THE MATHEMATICAL MODELLING OF TUMOUR ANGIOGENESIS AND INVASION

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ABSTRACT

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 comprising, at minimum, of a degradation of their basement membrane, migration and proliferation. Capillary sprouts are formed which migrate towards the tumour eventually penetrating it and permitting vascular growth to take place. It is during this stage of growth that the insidious process of invasion of surrounding tissues can and does take place. A model mechanism for angiogenesis 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. Numerical simulations of the model are shown to compare very well with experimental observations. The subsequent vascular growth of the tumour is discussed with regard to a classical reaction-diffusion pre-pattern model.

1. INTRODUCTION

Solid tumours are known to progress through two distinct phases of growth - the avascular phase and the vascular phase (Folkman 1974, 1976). The initial avascular growth phase can be studied in the laboratory by culturing cancer cells in the form of three-dimensional *multicell spheroids* (Durand, 1990; Sutherland, 1988). It is well known that these spheroids, whether grown from established tumour cell lines or actual *in vivo* tumour specimens, possess growth kinetics which are very similar to *in vivo* tumours. Typically, these avascular nodules grow to a few millimetres in diameter. Cells towards the centre, being deprived of vital nutrients, die and give rise to a necrotic core. Proliferating cells can be found in the outer three to five cell layers. Lying between these two regions is a layer of quiescent cells, a proportion of which can be recruited into the outer layer of proliferating cells. Much experimental data has been gathered on the internal architecture of spheroids, and studies regarding the distribution of vital nutrients (e.g. oxygen) and metabolites within the spheroids have been carried out.

The transition from the dormant avascular state to the vascular state, wherein the tumour possesses the ability to invade surrounding tissue and metastasise to distant parts of the

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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. Once vascularized the tumours grow rapidly as exophytic masses. In certain types of cancer, e.g. carcinoma arising within an organ, this process typically consists of columns of cells projecting from the central mass of cells and extending into the surrounding tissue area. The local spread of these carcinoma often assume an irregular jagged shape.

In order to accomplish this vascularization, 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 out into the nature of TAF and its effect on endothelial cells (EC) since initial research began in the early 1970's 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 (Folkman & Klagsbrun, 1987). 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, for example, the reviews of Folkman & Klagsbrun, 1987, and Paweletz & Knierim, 1989).

The first events of angiogenesis are rearrangements and migration of EC situated in nearby vessels. The main function of EC 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 EC in order to leave the respective vessels. These cells are the principal characters in the drama of angiogenesis and are always centre stage (Paweletz & Knierim, 1989).

In response to the angiogenic stimulus, EC 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 EC which are recruited from the parent vessel. The sprouts grow in length by migration of the endothelial cells (Sholley *et al.*, 1984). The experimental results of Sholley *et al.* (1984) demonstrated that EC 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 EC divide and proliferate to contribute to the number of migrating EC. 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 EC 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 recognised. From the primary loops, new buds and sprouts emerge and the process continues until the tumour is eventually penetrated.

In the first half of this paper we develop and extend a model developed by Chaplain and

Stuart (1993) which describes the response of the EC to the chemotactic stimulus of tumour angiogenesis factor. In the second half of the paper we discuss the vascular, exophytic growth of certain cancers vis-à-vis reaction-diffusion pre-pattern theory.

2. A MATHEMATICAL MODEL FOR TUMOUR ANGIOGENESIS

Chaplain & Stuart (1993) develop a model for the response of the EC to the chemotactic stimulus of the TAF which consists of two equations, one for the endothelial cell density per unit area of capillary sprout (n) and the other for the concentration (c) of TAF in the host tissue. Using conservation principles, the system of equations considered takes on the general form

rate of increase of TAF	=	diffusion of TAF	−	loss due to cells	−	decay of chemical
rate of increase of cell density	=	cell migration	+	mitotic generation	−	cell loss

We now briefly discuss each of the above equations in turn, deriving the mathematical representation of each term. A fuller description can be found in Chaplain & Stuart (1993).

2.1. Tumour Angiogenesis Factor

We assume that the TAF diffuses throughout the neighbouring tissue until it reaches any neighbouring EC which are then stimulated and begin migration. Once the capillary sprouts are formed, EC near the sprout tips begin to proliferate. Ausprunk & Folkman (1977) hypothesised that the reason for this proliferation was that these cells or vessels at the sprout tips were *acting as sinks* for the TAF. Following Chaplain & Stuart (1991, 1993), we thus incorporate a sink term, $f(c)$, say, for the TAF *in addition to* a natural decay term for the TAF and we assume that the local rate of uptake of TAF by the EC is governed by Michaelis-Menten kinetics (cf. Chaplain & Stuart, 1991, 1993). We also assume that this uptake rate depends on the cell density through some function $g(n)$, say, i.e. the greater the density of EC, the more TAF will be removed by the cells acting as sinks (cf. Ausprunk & Folkman, 1977; Chaplain & Stuart, 1991, 1993). We also assume that the decay of TAF with time is governed by first-order kinetics, a standard assumption (cf. Sherratt & Murray, 1990), hence a term $-dc$ is included. This leads to the following equation for the TAF in the external tissue

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c - \frac{Qcn}{(K_m + c)n_0} - dc \tag{2.1}$$

