecting the trivial and positive steady states. This is consistent with the observed result of one particular steady state c^* being picked out when diffusion is added to the system. The existence of a positive eigenvalue at the trivial steady state implies that any phase trajectory arbitrarily close to the heteroclinic connection between

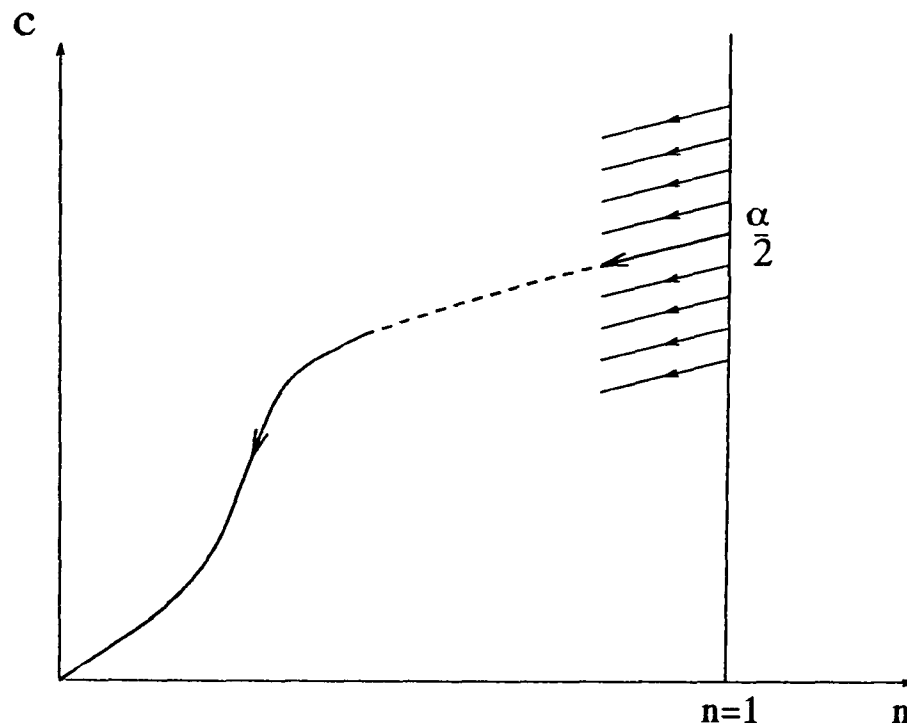


Figure 8.8: Schematic representation of possible trajectories from the line of positive steady states to the zero steady state, in the (n, c) plane.

$(0, 0, 0)$ and $(1, 0, c^*)$ will diverge. For the single reaction diffusion equation, one can show rigorously that there is a trajectory joining the non-zero steady state to the zero steady state. However, as we are in a phase space of dimension 3, this proof is not applicable.

The magnitude of the eigenvalues depend on the wave speed, a , and this can be altered by considering exponentially decaying initial conditions. However, this change in initial conditions simply results in a new system of ordinary differential equations with the same structure and hence one particular steady state is still selected.

8.2.4 Asymptotic Solutions of Model Equations

In this section, we determine an asymptotic solution for the nonlinear reaction diffusion equations (8.1). Phase plane analysis shows that travelling wave solutions exist for all wave speeds $a \geq 2\sqrt{D_1}$. These wave solutions are invariant to any shift in the origin of the coordinate system and we can thus impose the boundary condition

$N(0) = 1/2$. Following the work of Canosa (1973), we stretch the variable near the front by letting $\zeta = z/a$ and note that $\epsilon = 1/a^2$ is a small parameter, so that we can find the solution as an asymptotic expansion in ϵ . The transformed equations become

$$\epsilon D_1 \frac{d^2 N}{d\zeta^2} + \frac{dN}{d\zeta} + N(1 - N) = 0 \quad (8.9a)$$

$$\frac{dC}{d\zeta} + \alpha N(1 - N) - (1 - N)C = 0. \quad (8.9b)$$

We look for solutions as a regular perturbation series in ϵ , such that

$$N(\zeta; \epsilon) = N_0(\zeta) + \epsilon N_1(\zeta) + \epsilon^2 N_2(\zeta) + \dots \quad (8.10a)$$

$$C(\zeta; \epsilon) = C_0(\zeta) + \epsilon C_1(\zeta) + \epsilon^2 C_2(\zeta) + \dots \quad (8.10b)$$

with boundary conditions

$$N_0(-\infty) = 1, \quad N_0(\infty) = 0, \quad N_0(0) = 1/2, \quad (8.11a)$$

$$N_i(\pm\infty) = 0, \quad N_i(0) = 0 \quad \text{for } i = 1, 2, \dots \quad (8.11b)$$

$$C_0(-\infty) = c_0^*, \quad C_0(\infty) = 0, \quad (8.11c)$$

where the steady state is given by $c^*(\epsilon) = c_0^* + \epsilon c_1^* + \epsilon^2 c_2^* + \dots$. Substituting these expansions into equations (8.10) and equating powers of ϵ , we find

$$O(1) : \quad \frac{\partial N_0}{\partial \zeta} + N_0(1 - N_0) = 0 \quad (8.12a)$$

$$\frac{\partial C_0}{\partial \zeta} - (1 - N_0)C_0 = \alpha N_0(1 - N_0) \quad (8.12b)$$

$$O(\epsilon) : \quad \frac{\partial N_1}{\partial \zeta} + N_1(1 - 2N_0) = -D_1 \frac{\partial^2 N_0}{\partial \zeta^2} \quad (8.12c)$$

$$\frac{\partial C_1}{\partial \zeta} - C_1(1 - N_0) = \alpha N_1(1 - 2N_0) + N_1 C_0, \quad (8.12d)$$

with further equations for higher orders in ϵ . Since the N equations decouple from the C equations, we solve these first and use the boundary conditions (8.11) to obtain the solutions

$$N_0(\zeta) = \frac{1}{1 + e^\zeta}$$

$$N_1(\zeta) = \frac{e^\zeta D_1}{(1 + e^\zeta)^2} \log \left(\frac{4e^\zeta}{(1 + e^\zeta)^2} \right).$$

On substituting these expressions into the equations for C_0 and C_1 , we use the boundary conditions to get

$$C_0(\zeta) = \frac{\alpha}{2(1 + e^\zeta)}$$

$$C_1(\zeta) = \frac{1}{12(1 + e^\zeta)^2} \left(\alpha D_1 - 3\alpha D_1 e^\zeta + 6\alpha e^\zeta D_1 \log \left(\frac{4e^\zeta}{(1 + e^\zeta)^2} \right) \right)$$

and note that, to leading order, $C = \frac{\alpha}{2}N$.

Hence, in terms of the original variable z , the asymptotic solution is given by

$$\begin{aligned} N(z; \epsilon) &= \frac{1}{1 + e^{z/a}} + \epsilon \frac{e^{z/a} D_1}{(1 + e^{z/a})^2} \log \left(\frac{4e^{z/a}}{(1 + e^{z/a})^2} \right) + O(\epsilon^2), \\ C(z; \epsilon) &= \frac{\alpha}{2(1 + e^{z/a})} \\ &+ \frac{\epsilon}{12(1 + e^{z/a})^2} \left(\alpha D_1 - 3\alpha D_1 e^{z/a} + 6\alpha e^{z/a} D_1 \log \left(\frac{4e^{z/a}}{(1 + e^{z/a})^2} \right) \right) \\ &+ O(\epsilon^2). \end{aligned}$$

This asymptotic solution satisfies the boundary conditions (8.11) and we can deduce that

$$C(z) \rightarrow c^* = \frac{\alpha}{2} + \frac{\alpha D_1}{12} + O(\epsilon^2) \text{ as } z \rightarrow -\infty. \quad (8.13)$$

In terms of the wave speed, this expression gives us $c^* = \frac{\alpha}{2} + \frac{\alpha D_1}{12a^2} + O(a^{-4})$, and hence the steady state value depends on the wave speed but is uniquely determined for a given wave speed. Once again we look at ways of changing the speed of the fronts by altering the initial conditions.

We first consider step function initial conditions

$$n(x, 0) = \begin{cases} 1 & \text{if } x \leq 0 \\ 0 & \text{if } x > 0. \end{cases}$$

Kolmogorov *et al.* (1937) showed that the solution $n(x, t)$ evolves to a travelling wave solution with minimum wave speed $a_{min} = 2\sqrt{D_1}$. On substitution into the expression (8.13) for c^* , we find

$$c^* \approx \alpha/2 + \alpha/48 = 25\alpha/48.$$

We now consider exponentially decaying initial conditions

$$n(x, 0) \sim e^{-Rx} \text{ as } x \rightarrow \infty,$$

where R is a positive constant. We focus on the leading edge of the travelling wave so n is small and we can neglect n^2 in comparison with n . The parabolic partial differential equation thus becomes

$$\frac{\partial n}{\partial t} = D_1 \frac{\partial^2 n}{\partial x^2} + n.$$

By looking for the leading edge form of wavefront solutions, $n(x, t) \sim e^{-R(x-at)}$, Rothe (1978) showed that

$$\text{if } R \leq \frac{1}{\sqrt{D_1}}, \quad \text{then } a = \frac{1}{R} + D_1 R \quad (8.14a)$$

$$\& \text{ if } R > \frac{1}{\sqrt{D_1}}, \quad \text{then } a = 2\sqrt{D_1}. \quad (8.14b)$$

In the former case, $\epsilon = \frac{R^2}{(1+D_1 R^2)^2}$, and on substitution into (8.13) we find

$$c^* \approx \alpha/2 + \frac{\alpha D_1 R^2}{12(1 + D_1 R^2)^2}.$$

Now $0 \leq D_1 R^2 \leq 1$ so $1/4 \leq 1/(1 + D_1 R^2)^2 \leq 1$ and hence $c^* < 7\alpha/12$. We note that as $D_1 R^2 \rightarrow 1$, the second term in the above expression for c^* tends towards $\alpha/48$, as predicted for the step function initial conditions.

These predictions agree well with the numerical results, as shown in Figure 8.9 where we plot the numerically and analytically determined change in steady state with the wave speed. This analysis shows that the wave speed varies with the initial conditions and uniquely determines the steady state behind the front, but the speed only affects the equilibrium value, c^* , to order ϵ and, to leading order, the steady state is still $\alpha/2$.

