

A Mathematical Model for the Diffusion of Tumour Angiogenesis Factor into the Surrounding Host Tissue

M. A. J. CHAPLAIN AND A. M. STUART

*School of Mathematical Sciences, University of Bath, Claverton Down,
Bath BA2 7AY, UK*

[Received 18 May 1991 and in revised form 6 September 1991]

Unless they are furnished with an adequate blood supply and a means of disposing of their waste products by a mechanism other than diffusion, solid tumours cannot grow beyond a few millimetres in diameter. It is now a well-established fact that, in order to accomplish this neovascularization, solid tumours secrete a diffusable chemical compound known as tumour angiogenesis factor (TAF) into the surrounding tissue. This stimulates nearby blood vessels to migrate towards and finally penetrate the tumour. Once provided with the new supply of nutrient, rapid growth takes place. In this paper, a mathematical model is presented for the diffusion of TAF into the surrounding tissue. The complete process of angiogenesis is made up of a sequence of several distinct events and the model is an attempt to take into account as many of these as possible. In the diffusion equation for the TAF, a decay term is included which models the loss of the chemical into the surrounding tissue itself. A threshold distance for the TAF is incorporated in an attempt to reflect the results from experiments on corneal implants in test animals. By formulating the problems in terms of a free boundary problem, the extent of the diffusion of TAF into the surrounding tissue can be monitored. Finally, by introducing a sink term representing the action of proliferating endothelial cells, the boundary of the TAF is seen to recede, and hence the position and movement of the capillaries can be indirectly followed. The changing concentration gradient observed as the boundary recedes may offer a possible explanation for the initiation of anastomosis. Several functions are considered as possible sink terms and numerical results are presented. The situation where the tumour (i.e. the source of TAF) is removed is also considered.

Keywords: tumour angiogenesis factor; endothelial cells; free boundary; anastomosis.

1. Introduction

The progress and development of a solid tumour from a small dormant mass of cells, a few millimetres in diameter, into an invading metastatic cancerous growth, depends upon its ability to induce endothelial cells of neighbouring capillaries in the surrounding tissue to sprout towards and eventually penetrate the tumour, thus providing it with an adequate blood supply and microcirculation. Unless this neovascularization is achieved, the solid tumour will remain in a diffusion-limited avascular state (i.e. without its own blood supply and network of blood vessels). During this initial avascular phase of its growth, nutrients are obtained and waste products are disposed of via diffusion processes alone. Cells at the centre of the tumour are starved of vital nutrients and so die. A central necrotic core is formed, surrounded by a thin outer layer of live, proliferating cells. Avascular nodules can

be cultivated in the laboratory (Folkman, 1976) or can be found *in vivo* (carcinomas *in situ* being a good example). Models describing this avascular growth can be found in, for example, Greenspan (1976), Chaplain (1990), and Adam & Maggelakis (1990), and references therein.

It is now a well-established fact that, in order to initiate and make the transition from the avascular phase to the vascular phase, solid tumours secrete a diffusible chemical compound known as *tumour angiogenesis factor* (TAF) into the surrounding host tissue and extracellular matrix (ECM). Since initial research into the nature and effects of TAF by Folkman in the early 1970s, much subsequent work has been carried out. It has been demonstrated that TAF is found in tissues and parts of the body other than those in the vicinity of the tumour itself. For example, in blood serum (Lisniak & Sopotinskaia, 1989; Pawlikowski *et al.*, 1989), in cerebrospinal fluid (Romberger *et al.*, 1990), in pleural and peritoneal fluids (Deshpande & Shetna, 1989), in haematoma capsules (Nakamura & Tsubokawa, 1989), in ascitic fluid (Mills *et al.*, 1990), in the kidney (Bard *et al.*, 1986), in ECM (Reilly & McAuslan, 1988), and in tissue and urine (Shahabuddin *et al.*, 1984). Other related angiogenic compounds and growth factors are also known to exist (Shahabuddin & Kumar, 1983; Shahabuddin *et al.*, 1985; Baird & Walickē, 1989). The processes of neoangiogenesis and neovascularization have also been extensively studied and the sequence of events which takes place during the formation, growth, and development of the capillaries is now well documented. However, many questions still remain unanswered, and there are still parts of the process which are either not fully understood or reasons for their happening are unknown (cf. Paweletz & Knierim, 1990). Currently, several angiogenic factors have been fully purified, their amino acid sequences determined and their genes cloned (Strydom *et al.*, 1985; Folkman & Klagsbrun, 1987; Deshpande & Shetna, 1989). The current literature on the subject is extensive (see e.g. the reviews of Folkman & Klagsbrun, 1987, and Paweletz & Knierim, 1990).

Research to date has led to the development of four main techniques for studying the various stages involved during angiogenesis.

- (i) Implantation of a section of solid tumour into the cornea of various test animals such as the rabbit (Gimbrone *et al.*, 1974) or mouse (Muthukkaruppan *et al.*, 1982). The dorsal airsac of the rat (Folkman *et al.*, 1971) and the cheek pouch of a hamster (Warren & Shubik, 1966; Eddy & Casarett, 1973) have also been used as implantation sites.
- (ii) Biocompatible polymers which release angiogenic factors in a sustained manner *in vivo* have been developed (Langer & Folkman, 1976).
- (iii) Observing the effect partially purified fractions from tumour extracts have on the chick embryo chorioallantoic membrane with regard to angiogenic activity (Ausprunk *et al.*, 1974; Klagsbrun *et al.*, 1976; Ishiwata *et al.*, 1988).
- (iv) Vascular endothelial cells have been cultured from various sites of the body, e.g. the umbilical vein (Jaffe *et al.*, 1973), the aorta (Birdwell *et al.*, 1977), capillaries (Madri & Pratt, 1986), and used to guide the purification of endothelial cell growth factors.