The initial condition is given by

$$c(x,0) = c_0(x)\,, \tag{2.2}$$

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, 1993). The TAF is assumed to have a constant value c_b on the boundary of the tumour ($\partial\Omega_{tumour}$) and to have decayed to zero at the other boundaries ($\partial\Omega_i$) of the domain (cf. Chaplain & Stuart, 1991, 1993) giving the boundary conditions as

$$c(x,t) = c_b,\ \ x \in \partial\Omega_{tumour},\ \ c(x,t) = 0,\ \ x \in \partial\Omega_i. \tag{2.3}$$

2.2. Endothelial Cell Population Balance Equation

The main events we model are the *migration* and the *proliferation* of the EC and the important processes of *anastomosis* and secondary *budding/sprouting*. The latter two processes will be incorporated explicitly representing an extension of the model of Chaplain & Stuart (1993). We note that the migration and replication of EC 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. The general conservation equation for the endothelial cell density $n(x,t)$ may be written (cf. Chaplain & Stuart, 1993)

$$\frac{\partial n}{\partial t} + \nabla . \boldsymbol{J} = F(n)G(c) + H(n,x), \tag{2.4}$$

where $\boldsymbol{J}$ is the cell flux, $F(n)$ and $H(n,x)$ are functions representing a normalised growth term and a loss term respectively for the EC. We assume that mitosis is governed by logistic type growth and that natural cell loss is a first order process (cf. Stokes & Lauffenburger, 1991). The function $H(n,x)$ must also incorporate a term which models cell loss due to secondary sprouting of tips from an existing capillary and at the same time model the effect of anastomosis (tip-tip or tip-branch) on a capillary. It is assumed that the net effect of anastomosis will be to increase the cell density at that particular location of the capillary where fusion takes place. Thus

$$F(n) = rn\left(1 - \frac{n}{n_0}\right), \tag{2.5}$$

$$H(n,x) = -k_p n - s(x)n, \tag{2.6}$$

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). The function $s(x)$ will be taken to be some appropriate periodic function of the spatial variable x, (e.g. $\cos(a\pi x)$) whose *average* value over a period is zero. Such a function will thus represent the effect of anastomosis for half its period (net increase in cell density due to cell gain) and the effect of secondary budding for the other half period (net reduction in cell density due to cell loss). This means that we assume secondary budding and anastomosis occur in a consecutive, periodic manner and is a reasonable approximation to what are essentially two random processes.

As stated previously, the initial response of EC 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

$$G(c) = \begin{cases} 0, & c \le c^* \\ \dfrac{c - c^*}{c_b}, & c^* < c \end{cases} \tag{2.7}$$

where $c^* \le c_b$.

We assume that the flux $\boldsymbol{J}$ of EC consists of two parts, one representing random motion and the other chemotactic motion of the cells. We also assume that the diffusion of the EC

is density dependent which generalises the model of Chaplain & Stuart (1993). The flux J can be written

$$J = -D_n \left(\frac{n}{n_0}\right)^{\sigma} \nabla n + n\chi_0 \nabla c. \tag{2.8}$$

where D_n is the diffusion coefficient of the EC and χ_0 (constant) chemotactic coefficient. Simple Fickian diffusion is recovered on taking $\sigma = 0$. With the above assumptions we thus have the following population diffusion-chemotaxis equation for the EC

$$\frac{\partial n}{\partial t} = D_n \nabla . \left(\left(\frac{n}{n_0}\right)^{\sigma} \nabla n \right) - \chi_0 \nabla . (n \nabla c) + rn \left(1 - \frac{n}{n_0}\right) G(c) - k_p n - s(x) n \,, \tag{2.9}$$

where $G(c)$ is given by (2.7). Suitable initial and boundary conditions for the EC are prescribed depending upon the precise domain in which the above equations (2.1) (2.9) are to be solved.

However, we note that the numerical simulations of the model of Chaplain & Stuart (1993) indicate that the concentration profile for c rapidly reaches a steady state profile. This is not entirely unexpected since the TAF diffuses much faster than the endothelial cells. Thus a reasonable approximation to the TAF concentration profile may be obtained by solving

$$\nabla^2 c - \lambda c = 0 \,, \tag{2.10}$$

subject to $c = 1$ at tumour boundary, $c = 0$ at all other boundaries. This simplification means that now our model is reduced to a single partial differential equation, which after a suitable normalization (cf. Chaplain & Stuart, 1993) is

$$\frac{\partial n}{\partial t} = D_n \nabla . (n^{\sigma} \nabla n) - \kappa \nabla . (n \nabla c) + \mu n (1-n) G(c(x)) - \beta n - s(x) n \,, \tag{2.11}$$

with appropriate initial and boundary conditions for n. This equation is solved numerically and the results are presented in the following section where the exact form of the boundary and initial conditions will also be given. As far as possible parameter values are chosen to correspond to available experimental data (see Chaplain & Stuart, 1993, for details).

