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A model mechanism for the chemotactic response of endothelial cells to tumour angiogenesis factor

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In order to accomplish the transition from avascular to vascular growth, solid tumours secrete a diffusible substance known as tumour angiogenesis factor (TAF) into the surrounding tissue. Endothelial cells which form the lining of neighbouring blood vessels respond to this chemotactic stimulus in a well-ordered sequence of events consisting, at minimum, of a degradation of their basement membrane, migration, and proliferation. A model mechanism is presented which includes the diffusion of the TAF into the surrounding host tissue and the response of the endothelial cells to the chemotactic stimulus. The model accounts for the main observed events associated with the endothelial cells during the process of angiogenesis (i.e. cell migration and proliferation); the numerical results compare very well with experimental observations. The situation where the tumour (i.e. the source of TAF) is removed and the vessels recede is also considered.

Keywords: tumour angiogenesis factor; endothelial cells; chemotaxis.

1. Introduction

Unless furnished with an adequate blood supply and a means of disposing of waste products by a mechanism other than diffusion, a solid tumour cannot grow beyond a few millimetres in diameter and remains in an *avascular* state. Avascuar nodules can be cultivated in the laboratory (Folkman, 1976) or can be found *in vivo* (carcinomas *in situ* being a good example) and typically consist of a central necrotic core surrounded by a thin outer layer of live proliferating cells. Mathematical models describing this avascular growth can be found in, for example, Greenspan (1976), Chaplain (1990), and Adam & Maggelakis (1990), and references therein.

Transition from this dormant avascular state to the vascular state, wherein the tumour possesses the ability to invade surrounding tissue and metastasize to distant parts of the body, depends upon its ability to induce new blood vessels from the surrounding tissue to sprout towards and then gradually penetrate the tumour, thus providing it with an adequate blood supply and microcirculation. In order to accomplish this *neovascularization*, it is now a well-established fact that tumours secrete a diffusible chemical compound known as *tumour angiogenesis factor* (TAF) into the surrounding tissue and extracellular matrix. Much work has been carried

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out into the nature of TAF and its effect on endothelial cells since initial research began in the early 1970s with Folkman, culminating quite recently in the purification of several angiogenic factors, the determination of their amino acid sequences and the cloning of their genes (Strydom *et al.*, 1985; Folkman & Klagsbrun, 1987; Deshpande & Shetna, 1989). The extensive current literature on the subject is testimony to its importance in our understanding of the mechanisms by which solid tumours develop and grow (see e.g. the reviews of Folkman & Klagsbrun, 1987, and Paweletz & Knierim, 1989).

Several experimental techniques have been developed and employed for studying the various events involved during angiogenesis (see the references in Chaplain & Stuart, 1991) and it is well documented that there are three main events concerning the endothelial cells which go to make up the process of angiogenesis after the release of TAF by the tumour cells, namely:

(1) degradation of the basement membrane by enzymes secreted by the cells;

(2) migration of the endothelial cells;

(3) proliferation of the endothelial cells.

(A comprehensive description of the above events can be found in the extensive review of Paweletz & Knierim, 1989.)

It should be noted that the second and third of these stages—endothelial cell migration and endothelial cell proliferation—are not linked together. They are *distinct* events and *different* types of stimuli are necessary for each of them. Indeed, the first steps of angiogenesis can be performed without any cell division at all (Sholley *et al.*, 1984), and it is well known that mitotic figures can only be found once the sprouts have already started to grow. Thus cell division is a *sine qua non* event for the successful completion of angiogenesis. Endothelial cell migration *together with* endothelial cell proliferation are crucial to neovascularization. Angiogenic factors must therefore induce all of the above three events in a well-ordered sequence.

The main aim of this paper is to develop and extend the mathematical model of Chaplain & Stuart (1991), where the model for the process of angiogenesis was formulated in terms of a free boundary problem for the concentration of TAF. By introducing a sink term into the diffusion equation to represent the action of the proliferating endothelial cells at the capillary sprout tips, the boundary of the TAF receded and hence the position and subsequent movement of the sprout tips was indirectly followed. Given the relative simplicity of the model, the results obtained showed a good qualitative agreement with the experimental evidence available. However, there were a few shortcomings of the model, notably the lack of an equation modelling the endothelial cell density (this was accounted for indirectly in the sink term) and, as a result, the breakdown of the model when considering the removal of the tumor (i.e. the source of the TAF). In this paper we address both of the above problems by incorporating a population balance equation for the endothelial cells coupled with the diffusion equation for the TAF. In this way the progress of the endothelial cells can be monitored *directly* as they move from their source, e.g. the limbal vessels (cf. Gimbrone *et al.*, 1974; Muthukkaruppan *et al.*, 1982), and migrate

across the extracellular matrix to the angiogenic source, e.g. the tumour implant. This approach will also permit direct tracking of the cells when the tumour implant is removed. In the following section we describe the new model mechanism, and in Section 3 we present the results of the numerical simulation of the model and discuss these in the light of the experimental data. In section 4 various concluding remarks are made.

2. The mathematical model

In this section, we present a model mechanism for the diffusion of TAF into the surrounding tissue and its effect on the endothelial cells of the neighbouring blood vessels. We attempt to incorporate in it two of the three main events associated with angiogenesis described in the introduction, i.e. endothelial cell migration and proliferation. Therefore, throughout the paper, attention is principally focused on the endothelial cells, since they play the major role in the sequence of events described in the previous section and are always in the focus of action (cf. Paweletz & Knierim, 1989). The main function of endothelial cells is in the lining of the different types of vessels such as venules and veins, arterioles and arteries, small lymphatic vessels, and the thoracic duct. They form a single layer of flattened and extended cells and the intercellular contacts are very tight. Large intercellular spaces are not visible and any easy penetration of the established layer of cells is impossible. Special processes must take place for the intra- and extravasation of different cellular elements of the blood or the lymphatic fluids and tumour cells. Even intravascular tumour cells have to induce the formation of gaps in the single layer of endothelial cells in order to leave the respective vessels (Paweletz & Knierim, 1989).

As stated in the introduction, this paper develops and extends the model of Chaplain & Stuart (1991), where attention was focused on the concentration profile of TAF and the extent to which it had diffused into the external tissue after it was secreted by the tumour. It also develops the model of Balding & McElwain (1985), where the modelling of the formation and growth of the capillaries was undertaken based on the fungal growth model of Edelstein (1982).