We can conclude from this section that the caricature model mimics the full model in that there exists a continuum of positive steady states but a heteroclinic trajectory between the trivial and only one of these positive equilibrium values. In

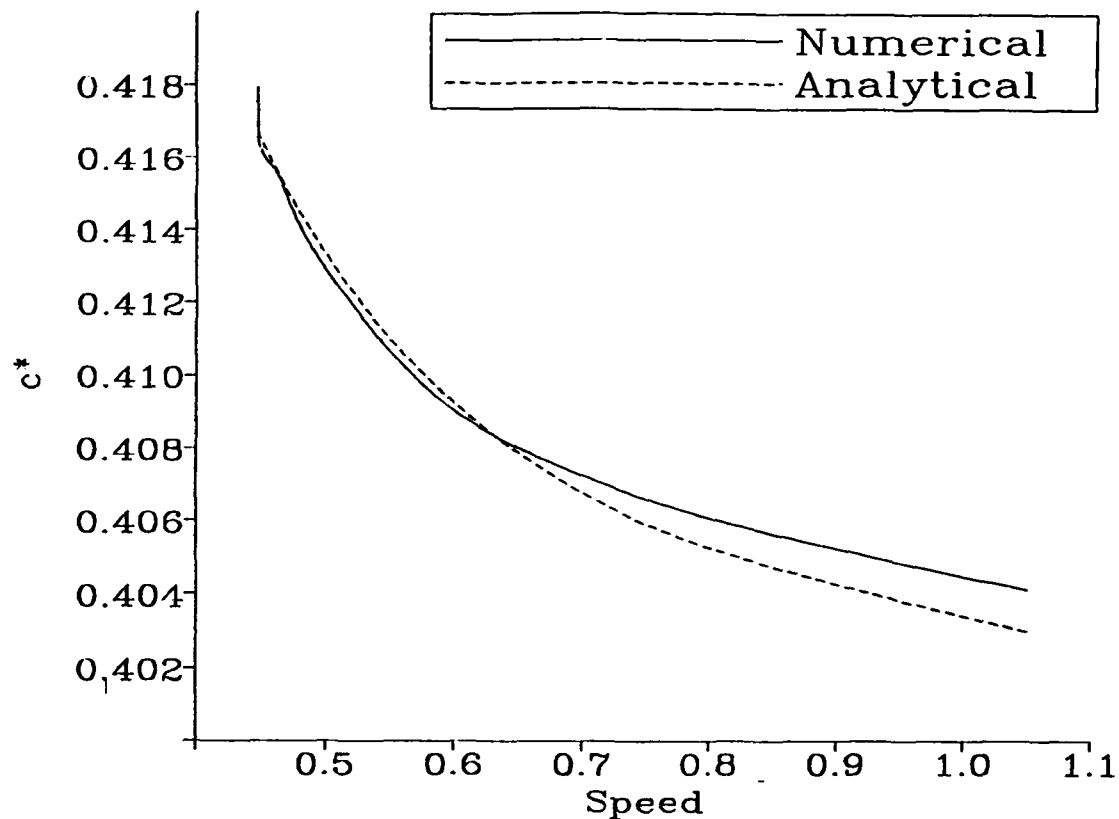


Figure 8.9: The numerically and analytically determined change in the steady state c^* as the wave speed a is altered by imposing exponentially decaying initial conditions.

the next section we look at possible changes to the caricature model. Our aim is to find a variation of the model in which we can drive the system to a new positive steady state by transient external influences and relate the findings to the full model.

8.3 Extensions to the Model

In this section, we consider extensions to both the caricature and full model, in an attempt to gain a further insight into why one particular steady state is being picked out of the continuum of possible values. In each subsection, we discuss the changes to the caricature model first and then apply the result to the full model.

8.3.1 Haptotaxis

Haptotaxis is the movement of cells up an adhesive gradient and we hypothesise that fibroblasts move up a collagen gradient as well as migrating into the wound by diffusion at a constant rate. We thus include a haptotactic term on the right hand side of the equation for n in the caricature model (8.1) so that

$$\frac{\partial n}{\partial t} = D_1 \frac{\partial^2 n}{\partial x^2} - \overbrace{\frac{\partial}{\partial x} \left(\gamma n \frac{\partial c}{\partial x} \right)}^{\text{Haptotaxis}} + n(1 - n) \quad (8.15a)$$

$$\frac{\partial c}{\partial t} = \alpha n(1 - n) - (1 - n)c \quad (8.15b)$$

where γ is a positive constant. Numerical simulations of the haptotactic caricature model, with step function initial conditions and zero flux boundary conditions, show that when γ is increased, there is a change in the shape of the waves, particularly at the edge $x = 0$ (Figure 8.10). Moreover, the front speed increases as γ increases and, in the notation of Section 8.2.4, the order ϵ term for equilibrium c^* increases slightly. We note that there are numerical problems at $x = 0$ if the haptotactic coefficient γ becomes too large.

To investigate the haptotactic term further, we perform some travelling wave analysis, using the techniques described previously. After much algebraic manipulation, we find that the eigenvalues at the trivial steady state $(0, 0, 0)$ are identical to those in the absence of haptotaxis, with one positive and two negative roots. Similarly, the eigenvalues at the positive steady state $(1, 0, C^*)$ are

$$\lambda = 0, \quad \lambda = \frac{-(a + \frac{3\gamma\alpha}{2} + \frac{\gamma C^*}{a}) \pm \sqrt{(a + \frac{3\gamma\alpha}{2} + \frac{\gamma C^*}{a})^2 + 4D_1}}{2D_1}.$$

Hence although the eigenvalues differ in magnitude, there is still only one positive root and hence only one possible outward trajectory. The presence of haptotaxis in the model simply changes the speed of convergence to the steady state but does not alter the equilibrium values. Again qualitatively similar results are obtained in numerical solutions of the full model with a fibroblast haptotactic term depending on both collagen I and III.

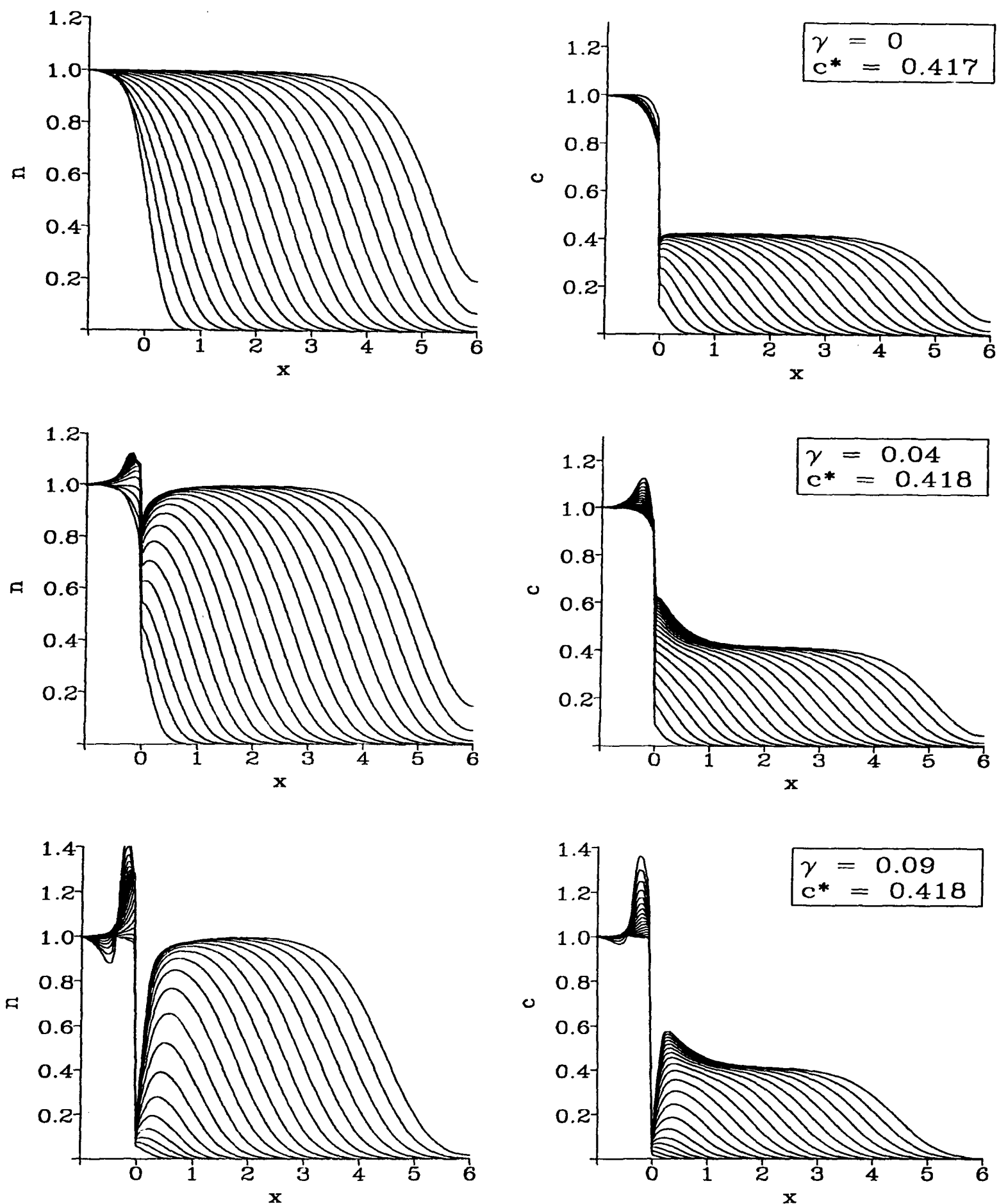


Figure 8.10: Numerical solutions of the caricature model (8.1) including a haptotactic term, showing the change in the shape of the wave front but no significant change in steady state as the haptotactic parameter γ is increased.