These above techniques have made it possible to observe the following sequence of events taking place during the process of angiogenesis. In response to the

angiogenic stimulus (i.e. the TAF), which has been secreted into the surrounding host tissue by the tumour cells, the endothelial cells of neighbouring capillaries first release proteolytic and collagenolytic enzymes that degrade and disintegrate the basal lumina and intercellular matrix through which they must move. They then migrate towards the solid tumour, the source of the angiogenic stimulus. Solid sprouts are formed as the endothelial cells elongate and align with one another. As the cells immediately behind those at the tip proliferate and divide, the lumen is pushed forward and the development of inter- and intralumina occurs. Cell proliferation at the tip also increases the sprout length. Anastomosis (i.e. the process whereby reconnections and fusions form a closed network) occurs between the sprouts to form loops which connect with the blood circulation. Pericytes appear at the base of the loops and the endothelial cells form a basal lamina. New sprouts can now grow from the loops, thus continuing the angiogenic process until the endothelial cells finally penetrate the tumour. Neovascularization now takes place, and the tumour can then continue to grow. During this vascular phase of growth, invasion of the surrounding tissue by tumour cells and metastasis may now take place. A comprehensive description of all the above events can be found in the extensive review of Paweletz & Knierim (1990). There are thus three main events which go to make up the neoangiogenesis after the release of TAF.

- (1) degradation of the basement membrane by enzymes.
- (2) migration of the endothelial cells.
- (3) proliferation of the endothelial cells.

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.

Although neoangiogenesis may initially appear to be a particularly insidious facet of tumour growth, this apparent strength has been exploited in an attempt to control, or even stop altogether, any subsequent growth by developing an *anti-angiogenesis* strategy (Folkman, 1972). Drugs have been sought which act in such a way as to prevent the formation of any new capillary growth (Langer *et al.*, 1980; Gross *et al.*, 1981). Indeed the clear differences between normal blood vessels and those present in neovascularized tumours (Kumar *et al.*, 1985; Erroi *et al.*, 1986) are now being exploited in order to develop drugs which can recognize and distinguish between normal tissue and the solid tumour. This specific targeting of the tumour itself has the potential to be of great value for patient chemotherapy (Willmott *et al.*, 1991).

The objective of this paper is to describe and evaluate a mathematical model for the diffusion of the TAF into the surrounding tissue and thereby to determine its effect on cell migration and proliferation; it is hoped that the results obtained will

stimulate further investigation of the TAF concentration profiles in the external tissue. In the following section, we describe the model which is formulated as a free boundary problem for the TAF concentration. New features that are included in the model are the consideration of finite boundaries and a critical distance between the tumour/tumour implant and the neighbouring capillary vessels (e.g. in the limbus), a natural decay term for the TAF and a sink term for TAF, *modelling the action of proliferating endothelial cells*. Section 3 contains a description and discussion of the results obtained from numerical simulation of the model, and in Section 4 various concluding remarks are made. Finally, there are three appendices containing various mathematical details omitted from the main text.

2. The mathematical model

In this section, we present a theoretical mathematical model for the diffusion of TAF into the surrounding tissue in which we attempt to reflect all of the main events associated with angiogenesis described in the introduction and offer an explanation for the occurrence of anastomosis. Attention is principally focused on the concentration of TAF and its profile in the external tissue *after* it has been secreted into the surroundings by the tumour. We seek to model the changes in its concentration as the endothelial cells migrate toward the tumour, forming capillary sprouts and undergoing mitosis. Thus, we hope that this paper in some sense provides a link between two previous models dealing with tumour angiogenesis and neovascularization: those of Balding & McElwain (1985) and Chaplain & Sleeman (1990). In the former the modelling of the formation and growth of the capillaries was undertaken based on the fungal growth model of Edelstein (1982), while in the latter attention was focused primarily on the production of TAF within the tumour *prior* to its secretion into the host tissue. This utilized the recent results of Sekiya *et al.* (1986), Oosaki *et al.* (1987) and Lisniak & Sopotinskaia (1989), linking TAF concentration with tumour size and blood vessel formation and growth.

The model is divided into two phases in order to take into account the experimental situation both *in vitro* and *in vivo*. In the *first phase*, TAF is secreted by the tumour from the thin layer of live cells at the tumour boundary and diffuses towards the limbal vessels. Following the findings and experiments of Gimbrone *et al.* (1974) and Muthukkaruppan *et al.* (1982) (where solid tumour extracts were placed at distances of 1–3 mm from the limbal vessels in the cornea of test animals) and Folkman (1976), and also in view of the nature of solid tumours *in situ*, we consider finite boundaries for the extent of the TAF into the surrounding tissue, and these finite boundaries are allowed to advance or recede according to the behaviour of the TAF and the action of the proliferating endothelial cells on the TAF. As we have seen in the previous section, TAF has been found in tissues and parts of the body other than those in the vicinity of the tumour. Also, whenever the tumour extracts are removed (cf. Gimbrone *et al.*, 1974), the capillary sprouts are seen to regress. In modelling the regression of the tips, Balding & McElwain (1985) assumed that the TAF concentration level had decayed to zero. Thus, in this first phase of the model, a decay or sink term is included in a diffusion equation for the TAF concentration. The inclusion of this term models accurately the experimental evidence and observations. We note