We assume that the tumour implant has been placed sufficiently close to the vessels of, for example, the corneal limbus (cf. Gimbrone *et al.*, 1974; Muthukkaruppan *et al.*, 1982) so as to be within the critical threshold distance observed by Gimbrone *et al.*, (1974) (in these experiments it was found that no vascularization of the tumour occurred, or that the time for vascularization was substantially increased, when the tumour implant was placed at a distance of more than 2.5 mm from the limbal vessels). Once the endothelial cells have begun to migrate towards the tumour, at a certain point they begin to proliferate. Ausprunk & Folkman (1974) hypothesized that the reason for this was that the cells at the tips of the capillary sprouts were acting as sinks for the TAF. In order to account for this behaviour, Chaplain & Stuart (1991) included an extra sink term in their equation to model the uptake of TAF by the proliferating endothelial cells. The model equation used was

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - m - p(c)q(x/s), \qquad (x, t) \in (0, s(t)) \times (0, T), \qquad (2.1)$$

together with the boundary conditions

$$c = c_b \quad \text{on } x = 0, \qquad c = \frac{\partial c}{\partial x} = 0 \quad \text{on } s(t), \tag{2.2}$$

and the initial conditions

$$c(x, 0) = c_0(x), \qquad s(0) = s_0 = L. \tag{2.3}$$

The sink function in (2.1) is composed of two terms, $p(c)$ representing a normalized rate or removal of TAF and $q(x/s)$ representing the *spatial* dependence of this removal rate. The function q was taken to be a continuous approximation to the delta-function and was also introduced to serve as a measure of the proliferating endothelial cell density at the sprout tips. By assuming that the proliferating cells were confined to the capillary sprout tips, following the development of the free boundary in effect meant following (indirectly) the endothelial cells as they made their way across the extracellular matrix towards the tumour. However, this formulation breaks down when the source of TAF (i.e. the tumour) is removed.

The results of Chaplain & Stuart (1991) were encouraging enough to warrant improving them by formulating the problem explicitly in terms of the endothelial cell density. To this end, the mathematical model here consists of two conservation equations, one for the concentration c of the TAF and the other for the density n per unit area (of capillary sprout) of the endothelial cells. We now derive the two conservation equations and justify the terms in each.

2.1 *Tumour angiogenesis factor*

Tumour angiogenesis factor having concentration $c(\boldsymbol{x}, t)$ is secreted by the solid tumour and diffuses into the surrounding tissue. Upon reaching neighbouring endothelial cells situated in, for example, the limbal vessels, the TAF stimulates the release of enzymes by the endothelial cells which degrade their basement membrane. As described in the introduction, after degradation of the basement membrane has taken place, the initial response of the endothelial cells is to begin to migrate towards the source of angiogenic stimulus. Capillary sprouts are formed and cells subsequently begin to proliferate at a later stage. Once the capillary sprouts have formed, mitosis is largely confined to a region a short distance behind the sprout tips (Ausprunk & Folkman, 1977; Sholley *et al.*, 1984; Paweletz & Knierim, 1989; Stokes & Lauffenburger, 1991). Ausprunk & Folkman (1977) hypothesized that the reason for this proliferation was that these cells or vessels at the sprout tips were *acting as sinks* for the TAF. Balding & McElwain (1985) also suggested that a sink term could be included in their model of capillary growth. Following Chaplain & Stuart (1991), we thus incorporate a sink term for the TAF *in addition to* a natural decay term for the TAF. The conservation equation for the TAF concentration is thus given by

rate of increase of TAF = diffusion of TAF − loss due to cells − decay of chemical,

which, under the assumption of linear Fickian diffusion, can be written mathematically as

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c - f(c)g(n) - h(c), \tag{2.4}$$

where D_c is the TAF diffusion coefficient. We assume that the local rate of uptake of TAF by the endothelial cells (modelled by the function $f(c)$) is governed by Michaelis–Menten kinetics (cf. Lin, 1976; McElwain, 1978; Hiltman & Lory, 1983; Chaplain & Stuart, 1991) and that it also depends on the cell density, hence the inclusion of the function $g(n)$, i.e. the greater the density of endothelial cells, the more TAF will be removed by the cells acting as sinks (cf. Ausprunk & Fulkman, 1977; Chaplain & Stuart, 1991). In general, the function $g(n)$ can therefore be chosen to be some strictly increasing function to account for this. For simplicity the actual function used in the model is given by $g(n) = n/n_0$, a simple linear function. More complicated choices are also possible, however, and do not affect the model qualitatively. We also assume that the decay of TAF with time is governed by first-order kinetics, a standard assumption (cf. Sherratt & Murray, 1990). This leads to the following equation for the TAF in the external tissue:

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c - \frac{Qcn}{(K_m + c)n_0} - dc. \tag{2.5}$$

The initial condition is

$$c(x, 0) = c_0(x), \tag{2.6}$$

where $c_0(x)$ is a prescribed function chosen to describe qualitatively the profile of TAF in the external tissue when it reaches the limbal vessels (cf. Chaplain & Stuart, 1991). The TAF is assumed to have a constant value c_b on the boundary of the tumour and to have decayed to zero at the limbus (cf. Chaplain & Stuart, 1991) giving the boundary conditions as

$$c(0, t) = c_b, \qquad c(L, t) = 0. \tag{2.7}$$

2.2 *Endothelial cell population balance equation*

The endothelial cells are the principal characters in the drama of angiogenesis and are always centre stage (cf. Paweletz & Knierim, 1989). It is therefore highly desirable and logical to include in our model an equation modelling the endothelial cells. The cascade of events which goes to make up the complex process of angiogenesis is essentially driven by the endothelial cells. We will thus follow the route of the endothelial cells from their origin in their parent vessel (e.g. the limbus), their crossing of the extracellular matrix and other material in the surrounding host tissue, to their destination within the tumour.

The first events of angiogenesis are rearrangements and migration of endothelial cells rather than induction of cell division (Paweletz & Knierim, 1989). In response to the angiogenic stimulus, endothelial cells in the neighbouring normal capillaries which do not possess a muscular sheath are activated to stimulate proteases and

collagenases. The endothelial cells destroy their own basal lamina and start to migrate into the extracellular matrix. Small capillary sprouts are formed by accumulation of endothelial cells which are recruited from the parent vessel. The sprouts grow in length by migration of the endothelial cells (Cliff, 1963; Schoefl, 1963; Warren, 1966; Sholley *et al.*, 1984). The experimental evidence of Sholley *et al.* (1984) demonstrated that endothelial cells are continually redistributed among sprouts, moving from one sprout to another. This permits the significant outgrowth of a network of sprouts even when cell proliferation is prevented (Sholley *et al.*, 1984). At some distance from the tip of the sprout, the endothelial cells divide and proliferate to contribute to the number of migrating endothelial cells. The mitotic figures are only observed once the sprout is already growing out and cell division is largely confined to a region just behind the sprout tip. Solid strands of endothelial cells are formed in the extracellular matrix. Lumina develop within these strands and mitosis continues.