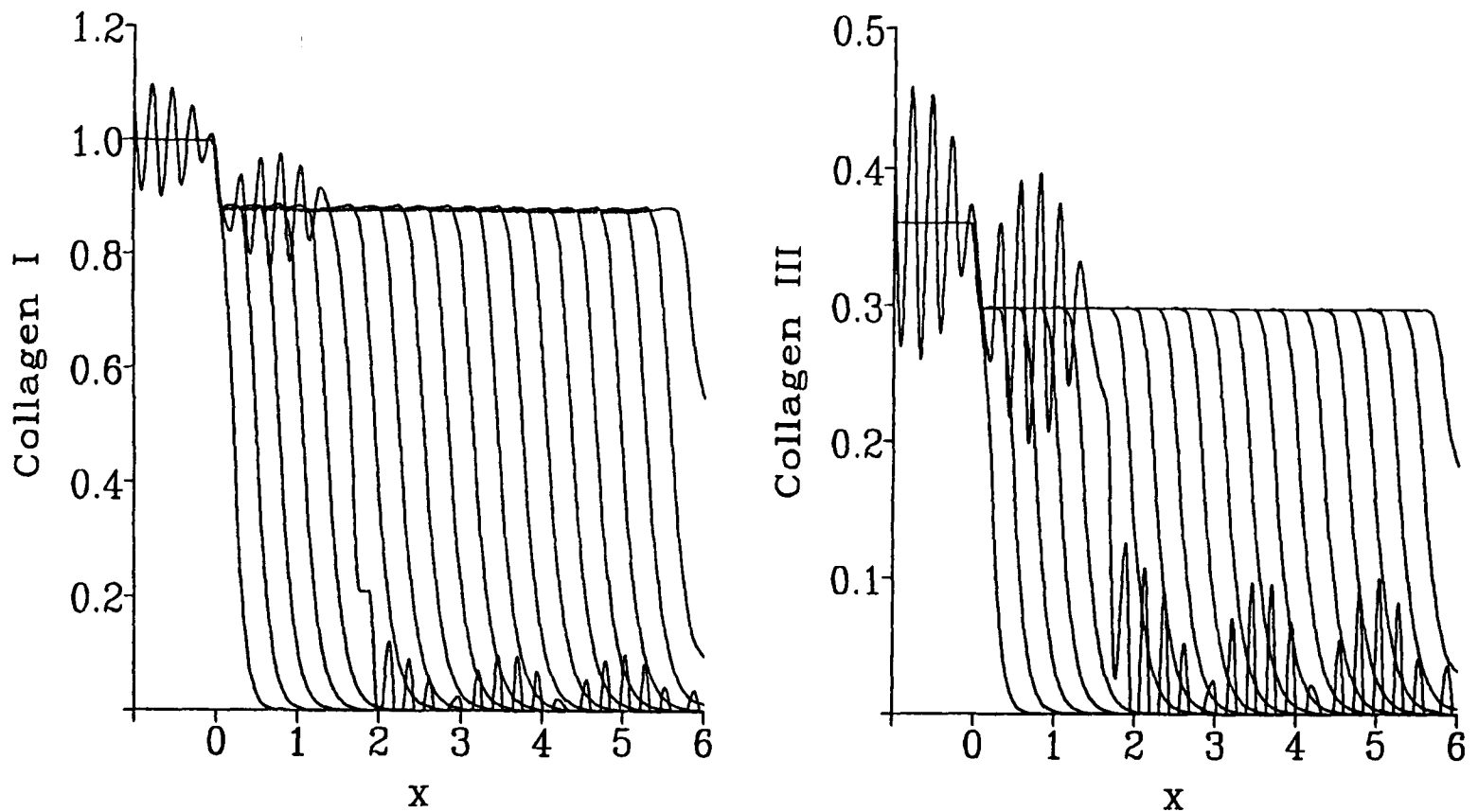


Figure 8.11: Fronts of collagen I and III moving into the wound, with noise added at time 216 hours. We take $\delta = 0.1$ and use the parameters in Table 5.3. The perturbation is smoothed out immediately and the final levels of collagen are unaltered.

We thus conclude from this subsection that the inclusion of a term to model the movement of fibroblasts up a collagen gradient does not significantly affect the final collagen levels, although the process is biologically plausible. We hence omit this term from any further extensions to the model.

8.3.2 Robustness

In this subsection we perturb the travelling waves by adding noise to the system, representing some external influence. This is achieved in the caricature model by introducing the perturbation at a particular time step, using the functional form

$$c_{new}(x, t) = c_{old}(x, t) + \delta \cos(500x), \quad (8.16)$$

thus causing an oscillation of amplitude δ , throughout the wave. There is no special significance in our use of a wave number of 500. Numerical solutions of the caricature

model with noise, using step function initial conditions and zero flux boundary conditions, show that the noise decays extremely rapidly.

We introduce noise into the full system by adding perturbations with functional form as in (8.16) to the collagen I and III equations, at one particular time step. Figure 8.11 shows the numerical solutions for collagen I and III, with $\delta = 0.1$, illustrating the smoothing out of the perturbation within one time step and no change in the levels of collagen I and III. The robustness of this result was investigated by changing the amplitude and time of perturbation and the timestep in the simulations, with qualitatively similar results being obtained. Hence, the collagen levels are unaltered by perturbations due to external factors, at any time during the healing process.

8.3.3 Diffusion of c

In this subsection we consider diffusion of c in the caricature model

$$\frac{\partial n}{\partial t} = D_1 \frac{\partial^2 n}{\partial x^2} + n(1 - n) \quad (8.17a)$$

$$\frac{\partial c}{\partial t} = \underbrace{D_2 \frac{\partial^2 c}{\partial x^2}}_{\text{Diffusion}} + \alpha n(1 - n) - (1 - n)c, \quad (8.17b)$$

where D_2 is a positive constant. We impose step-function initial conditions $n(x, 0) = c(x, 0) = 1$ if $x < 0$, $n(x, 0) = c(x, 0) = 0$ if $x > 0$ and specify zero flux boundary conditions. Figure 8.12 shows numerical solutions over a finite domain $[-1.5, 1.5]$, at equal time steps. After 3 time steps, n has reached its equilibrium value and thus the kinetics for c goes to zero, leaving a simple diffusion equation. The profiles for c thus show constant diffusion into the domain and we observe a new equilibrium value c^* . We now try to drive c^* to a different value by forcing the initial conditions so that $c(x, 0) = \sigma$ if $x < 0$, where σ is a positive constant. By varying σ we find that the final value c^* does indeed change.

We now solve the same system of equations on an infinite domain, in an attempt

to gain more insight into the shape of the fronts. Using the same boundary conditions and forced initial conditions, the solution profiles show waves of n and c travelling at constant speed and shape, with the equilibrium value c^* remaining at approximately $\alpha/2$, the level attained in the absence of c diffusion. However, once the steady state $n = 1$ is attained, the c equation again becomes the diffusion equation and, over a long period of time, the steady state c^* changes its value, in agreement with the finite domain solutions. By varying σ , the initial value of c , different steady states are attained. It thus seems that for each value of σ there are 2 possible steady state values c^* , one identical to the level attained in the absence of diffusion and the other being dependent on σ . To investigate this, we look at the system in travelling wave co-ordinates.

We again introduce the variable $z = x - at$ and carry out the usual phase plane analysis. The eigenvalues of the Jacobian matrix can be shown to satisfy the quartic equation

$$0 = \lambda^2\left(\lambda + \frac{a}{D_1}\right)\left(\lambda + \frac{a}{D_2}\right) - \lambda\left(\lambda + \frac{a}{D_1}\right)\left(\frac{1-N}{D_2}\right) + \lambda\left(\lambda + \frac{a}{D_2}\right)\left(\frac{1-2N}{D_1}\right) + \frac{(1-2N)(N-1)}{D_1 D_2}. \quad (8.18)$$

At the trivial steady state, $N = 0$, we have one positive and three negative eigenvalues, namely

$$\lambda = \frac{-a \pm \sqrt{a^2 - 4D_1}}{2D_1}, \quad \lambda = \frac{-a \pm \sqrt{a^2 + 4D_2}}{2D_2}.$$

In order to prevent the existence of a spiral, resulting in negative values of N , we determine a minimum wave speed, $a_{min} = 2\sqrt{D_1}$. At the positive steady state, $N = 1$, the eigenvalues are

$$\lambda = 0, \quad \lambda = -\frac{a}{D_2}, \quad \lambda = \frac{-a \pm \sqrt{a^2 + 4D_1}}{2D_1},$$

the single positive value implying that there is only one possible trajectory in the outward direction. Focusing on the negative eigenvalues at the zero steady state,

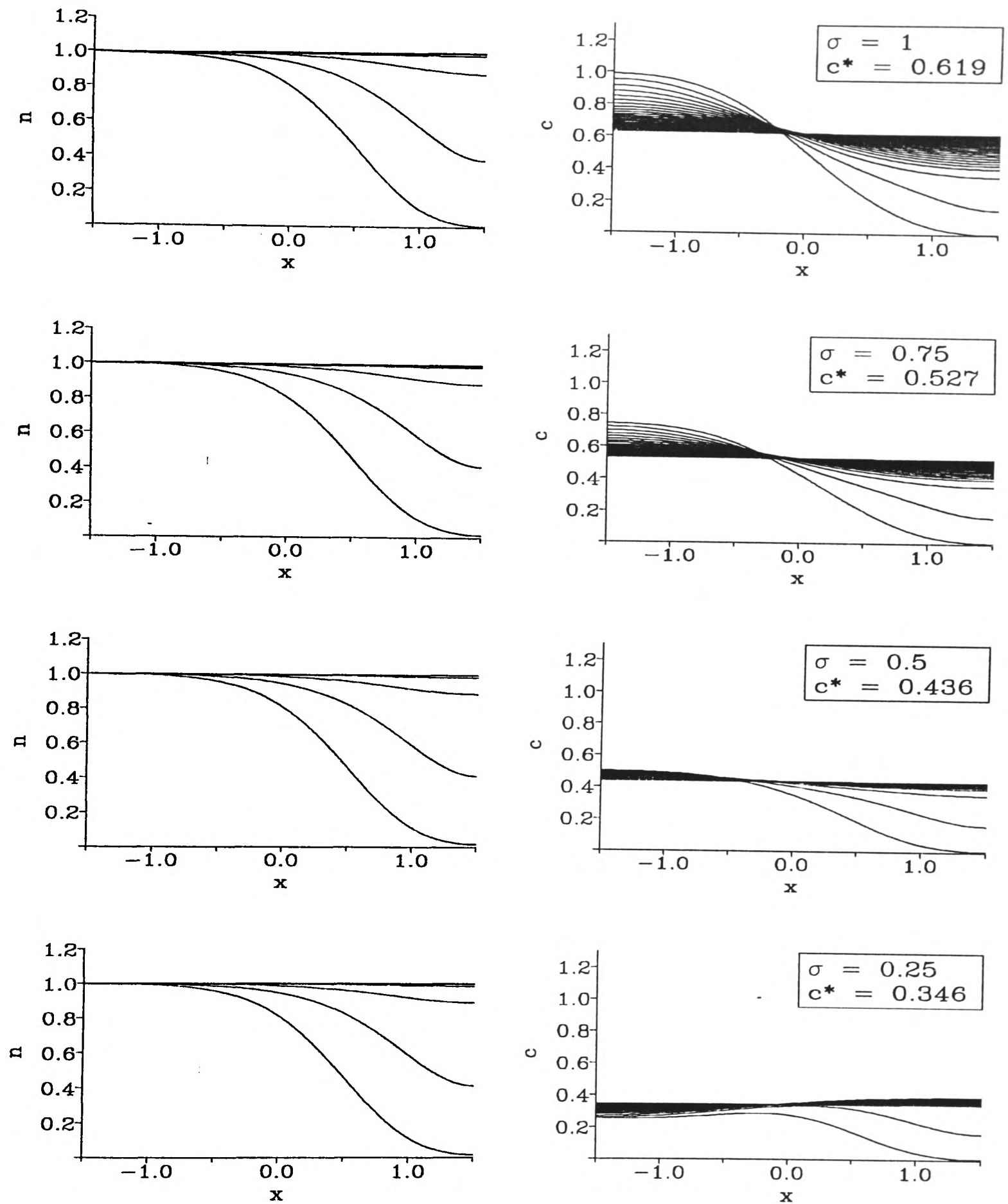


Figure 8.12: Numerical solutions of the caricature model with diffusion of both variables (8.17), over a finite domain $[-1.5, 1.5]$, at equally spaced time intervals. By varying the initial value of c , we can change the equilibrium value c^* . We take $\alpha = 0.8$ and $D_1 = D_2 = 0.05$, with σ as shown.

two are identical to the case of no c diffusion, whereas the other is dependent on D_2 . Numerical simulations show that the steady state attained depends on the diffusion coefficient D_2 , but this dependence is simply due to n reaching its equilibrium value, resulting in zero kinetics for c . Hence we are simply solving the diffusion equation on a finite domain. By varying σ , we are in fact altering the initial conditions, but since n attains its steady state value more rapidly than c , the restriction of the domain size results in boundary effects.