3. NUMERICAL SIMULATIONS

3.1 Simulations in One Spatial Dimension

We firstly solve our equation in a one dimensional domain, normalized to be [0,1]. We assume the tumour implant is situated at $x = 0$ and the EC at $x = 1$. The solution to (2.10) (in one space dimension) is given by

$$c(x) = \sinh[(1-x)\sqrt{\lambda}]/\sinh\sqrt{\lambda} \,.$$

It can be shown that a good approximation to the TAF concentration profile with $\lambda = 1$ can be obtained on taking $c = 1 - x$. The equation to be solved is thus

$$\frac{\partial n}{\partial t} = D \frac{\partial}{\partial x} \left(n^{\sigma} \frac{\partial n}{\partial x} \right) + \kappa \frac{\partial n}{\partial x} + \mu (1-x) n (1-n) - \beta n - s(x) n \,, \tag{3.1}$$

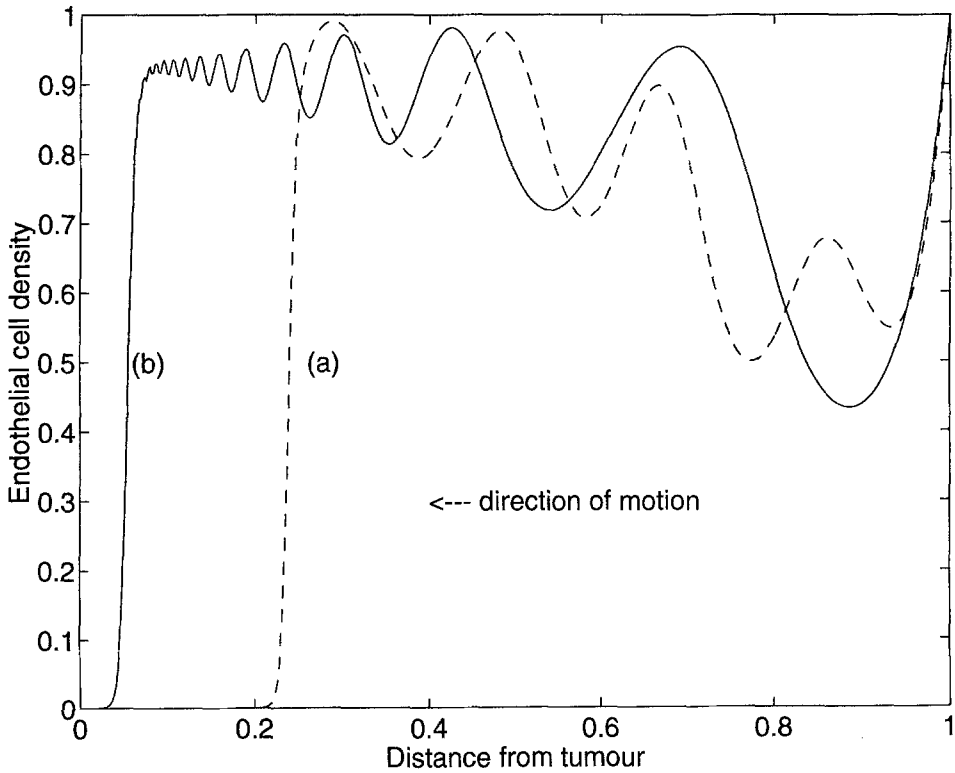

Fig.1. Profile of the endothelial cell density in the host tissue at a time corresponding to 12 days. Profile (a) (- - -) corresponds to regular anastomosis/side-branching with $s(x) = 5 \cos(10\pi x)$, profile (b) (—) corresponds to more frequent anastomosis/side-branching with $s(x) = 5 \cos(2.25\pi x)$. Parameter values: $D = 0.001$, $\sigma = 4$, $\kappa = 0.65$, $\mu = 75$, $\beta = 5$.

with initial conditions

$$n(x,0) = \begin{cases} 1, & x = 1 \\ 0, & \text{elsewhere} \end{cases} \tag{3.2}$$

and boundary conditions $n(1,t) = 1$, $n(0,t) = 0$. Figure 1 shows the endothelial cell density profile in the external tissue for two different functions $s(x)$. As can clearly be seen in each case, the results capture qualitatively both the effects of anastomosis (local maxima) and the formation of side branches (local minima) as the capillary sprout migrates towards the tumour. For profile (a) (hatched line - - -) we chose $s(x) = 5 \cos(10\pi x)$ representing a regular spatial occurrence of both anastomosis and side-branching. However in profile (b) (solid line —) $s(x) = 5 \cos(2.25\pi/x)$ which represents more faithfully the fact that as the capillary network progresses, anastomosis and side-branching occur more frequently. This captures more faithfully the so-called brush-border effect reported by Muthukkaruppan *et al.* (1982).