Initially the sprouts arising from the parent vessel grow in a more or less parallel way to each other. They tend to incline toward each other at a definite distance from the origin when neighbouring sprouts run into one another and fuse to form loops or *anastomoses*. Both tip–tip and tip–branch anastomosis occur and the first signs of circulation can be recognized. From the primary loops, new buds and sprouts emerge and the process continues until the tumour is eventually penetrated.

We now attempt to account for the above sequence of events using a population balance equation for the endothelial cells and to interpret the processes described above by analysing the endothelial cell density profile (cf. Stokes & Lauffenburger, 1991). The main events we model are the *migration* and the *proliferation* of the endothelial cells (the processes of anastomosis and budding will be accounted for implicitly in the model). We note that the migration and replication of endothelial cells are not linked together. Different types of stimuli are necessary for these two processes and we take this important fact into account in our model. We begin then with a general conservation equation for the endothelial cell density $n(x, t)$ which is of the form (cf. Maini *et al.*, 1991)

$$\frac{\partial n}{\partial t} + \nabla \cdot J = F(n)G(c) - H(n), \tag{2.8}$$

where J is the cell flux, and $F(n)$ and $H(n)$ are functions representing a normalized growth term and a loss term respectively for the endothelial cells. We assume that mitosis is governed by logistic type growth and that cell loss is a first order process (cf. Stokes & Lauffenburger, 1991). Thus

$$F(n) = rn\left(1 - \frac{n}{n_0}\right), \tag{2.9}$$

$$H(n) = -k_p n, \tag{2.10}$$

where r is a positive constant related to the maximum mitotic rate and k_p is the proliferation rate constant which is taken to be the reciprocal of the endothelial cell doubling time (cf. Sherratt & Murray, 1990; Stokes & Lauffenburger, 1991). We note that (2.9) contains a second-order loss term while (2.10) is a first-order loss term. Balding & McElwain (1985) considered cell loss due to anastomosis (both tip–tip

and tip–branch) as essentially a second-order process, while Stokes & Lauffenburger (1991) modelled endothelial cell loss due to budding as a first-order loss term. Moreover, they assumed that the probability of budding was uniform in all sprouts for all positions and all times. Thus these two terms implicitly account for endothelial cell loss due to anastomosis and budding respectively. Further we assume that the endothelial cell proliferation is controlled in some way by the TAF (Paweletz & Knierim, 1989) and this is reflected by the inclusion of the function $G(c)$ which is assumed to be nondecreasing. As stated previously, the initial response of endothelial cells to the angiogenic stimulus is one of migration (Paweletz & Knierim, 1989). Proliferation is a crucial but secondary response. In order to account for this through the function $G(c)$, we assume that there is a threshold concentration level of TAF below which proliferation does not occur. Thus, in the present model, we chose $G(c)$ to be of the form

$$G(c) = \begin{cases} 0 & \text{if } c \leqslant c^*, \\ \dfrac{c - c^*}{c_b} & \text{if } c^* < c, \end{cases} \tag{2.11}$$

where $c^* \leqslant c_b$. We note that a similar approach was used by Sherratt & Murray (1990) in modelling the chemical control of epithelial cells during wound healing.

There is substantial evidence that the response of the endothelial cells to the presence of the TAF is a chemotactic one (Ausprunk & Folkman, 1977; Terranova *et al.*, 1985; Balding & McElwain, 1985; Stokes *et al.*, 1990; Stokes & Lauffenburger, 1991), and following Balding & McElwain (1985) we assume that the flux $\boldsymbol{J}$ of endothelial cells consists of two parts, one representing random motion and the other chemotactic motion of the cells. Thus

$$\boldsymbol{J} = \boldsymbol{J}_{\text{diffusion}} + \boldsymbol{J}_{\text{chemotaxis}}. \tag{2.12}$$

Once again we assume linear diffusion, so that

$$\boldsymbol{J}_{\text{diffusion}} = -D_n \nabla n, \tag{2.13}$$

where D_n is the diffusion coefficient of the endothelial cells, and

$$\boldsymbol{J}_{\text{chemotaxis}} = n\chi(c)\nabla c, \tag{2.14}$$

the well-known form for the chemotactic flux (cf. Keller & Segel, 1971; Balding & McElwain, 1985). Various functional forms have been proposed for $\chi(c)$ including a logarithmic law

$$\chi(c) = \chi_0/c,$$

a receptor kinetic law

$$\chi(c) = \chi_0 k/(k + c)^2,$$

and a constant law

$$\chi(c) = \chi_0 \quad \text{(a constant)}.$$

For mathematical simplicity, throughout this paper we adopt the latter, i.e. we take $\chi(c) = \chi_0$, a constant (cf. Balding & McElwain, 1985; Murray, 1989; Maini

et al., 1991). We note, however, that the present model could easily be adapted to incorporate either the logarithmic law or the receptor kinetic law. The cell conservation equation can be written

rate of increase of cell density = cell migration + mitotic generation − cell loss.

With the above assumptions, we thus have the following population diffusion–chemotaxis equation for the endothelial cells:

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \chi_0 \nabla \cdot (n \nabla c) + rn\left(1 - \frac{n}{n_0}\right) G(c) - k_p n, \tag{2.15}$$

where $G(c)$ is given by (2.11). We assume that initially the endothelial cell density at the limbus is a constant n_0 and zero elsewhere, giving initial condition

$$n(\boldsymbol{x}, 0) = \begin{cases} n_0 & \text{if } x = L, \\ 0 & \text{if } x < L. \end{cases} \tag{2.16}$$

We assume that throughout the subsequent motion, the cell density remains constant at the limbus, and hence the boundary condition here becomes

$$n(L, t) = n_0. \tag{2.17}$$

As stated previously, the main aim of the model is to monitor the progress of the endothelial cells (in particular those at the sprout tips) as they cross the extracellular matrix and eventually reach the tumour. Once they reach the tumour and penetrate it, interactions with the tumour cells become important (Paweletz & Knierim, 1989) and the assumptions of the present model no longer hold. The modelling of this stage of the process is considered by Liotta *et al.* (1977). Thus, within the assumptions and limitations of the present model, we consider either of the following two boundary conditions at $\boldsymbol{x} = \boldsymbol{0}$:

$$\hat{\boldsymbol{n}} \cdot \nabla n = 0 \quad \text{at } \boldsymbol{x} = \boldsymbol{0}, \tag{2.18}$$

or

$$n = 0 \quad \text{at } \boldsymbol{x} = \boldsymbol{0}, \tag{2.19}$$

where $\hat{\boldsymbol{n}}$ is the unit outward normal at $\boldsymbol{x} = \boldsymbol{0}$. Under the assumptions of the model these boundary conditions will remain valid for all times such that n (in the case of (2.18) being imposed) or $|\nabla n|$ (in the case of (2.19) being imposed) remain very small at $\boldsymbol{x} = \boldsymbol{0}$, i.e. the solution will remain valid with these boundary conditions up to the time when the endothelial cells at the sprout tips first reach the tumour.