Biological evidence suggests that fibroblasts migrate into the wound and secrete procollagen fibres, which are activated by specific enzymes to form collagen fibrils. There is little research into the movement of these fibrils, but the general opinion is that the fibrils re-orientate themselves but do not migrate within the wound space. However, we now consider the possibility that the collagen fibres diffuse into the wound at a constant rate, in an attempt to drive the healed levels of collagen I and III to new values. As expected, we obtain a similar result to the caricature model, with diffusion of collagen I and III causing a shift in healed levels once the collagenases and enzyme 2 reach their equilibrium values, the collagen equations thus becoming the basic diffusion equation. The healed collagen levels can also be changed by forcing the initial conditions. However, it is the experimentally determined result of addition of $TGF\beta$ affecting scar tissue that we need to investigate. By numerically solving the full model with collagen diffusion, no change in collagen levels is observed when the initial conditions of $TGF\beta$ are altered, as discussed previously. Hence, this leads us to conclude that diffusion of collagen does allow new collagen steady state levels to be attained but this is purely due to the collagen equations changing to basic diffusion equation and is not related to the exogenous application of growth factors, as experimentally predicted.

8.4 Improved Caricature Model

In this section, we extend the caricature model (8.1) to further mimic the full system. A key feature of the full system is the transient change in the level of collagen when TGF β is added to the system. In the previous caricature model, neither n nor c mimicked TGF β and we thus introduce a third variable, b , which diffuses, has a saturating kinetic term and linear degradation. One possible system is

$$\frac{\partial n}{\partial t} = D_1 \frac{\partial^2 n}{\partial x^2} + n(1 - n) \quad (8.19a)$$

$$\frac{\partial b}{\partial t} = D_2 \frac{\partial^2 b}{\partial x^2} + \frac{2nb}{1 + b} - b \quad (8.19b)$$

$$\frac{\partial c}{\partial t} = (1 - n)[n(1 + \beta b) - c], \quad (8.19c)$$

where β is a non-negative constant, and the other constants in the kinetic terms are chosen so that the steady states are $(0, 0, 0)$ and $(1, 1, c^*)$, where $c^* > 0$.

We first solve the system in the absence of diffusion, with initial conditions $n = n_0, b = b_0, c = c_0$ at $t = 0$. Numerical solutions indicate that the final level c^* depends on the initial values b_0 and c_0 . We then solve the reaction diffusion equations (8.19) and impose step function initial conditions

$$\begin{aligned} n(x, 0) &= 1, \quad b(x, 0) = 1, \quad c(x, 0) = \sigma_1 \quad \text{if } x < 0 \\ n(x, 0) &= 0, \quad b(x, 0) = \sigma_2, \quad c(x, 0) = 0 \quad \text{if } x > 0, \end{aligned}$$

with zero flux boundary conditions. Numerical solutions with $\sigma_2 = 0$ evolve to travelling waves with constant speed and shape, with one particular level c^* being picked out, independent of σ_1 . To mimic the addition of TGF β , we increase σ_2 and observe only a transient change in the final level c^* , the same phenomenon as in the full model (Figure 8.13).

This phenomenon can be explained by the existence of either only one trajectory from the line of non-trivial steady states to $(0, 0, 0)$, or an infinite number of trajectories leaving the line $(1, 1, c^*)$ but only one terminating at $(0, 0, 0)$ and the others tending to infinity. A final possibility is that there are many different connections

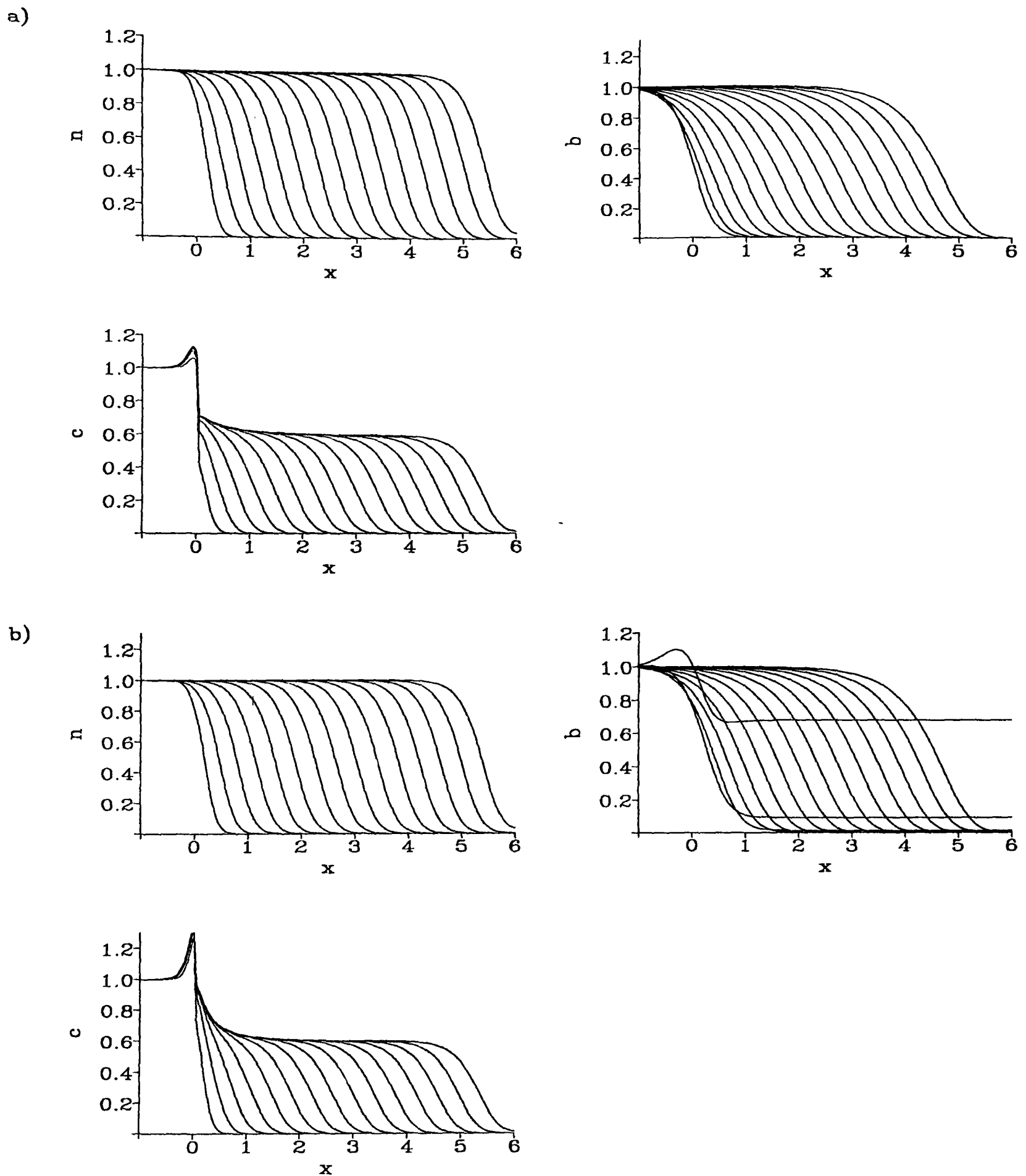


Figure 8.13: Numerical solutions of (8.19) with $D_1 = 0.01$, $D_2 = 0.05$ and $\beta = 0.8$. In a) $\sigma_2 = 0$ and in b) $\sigma_2 = 5$, corresponding to addition of b . We note the transient change in the fixed level, c^* , before attaining the level $c^* = 0.61$.

to different c^* , with only one being stable as a reaction-diffusion solution. We investigate these possibilities by introducing travelling wave coordinates, $z = x - at$, as discussed previously, to obtain the system of ordinary differential equations

$$D_1 N'' + aN' + N(1 - N) = 0 \quad (8.20a)$$

$$D_2 B'' + aB' + \frac{2NB}{1+B} - B = 0 \quad (8.20b)$$

$$aC' + (1 - N)[N(1 + \beta B) - C] = 0. \quad (8.20c)$$

We linearise about the positive steady state to determine the eigenvalues

$$\lambda = 0, \quad \lambda = \frac{-a \pm \sqrt{a^2 + 2D_2}}{2D_2}, \quad \lambda = \frac{-a \pm \sqrt{a^2 + 4D_1}}{2D_1}.$$

Since we have two positive eigenvalues, there are an infinite number of trajectories leaving the positive steady state. Extensive numerical solutions suggest that one particular level c^* is selected and we thus seek an explicit solution for c^* to investigate the dependence on initial conditions. We first look for asymptotic solutions to the travelling wave equations (8.20), by introducing a change of variables $\zeta = z/a$ and defining a small parameter $\epsilon = 1/a^2$, as in Section 8.2.4. In this case, we are able to find the first order term for N , but the equation for the first order term for B involves transcendental functions and an explicit solution cannot be determined. Hence we are unable to derive an explicit solution for c^* for this improved caricature model.

Numerical calculation of the eigensolutions for (8.20) at the zero steady state shows that there is a change in character of two eigenvalues from complex to real at a wave speed which is approximately $2\sqrt{D_1}$. Furthermore, we verify the existence of 2 positive eigenvalues at the non-trivial steady state, which suggests that there is more than one outward trajectory and we thus conjecture that there are an infinite number of trajectories leaving the line of positive steady states, with all but one tending to infinity. Dunbar (1984) proved the existence of a heteroclinic connection in a travelling wave system in $\mathbb{R}^4$, but, to our knowledge, no analogous results have

been derived for systems in higher dimensions. Hence, since we are considering 5-dimensional phase space, we do not attempt to determine these connections.

We thus conclude that the improved caricature model has a continuum of steady states $(1, 1, c^*)$, but numerical solutions suggest that the final level c^* does not depend on the initial conditions. Although there are 2 positive eigenvalues at the positive steady state, there is only one trajectory which connects with the origin and hence one particular value c^* is always picked out.

8.5 General Caricature Model

In this section, we consider a caricature model which has general kinetic and diffusion terms, and exhibits the phenomenon of a continuum of steady states. By determining the eigenvalues of the resulting travelling wave equations we investigate the dependence of the steady states on the initial conditions and show that it is not possible to have two positive eigenvalues at the non-trivial steady state. We consider the equations

$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial x} \left((D_1 + \gamma_1 c) \frac{\partial n}{\partial x} + \gamma_2 n \frac{\partial c}{\partial x} \right) + f(n)g(n) \quad (8.21a)$$

$$\frac{\partial c}{\partial t} = f(n)h(n, c), \quad (8.21b)$$

where $f(1) = 0$, $f(0) \neq 0$, $g(0) = 0$, $g(1) \neq 0$, $h(0, 0) = 0$ and $h(1, c^*) \neq 0$, $c^* > 0$.