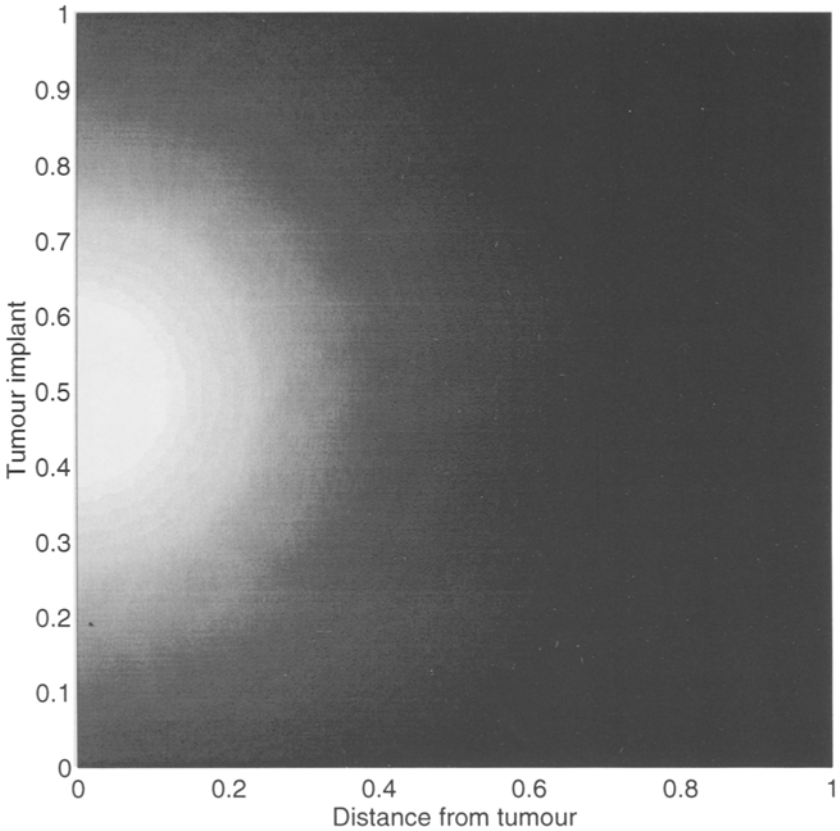

Fig.2. Profile of the steady state TAF concentration profile given by (3.3) used in the 2 dimensional simulations. White = high concentration, black = low.

3.2 Simulations in Two Spatial Dimensions

Experiments are often carried out in very thin regions (< 50μm) of the cornea or ear chamber of test animals (Stokes & Lauffenburger, 1991) and so a two dimensional domain is a good approximation of the sprout growth. Using normalized variables, we work on the unit square $[0,1] \times [0,1]$ and assume that the tumour implant occupies the region $(x-0.5)^2 + y^2 \leq 0.1$. We assume that the TAF diffuses in a radially symmetric manner and it can easily be seen that the corresponding solution to (2.10) (in polar coordinates with radial symmetry) is given in terms of Bessel functions but once again we find that a very good approximation is given by

$$c(x,y) = c(r) = \begin{cases} 1, & r \leq 1 \\ (1-r)^2/0.81, & r > 1 \end{cases} \tag{3.3}$$

where $r^2 = (x-0.5)^2 + y^2$. This profile is illustrated in figure (2). The single equation we now have to solve is given by

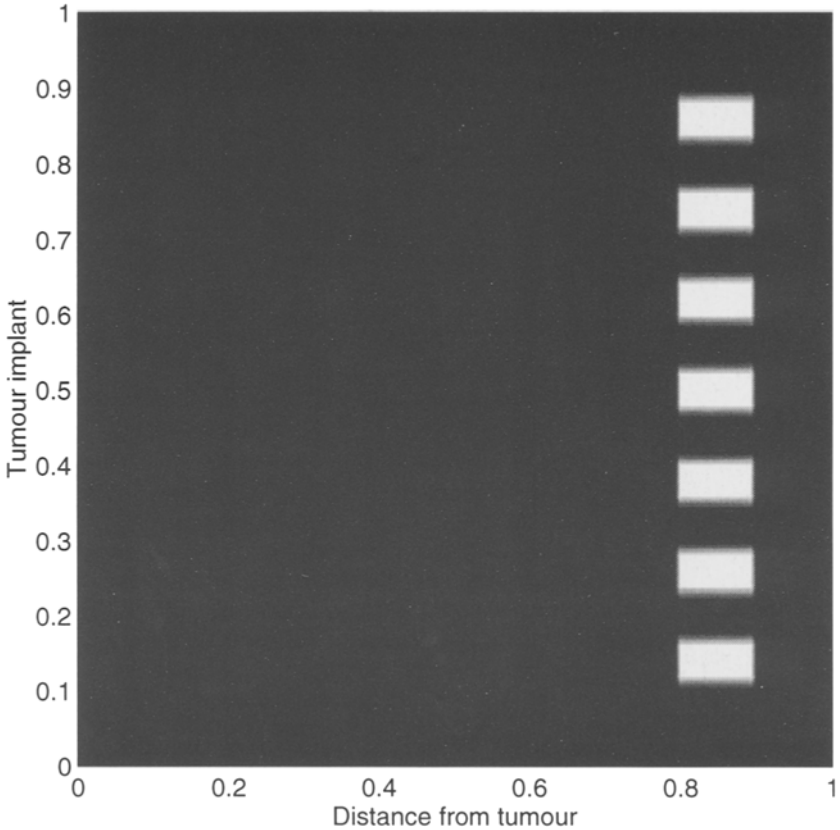

Fig.3. Profile of the initial conditions used for the endothelial cell density simulating the formation of several capillary sprouts. White = high density, black = low.

$$\frac{\partial n}{\partial t} = D\frac{\partial}{\partial x}\left(n^{\sigma}\frac{\partial n}{\partial x}\right) + D\frac{\partial}{\partial y}\left(n^{\sigma}\frac{\partial n}{\partial y}\right) - \kappa\frac{\partial}{\partial x}\left(n\frac{\partial c}{\partial x}\right) - \kappa\frac{\partial}{\partial y}\left(n\frac{\partial c}{\partial y}\right) \quad (3.4)$$

$$+ \mu c(x,y)n(1-n) - \beta n - s(x,y)n \ ,$$

We assume that the initial phase of capillary sprout growth has taken place i.e. the cells have secreted enzymes which degrade their basement membrane permitting the formation of small buds (cf. Paweletz \& Knierim, 1990). Indeed, Muthukkaruppan *et al.* (1982) do not consider tumour-induced angiogenesis to be initiated until 2 days after implant and only designate a stage I of angiogenesis after 4 days recognising that there may well be some limbal response to the actual preparation of the implant site. Initially then we assume that there are several buds formed and we focus attention exclusively upon those endothelial cells which are close to the sprout tips since only these cells proliferate. Here the endothelial cell density is initially a constant $n_0 = 1$ and zero elsewhere, giving initial conditions