Following Chaplain & Stuart (1991) we normalize the equations using the following reference variables:

- reference TAF concentration: c_b, the value of the TAF concentration at the tumour boundary;
- reference cell density: n_0, the value of the endothelial cell density at the limbus;
- reference length: L, the distance from the tumour boundary to the limbal vessels;
- reference time unit: $\tau = L^2/D$.

We thus define new variables:

$$\tilde{c} = c/c_b, \quad \tilde{n} = n/n_0, \quad \tilde{x} = x/L, \quad \tilde{t} = t/\tau.$$

Dropping the tildes and specializing to a one-dimensional geometry (cf. Liotta *et al.*, 1977; Balding & McElwain, 1985; Sherratt & Murray, 1990; Chaplain & Sleeman, 1990; Chaplain & Stuart, 1991), the equations now become

$$\frac{\partial c}{\partial t} = \frac{\partial^2 c}{\partial x^2} - \frac{\alpha n c}{\gamma + c} - \lambda c, \tag{2.20}$$

$$\frac{\partial n}{\partial t} = D\frac{\partial^2 n}{\partial x^2} - \kappa\frac{\partial}{\partial x}\left(n\frac{\partial c}{\partial x}\right) + \mu n(1-n)G(c) - \beta n, \tag{2.21}$$

where

$$G(c) = \begin{cases} 0 & \text{if } c \leqslant c^*, \\ c - c^* & \text{if } c^* < c, \end{cases} \tag{2.22}$$

and

$$\alpha = \frac{L^2 Q}{D_c c_b}, \quad \gamma = \frac{K_m}{c_b}, \quad \lambda = \frac{L^2 d}{D_c}, \quad D = \frac{D_n}{D_c}, \quad \kappa = \frac{c_b \chi_0}{D_c}, \quad \mu = \frac{L^2 r}{D_c}, \quad \beta = \frac{L^2 k_p}{D_c}. \tag{2.23}$$

The initial and boundary conditions become respectively

$$c(x, 0) = c_0(x), \tag{2.24}$$

$$n(x, 0) = \begin{cases} 1 & \text{if } x = 1, \\ 0 & \text{if } x < 1, \end{cases} \tag{2.25}$$

$$c(0, t) = 1, \qquad c(1, t) = 0, \tag{2.26}$$

$$n(1, t) = 1, \tag{2.27}$$

$$\frac{\partial n}{\partial x} = 0 \quad \text{at } x = 0 \tag{2.28}$$

or

$$n = 0 \quad \text{at } x = 0. \tag{2.29}$$

As we have mentioned previously, angiogenic factors must be able to provoke three main activities of the endothelial cells, namely (1) production and secretion of enzymes capable of digesting extracellular matrix, (2) the initiation of *migration*, and (3) cell *proliferation*. It is clear from experimental evidence (Sholley *et al.*, 1977, 1984; Reidy & Schwartz, 1981) that *different* types of stimuli are necessary for (2) and (3), and we note that this is accounted for in the model, i.e. migration is governed by chemotaxis while proliferation is governed by the function $G(c)$, which essentially only depends on the TAF concentration. As stated previously, the processes of anastomosis and budding are also (implicitly) accounted for through the second- and first-order loss terms respectively in (2.21). Moreover, the form of the function $G(c)$ ensures that the second-order loss term implicitly modelling anastomosis will only take effect after the sprouts have reached a certain distance

into the extracellular matrix, which is precisely what is observed experimentally (cf. Paweletz & Knierim, 1989).

2.3 *Removal of angiogenic source*

When the tumour implant is removed, the TAF diffuses away naturally over a certain period of time (Balding & McElwain, 1985) and the capillary sprouts regress (Gimbrone *et al.*, 1974). The main effect of this on the model is to change the boundary condition for the TAF concentration c at $x = 0$. Instead of the TAF concentration here being kept constant, we now adopt the condition that there is no flux of TAF at $x = 0$ (cf. Chaplain & Stuart, 1991), since once the tumour has been removed TAF is no longer produced, i.e. $\partial c/\partial x = 0$ at $x = 0$. The system to be solved in this particular case is therefore (2.20, 2.21) with the boundary conditions

$$\frac{\partial c}{\partial x} = 0 \quad \text{at } x = 0, \qquad c(1, t) = 0 \tag{2.30}$$

replacing (2.26). The initial conditions

$$c(x, 0) = c_0(x), \tag{2.31}$$

$$n(x, 0) = n_0(x), \tag{2.32}$$

(replacing (2.24) and (2.25)) are taken to be the TAF concentration profile and the cell density profile, respectively, at the instant of removal of the tumour and can be obtained from the numerical solution to (2.20–2.29).

All of the above equations are solved numerically using a finite-difference scheme and the results are presented in the following section.

3. Results

In this section, we present the results from the numerical simulation of (2.20–2.29) with appropriate boundary and initial conditions. As far as possible, parameter values are chosen to correspond to available experimental data. Unfortunately, data are not available for all parameters, in particular those which relate to the concentration of TAF. However, most of the parameters used in (2.23) can be estimated from actual experimental data, while, for those which as yet cannot, we choose values estimated and used in other models (e.g. Stokes & Lauffenburger, 1991; Balding & McElwain, 1985).

Diffusion coefficient D

For a reference length we choose $L = 2$ mm, an average distance between a tumour implant and the limbal vessels, and the value for τ is taken to be 14 days, an average time for neovascularization to occur (cf. Balding & McElwain, 1985; Chaplain & Stuart, 1991; Stokes & Lauffenburger, 1991). According to the nondimensionalization in (2.23), this gives a value for D_c of 3.3×10^{-8} cm^2 s^{-1}. Sherratt & Murray (1990) found values of 3.1×10^{-7} and 5.9×10^{-6} cm^2 s^{-1} as estimates for diffusion

coefficients of chemicals. Correspondingly, they estimated the diffusion coefficients D_n of the epithelial cells under consideration in their model as 3.5×10^{-10} and 6.9×10^{-11} cm^2 s^{-1}. Using these values, the ratio D_n/D_c thus varies between 1.1×10^{-3} and 1.2×10^{-5}. In accordance with this range, we choose D to be 10^{-3}.