that the recent model of wound healing via a reaction–diffusion mechanism of Sherratt & Murray (1990) also includes a natural decay term for the chemoattractant. This is similar in nature to, and plays a similar role as, the TAF. We assume that this first phase of the problem continues, with the TAF being secreted, until a steady state has been reached. At steady state, the TAF extends a finite distance into the external tissue and does not penetrate it any further. This models the situation and experimental results of Gimbrone *et al.* (1974) (see also Ausprunk & Folkman, 1977, and Balding & McElwain, 1985), where a critical distance for the TAF was found to exist, and also the hypothesis of Folkman (1976), where a parallel was drawn between the finite extent oxygen can diffuse into tissue and a proposed similar distance for TAF (see also Folkman & Klagsbrun, 1987). The critical distance is given in terms of the parameters involved in the model. Gimbrone *et al.* (1974) found that no vascularization of the tumour occurred or that the time for vascularization was substantially increased (3–4 weeks as opposed to 10–12 days) when the tumour implant was placed at a distance of more than 2.5 mm from the limbal vessels.

Once a steady state has been reached, two possibilities arise. The first is that the distance between the tumour and the neighbouring vessels is below the critical threshold distance (Gimbrone *et al.* 1974), and so the endothelial cells cannot react to the angiogenic stimulus, and thus no cell migration and sprouting takes place. The second possibility, and the one which we shall concentrate upon, is that the tumour is within the critical distance and hence capillary growth can take place. This constitutes the *second phase* of the model. As described in the introduction, after degradation of the basement membrane, the initial response of the endothelial cells is to begin to migrate towards the source of angiogenic stimulus. Cells subsequently begin to proliferate and capillary sprouts are formed at a later stage. Initially, cell proliferation is seen only in the area of the parent vessel at the base of the outgrowing capillaries (cf. Paweletz & Knierim, 1990). However, once the capillary sprouts have formed, it is only cells at the tips of the sprouts that are actively migrating and reproducing. Ausprunk & Folkman hypothesized that the reason for this *restricted proliferation* was that these cells or vessels at the sprout tip 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. In order to account for this behaviour, we thus *incorporate a further sink term* for the TAF which comes into effect during this second phase of the model once the threshold distance has been reached. This has the effect of forcing the boundary of the TAF to recede towards the tumour. Thus, the external tissue continues to absorb the TAF, but now *so also* do the proliferating endothelial cells which initially may appear either near the parent vessel or at the sprout tips. The boundary, which marks the depth of penetration in the steady state, recedes towards the tumour surface as the sprouts begin to grow and the cells act as sinks. The problem is thus to track the movement of the boundary of the TAF, hence determining the distribution of the TAF concentration as a function of time. In the model, we consider several possible sink functions which could describe the (spatially restricted) action of the proliferating endothelial cells. Since the results are qualitatively the same in each particular case, we give numerical solutions for one possible choice. By following the movement of the boundary of the TAF as it recedes, we also have a way of (*indirectly*) tracking the movement of the capillary

tips as they make their way across the extracellular matrix *moving up the TAF concentration gradient* towards the source of the angiogenic stimulus, the solid tumour itself. As this process continues, a second steady state for the TAF is reached. This corresponds well with the occurrence of an important but poorly studied event of angiogenesis—that of *anastomosis*. Initially, as we have seen, the capillary sprouts which arise from their parental vessels (e.g. the limbal vessels of the cornea) grow in a more or less parallel way to each other. They then incline towards each other *at a definite distance* from their origin. It is not known why anastomosis occurs *only* at a definite distance from the parental vessels or what signal ‘decides’ whether elongation of an existing loop or formation of new sprouts is realized. We hope that the results which arise from our model with regard to the *changing TAF concentration gradient* may shed light upon this matter. Finally, we consider the effects of removing the tumour (i.e. the source of angiogenic stimulus) altogether. In this situation, changing concentration gradients may also play a role, helping to explain the regression of the capillary sprouts.

Initially, our model is formulated in a general setting with an arbitrary geometry, as is the case *in vivo*. Subsequently, following the example of previous papers on the subject (Balding & McElwain, 1985; Chaplain & Sleeman, 1990), we specialize to geometries which are more amenable to straightforward numerical analysis but which capture the qualitative features of the model; we also present some numerical results and compare them with those of Balding & McElwain (1985). There is little experimental data on the profile of TAF concentration in the external tissue, and so we hope that this paper may stimulate some research into this aspect of angiogenesis, thus providing a more complete picture of this important process.

We begin with some definitions for our model. Let Ω be an open bounded region in \mathbb{R}^n denoting the exterior of the solid tumour, which we take to have a smooth boundary $\partial\Omega$. We suppose that TAF, with concentration denoted by $c(x, t)$, is being secreted by the solid tumour and diffuses into Ω . The concentration of TAF at the tumour surface, where there is assumed to be a thin layer of live proliferating cells (cf. Greenspan, 1976; Chaplain & Sleeman, 1990), is maintained at a constant level c_b . In this first phase the TAF is absorbed *only* by the external tissue, while in the second phase absorption occurs not only through the external tissue but *also* by the endothelial cells at the tips of the sprouts which have formed as a result of the chemotactic stimulus of the TAF. The precise forms of the absorption rate by the tissue and the sink function, representing the action of the proliferating endothelial cells, will be discussed and given later.