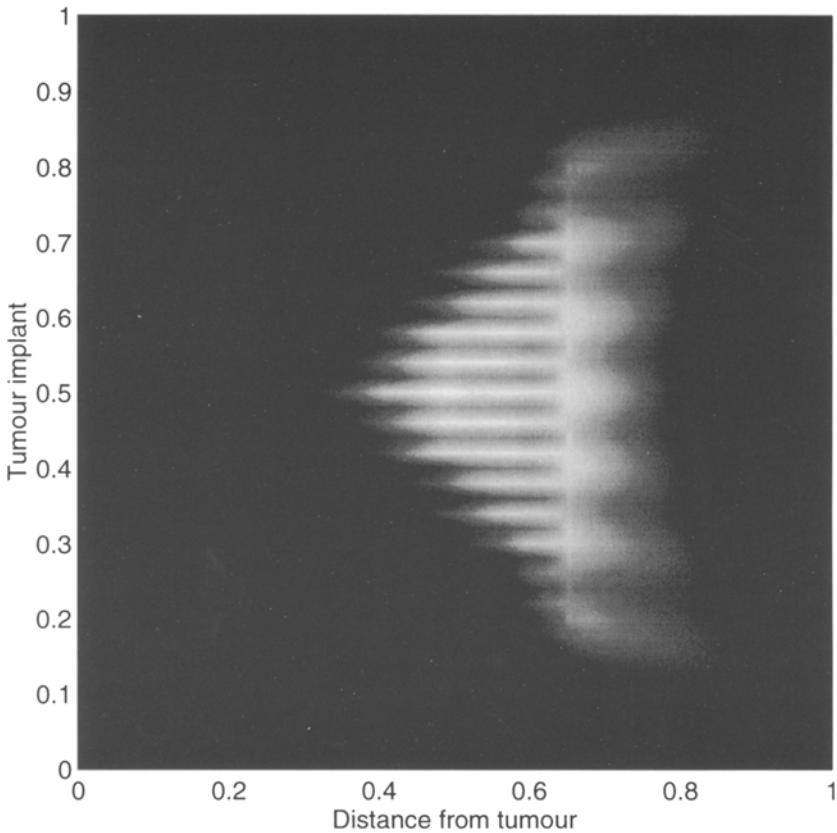

Fig.4. Profile of the endothelial cell density after the capillary network has been established and anastomosis has taken place corresponding to a real time of 7-8 days after initial sprouts have formed as per figure 3. Brush-border effect is clearly visible at the vascular front. Parameter values: $D = 0.001$, $\sigma = 1$, $\kappa = 0.65$, $\mu = 75$, $\beta = 0.5$.

$$n(x,0) = \begin{cases} n_0 = 1, & x \text{ near sprout tip} \\ 0, & x \text{ elsewhere} \end{cases} \tag{3.5}$$

The initial profile chosen for the EC is illustrated in figure (3). Since we are attempting to focus attention on the EC near to the sprout tips (since these are the cells which proliferate) and also since all the EC are confined to within the capillary sprouts, we take the EC density to be zero ($n = 0$) on all boundaries.

Figure (4) shows the EC density profile within the capillary sprouts as they migrate towards the tumour. In this case we chose $s(x,y) = 10\cos^6(25\pi x)$, $y < 0.65$; $s(x,y) = 0$, $y \geq 0.65$, representing explicitly the effect of the brush-border once the initial sprouts/loops have all anastomosed (cf. Muthukkaruppan *et al.*, 1982).

4. THE VASCULAR GROWTH PHASE

While the process of angiogenesis is taking place, the avascular tumour although dormant (or quasi-dormant) with regard to its growth is still in a dynamic state. In this section we consider the possibility of the development of a heterogeneous cellular pre-pattern which takes place prior to successful angiogenesis and which then facilitates the vascular, invasive growth. Experimental results have demonstrated that tumour cells secrete both growth-activating and growth-inhibiting chemicals. Several recent papers, Chaplain & Britton (1993), Chaplain *et al.*, (1994) (and references therein) have focused attention on the chemical inhibition of mitosis within multicell spheroids, the main assumption of the modelling being that a growth inhibitory factor (GIF) is produced within the spheroid in some prescribed spatially-dependent manner to reflect the observed cellular heterogeneity within spheroids. The existence and properties of chemicals which inhibit mitosis are very well documented, e.g. Harel *et al.* (1984), Iverson (1991), Parkinson & Balmain (1990). However, there is also much evidence to demonstrate that tumour cells also secrete growth-promoting factors, e.g. Chen *et al.* (1993), Kawase *et al.* (1994), Wu *et al.* (1993).

We consider then the production of growth promoting factors (GPF) and growth inhibitory factors (GIF) by tumour cells during the avascular phase of growth. The theory will be presented for solid spherical tumours, but the analysis goes over equally well to other similar geometries e.g. oblate spheroid, prolate spheroid, ellipsoid etc. It is known, for example, that carcinoma arising in the breast tend to be ellipsoidal in shape. We consider the case whereby the growth factors are produced throughout the interior of the tumour and also the case whereby production of the factors is restricted to the thin layer of live, proliferating cells at the tumour surface. We will discuss the relevance of the resulting spatially inhomogeneous patterns to the experimentally observed cellular heterogeneity within carcinoma and multicell spheroids and also to the well known invasion characteristics of carcinoma.