Chemotactic parameter κ

Stokes *et al.* (1990) have measured the value for the chemotaxis coefficient χ_0 of endothelial cells migrating in a medium containing unpurified acidic fibroblast growth factor (aFGF) as 2600 cm^2 s^{-1} M^{-1}. As above, the value for D_c is estimated at 3.3×10^{-8} cm^2 s^{-1}. In the numerical simulations carried out, the (nonzero) values for κ varied between 0.3 and 1.0, which from (2.23) gives a value for c_b of approximately 10^{-11} M, which is not unreasonable.

Cell proliferation parameter μ

From equation (2.15), we assume that the parameter r is a positive constant related to the maximum mitotic rate. Using data based on epidermal wound healing (Winter, 1972), Sherratt & Murray (1990) estimated that the maximum value r_{max} for this parameter was 10 times the proliferation rate constant. Based on *in vitro* experiments on endothelial cell proliferation (Williams, 1987), Stokes & Lauffenburger (1991) estimated the proliferation rate constant k_p of endothelial cells to be 0.056 h^{-1} under the assumption that all cells proliferate. However, cell mitosis in the sprouts is mainly confined to a region close to the tips. To compensate for this, in most of their simulations Stokes & Lauffenburger (1991) reduced the value of k_p to 0.02 h^{-1} and assumed that all cells in a sprout may proliferate. Thus, in the following numerical simulations, we assume a range for r_{max} of 0.2–0.56 h^{-1}. Under this assumption, we obtain a range of values for the cell proliferation parameter μ of approximately 70–190.

Cell loss parameter β

The parameter k_p is the reciprocal of the endothelial cell doubling time (cf. Sherratt & Murray, 1990). As seen above, Stokes & Lauffenburger (1991) estimated this to lie within the range 0.02–0.056 h^{-1}. Also, from experimental data on epidermal cells (Wright, 1983), Sherratt & Murray (1991) estimated this to be 0.01 h^{-1}. This gives a range of values for the cell loss parameter β of approximately 3–18.

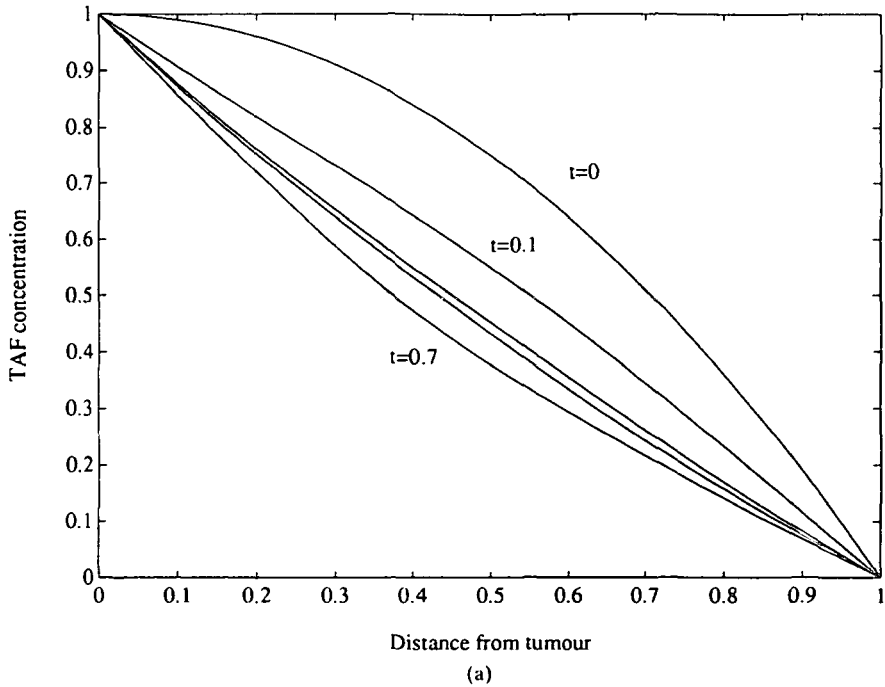
Initial conditions

The initial condition (2.24) was taken to be $c(x, 0) = c_0(x) = \cos \frac{1}{2}\pi x$, which is of the correct qualitative shape for the TAF profile in the external tissue, i.e. a constant value of 1 at the tumour edge decaying away to 0 at the limbus. Other initial profiles chosen were, for instance, $c_0(x) = 1 - x^2$ and $c_0(x) = 1 - x$, and once again the numerical results were very similar to those obtained with the cosine function.

3.1 *Numerical simulations of the model*

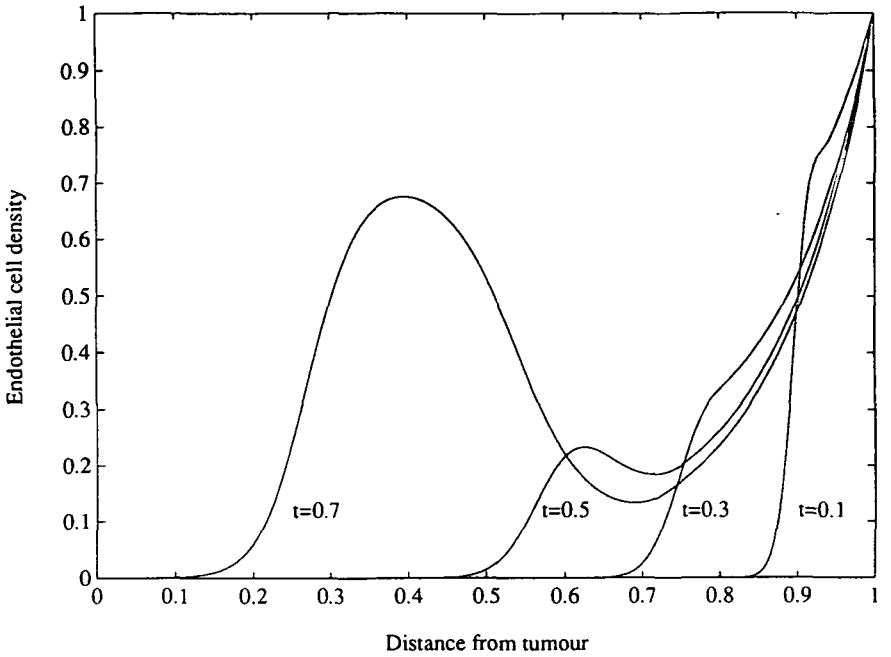
Figure 1(a, b) shows the profiles of the TAF concentration and the endothelial cell density in the external host tissue at various different times. In this simulation, the boundary condition used at $x = 0$ is $n_x = 0$. As has been explained in the previous section, the solution remains valid while the endothelial cells are crossing the extracellular matrix and have not yet reached the tumour, i.e. so long as n remains very small at $x = 0$. As can be seen from Fig. 1(b), shortly after $t = 0.7$ this condition no longer holds and the model loses its validity since the endothelial cells reach the tumour and interactions between tumour cells and endothelial cells become important. Figure 1(c) shows the profile of the endothelial cell density in the external host tissue at various different times with boundary condition $n = 0$ at $x = 0$. In this case the solution remains valid so long as n_x remains very small at $x = 0$, for the same reason as given above. Once again it can be seen from the figure that this condition is violated shortly after $t = 0.7$. As can be seen from Fig. 1(b, c), both boundary conditions give very similar results. The time taken for the endothelial cells to first reach the tumour corresponds to a real time of approximately 11 days, which is within the experimentally observed timescale (cf. Balding & McElwain, 1985). By varying the parameters μ and κ, the time taken for the endothelial cells (and hence the capillary sprouts) to reach the tumour can also be varied.