8.5.1 Travelling wave analysis

We look for travelling wave solutions of (8.21) with $u(x, t) = U(z)$, $z = x - at$, and obtain

$$(D_1 + \gamma_1 C)N'' + \left[a - \frac{(\gamma_1 + \gamma_2)}{a}fh - \frac{\gamma_2 N}{a} \left(\frac{df}{dN}h + f \frac{\partial h}{\partial N} \right) \right] N' \quad (8.22a)$$

$$+ \frac{\gamma_2 N}{a^2} f^2 h \frac{\partial h}{\partial c} + fg = 0$$

$$aC' = -fh, \quad (8.22b)$$

where ' denotes d/dz . We let $M = N'$, and since $C(Z)$ is monotonic, we can remove the resulting singularity by defining a new variable ζ such that

$$(D_1 + \gamma_1 C) \frac{d}{dz} = \frac{d}{d\zeta}.$$

The critical points in the (N, M, C) phase space are $(0, 0, 0)$ and $(1, 0, C^*)$, where C^* can take any value. Standard linear analysis about $(0, 0, 0)$ yields the eigenvalues

$$\lambda = -\frac{D_1}{a} f \frac{\partial h}{\partial C}, \quad 2\lambda = -a \pm \sqrt{a^2 - 4D_1 f \frac{dg}{dN}} \quad (8.23)$$

where the functions and derivatives are all evaluated at $N = C = 0$. Similarly the eigenvalues at $(1, 0, C^*)$ are determined to be

$$\lambda = 0, \quad 2\lambda = -\left(a - \frac{\gamma_2 h}{a} \frac{df}{dN}\right) \pm \sqrt{\left(a - \frac{\gamma_2 h}{a} \frac{df}{dN}\right)^2 - 4(D_1 + \gamma_1 C^*) g \frac{df}{dN}}. \quad (8.24)$$

As discussed previously, in order to obtain a continuum of possible steady states, we require either at least two positive eigenvalues at $(1, 0, C^*)$ and thus two trajectories leaving $(1, 0, C^*)$ into the space $N, M, C > 0$. It is clear from (8.24) that there is at most one positive eigenvalue at $(1, 0, C^*)$ and thus only one trajectory leaving the line of positive steady states.

Eigenvalues at $(0, 0, 0)$

We use the eigenvalues at the trivial steady state to determine the wave speed. We note from (8.23) that, provided $f(0) \frac{dg}{dN}\big|_0 > 0$, there is either a stable spiral or node at $(0, 0, 0)$, depending on the sign of $a^2 - 4D_1 f(0) \frac{dg}{dN}\big|_0$. For the existence of a wave, we require positive kinetics for all $N \in (0, 1)$ and hence the simplest functions f and g are

$$f(N) = \beta_0 N(1 - N) \quad \& \quad g(N) = \alpha_1 N.$$

The minimum wave speed is thus determined to be $2\sqrt{D_1 f(0) \frac{dg}{dN}\big|_0}$.

Eigenvalues at $(1, 0, C^*)$

The expression (8.24) implies that there is always one zero eigenvalue and at most one positive eigenvalue, depending on the magnitude of the discriminant. We now consider the conditions for a positive eigenvalue at the steady state $(1, 0, C^*)$, and thus the existence of a travelling wave. Using (8.24), the necessary condition is

$$(D_1 + \gamma_1 C^*)g(1)\frac{df(1)}{dN} < 0,$$

from which we derive four possible cases

$$g(1) > 0, \quad \frac{df(1)}{dN} > 0, \quad \& \quad (D_1 + \gamma_1 C^*) < 0 \quad (8.25a)$$

$$g(1) > 0, \quad \frac{df(1)}{dN} < 0, \quad \& \quad (D_1 + \gamma_1 C^*) > 0 \quad (8.25b)$$

$$g(1) < 0, \quad \frac{df(1)}{dN} < 0, \quad \& \quad (D_1 + \gamma_1 C^*) < 0 \quad (8.25c)$$

$$g(1) < 0, \quad \frac{df(1)}{dN} > 0, \quad \& \quad (D_1 + \gamma_1 C^*) > 0. \quad (8.25d)$$

Again, we require positive kinetics for all $N \in (0, 1)$ and hence the necessary condition for a positive eigenvalue, with travelling waves for both N and C , is

$$D_1 + \gamma_1 C^* > 0. \quad (8.26)$$

Thus, if $D_1 + \gamma_1 C^* < 0$, there is no positive eigenvalue and hence no wave.

We now consider the system (8.21), letting $\gamma_2 = 0$ since it does not affect (8.26), and derive the conditions for diffusion driven instability. We linearize about the steady state by defining

$$\underline{w} = \begin{pmatrix} n - n_0 \\ c - c_0 \end{pmatrix},$$

where n_0 and c_0 are the steady states, and look for solutions of the form

$$\underline{w}(\underline{r}, t) = \sum_k p_k e^{\lambda_k t} \underline{W}_k(\underline{r}) \quad (8.27)$$

where p_k are Fourier constants, $\underline{W}_k(\underline{r})$ is the eigenfunction corresponding to the wave number k , with zero flux boundary conditions, and λ is the eigenvalue which

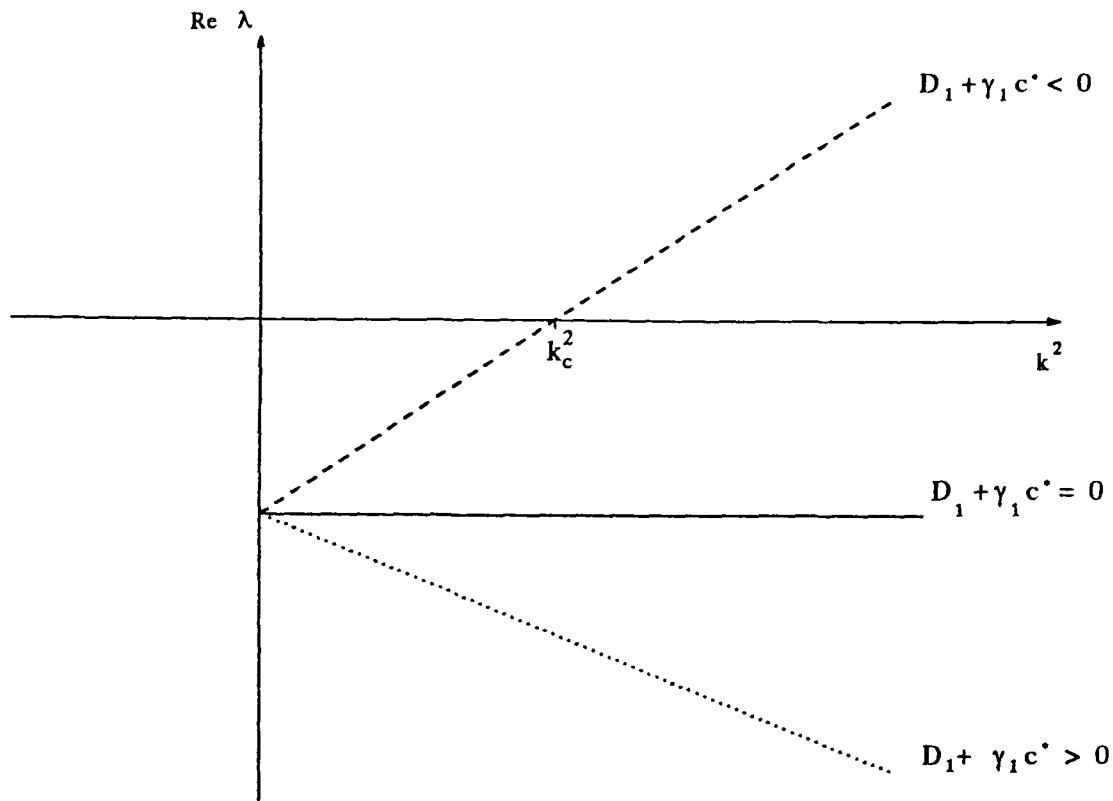


Figure 8.14: Dispersion relation for the eigenvalue, λ , as a function of wave number, k .

determines temporal growth. By considering the positive steady state, $(1, c^*)$, we derive a dispersion relation for λ , which is a linear function of k^2 , namely

$$\lambda = -1 - (D_1 + \gamma_1 c^*)k^2.$$

By plotting the real part of λ against k^2 (Figure 8.14), it is clear that for the positive steady state to be driven unstable by the presence of diffusion, a necessary condition is $D_1 + \gamma_1 c^* < 0$. However, this violates the condition for the existence of a positive eigenvalue and thus the existence of a wave.

8.5.2 Numerical Simulations

To verify this last result numerically, we define functional forms for $f(n)$, $g(n)$, and $h(n, c)$ and solve the system for different values of γ_1 . The model equations are taken to be

$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial x} \left((D_1 + \gamma_1 c) \frac{\partial n}{\partial x} \right) + n(1 - n) \quad (8.28a)$$

$$\frac{\partial c}{\partial t} = (1 - n)(\alpha n - c), \quad (8.28b)$$

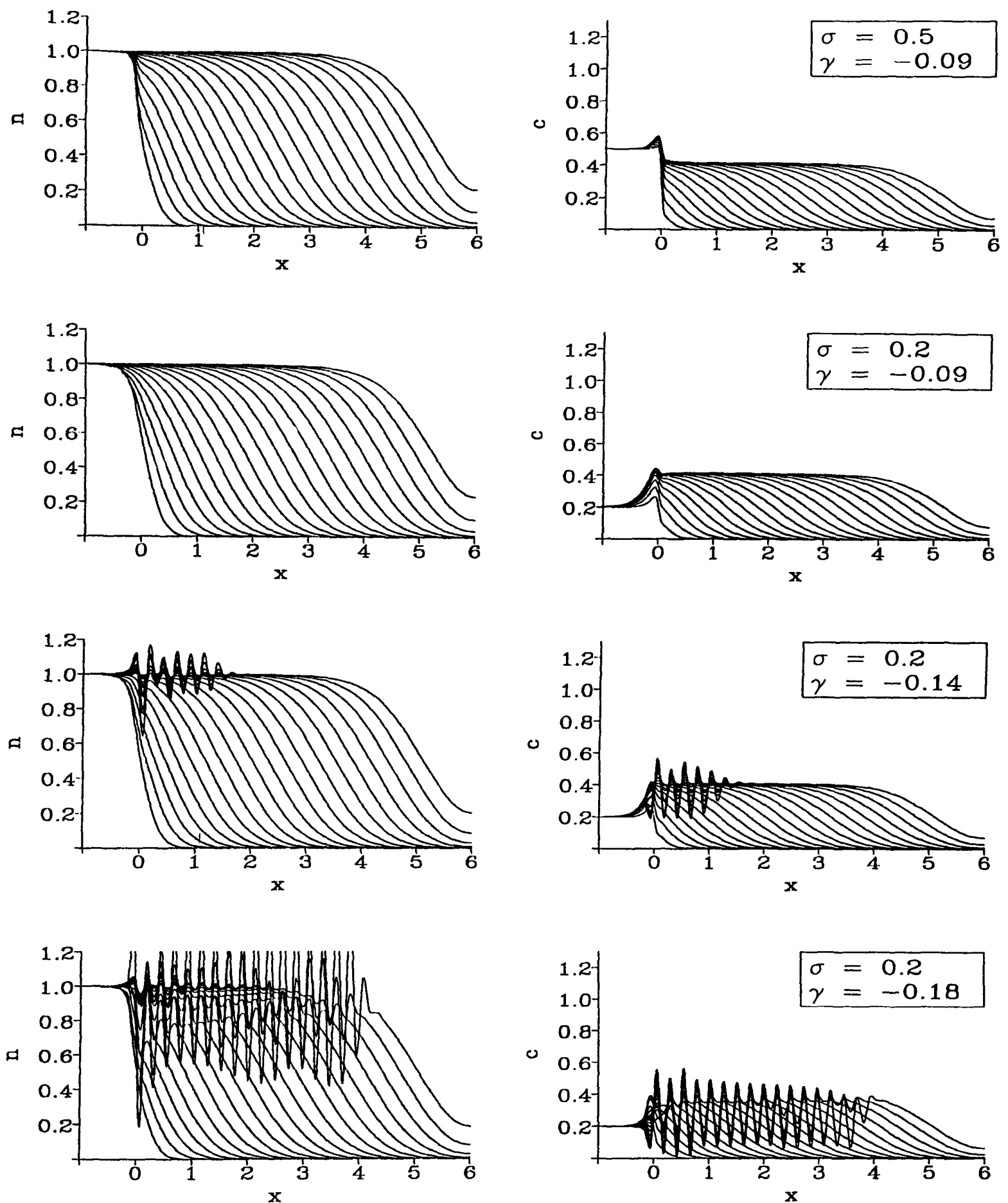


Figure 8.15: Numerical solutions of (8.28) for a range of initial conditions, σ , and density dependent diffusion coefficients, γ_1 . The level c^* is shown to be independent of σ and decreasing the values of γ_1 results in instability.