The system we study is the set of nonlinear partial differential equations which we write in the general form familiar from the theory of reaction-diffusion equations:

$$\frac{\partial \boldsymbol{c}}{\partial t} = \boldsymbol{f}(\boldsymbol{c}) + D\nabla^2 \boldsymbol{c} \tag{4.1}$$

where $\boldsymbol{c}$ is the vector of growth factor concentrations, $\boldsymbol{f}$ represents the reaction kinetics of the chemicals and D is the diagonal matrix of positive constant diffusion coefficients cf. Dillon *et al.* (1994), Hunding (1980), Murray (1982). If we denote the permeability of the tumour tissue at the tumour surface by P then appropriate boundary conditions for the above system are of the form

$$D(\boldsymbol{n}.\nabla)\boldsymbol{c} = -P\boldsymbol{c}, \quad \boldsymbol{r} \text{ on } \partial\Omega \tag{4.2}$$

where $\partial\Omega$ is the closed boundary of the domain i.e. the tumour surface and $\boldsymbol{n}$ is the outward unit normal. A derivation of these boundary conditions may be found in Chaplain & Britton (1993), Chaplain *et al.* (1994) and references therein. Appropriate initial conditions will also be prescribed. For our purposes we consider only two chemical species whose concentrations are c_1 and c_2, say, representing some growth-promoting factor (GPF) and some growth-inhibiting factor (GIF) respectively. The reaction kinetics governing the system may either be a pure or cross activator-inhibitor mechanism (Dillon *et al.*, 1994; Murray,

1982). The distinction between these two types of kinetics lies in whether the self-activating chemical either activates (pure) or inhibits (cross) the second species. There is experimental evidence which suggests both mechanisms may be applicable in the case of growth factors secreted by tumour cells (Michelson & Leith, 1991). For our purposes throughout this paper we shall assume that the kinetics are a cross activator-inhibitor mechanism e.g. the prototypical Schnakenberg system (Murray, 1982). Under appropriate scaling, it can be shown (Dillon *et al.*, 1994; Murray, 1982) that the above system of equations then reduces to the following:

$$\frac{\partial u}{\partial t} = \gamma f(u,v) + \nabla^2 u, \quad 0 \leq r < 1, \tag{4.3}$$

$$\frac{\partial v}{\partial t} = \gamma g(u,v) + d\nabla^2 v, \quad 0 \leq r < 1, \tag{4.4}$$

where, with R an appropriate tumour radius, k an appropriate reaction parameter and D_1, D_2 the diffusion coefficients of the GPF and GIF respectively, $\gamma = R^2k/D_1$ and $d = D_2/D_1$. The boundary conditions for the above system are then given by

$$\frac{\partial u}{\partial r} = -\rho_1 u, \quad \text{on } r = 1 \tag{4.5}$$

$$\frac{\partial v}{\partial r} = -\rho_2 v, \quad \text{on } r = 1 \tag{4.6}$$

where $\rho_i = RP/D_i$. The homogeneous steady-state (u_0,v_0) of the above equation is the positive solution of

$$f(u,v) = 0, \quad g(u,v) = 0. \tag{4.7}$$

Using standard linear stability theory (Dillon *et al.*, 1994; Hunding, 1980), it can be shown that the stability of the above steady state is governed by the nature of the eigenvalues λ satisfying the following characteristic equation:

$$\lambda^2 + \lambda\,[\kappa_{nl}^2(1+d) - \gamma\,(f_u+g_v)] + [d\kappa_{nl}^4 - \gamma\,(df_u+g_v)\kappa_{nl}^2 + \gamma^2(f_u g_v - f_v g_u)] = 0. \tag{4.8}$$

The steady state will be unstable with respect to spatial disturbances if $\mathrm{Re}(\lambda) > 0$. The parameters κ_{nl} are such that they satisfy the eigenvalue problem

$$\nabla^2\psi + \kappa^2\psi = 0, \quad r \in \Omega \tag{4.9}$$

subject to

$$\frac{\partial \psi}{\partial n} + \rho\psi = 0, \quad r \text{ on } \partial\Omega. \tag{4.10}$$

Since we are considering the production of the GPF and GIF within a sphere, then the eigenfunctions ψ are given by

$$\psi_{nlm}(r,\theta,\phi) = j_n(\kappa_{nl}r)Y_{nm}(\theta,\phi) \tag{4.11}$$

where $j_n(\kappa_{nl}r)$ are the spherical bessel functions and $Y_{nm}(\theta,\phi)$ are the (surface) spherical harmonics. From the boundary condition at $r = 1$ the wavenumbers κ_{nl} satisfy

$$\kappa_{nl} j_n'(\kappa_{nl}) + \rho j_n(\kappa_{nl}) = 0. \tag{4.12}$$

It is well known that for certain conditions on the reaction kinetics and $d > 1$ (Dillon *et al.*, 1994; Murray, 1982) there exists a range of unstable wavenumbers $\kappa_1^2 < \kappa^2 < \kappa_2^2$ for which $\mathrm{Re}(\lambda) > 0$. Within this unstable range $\mathrm{Re}(\lambda)$ will have a maximum which indicates a fastest growing mode which will eventually dominate over time. This will give rise to a spatially heterogeneous pattern.