Figure 2 shows the endothelial cell density profile when the chemotactic response

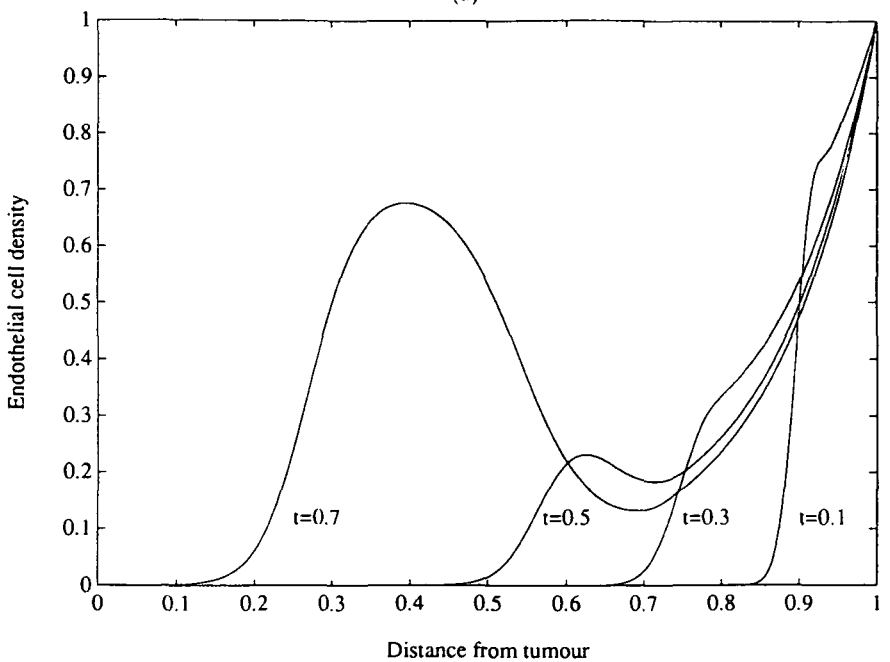


(a)

FIG. 1. (a) Profile of the TAF concentration in the external host tissue at times $t = 0$, 0.1, 0.3, 0.5, 0.7 showing changing gradient profile.



(b)



(c)

FIG. 1. (*continued*) (*b*) Profile of the endothelial cell density in the external host tissue at times $t = 0.1, 0.3, 0.5, 0.7$. The boundary condition imposed at $x = 0$ is $n_x = 0$. Shortly after $t = 0.7$ the endothelial cells reach the tumour and the assumptions of the model no longer hold. (c) Profile of the endothelial cell density in the external host tissue at times $t = 0.1, 0.3, 0.5, 0.7$. The boundary condition imposed at $x = 0$ is $n = 0$. Shortly after $t = 0.7$ the endothelial cells reach the tumour and the assumptions of the model no longer hold. Parameter values: $\alpha = 10$, $\gamma = 1$, $\lambda = 1$, $D = 0.001$, $\kappa = 0.75$, $\mu = 100$, $\beta = 4$; $c^* = 0.2$.

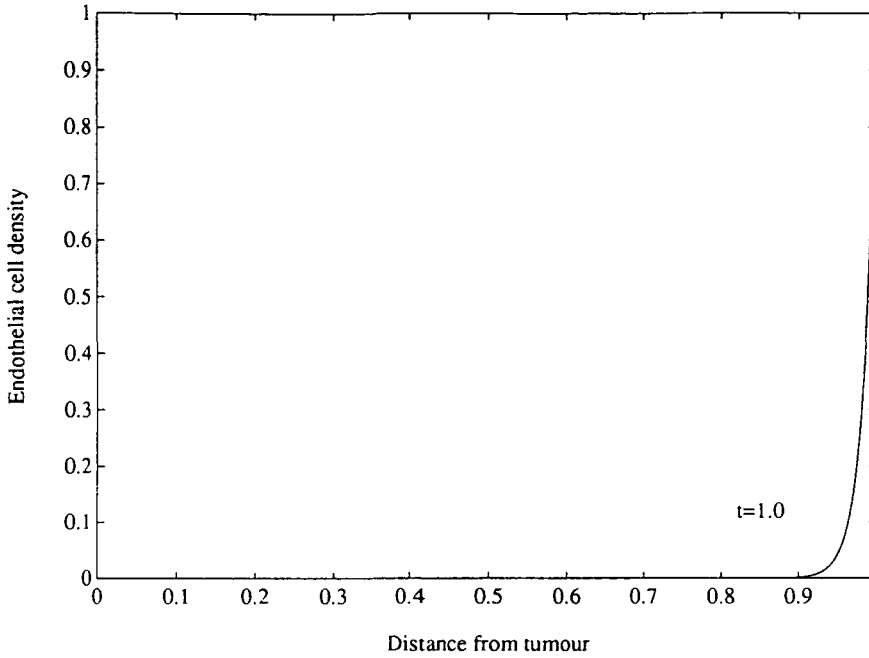


FIG. 2. Profile of the endothelial cell density in the external host tissue at $t = 1.0$ when the cell chemotaxis coefficient κ is set to zero. The figure clearly shows very little cell, and hence sprout, outgrowth. This shows that cell migration is vital to the complete process of angiogenesis. Parameter values: $\alpha = 10$, $\gamma = 1$, $\lambda = 1$, $D = 0.001$, $\kappa = 0.0$, $\mu = 100$, $\beta = 4$, $c^* = 0.2$.

parameter $\kappa = 0$, and, as can clearly be seen, there is very little cell response indicating that chemotaxis, and therefore cell migration, plays a major initiating role in the angiogenic process.