Phase 1

The TAF is assumed to diffuse with rate constant D and to be absorbed by the surrounding host tissue at a rate $g(c)$ (cf. oxygen diffusion in muscle tissue; Galib *et al.*, 1981). On the boundary $\partial\Omega$ of the tumour, the concentration of TAF is assumed to be maintained at a constant level c_b (cf. Balding & McElwain, 1985). The free boundary determining the furthest extent of the TAF outside the tumour into the surrounding tissue is denoted by Γ and, at that boundary, the concentration and gradient of concentration are taken to be zero (cf. Crank & Gupta, 1972). The free

boundary is allowed to move as the TAF advances or recedes into the tissue; such free or moving boundary problems are described in Crank (1984). We also present results (which show no qualitative differences) for the case where the TAF concentration here is taken to be nonzero. Under these assumptions, the concentration satisfies the following equation:

$$\frac{\partial c}{\partial t} = D\nabla^2 c - g(c), \quad (x, t) \in \Omega \times (0, T),$$

together with the boundary conditions

$$c = c_b \quad \text{on } \partial\Omega, \quad c = \nabla c = 0 \quad \text{on } \Gamma(t).$$

and the initial conditions

$$c(\mathbf{x}, 0) = c_0(\mathbf{x}), \quad \Gamma(0) = \Gamma_0.$$

Here ∇c is in the direction normal to the boundary of Γ .

We first of all specialize to a one-dimensional geometry (cf. Balding & McElwain, 1985; Chaplain & Sleeman, 1990). This will enable the steady state to be calculated in a straightforward manner and also facilitate the numerical solution of the equation when required. Thus, in this phase of the model, we take the boundary of the tumour to be situated at $x = 0$ and the capillary vessels (e.g. in the limbus) to be a distance L from the tumour. The free boundary $\Gamma(t)$ is denoted by $s(t)$. The above equations now become

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} - g(c), \quad (x, t) \in (0, s(t)) \times (0, T), \tag{2.1}$$

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

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

Various choices are possible for the function $g(c)$, which represents the natural loss of TAF to the external tissue. However, we shall concentrate on the simple choice $g(c) = m$, where m is a constant (cf. Galib *et al.*, 1981) since it captures the right qualitative features. This gives a linear decay with time in the absence of the diffusion term $D\nabla^2 c$. We also briefly examine the choice $g(c) = kc + m$, which gives exponential decay with time in the absence of the diffusion term (cf. Sherratt & Murray, 1990).

Equations (2.1)–(2.3) are assumed to hold until a steady state is reached. Thus it is important to determine the maximum extent of TAF from the boundary of the tumour to discover whether the critical distance L is reached. To do this, we determine the extent of the free boundary s in the steady-state configuration which it is assumed to approach as the diffusion process governed by (2.1)–(2.3) occurs. The above equations then reduce to the following problems for the concentration $c(x)$ and the extent of the boundary s :

$$D \frac{d^2 c}{dx^2} = m, \quad c(0) = c_b, \quad c(s) = \frac{dc}{dx}(s) = 0.$$

This has solution

$$c = \frac{m}{2D}(x - s)^2, \quad s = (2Dc_b/m)^{1/2}. \quad (2.4)$$

From a qualitative analysis of the above solution, we can see that we have the following features in the steady-state solution.

(i) The larger the value of c_b , the greater the extent of TAF into the host tissue, which is not unexpected. A high value for c on the boundary of the tumour also fits in well with the proposed hypothesis of Chaplain & Sleeman (1990). Utilizing the experimental results of Sekiya *et al.* (1986), Oosaki *et al.* (1987), and Lisniak & Sopotinskaia (1989), which showed there to be a direct correlation between TAF activity, TAF concentration level, and tumour size, Chaplain and Sleeman (1990) formulated a mathematical model which linked a critical tumour size to a critical TAF concentration level. Thus, we assume that the tumour is larger than the critical size and is secreting TAF at a higher concentration than the critical level. This is also consistent with the experimental results of Gimbrone *et al.* (1974).

(ii) A large value of D also indicates that the TAF has penetrated the tissue to a large extent, while conversely a small value for D indicates a lesser penetration. Several TAFs with varying molecular weights have been isolated, and so we can see that different angiogenic factors will diffuse different distances (cf. Errol *et al.*, 1986; Sekiya *et al.*, 1986; Folkman & Klagsbrun, 1987; Ishiwata *et al.*, 1987; Oosaki *et al.*, 1987; Ishiwata *et al.*, 1988).

(iii) As m decreases, s increases and vice versa. Once again, we see that this gives the correct qualitative behaviour. We note that $s \rightarrow \infty$ as $m \rightarrow 0$; i.e. the smaller the absorption rate, the greater the extent of the TAF penetration. In the limit $m \rightarrow 0$, we retrieve the solution of Balding & McElwain (1985). At the opposite extreme, $s \rightarrow 0$ as $m \rightarrow \infty$, which can be thought of as modelling the effect of an *anti-angiogenesis strategy*, whereby the effect of the TAF is countered by a drug which neutralizes it.

We note that a similar solution, with similar qualitative features, is obtained when the decay term $g(c) = kc + m$ is used:

$$c = \frac{m}{k} [\cosh(k/D)^{1/2}(x - s) - 1], \quad s = (D/k)^{1/2} \cosh^{-1}(kc_b/m + 1).$$

Solutions for circular and spherical geometries can be found in Appendix 1.