and we fix $D_1 = 0.05$ and $\alpha = 0.8$. We impose step function initial conditions

$$\begin{aligned} n(x, 0) &= 1, \quad c(x, 0) = \sigma \quad \text{if } x < 0 \\ n(x, 0) &= 0, \quad c(x, 0) = 0 \quad \text{if } x > 0 \end{aligned}$$

and aim to force the value of c^* is a new level by changing σ . Figure 8.15 shows solutions for n and c for a range of different values of σ and γ_1 . We note that the steady state level c^* does not depend on σ and, furthermore, by decreasing γ_1 below the critical value, $-D_1/c^*$, we observe instabilities in the travelling waves.

8.6 Summary

In this chapter we have developed a caricature model which mimics the behaviour of the full system. By considering a temporal model, we have derived explicit solutions for the steady state values, in terms of the initial conditions. Furthermore, asymptotic analysis for the reaction diffusion equations reveals that, to first order, the steady state is independent of wave speed and diffusion coefficient. Numerical simulations of the caricature model again evolve to travelling wave fronts, with a continuum of steady states but only one always being picked out. By introducing exponentially decaying initial conditions, we have altered the wave speed but again the change in collagen levels was less than 2%. Extensions to this model to include different diffusion terms and the addition of noise to try and perturb the system, result in the same steady state solution. To investigate this phenomenon, we have considered a caricature model with general kinetics, which has a zero steady state $(0, 0)$ and a continuum of steady states $(1, c^*)$, where $c^* > 0$. By focussing on the eigenvalues of the resulting travelling wave system, we have shown that there can only be one positive eigenvalue and thus only one trajectory leaving the positive steady state. Hence, we conjecture that in any reaction diffusion system of the form (8.1) there is only one travelling wave trajectory from $(1, 0, c^*)$ to $(0, 0, 0)$ and therefore one particular steady state is always picked out in a travelling wave of a given speed, independent of initial conditions.

Chapter 9

Discussion on Dermal Wound Healing

Scar tissue formation following trauma or surgery is a major clinical problem, often resulting in defective growth or functional impairment. Adult wound healing entails a complex series of events, involving many cell types and products that ultimately end in scar formation. In contrast, foetal wounds have been shown to heal more rapidly, with complete regeneration of the tissue and no scar. The ultimate aim of foetal wound healing studies is thus to manipulate the adult wound so that they heal in a scarless, foetal-like, fashion. Experimentalists have defined scar tissue in terms of the density, diameter and orientation of the collagen fibres within the wound domain. In adult wounds, thinner, more densely packed collagen fibres are observed, with the fibres being orientated along the line of tension, rather than the basket-weave orientation of normal tissue. The diameter of the collagen fibrils is related to the ratio of types I to III, with higher levels of type III resulting in thinner fibres. Here, we have presented a model which aims to explain the difference between adult and foetal repair, by focussing on the final levels and the ratio of collagen I to III. Some parameters were estimated from the limited amount of quantitative data available, with the rest being fixed by comparing model solutions with experimental results.

Specifically, we have concentrated on the role of transforming growth factor β (TGF β), and investigated the effect of addition of isoform 1 or 3. We first considered a temporal model, in which there were a few fibroblasts and growth factors scattered uniformly throughout the wound, corresponding to the assumption that the main source of cells and TGF β is from the subcutaneous tissue. An important feature of the model is the presence of a continuum of possible collagen steady states. Numerical simulations of the resulting model for the foetal case showed a 3 : 1 ratio of collagen I to III, indicating normal fibril diameters. By changing the initial conditions, we modelled the addition of TGF β 1 and 3, with TGF β 1 decreasing the ratio, resulting in thinner fibres and TGF β 3 causing thicker fibres. We changed a number of the parameters to model adult wound healing, and again numerically solved the temporal model. The healed levels of collagen I and III corresponded to thinner fibres, as observed experimentally. Furthermore, addition of TGF β 3 reduced the amount of collagen III and hence resulted in thicker fibres and less scarring.

One of the major biological controversies in dermal wound healing is the source of fibroblasts and growth factors. To model the influx of these substances from the neighbouring unwounded tissue, we included diffusion terms, with constant coefficients. Numerical solutions of the foetal model evolved to fronts of fibroblasts, TGF β and collagen moving into the wound, with constant shape and a wave speed which indicates that a 0.1mm foetal wound heals in 5 days, in good agreement with experimental data. The ratio of collagen I to III was maintained at 3 : 1. The important question of the effect of addition of TGF β was addressed by appropriately changing the initial conditions. Numerical simulations indicated a transient change in the collagen levels, before settling down to the levels attained in the untreated case. Extensive numerical simulations indicated that, although there exists a continuum of possible steady states, the collagen levels are insensitive to the amount of TGF β added and this holds for a large range of parameter values.

The system of equations was too complex to analyse in detail, so to gain more mathematical insight into the result that, regardless of the amount of TGF β added,

only one specific steady state is selected from a possible continuum of steady states, we developed a caricature model. Numerical simulations again evolved to travelling wave fronts, with a particular steady state selected behind the wave, even though there is a continuum of possible steady states. To investigate this phenomenon, we calculated the number of positive eigenvalues at the non-trivial steady state. For a caricature model with general reaction and diffusion terms, we showed that it is not possible to develop a model which has two positive eigenvalues at the non-trivial steady state, and thus we conjectured that there is only one trajectory connecting the wounded and healed steady states. Hence one particular steady state is always picked out.

Some important conclusions from this model are:

1. *The main source of fibroblasts is the subcutaneous tissue.*

There is biological controversy over the source of fibroblasts and growth factors in wound healing. The reaction diffusion model suggests that migration into the wound from the surrounding dermis cannot account for the experimentally observed change in collagen levels, on addition of $TGF\beta$. We conjecture that the main source of cells and growth factor is the underlying subcutaneous tissue, corresponding to the temporal model.

2. *Addition of $TGF\beta 3$ decreases scarring.*

Numerical solutions of the model equations indicate that addition of $TGF\beta 1$ results in higher levels of collagen III and thus thinner fibres and more scarring. In contrast, addition of $TGF\beta 3$ increases the ratio of collagen I to III, thus indicating thicker fibres. Furthermore, the maximum increase in fibril diameters is obtained for concentrations of $TGF\beta 3$ of approximately $1 \mu\text{g ml}^{-1}$ for adult healing.

3. *Modulation of $TGF\beta 1$ in adult wounds decreases scarring.*

By removing the term which represents the modulation of $TGF\beta 1$ by $TGF\beta 3$,

the ratio of collagen I to III decreases in the adult case but there is no significant change in foetal healing. This is due to the intrinsically higher levels of growth factors in the adult wound environment. We hypothesize that a viable alternative to the addition of growth factors to increase fibril diameters is to increase the modulation of $TGF\beta 1$.

4. *Early application of $TGF\beta$ is essential.*

Little experimental research has been done of the time dependence of addition of $TGF\beta$. The model predicts that early addition of growth factor is essential for successful treatment. In the adult case, addition after day 15 of healing has little effect of the final fibril diameters.

The model also suggests further experimental work to address the following questions

1. *“What are the key parameters?”.*

Parameter sensitivity analysis reveals that the model is sensitive to changes in certain key parameters. These are the natural decay rates of latent $TGF\beta 1$ and 3, the rates of activation of procollagen I and III to collagen I and III, respectively, and the modulation of $TGF\beta 1$ by $TGF\beta 3$. These are valuable areas of experimental work which would help understand the difference between foetal and adult healing.

2. *“Should $TGF\beta 3$ be applied to dermal wounds in young adults?”.*

Experimental research shows that there is a gradual change from scar free to scar type wound healing. Our model does not take into account the transition phase but does indicate problems with the addition of $TGF\beta 3$ in young adults, with a fine line between either really good or abnormal healing, such as hypertrophic scarring. This would be another valuable area of research. This transition phase indicates the importance of timing of human foetal surgery if scarring is to be minimized and optimal results achieved.

In this part of the thesis, we have concentrated on $\text{TGF}\beta 1$ and 3 as the important regulators of collagen fibril levels. We now briefly discuss other factors which play some role in the healing process. Platelet derived growth factor (PDGF) is a glycoprotein which is released by platelets in abundance at the time of wounding. There are no PDGF receptors on epithelial cells or endothelial cells but mesenchymal cells have a high affinity membrane receptor and PDGF has been shown to initiate angiogenesis. High concentrations of PDGF can activate granulocytes whereas fibroblasts are attracted to low concentrations of PDGF, which also induces proliferation (Pierce *et al.*, 1991). Studies suggest that wound tissues are continuously bathed in and respond to PDGF throughout the healing process. However, research has shown that the effect of PDGF on collagen deposition is small compared to the effect of $\text{TGF}\beta$ (Pierce *et al.*, 1991). Thus, the inclusion of PDGF in the model would have little effect on the final levels and ratio of the collagens and we choose to neglect it in our model. Fibroblast growth factor (FGF) has both a basic and an acidic form and can act as an angiogenic factor *in vivo* and *in vitro*, directing endothelial cell migration, proliferation and plasminogen activator synthesis. Basic FGF triggers normal granulation tissue formation, with the secretion of fibronectin and collagen, but has been shown to most significantly enhance neovascularization and re-epithelialization (reviewed in Martin *et al.*, 1992). Again, we can legitimately neglect the effect of FGF compared with $\text{TGF}\beta$. Fibronectins are complex glycoprotein matrix molecules, produced by fibroblasts which are involved in wound contraction, cell migration, collagen matrix deposition and re-epithelialization (Whitby & Ferguson, 1992). It acts as a “scaffold” for collagen deposition; granulation tissue fibroblasts are coated with a layer of fibronectin matrix and myofibroblasts are covered with fibronectin which attaches with stress fibres in the wound (Clark, 1989; McDonald, 1988). Inclusion of fibronectin is one possible extension to the model, but since it primarily effects the arrangement of fibres and not the density or diameter, our model solutions will not be affected by the absence of this term. A current area of research is the role played by water in the process of collagenolysis; recent

reports have suggested that in the scarcity of water, the rate of collagenase activity is slowed considerably, a fact that could affect scarring but which we have chosen to neglect in our model.