Figure 3 shows the endothelial cell density profile when the cell proliferation parameter $\mu = 0$, and once again the effect of this is significant. The figure shows that cell outgrowth, and hence sprout outgrowth, has virtually ceased after $t = 0.4$, which corresponds to a real time of 3.5 days. This is in very good agreement both with experimental evidence (Sholley *et al.*, 1984) and with the model of Stokes & Lauffenburger (1991). The previous two figures demonstrate that neither cell migration (via chemotaxis) nor cell proliferation alone is sufficient for a completion of angiogenesis, which is in agreement with experimental observations. In order for a completion of angiogenesis, both events must be included in the model, which is what is observed experimentally.

Comparing Fig. 1(b, c) with Fig. 3 demonstrates a good qualitative agreement with the available experimental evidence. Both simulations give the same profile of cell density for times $0 \leqslant t \leqslant 0.4$. This shows that the initial response of the endothelial cells is essentially one of migration with proliferation of the cells occurring at a later time (cf. Paweletz & Knierim, 1989). The form of the function $G(c)$ ensures that there is always a region within the sprouts where there is zero cell proliferation and also that, once cells have started to proliferate, the proliferation is mainly confined to a

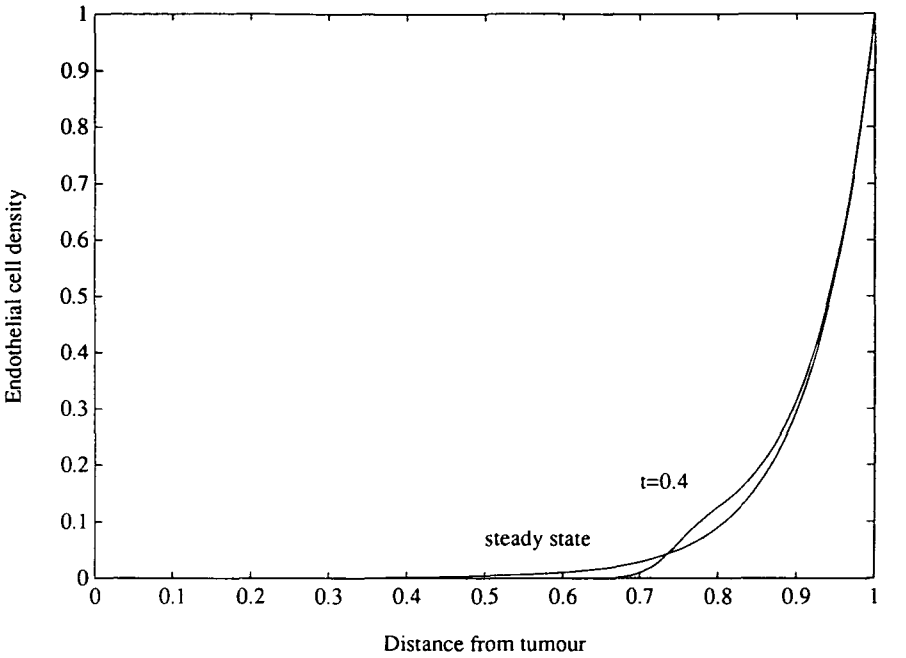


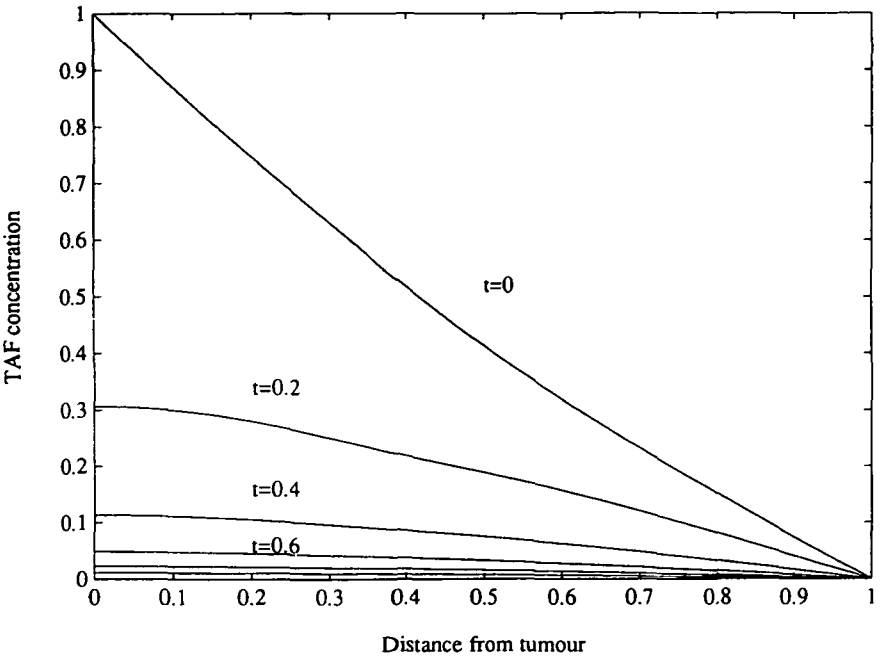
FIG. 3. Profile of the endothelial cell density in the external host tissue at time $t = 0.4$ and at steady state (when all outgrowth had stopped completely) when the cell proliferation parameter μ is set to zero. The figure clearly shows that at $t = 0.4$ sprout outgrowth has virtually ceased, which is in agreement with the experimental evidence. This shows that cell proliferation is essential for the completion of angiogenesis. Parameter values: $\alpha = 10$, $\gamma = 1$, $\lambda = 1$, $D = 0.001$, $\kappa = 0.6$, $\mu = 0.0$, $\beta = 6.0$, $c^* = 0.2$.

region a short distance behind the sprout tips. Thus the model distinguishes, as far as is possible within its limitations, between proliferating cells near the sprout tip and nonproliferating cells within the rest of the sprout. Also, once the cells have started to proliferate, the endothelial cell density is greatest (locally) a short distance behind the sprout tips, which is what is observed experimentally (Ausprunk & Folkman, 1977; Sholley *et al.*, 1984; Pawletz & Knierim, 1989). Similar results were obtained for different functions $G(c)$ which were of the same qualitative form as (2.11).