Phase 2

At steady state, the extent of the TAF penetration into the surrounding tissue is given by (2.4). There are thus two possibilities which can now arise. It is possible that the tumour is situated at a distance *less* than the critical distance for stimulating angiogenesis (Gimbrone *et al.*, 1974), and so consequently s is not large enough for the TAF to have reached the limbus and act as a chemoattractant for the endothelial

cells. Alternatively the tumour may be sufficiently close for s to have just reached or be beyond the boundary of the neighbouring vessels (e.g. at the limbus), thus providing a suprathreshold concentration of TAF to the vessels and allowing the endothelial cells to be stimulated chemotactically by the TAF (Gimbrone *et al.*, 1974). The process of angiogenesis can then begin. We now focus our attention on this latter possibility and assume for simplicity that the free boundary s has just reached the limbal vessels, so that we may write

$$L = s = (2Dc_b/m)^{1/2}. \quad (2.5)$$

Having degraded their basal lamina, the endothelial cells migrate across the extracellular matrix, moving up the gradient of TAF. Proliferation occurs some time after migration and, following the hypothesis of Ausprunk & Folkman (1977) and Balding & McElwain (1985), we assume that the *proliferating* endothelial cells act as sinks for the TAF. This necessitates the *inclusion of an extra sink term* in our equation to model the uptake of TAF by the proliferating cells and vessels in the sprouts, and as our model we take

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} - m - f(x/s, c), \quad (x, t) \in (0, s(t)) \times (0, T), \quad (2.6)$$

together with the boundary conditions

$$c = c_b \quad \text{on } x = 0, \quad c = \frac{\partial c}{\partial x} = 0 \quad \text{on } s(t), \quad (2.7)$$

and the initial conditions

$$c(x, 0) = c_0(x), \quad s(0) = s_0 = L. \quad (2.8)$$

The function $f(x/s, c)$ is the corrective sink term representing the action of the proliferating endothelial cells and the initial condition $c(x, t) = c_0(x)$ is taken to be the configuration of the TAF boundary in its steady state from phase 1 given by (2.4).

In formulating the function representing the sink term in equation (2.6), we have incorporated two aspects—first, the dependence of the rate at which the TAF is being removed by the cells and vessels in the capillary tips on the TAF concentration itself; second, the fact that this removal rate is *spatially dependent* (cf. Ausprunk & Folkman, 1977; Balding & McElwain, 1985; Paweletz & Knierim, 1990) and occurs predominantly near the capillary tips. The sink function f is thus proportional to the spatial concentration of the *proliferating* endothelial cells, as hypothesized by Ausprunk & Folkman (1977).

We assume that the sink term $f(x/s, c)$ may be written as the product of two functions: the first dependent only on the concentration c of TAF, and the second dependent only on the distance from the tumour as a proportion of distance of the free boundary from the tumour, x/s . Hence,

$$f(x/s, c) = p(c)q(x/s). \quad (2.9)$$

For $p(c)$ we consider three possible functions which might model the uptake rate of the TAF by the proliferating cells and which have been used in previous models of

this nature for the (nonlinear) uptake of chemicals by cells (cf. Lin, 1976; McElwain, 1978; Hiltman & Lory, 1983; Balding & McElwain, 1985; Murray, 1989).

(i) Michaelis–Menten kinetics:

$$p(c) = K_{\max}c/(K_n + c). \quad (2.10)$$

(ii) A logarithmic law:

$$p(c) = \chi_0/(1 + c). \quad (2.11)$$

(iii) A receptor kinetic law:

$$p(c) = \chi_0k/(k + c)^2. \quad (2.12)$$

To allow for the spatial dependence of f (i.e. to model the fact that this sink term should in some way be proportional to the *spatial concentration* of the *proliferating* endothelial cells in the capillary sprouts), we take

$$q(X) = \frac{d}{1 + d^2(a - X)^2}. \quad (2.13)$$

For d large, the function $q(X)$ is a continuous approximation to the delta-function $\delta(X - a)$ (cf. Adam, 1991a, b). Thus the removal of TAF occurs predominantly at and near $X = a$, that is, where $x = as(t)$. We assume that $0 < a \leq 1$; the choice $a = 1$ corresponds to the assumption that the boundary marking the maximum extent of the TAF coincides exactly with the position of the proliferating endothelial cells as they migrate across the extracellular matrix. The choice $a < 1$ corresponds to the assumption that the proliferating endothelial cells are closer to the tumour than is the boundary marking the maximum extent of the TAF into the external tissue. Both these cases are consistent with experimental observations. Initial endothelial cell proliferation has been observed both in the parent vessel (Paweletz & Knierim, 1990) and also at some distance from the parent vessel, at the tips of the capillary sprouts (Ausprunk & Folkman, 1977). Thus a choice $a = 1$ corresponds to the case whereby proliferating endothelial cells appear initially in the parent vessel and in the subsequent motion the extent of the TAF boundary coincides exactly with their position. A choice of $a < 1$ corresponds to the endothelial cells initially proliferating at some distance in from the parent vessel (e.g. at or near the capillary sprout tips), and then subsequently remaining ‘slightly ahead’ of the boundary marking the extent of the TAF into the external tissue, as the tips migrate towards the tumour. The precise choice of the value for a does not affect the qualitative results in this paper (i.e. the TAF boundary recedes in each case) and gives the model a degree of flexibility in the choice of the exact position of the sink term modelling the action of the proliferating endothelial cells. The important feature to capture is that the *qualitative* shape of the sink function f is spatially restricted, thus mirroring the profile of the proliferating endothelial cells.

For convenience and simplicity, we now nondimensionalize the variables in the previous equations (cf. Balding & McElwain, 1985). As reference variables, we choose the following:

- (i) reference TAF concentration: c_b , the value of the TAF concentration at the tumour boundary;
- (ii) reference length: L , the distance from the tumour boundary to the limbal vessels;
- (iii) reference time unit: $\tau = L^2/D$.