We have presented a mathematical model which captures the essential features of foetal and adult dermal wound healing. We have chosen to measure the extent of scarring in terms of the ratio and levels of collagen I and III. Another essential element of scar tissue, which we have neglected, is the orientation of the fibres and future extensions to the model should take into account this process. Further possible work includes the modelling of $\text{TGF}\beta 1$ and 2 as separate variables, and introducing a time dependency to explain the transition phase between scarless healing and scar tissue formation.

References

Adzick, N.S., Longaker, M.T.: Characteristics of fetal tissue repair. In: Adzick, N.S., Longaker, M.T. (eds.) Fetal wound healing. Elsevier, New York, pp. 53-70 (1992)

Appling, W.D., O'Brien, W.R., Johnston, D.A., Duvie, M.: Synergistic enhancement of type I and III collagen production in cultured fibroblasts by transforming growth factor - β and ascorbate. *FEBS Letters* **250**: 541-544 (1989)

Asmussen, P.D., Söllner, B.: Wound care: Principles of wound healing. *Beierdorf Medical Bibliothek* (1993)

Assoian, R.K., Komoriya, A., Meyers, C.A., Miller, D.M., Sporn, M.B.: Transforming growth factor- β in human platelets. *J. Biol. Chem.* **258**: 7155-7160 (1983)

Bard, J.B.L., Hay, E.D.: The behaviour of fibroblasts from the developing avian cornea. Morphology and movement *in situ* and *in vitro*. *J. Cell Biol.* **67**: 400-418 (1975)

Barrandon, Y., Green, H.: Cell migration is essential for sustained growth of keratinocyte colonies: The roles of transforming growth factor- α and epidermal growth factor. *Cell* **50**: 1131-1137 (1987)

Barrow, G.: Physical chemistry for the life sciences. *McGraw-Hill Inc.*, New York (1981)

Berg, O.G., Von Hippel, P.H.: Diffusion controlled macromolecular interactions. *Ann. Rev. Biophys. Chem.* **14**: 131-160 (1985)

Boyarsky, A.: A stochastic model for wound healing. *Math. Modelling* **7**: 371-375 (1986)

Bramson, M.: Convergence of solutions of the Kolmogorov equation to travelling waves. *Mem. Am. Math. Soc.* **44**: 285 (1983)

Brazell, R.K., Stern, M.E., Aquavella, J.V., Beuerman, R.W., Baird, L.: Human recombinant epidermal growth factor in experimental corneal wound healing. *Invest. Ophthalmol. Vis. Sci.* **32**: 336-340 (1991)

Buck, R.C.: Cell migration in repair of mouse corneal epithelium. *Invest. Ophthalmol. Vis. Sci.* **18**: 767-784 (1979)

Canosa, J.: On a nonlinear diffusion equations describing population growth. *IBM J. Res. & Dev.* **17**, 307-313 (1973)

Carpenter, G., Cohen, S.: Human epidermal growth factor and the proliferation of human fibroblasts. *J. Cell Physiol.* **88**: 227-238 (1976)

Chan, K.Y., Patton, D. L., Cosgrove, Y.T.: Time-lapse videomicroscopic study of *in vitro* wound closure in rabbit corneal cells. *Invest. Ophthalmol. Vis. Sci.* **30**(12): 2488-2498 (1988)

Chan, K.Y., Lindquist, T.D., Edenfield, M.J., Nicolson, M.A., Banks, A.R.: Pharmacokinetic study of recombinant human epidermal growth factor in the anterior eye. *Invest. Ophthalmol. Vis. Sci.* **32**: 3209-3215 (1991)

Chen, R.H., Ebner, R., Derynck, R.: Inactivation of the type II receptor reveals two receptor pathways for the diverse TGF β activities. *Science* **260**: 1335-1338 (1993)

Clark, R.A.F.: Overview and general considerations of wound repair. In: Clark, R.A.F., Henson, P.M. (eds.) *The molecular and cellular biology of wound repair*. Plenum press, New York, pp. 3-34 (1988)

Clark, R.A.F.: Wound repair. *Curr. Op. Cell Biol.* **1**: 1000-1008 (1989)

Clore, J.N., Cohen, I.K., Diegelmann, R.F.: Quantitation of collagen types I and III during wound healing in rat skin. *Proc. Soc. Exp. Biol. Med.* **161**: 337-340 (1979)

Coffey, R. J., Derynck, R., Wilcox, J. N., Bringman, T. S., Goustin, A.S., Moses, H.L., Pittelkow, M.R.: Production and auto-induction of transforming growth factor $-\alpha$ in human keratinocytes. *Nature* **328**: 817-820 (1987)

Cohen, I.K., McCoy, B.J.: Wound healing. In: Goldsmith, L.A. (ed.) *Biochemistry and physiology of the skin*. Oxford Univ. Press, Oxford (1983)

Crank, J.: *The Mathematics of Diffusion*. Oxford Univ. Press, Oxford (1956)

Crosson, C.E., Klyce, S.D., Beuerman, R.W.: Epithelial wound closure in rabbit cornea. *Invest. Ophthalmol. Vis. Sci.* **27**: 464-473 (1986)

Danjo, S., Friend, J., Thoft, R.A.: Conjunctival epithelium in healing of corneal epithelial wounds. *Invest. Ophthalmol. Vis. Sci.* **28**: 1445-1449 (1989)

Dunbar, S.R.: Travelling wave solutions of diffusive Lotka-Volterra equations. *J. Math. Biol.* **17**: 11-32 (1983)

Dunbar, S.R.: Travelling wave solutions of diffusive Lotka-Volterra equations: a heteroclinic connection in $\mathbb{R}^4$. *Trans. AMS* **268**: 557-594 (1984)

Dunn, G.A., Ireland, G.W.: New evidence that growth in 3T3 cell cultures is a diffusion-limited process. *Nature* **312**: 63-65 (1984)

Dvorak, H.F., Kaplan, A.P., Clark, R.A.F.: Potential functions of the clotting system in wound repair. In: Clark, R.A.F., Henson, P.M. (eds.) *The molecular and cellular biology of wound repair*. Plenum Press, New York, pp. 57-86 (1988)

Ferguson, M.W.J., Howarth, G.F.: Marsupial models of scarless fetal wound healing. In: Adzick, N.S., Longaker, M.T. (eds.) *Fetal wound healing*. Elsevier, New York, pp. 95-124 (1992)

Fisher, R.A.: The wave of advance of advantageous genes. *Ann. Eugenics* **7**: 353-369 (1937)

Flint, M.H., Craig, A.S., Reilly, H.C., Gillard, G.C., Parry, D.A.D.: Collagen fibril diameters and glycosaminoglycan content of skins - indices of tissue maturity and function. *Conn. Tiss. Res.* **13**: 69-81 (1984)

Fredj-Reygrobellet, D., Plouet, J., Delayre, T., Baudouin, C., Bourret, F., Lapalus, P.: Effects of aFGF and bFGF on wound healing in rabbit corneas. *Curr. Eye Res.* **6**: 1205-1209 (1986)

Furthmayr, H.: Basement membranes. In: Clark, R.A.F., Henson, P.M. (eds.) *The molecular and cellular biology of wound repair*. Plenum press, New York, pp. 525-558 (1988)

Gibbs, R.G.: Travelling waves in the Belousov-Zhabotinski reaction. *SIAM J. Appl. Math.* **38**: 422-444 (1980)

Goss, R.J.: Regeneration versus repair. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) Wound Healing: Biochemical & Clinical Aspects. W. B. Saunders Co., Philadelphia, pp. 20-39 (1992)

Grindrod, P.: Patterns and waves. Oxford Univ. Press, Oxford (1991)

Green, M.R., Couchman, J.R.: Distribution of EGF receptors in rat tissues during embryonic skin development, hair formation and adult hair growth cycle. *J. Invest. Dermatol.* **83**: 118-123 (1984)

Hanna, C., Bicknell, D.S., O'Brien, J.E.: Cell turnover in the adult human eye. *Arch. Ophthalmol.* **65**: 695-698 (1961)

Harris, A.L., Nicholson, S.: Epidermal growth factor receptors in human breast cancer. In: Lippman, M.E, Dickson, R.B. (eds.) Breast Cancer: Cellular and molecular biology. Kluwer Acad. Publ., Boston, pp. 93-118 (1988)

Jennings, R.W., Hunt, T.K.: Overview of postnatal wound healing. In: Adzick, N.S., Longaker, M.T. (eds.) Fetal wound healing. Elsevier, New York, pp. 25-52 (1992)

Jeffrey, J.J.: Collagen degradation. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) Wound healing: Biochemical & clinical aspects. W. B. Saunders Co., Philadelphia, pp. 177-194 (1992)

Kevorkian, J., Cole, J.D.: Perturbation methods in applied mathematics. Springer Verlag, Berlin (1981)

Kitazawa, T., Kinoshita, S., Fujita, K., Araki, K., Watanabe, H., Ohashi, Y., Manabe, R.: The mechanism of accelerated corneal epithelial healing by human epidermal growth factor. *Invest. Ophthalmol. Vis. Sci.* **31**: 1773-1778 (1990)

Klyce, S.D., Beuerman, R.W.: Structure and function of the cornea. In: Kaufman, H.E., Barron, B.A., McDonald, M.B., Waltman, S.R. (eds.) *The Cornea*. Churchill Livingstone (1988)

Kolmogorov, A., Petrovsky, I., Piscounoff, N.: Etude de l'équation de la diffusion avec croissance de la quantité de matière et son application à un problème biologique. *Moscow Univ. Bull. Math.* **1**: 1-25 (1937)

Krummel, T.M., Michna, B.A., Thomas, B.L., Sporn, M.B., Nelson, J.M., Salzberg, A.M., Cohen, I.K., Diegelmann, R.F.: Transforming growth factor beta induces fibrosis in a fetal wound model. *J. Pediatric Surgery* **23**: 647-652 (1988)

Kuwabara, T., Perkins, D.G., Cogan, D.G.: Sliding of the epithelium in experimental corneal wounds. *Invest. Ophthalmol. Vis. Sci.* **15**: 4-14 (1976)

Kwok, L.S.: Kinematics of epithelial wound closure in the rabbit cornea. *Docu. Ophthalmol.* **77**: 1-38 (1991)

Lapierre, Ch.M., Nusgens, B.V., Pierard, G.E.: The architectural organisation and function of the macromolecules in the dermis. In: Marks, R.M., Barton, S.P., Edwards, C. (eds.) *The physical nature of the skin*. MTP Press Ltd, Lancaster, pp. 163-178 (1988)

Longaker, M.T., Whitby, D.J., Jennings, R.W., Duncan, B.W., Ferguson, M.W.J., Harrison, M.R., Adzick, N.S.: Fetal diaphragmatic wounds heal with scar formation. *J. Surg. Res.* **50**: 375-385 (1991)

Maini, P.K., Myerscogh, M.R., Winters, K.H., Murray, J.D.: Bifurcating spatially heterogeneous solutions in a chemotaxis model for biological pattern generation. *Bull. Math. Biol.* **53**: 701-719 (1991)

Martin, P., Hopkinson-Woolley, J., McCluskey, J.: Growth factors and cutaneous wound repair. *Prog. Growth Factor Res* 4: 25-44 (1992)