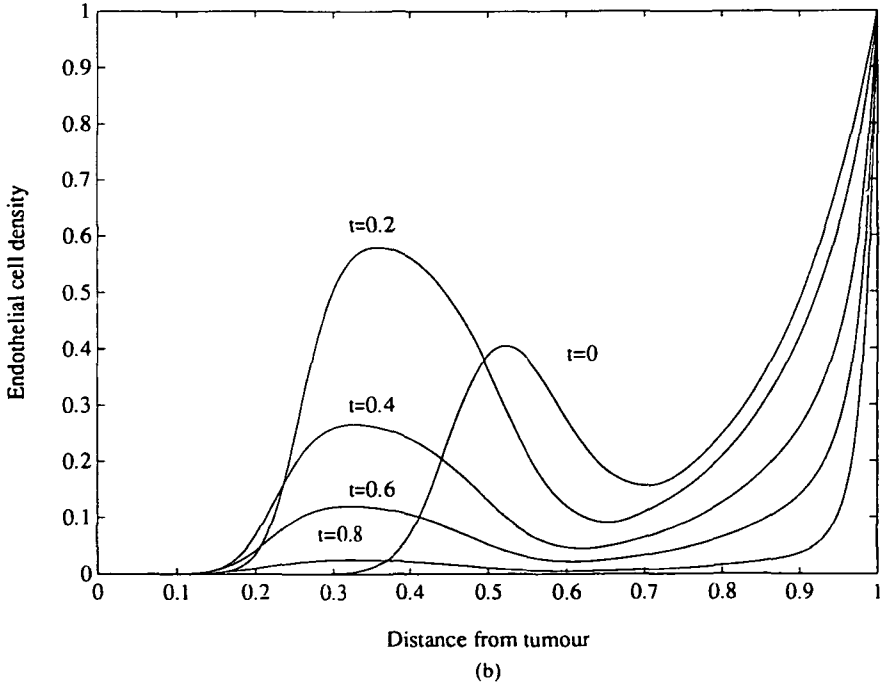
For all numerical simulations carried out, we took $c^* = 0.2$. Qualitatively similar results were obtained for various other values of c^* between 0.1 and 0.4. We note that other numerical simulations were carried out with $D = 0$ and $\alpha = 0$. In each case, the profiles of TAF concentration and endothelial cell density remained almost the same as when these parameters were nonzero. In the former case, this would seem to confirm the results of the models of Balding & McElwain (1985) and Stokes & Lauffenburger (1991), where diffusion was also seen to have a negligible effect on the results.

3.2 *Removal of TAF*

Figure 4(a, b) shows the numerical simulations of the model when the tumour is removed. Gimbrone *et al.* (1974) report, as is to be expected, a regression of sprouts


TAF concentration
Distance from tumour
t=0
t=0.2
t=0.4
t=0.6
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

(a)


Endothelial cell density
Distance from tumour
t=0.2
t=0
t=0.4
t=0.6
t=0.8
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

(b)

and vessels. As can be seen from the figures, the TAF concentration decays away to zero while the cell density initially increases, but then gradually decays to zero throughout the external tissue. After one time unit (another 14 days), there is zero cell density everywhere indicating a complete regression of the sprouts.

These results are an improvement upon the previous model of Chaplain & Stuart (1991) in two ways: (i) the present model permits direct tracking of the endothelial cells (and hence sprout tips) from their origin in the parent vessel until they reach the tumour; (ii) the regression of the endothelial cells (and hence sprout tips) can also be directly monitored when the source of TAF is removed.

4. Conclusions

As is apparent from its brief description in the introduction, the complete process of angiogenesis is a complicated one, involving several distinct, and not necessarily related, events. This in turn requires several separate mechanisms which can stimulate each event, e.g. vasodilation, endothelial cell migration, endothelial cell proliferation, and loop formation (anastomosis), to name a few. Despite much research and many advances, many questions still remain unanswered (cf. Paweletz & Knierim, 1989). To formulate a single mathematical model which would include all of these processes would be very difficult indeed. In this paper we have chosen to focus attention principally on the activity of the endothelial cells, since they are always at the heart of the angiogenic process, as well as on the TAF concentration profile. We have modelled in a simple but effective manner two of the three main events concerning the endothelial cells (migration and proliferation), and the results obtained are in good agreement with both experimental evidence and other models (Sholley *et al.*, 1984; Paweletz & Knierim, 1989; Stokes & Lauffenburger, 1991) and represent an improvement upon those obtained by Chaplain & Stuart (1991). The model reproduces the experimentally observed facts that the initial response of the endothelial cells is one of migration with proliferation occurring as a secondary response, which is nevertheless vital for the successful completion of angiogenesis. Given the above results concerning the endothelial cell density, the present model could be easily extended to include an extra equation for sprout tip density, for example. Work on a model of this type, which will also explicitly model the processes of anastomosis and sprout budding, is currently being carried out by the authors.

FIG. 4. (a) Profile of the TAF concentration in the external host tissue when tumour implant is removed showing decrease in TAF concentration at times $t = 0, 0.2, 0.4, 0.6, 0.8, 1.0$. The TAF concentration at $t = 0.8, 1.0$ is virtually zero. The initial profile of the TAF concentration here is taken to be the TAF concentration profile at $t = 0.6$ in the simulation of Fig. 1(b). (b) Profile of the endothelial cell density in the external host tissue when tumour implant is removed at times $t = 0, 0.2, 0.4, 0.6, 0.8, 1.0$. The profile at $t = 1.0$ is zero virtually everywhere. The initial profile of the cell density here is taken to be the cell density profile at $t = 0.6$ in the simulation of Fig. 1(b). The figures illustrate the decay of endothelial cells, and hence the capillary sprouts, once the tumour implant is removed. Parameter values: $\alpha = 10$, $\gamma = 1$, $\lambda = 1$, $D = 0.001$, $\kappa = 0.75$, $\mu = 100$, $\beta = 4$, $c^* = 0.2$.

The results also confirm previous assumptions and findings that the endothelial cell migration is almost certainly controlled by some form of taxis rather than diffusion. However, since endothelial cells are known to exhibit different levels of motility on different extracellular molecules (Terranova *et al.*, 1985; Ungari *et al.*, 1985; Young & Herman, 1985), it would certainly be possible to modify the model to account for this variable motility. More specifically, we could assume a more general form of (2.13), namely

$$J_{\text{diffusion}} = -D_n(c)\nabla n. \tag{4.1}$$

The diffusion, or motility, coefficient of the cells would now be dependent upon the concentration of the TAF. This means considering a nonlinear diffusion problem.

Although we have chosen to focus on chemotaxis as the underlying driving mechanism since there is much experimental evidence to support this, there is also experimental evidence that the interaction between the endothelial cells and the extracellular matrix, through which they must move to reach the tumour, may also have a part to play in the angiogenic process. From *in vitro* experiments, it is known that the mobilization of endothelial cells can be greatly enhanced by adding gangliosides to whatever medium they are cultivated upon. It has been shown that preincubating endothelial cells with trisialogangliosides and then culturing on the same substratum leads to their *binding* to fibronectin being greatly enhanced and their migration is increased fivefold (Ungari *et al.*, 1985; Alessandri *et al.*, 1986; Paweletz & Knierim, 1989). The structure of the present model permits these adhesive gradients, i.e. haptotaxis, to be included in the model in a relatively straightforward way. Analysis of travelling wave solutions for the model is also another area of possible research (cf. Myerscough & Murray, 1992).

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