We thus define new variables:

$$\tilde{c} = c/2c_b, \quad \tilde{x} = x/L, \quad \tilde{s} = s/L, \quad \tilde{t} = t/\tau.$$

Dropping the tildes and noting that L is given by (2.5), equations (2.6)–(2.8) now become

$$\frac{\partial c}{\partial t} = \frac{\partial^2 c}{\partial x^2} - 1 - f(x/s, c), \quad (x, t) \in (0, s(t)) \times (0, T), \quad (2.14)$$

$$c = \frac{1}{2} \quad \text{on } x = 0, \quad c = \frac{\partial c}{\partial x} = 0 \quad \text{on } x = s(t) \quad (t \geq 0), \quad (2.15)$$

$$c(x, 0) = c_0(x) = \frac{1}{2}(x - 1)^2 \quad (0 \leq x \leq 1), \quad s(0) = s_0 = 1. \quad (2.16)$$

The sink function is also suitably nondimensionalized and the exact details of this are given in Appendix 1. Figure 1 shows the profile of the (dimensionless) sink function f , with $a = 1$ and $a = 0.8$, at time $t = 0$ and initial conditions given by (2.16) (the profile is similar in the subsequent evolution of the free boundary). In each case, the dependence of the function f on the *spatial concentration* of the proliferating endothelial cells is clearly shown. The removal of the TAF occurs along a small ‘band’ corresponding to the spatial distribution of the proliferating endothelial cells which agrees well with experimental evidence (cf. Paweletz & Knierim, 1989). We note that in approximating the delta-function, an exact zero value for the sink term outside this band of proliferating cells is not obtained. However, the actual nonzero values obtained are sufficiently small compared with the dimensionless ‘background’ tissue absorption rate of unity. Using the values described in the following results section (with $a = 1$), we have $f(0) = 0.008$, $f(0.25) = 0.016$, $f(0.5) = 0.04$, $f(0.75) = 0.17$, $f(0.9) = 1.1$. It is thus clearly seen that the nonzero values outwith those representing the endothelial cells are of at least an order of magnitude smaller than unity and hence can be neglected (this also remains true in the subsequent evolution of the free boundary). The results of solving the above system numerically are given in the following section. In the numerical simulations, we shall also modify (2.15) to allow a nonzero value for the concentration ($c = 0.5$) on $x = s(t)$.

Circular and Spherical Geometries

We also consider the case of the problem in two and three dimensions, since in the experiments carried out by Gimbrone *et al.* (1974) and Muthukkaruppan *et al.* (1982) the tumour implant grew as a flat circular mass of cells and, in consideration of the *in vivo* situation, it is realistic to consider three-dimensional geometries also. Once again in order to simplify the problem, we assume radial symmetry for both cases and assume that the tumour boundary is at a radial distance b ; we thus have

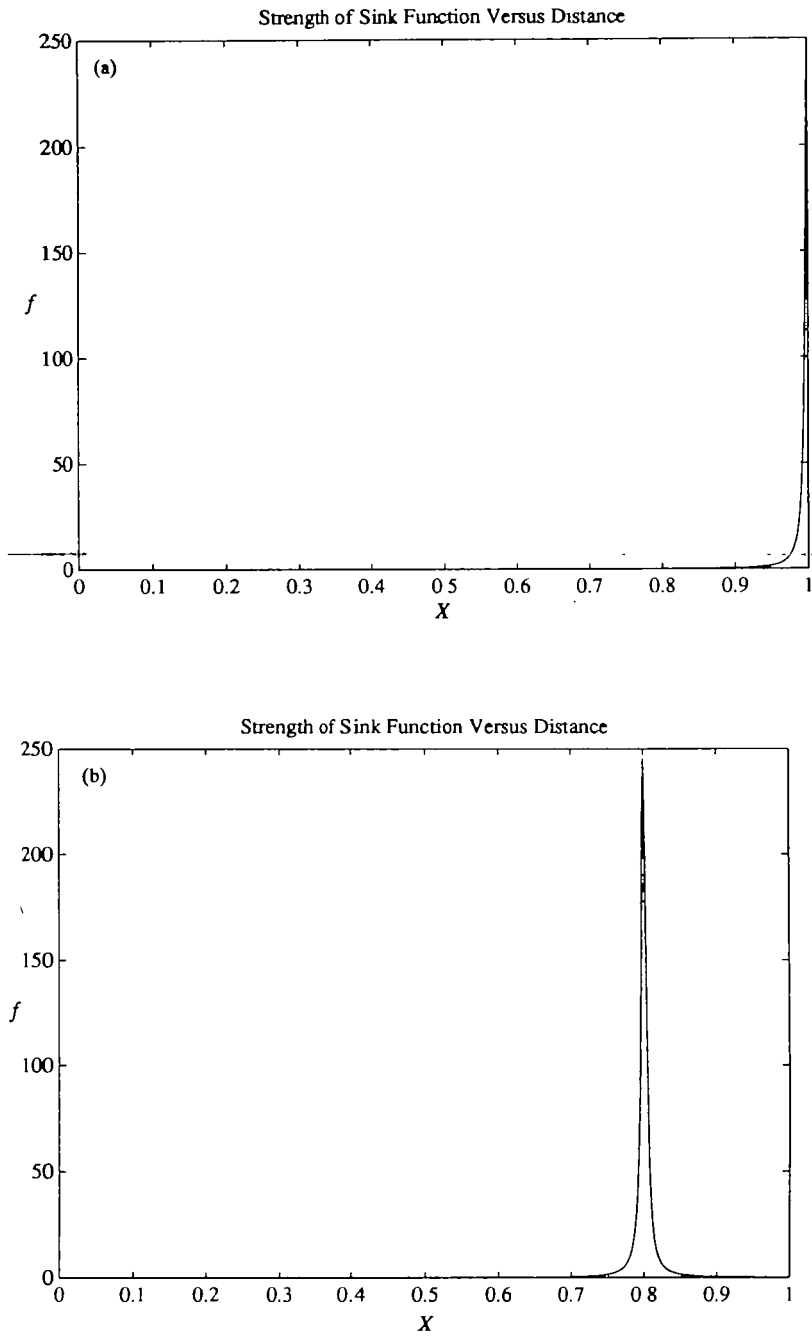


FIG. 1. (a) Profile of the sink function f representing the action of the proliferating endothelial cells ($a = 1$).
(b) Profile of the sink function f representing the action of the proliferating endothelial cells ($a = 0.8$).