5. RESULTS

From the results of the previous section we assume that all conditions necessary for diffusion driven instability to occur are satisfied and that there exists a dominant, fastest growing mode. As stated previously we also assume that the reaction kinetics are of a cross

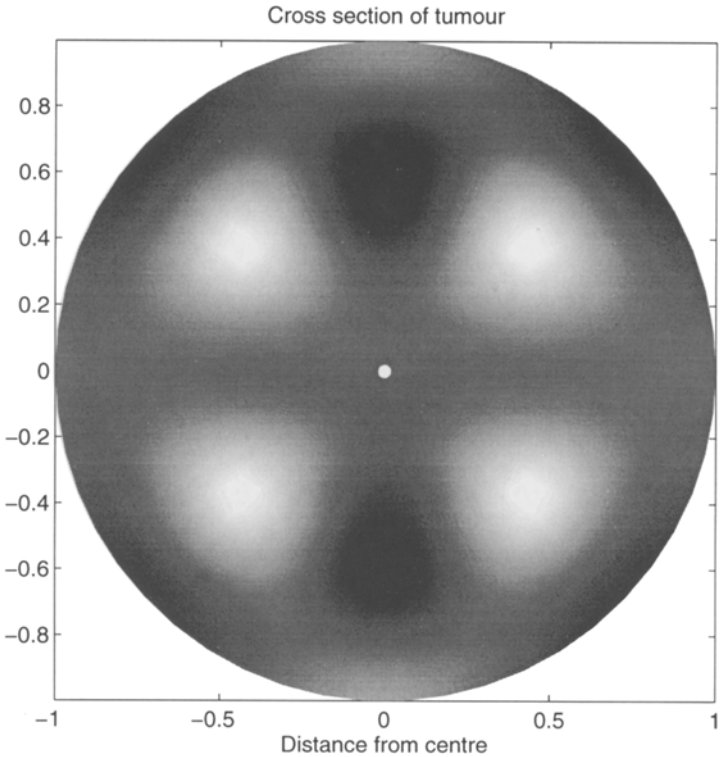

FIg.5. A cross section of a solid tumour showing the prepattern generated when the dominant mode is $j_4(\kappa_{42}r)Y_{42}(\theta,\phi)$, with $\kappa_{42} = 9.84$. The light regions are those where the local concentration of a growth promoting factor is high. It is from these regions that recruitment of the quiescent cells into the proliferating compartment is proposed to take place.

activator-inhibitor mechanism i.e. regions of high concentration of GPF correspond to regions of low concentration of GIF and vice versa (Dillon *et al.*, 1994). The following figures illustrate what relevance this could have for the growth of solid tumours. We denote the wavenumber of the fastest growing mode by κ^*_{nl} where from (4.12) we have

$$\kappa^*_{nl} j'_n(\kappa^*_{nl}) + \rho j_n(\kappa^*_{nl}) = 0 \tag{5.1}$$

We now give an estimate for the variable $\rho = RP/D$. In Chaplain & Britton (1993), Chaplain *et al.*, (1994) (and references therein) it was demonstrated that reasonable estimates for a range of values for the diffusion coefficient D and the permeability P were 10^{-6}–10^{-8} cm^2s^{-1} and 10^{-5}–$10^{-6}cms^{-1}$ respectively. The radius of an avascular multicell spheroid is at most a few millimetres i.e. of the order 10^{-1}–10^{-2}cm. Hence within this range of parameters we can choose $\rho = 1$ enabling us to use standard tables to estimate κ^*_{nl}. For illustrative purposes only, figure 5 shows a cross-section of a solid tumour in which the dominant mode prepattern is $j_4(\kappa_{42}r)Y_{42}(\theta,\phi)$, with $\kappa_{42} = 9.84$. This situation is illustrative of growth factors being produced throughout the tumour. As is clearly seen from the figure, there are regions in the interior of the tumour where the concentration of the GPF is high and the GIF low (the light regions). These regions are situated in the area of the interior of the tumour where the quiescent cells are likely to be situated. It is known that these quiescent cells can be recruited to repopulate the proliferating compartment (Durand, 1990; Sutherland, 1988; Wibe *et al.*, 1981). From figure

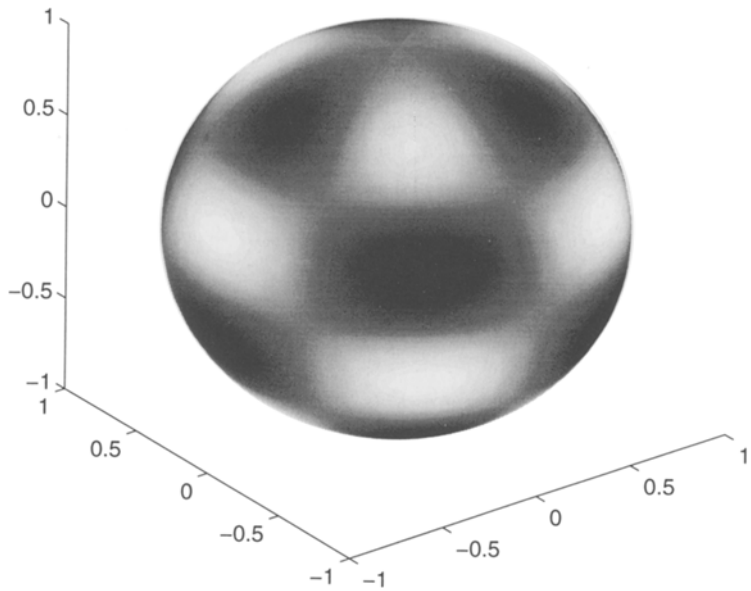

Fig.6. Prepattern observed on the surface of a solid tumour when the dominant mode is $Y_{63}(\theta,\phi)$. Once again the light regions are those in which the concentration of a growth promoting factor is high. It is from these regions that the columns of invading cells are expected to emerge when exophytic growth is taking place. These regions are also expected to influence the local environment through their secretion of growth factors.