Mast, B.A.: The Skin. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) Wound healing: Biochemical & clinical aspects. W. B. Saunders Co., Philadelphia, pp. 344-355 (1992)

Mast, B.A., Nelson, J.M., Krummel, T.M.: Tissue repair in the mammalian fetus. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) Wound healing: Biochemical & clinical aspects. W. B. Saunders Co., Philadelphia, pp. 326-343 (1992)

McDonald, J.A.: Fibronectin: a primitive matrix. In: Clark, R.A.F., Henson, P.M. (eds.) The molecular and cellular biology of wound repair. *Plenum press*, New York, pp. 405-436 (1988)

Menasche, M., Robert, L., Payrau, P., Hamada, R., Pouliquen, Y.: Comparative biochemical and morphometric studies on corneal wound healing. *Pathol. Biol. Paris*. 36: 781-789 (1988)

Merkel, J.R., DiPaolo, B.R., Hallcock, G.C., Rice, D.C.: Type I and type III collagen content of healing wounds in fetal and adult rats. *Proc. Soc. Exp. Biol. Med.* 187: 493-497 (1988)

Miller, E.J., Gay, S.: Collagen structure and function. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) Wound healing: Biochemical & clinical aspects. W. B. Saunders Co., Philadelphia, pp. 130-151 (1992)

Morgan, C.J., Pledger, W.J.: Fibroblast proliferation. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) Wound healing: Biochemical & clinical aspects. W. B. Saunders Co., Philadelphia, pp. 63-76 (1992)

Murray, J.D.: On traveling wave solutions in a model for the Belousov-Zhabotinskii reaction. *J. Theor. Biol.* **52**: 329-353 (1976)

Murray, J.D.: Mathematical Biology. Springer Verlag, Berlin (1991)

Nishida, T., Nakamura, M., Mishima, H., Otori, T.: Differential modes of action of fibronectin and epidermal growth factor on rabbit corneal epithelial migration. *J. Cell. Physiol.* **145**: 549-554 (1990)

Ockendon, J.R., Tayler, A.B.: Applied Partial Differential Equations. University of Oxford Lecture Notes (1982)

Odland, G.F.: Structure of the skin. In: Goldsmith, L.A. (ed.) Biochemistry and physiology of the skin. Oxford Univ. Press, Oxford, pp. 3-63 (1983)

Ohashi, Y., Morokura, M., Kinoshita, Y., Mano, T., Watanabe, H., Kinoshita, S., Manabe, R., Oshiden, K., Yanaihara, C.: Presence of epidermal growth factor in human tears. *Invest. Ophthalmol. Vis. Sci.* **30**: 1879-1882 (1989)

Ortonne, J.P., Loning, T., Scmitt, D., Thivolet, J.: Immunomorphological and ultrastructural aspects of keratinocyte migration in epidermal wound healing. *Virchows Arch. A* **392**: 217-230 (1981)

Peacock, E.E.: Wound Repair. W. B. Saunders Co., Philadelphia (1984)

Pierce, G.F., Brown, D., Mustoe, T.A.: Quantitative analysis of the inflammatory cell influx, procollagen type I synthesis, and collagen cross-linking in incisional wounds: influence of PDGF-BB and TGF- β 1 therapy. *J. Lab. Clin. Med.* **117**: 373-282 (1991)

Roberts, A.B., Sporn, M.B.: The transforming growth factor - β s. In: Sporn, M.B., Roberts, A.B. (ed.) Peptide growth factors and their receptors. Springer Verlag, Berlin, pp.419-472 (1990)

Rothe, F.: Convergence to travelling fronts in semilinear parabolic equations. *Proc. R. Soc. Edin.* **80A**: 213-234 (1978)

Rudolph, R., Vande Berg, J., Ehrlich, H.P.: Wound contracture and scar contracture. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) Wound healing: Biochemical & clinical aspects. W. B. Saunders Co., Philadelphia, pp. 96-114 (1992)

Schultz, G.S., White, M., Mitchell, R., Brown, G., Lynch, J., Twardzik, D.R., Todaro, G.J.: Epithelial wound healing enhanced by transforming growth factor α and vaccinia growth factor. *Science* **235**: 350-352 (1987)

Shah, M, Foreman, D.M., Ferguson, M.W.J.: Control of scarring in adult wounds by neutralising antibody to transforming growth factor β . *Lancet* **339**: 213-214 (1992)

Sherratt, J.A., Murray, J.D.: Models of epidermal wound healing. *Proc. R. Soc. Lond. B* **241**: 29-36 (1990)

Sherratt, J.A., Murray, J.D.: Mathematical analysis of a basic model for epidermal wound healing. *J. Math. Biol.* **29**: 389-404 (1991)

Sherratt, J.A., Murray, J.D.: Epidermal wound healing - A theoretical approach. *Comments Theor. Biol.* **2** (5): 315-333 (1992)

Sherratt, J.A., Murray, J.D.: Epidermal wound healing: The clinical implications of a simple mathematical model. *Cell Transplantation* **1**: 365-371 (1992)

- Sherratt, J.A., Sage, E.H., Murray, J.D.: Chemical control of eukaryotic cell movement: a new model. *J. Theor. Biol.* **162**: 23-40 (1993)
- Sherratt, J.A.: Cellular growth control and travelling waves of cancer. *SIAM J. Appl. Math.* **53**: 1713-1730 (1993)
- Sherratt, J.A.: Chemotaxis and chemokinesis in eukaryotic cells: the Keller-Segel equations as an approximation to a detailed model. *Bull. Math. Biol.* **56**: 129-146 (1994)
- Siebert, J.W., Burd, D.A.R., McCarthy, J.G., Weinzwieg, J., Ehrlich, H.P.: Fetal wound healing: a biochemical study of scarless healing. *Plast. Reconstr. Surg.* 495-504 (1990)
- Sinclair, R.D., Ryan, T.J.: Proteolytic enzymes in wound healing: the role of enzymatic debridement. *Australas. J. Dermatol.* **35**: 35-41 (1994)
- Soderquist, A.M., Carpenter, G.: Biosynthesis and metabolic degradation of receptors for epidermal growth factors. *J. Membrane Biol.* **90**: 97-105 (1986)
- Stenn, K.S., Malthorta, R.: Epithelialisation. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) Wound healing: Biochemical & clinical aspects. W. B. Saunders Co., Philadelphia, pp. 115-129 (1992)
- Stern, M.G., Longaker, M.T., Stern, R.: Hyaluronic acid and its Modulation in fetal and adult wounds. In: Adzick, N.S., Longaker, M.T. (eds.) Fetal wound healing. Elsevier, New York, pp. 189-198 (1992)
- Streuli, C.H., Schmidhauser, C., Kobrin, M., Bissell, M.J., Derynck, R.: Extracellular matrix regulates expression of the TGF- β 1 gene. *J. Cell Biol.* **120**: 253-260 (1993)

- Stricklin, G.P., Bauer, E.A., Jeffrey, J.J: Human skin collagenase: Chemical properties of precursor and active forms . *Biochemistry* **17**: 2331-2337 (1978)
- Sunada, H., Peacock, J., Mendelsohn, J.: Ligand induced intermediate of EGF receptors by A431 cells decreases at high cell density in culture. *Growth Factors* **5**: 45-55 (1991)
- Thompson, H.W., Malter, J.S., Steinemann, T.L., Beuerman, R.W.: Flow cytometry measurements of the DNA content of corneal epithelial cells during wound healing. *Invest. Ophthalmol. Vis. Sci.* **32**: 433-436 (1990)
- Toole, B.P.: Proteoglycans and hyaluronan in morphogenesis and differentiation. In: Hay, E.D. (ed.) *Cell biology of extracellular matrix*. Plenum press, New York, pp. 305-342 (1992)
- Tsutsumi, O., Tsutsumi, A., Oka, T.: Epidermal growth factor-like corneal wound healing substance in mouse tears. *J. Clin. Invest.* **81**: 1067-1071 (1988)
- Van Setten, G.B.: Epidermal growth factor in human tear fluid: increased release but decreased concentrations during reflex tearing. *Curr. Eye Res.* **9**: 79-83 (1990)
- Van De Water, L., French-Constant, C., Brown, L.: Fibronectin expression during cutaneous wound healing. In: Adzick, N.S., Longaker, M.T. (eds.) *Fetal wound healing*. Elsevier, New York, pp. 281-302 (1992)
- Wahl, L.M., Wahl, S.M.: Inflammation. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) *Wound healing: Biochemical & clinical aspects*. W. B. Saunders Co., Philadelphia, pp. 40-62 (1992)

Wakefield, L.M., Smith, D.M., Masui, T., Harris, C.C., Sporn, M.B.: Distribution and modulation of the cellular receptor for growth factor - β . *J. Cell Biology* **105**: 965-975 (1987)

Wakefield, L.M., Smith, D.M., Flanders, K.C., Sporn, M.B.: Latent transforming growth factor β from human platelets. *J. Biol. Chem.* **263**: 7646-7654 (1988)

Wakefield, L.M., Winokur, T.S., Hollands, R.S., Christopherson, K., Levinson, A.D.: Recombinant latent transforming growth factor beta 1 has a longer plasma half-life in rats than an active transforming growth factor beta 1, and a different tissue distribution. *J. Clin. Invest.* **86**: 1976-1984 (1990)

Wenstrup, R.J., Murad, S., Pinnell, S.R.: Collagen. In: Goldsmith, L.A. (ed.) *Biochemistry and physiology of the skin*. Oxford Univ. Press, Oxford, pp. 481-508 (1983)

Whalen, G.F., Zetter, B.R.: Angiogenesis. In: Cohen, I.K., Diegelmann, R.F., Lindblad, W.J. (eds.) *Wound healing: Biochemical & clinical aspects*. W. B. Saunders Co., Philadelphia, pp. 77-95 (1992)

Whitby, D.J., Ferguson, M.W.J.: The extracellular matrix of lip wounds in fetal, neonate and adult mice. *Development* **112**: 651-668 (1991)

Whitby, D.J., Ferguson, M.W.J.: Immunohistochemical localization of growth factors in fetal wound healing. *Dev. Biol.* **147**: 207-215 (1991)

Whitby, D.J., Longaker, M.T., Harrison, M.R., Adzick, N.S., Ferguson, M.W.J.: Rapid epithelialisation of fetal wounds is associated with the early deposition of tenascin. *J. Cell Sci.* **99**: 583-586 (1991)

Whitby, D.J., Ferguson, M.W.J.: Immunohistochemical studies of the extracellular matrix and soluble growth factors in fetal and adult wound healing. In: Adzick, N.S., Longaker, M.T. (eds.) Fetal wound healing. Elsevier, New York, pp. 161-176 (1992)

Wiegel, P.H., Fuller, G.M., LeBoeuf, R.D.: A model for the role of hyaluronic acid and fibrin in the early events during the inflammatory response and wound healing. *J. Theor. Biol.* **119**: 219-234 (1986)

Winter, G.D.: Epidermal regeneration studied in the domestic pig. In: Maibach, H.I., Rovee, D.T. (eds.): Epidermal Wound Healing. Year Book Med. Publ. Inc., Chicago, USA. pp. 71-112 (1972)