the following system to solve:

$$\frac{\partial c}{\partial t} = \frac{\partial^2 c}{\partial r^2} + \frac{(n-1)}{r} \frac{\partial c}{\partial r} - 1 - f\left(\frac{r-b}{s-b}, c\right), \quad (x, t) \in (0, s(t)) \times (0, T) \quad (n = 2, 3), \tag{2.17}$$

$$c = c_b \quad \text{on } r = b, \quad c = \frac{\partial c}{\partial r} = 0 \quad \text{on } x = s(t) \quad (t \geq 0), \tag{2.18}$$

$$c(r, 0) = c_0(r) \quad (b \leq r \leq s(0)), \quad s(0) = s_0. \tag{2.19}$$

Removal of Angiogenic Source

When the tumour implant is removed, the TAF diffuses away naturally over a certain period of time (Balding & McElwain, 1985). The main result of this on the model is to change the boundary condition 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 $x = 0$, since, once the tumour has been removed, TAF is no longer produced. This now gives us the following equations:

$$\frac{\partial c}{\partial t} = \frac{\partial^2 c}{\partial x^2} - 1 - f(x/s, c), \quad (x, t) \in (0, s(t)) \times (0, T), \tag{2.20}$$

$$\frac{\partial c}{\partial x} = 0 \quad \text{on } x = 0, \quad c = \frac{\partial c}{\partial x} = 0 \quad \text{on } x = s(t) \quad (t \geq 0), \tag{2.21}$$

$$c(x, 0) = c_0(x) \quad (0 \leq x \leq 1), \quad s(0) = s_0. \tag{2.22}$$

In this case, the initial conditions $c_0(x)$ and s_0 are taken to be the steady-state profile obtained from the numerical solution of equations (2.14)–(2.16). Equations (2.20)–(2.22) are only a valid model for a short time after initialization because the assumption that the capillary tips and the position of the free boundary move together becomes invalid for this boundary condition modelling removal of angiogenic source. This is discussed further in Section 3.

All of the above equations are solved numerically and the results are presented in the following section.

3. Results

In this section, we present results from numerical simulations of the models (2.14)–(2.16), (2.17)–(2.19), and (2.20)–(2.22). The numerical method employed is based on a front-tracking finite-difference scheme and is described in Appendix 2. Throughout this section, the choice of $p(c)$ is given by (2.11), a logarithmic law. The results are qualitatively the same if either a Michaelis–Menten (2.10) law or a receptor kinetic (2.12) law is used for the choice of $p(c)$. The only differences are that the boundary denoting the maximum extent of the TAF into the external tissue at the second steady state changes slightly and the precise shape of the concentration profiles is also modified slightly.

In Appendix 3, it is proved that equations (2.14)–(2.16) have a steady solution and

that the boundary of this steady solution is closer to the tumour surface than the boundary of the steady solution from phase 1. This is to be expected and indicates that the capillary tips move towards the tumour during phase 2 of the angiogenic process and eventually reach an equilibrium position close to the tumour. Similar results can be proved for equations (2.17)–(2.19) and (2.20)–(2.22).

In nondimensionalizing the model, we followed Balding & McElwain and chose for our reference length L a value of 2 mm, while the value for τ was taken to be 14 days, the average time for vascularization to occur. This gives an approximate value of $3.3 \times 10^{-8} \text{ cm}^2/\text{s}$ or $0.29 \text{ mm}^2/\text{day}$ as a value for D .

Figure 2(a) shows the results obtained by solving (2.14)–(2.16) numerically and then plotting the profile of TAF concentration in the external host tissue at regular intervals of time with the function $p(c)$ as given above. The value $x = 0$ denotes the tumour surface, while the value $x = 1$ denotes the position of the parent vessel (e.g. the limbus). The value chosen for a here was 1. Figure 3(a) shows the results obtained when the value for a is taken to be 0.8. We can see that the results obtained in each case are qualitatively the same, with the boundary of TAF moving farther in when $a = 0.8$, as is to be expected. As explained in the previous section, a value of $a = 1$ denotes the *initial* appearance of proliferating endothelial cells at the parent vessel (e.g. the limbus) and then subsequently their position and the extent of the TAF coincide. A value of $a = 0.8$ denotes the *initial* appearance of the proliferating endothelial cells at or near the tips of the capillary sprouts, once these have formed. In the subsequent motion in this case, therefore, the capillary tips will be slightly ahead of the extent of the TAF boundary. The important feature in both cases ($a = 1$, $a < 1$) is that the boundary, marking the extent of TAF into the external tissue, recedes. Thus, both *proximal* and *distal* endothelial cell proliferation can be accounted for in the model (cf. Muthukkaruppan *et al.*, 1982). Figures 4(a) and 5(a) show the results obtained from (2.17)–(2.19) when circular (i.e. 2-D) and spherical (i.e. 3-D) geometries are considered, respectively. In each case, the tumour is assumed to have radius 0.25, at which the layer of proliferating tumour cells is situated. Thus $r = 0.25$ is the edge of the tumour. Figure 6(a) shows the results of having a nonzero boundary condition on the concentration $c(x, t)$ at $x = s(t)$.