5 we suggest that a possible mechanism for this is that the quiescent cells are firstly stimulated into proliferating in those regions of high GPF and are then recruited into the proliferating compartment.

If we now consider growth factors being produced only by the live cells at the surface of the tumour, then the problem is essentially a 2-dimensional one in the variables θ and ϕ. The eigenfunctions are simply the surface harmonics $Y_{nm}(\theta,\phi)$ and the wavenumbers in this case are given by $\kappa_n^2 = n(n+1)$. In this case zero flux boundary conditions are assumed. Figure 6 illustrates the case when the dominant mode is given by $Y_{63}(\theta,\phi)$. As can clearly be seen once again there are regions on the surface of the tumour where the concentration of the GPF is locally high (dark regions). It is a well-known feature of carcinomas that they invade the surrounding local tissue with columns of cells projecting from the central mass (Gimbrone *et al.*, 1974). We suggest that while in its avascular dormant state a pre-pattern such as is illustrated in figure 6 is set up. Once vascularized the cells which are in regions of high GPF will begin to invade the local tissues. A prepattern of this type is also consistent with the observation that tumours can manipulate the local environment by secretion of growth factors. Thus this prepattern will not only prime the tumour cells but will be affecting the local surrounding tissue as well thus facilitating invasion (Keski-Oja *et al.*, 1988).

6. DISCUSSION

In this paper we have considered two mathematical models which describe different aspects of solid tumour growth and development - angiogenesis and vascular growth. In the first part of the paper the model of Chaplain & Stuart (1993) was modified, and improved qualitative results were obtained with simulations in 1 spatial dimension. The model was then extended to 2 spatial dimensions in order to take into account explicitly the key features of angiogenesis of anastomosis and secondary sprout formation (branching). The simulations show that the basic assumptions of the original model can capture most of the salient features in 2 spatial dimensions with only slight modifications necessary. However some further points of clarification remain. It was found difficult to simulate two capillary sprout tips moving towards each other (in opposite directions) and fusing together. The features captured by the model are on a rather coarser level. This is perhaps to be expected since certain simplifying assumptions have been made and certain biological details omitted. It is known for instance that the EC secrete chemicals which may give rise to haptotaxis (motion up a gradient of increased adhesiveness). Including haptotaxis within the model would not be difficult to do from a modelling point of view but would increase the computational time for the simulations drastically since several chemicals would perhaps need to be considered. Also we note that in simulating the brush-border effect, we have not prescribed the precise *mechanism* which gives rise to this event, only assumed that it occurs and incorporated appropriate term in the equation which accounts for this. Investigation of precise mechanisms which give rise to the brush-border and also to the side-branching is a possible line of further research. It may be that a combination of deterministic and stochastic modelling is necessary.

In the second part of the paper we applied standard reaction-diffusion theory (Turing-type models) to a novel situation - that of the growth of solid tumours e.g. carcinoma. We have shown that the spatially heterogeneous patterns which arise in the case

of a spherical geometry may play a part and help to explain certain observed phenomenon in carcinoma and multicell spheroids i.e. the characteristic invasive pattern and the recruitment of quiescent cells into the proliferating population. We note that similar results can be obtained for other quasi-spherical geometries which may also be appropriate for solid tumours e.g. oblate spheroid, prolate spheroid, ellipsoid (cf. Hunding, 1983; Hunding, 1984). Of course there are many other factors which are involved in tumour growth of this type e.g. nutrient supply, and which are also very important. We certainly do not claim that Turing systems can provide a complete answer in this particular case, but rather may be an important part of the complex overall mechanisms governing solid tumour growth.

We note that there are several features of solid tumour growth which lend themselves naturally to the consideration of a Turing-type model. There are actually present in this system chemicals which both promote and inhibit growth of cells. The time scale for the growth of a solid tumour is very slow in comparison to the time needed for a diffusing substance to reach steady state concentration hence there will be a genuine pre-pattern. There also appears to be a natural critical domain size in this system i.e. the size of carcinoma in its diffusion-limited, avascular state. While in this state no invasion can take place but once vascularized rapid exophytic growth does occur.

We also note that many of the standard problems inherent in Turing models regarding robustness i.e. the same pattern must be repeated faithfully time and time again with the requirement that only a small number of patterns are selected in a robust and controlled manner, are not present for solid tumour growth. The observed 'invasion patterns' of *in vivo* carcinoma vary greatly from individual to individual. It is also well known that cellular heterogeneity is not only observed within an individual tumour/multicell spheroid (Sutherland, 1988; Wibe *et al.*, 1981) but also varies from tumour to tumour. Also it is worth pointing out that the complex process of invasion is dependent upon many factors such as the actual make up of the surrounding tissue. In view of the latter point regarding the surrounding tissue this may well necessitate a reconsideration of the boundary conditions involved which may lead to even more complex patterning (Dillon *et al.*, 1994).

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