In each case considered above, we can see that the qualitative result is the same—the TAF boundary recedes and moves towards the tumour, thus depriving the cells in the vicinity of the parent vessel of TAF and hence no mitotic figures are observed once the sprouts have appeared and a steady state has been reached. This corresponds well with the experimental observations and hypothesis of Ausprunk & Folkman (1977). As the TAF boundary recedes, we also note the *changes* in the TAF concentration *gradient*. The endothelial cells which are migrating in the sprout tips (Ausprunk & Folkman, 1977) may, through their position, detect these changes in gradients, and hence may provide a signal for the beginning of anastomosis. The steady state also occurs at a definite distance from the parent vessels and this is in agreement with the *in vivo* situation. We propose that it is this change in TAF gradient which may be responsible for initiating anastomosis and then the subsequent formation of the brush border of blood vessels (this phenomenon was reported by both Gimbrone *et al.* (1974), who noted that the initial sprouts were converted into

'dense vascular brushes' and Muthukkaruppan *et al.* (1982), who observed the formation of a 'brush border of red blood cells'). Indeed, after anastomosis, the whole angiogenic process repeats itself once more, with the formation of new capillary loops and sprouts from the anastomoses. These can now move up the *increased* TAF concentration gradient and penetrate the tumour which becomes vascularized.

Figures 2(b)–6(b) show the corresponding profiles of the farthest extent of TAF into the external tissue plotted against time, showing how the boundary recedes under the action of the proliferating endothelial cells which behave as sinks. In all cases, a steady state is reached in a dimensionless time of $t \approx 0.2$ – 0.3 , which corresponds to a real time of approximately 3–4 days. When the time for mitotic figures to appear is also taken into account (36 hours; cf. Paweletz & Knierim, 1990), we see that steady state is reached approximately 5 or 6 days after the TAF has reached the neighbouring vessels (in the limbus, for example) and stimulated the angiogenic process. This timescale is in good agreement with the experimental evidence for the appearance of sprouts and the onset of anastomosis. We also note that the average speed of the receding boundary (which may be used as an indication of the speed of the migrating cells and the sprout tips) is approximately 0.36 mm/day. Once again this compares favourably with the experimental data available.

Finally, Fig. 7 gives the profile of TAF concentration in the external tissue and the farthest extent of the boundary of TAF plotted against time, once the tumour has been removed altogether (these are obtained from the numerical solution of (2.20)–(2.22)). The initial configuration for the TAF concentration profile is taken to be the steady state reached in Fig. 2(a). As can be seen from Fig. 7(a), initially, the boundary of the TAF moves *towards the parent vessel* (e.g. the limbus) at the boundary $x = 1$, with corresponding decreases in the TAF concentration *gradient* and TAF concentration *level*. Just as the endothelial cells and capillary sprouts reacted to the initial chemotactic stimulus by moving up the TAF concentration gradient (which increased as the TAF boundary receded) so, as the concentration gradient falls with the removal of the tumour, the tips begin to recede in response to the *falling* gradient. Under the same assumption that the initial capillary tip movement towards the tumour could be indirectly monitored in Figs. 2(a)–6(a) by following the extent of the TAF boundary as it receded (producing an increased TAF concentration gradient), so from Fig. 7(a) we can indirectly follow the *initial* regression of the capillary tips via the motion of the TAF boundary. However, as can be seen from Fig. 7(a), at $t \approx 0.03$, the TAF boundary begins to recede towards the tumour, in this case with falling concentration gradient, and the above assumption about the relationship between the free boundary and the capillary tips is no longer valid. Hence our model breaks down for $t \approx 0.03$. The profile of the TAF and its free boundary can still be monitored as it decays to zero, using the model of Crank & Gupta (1972) for the oxygen diffusion problem, but the position of the capillary tips can no longer be monitored under the above assumption. At this stage of the problem, a new approach would have to be tried in order to achieve this and we do not concern ourselves with this problem here. It is hoped that this can be investigated in a future paper.

4. Conclusions

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 (both *proximal*, in the limbus, for example, and *distal*, at the tips of the capillary sprouts and the brush border of new blood vessels), and loop formation (anastomosis), to name a few. Despite much research and many advances, many questions still remain unanswered (cf. Paweletz & Knierim, 1990). To formulate a single mathematical model which would include all of these processes would be very difficult indeed. In this paper, therefore, we have chosen to focus our attention on the profile of the TAF concentration in the external host tissue, after it has been secreted by the tumour cells. This is a novel approach to modelling angiogenesis, and we have been able to include in the model several important features from the experimental observations which are biologically relevant but which have not previously been used in other models of this kind. These are:

- finite boundaries, i.e. finite distance between the tumour and the neighbouring vessels (e.g. the limbus);
- critical distance between tumour and neighbouring vessels;
- natural decay term for the TAF;
- sink term for TAF to model the action of the *proliferating* endothelial cells, whether initially seen in the parent vessel or at the sprout tips; a feature of this sink function is that it is proportional to the *spatial concentration* of the proliferating endothelial cells.

The model is, in essence, a *qualitative* one since any relevant experimental data (e.g. TAF concentration, rate of uptake of TAF by both the host tissue and the proliferating endothelial cells) is difficult to obtain. As we have already stressed in the preceding sections, it is the qualitative shape of the profile of the sink function f that is important and we note that this is also the case in other successful qualitative models where experimental data has been difficult to find (cf. Liotta *et al.*, 1977; Balding & McElwain, 1985). Indeed we hope that our results may stimulate research into measurement of those parameters which remain unknown in our model. However, when parameter values are chosen to correspond with the experimental data that is available, the results obtained in the model are in good agreement with those observed experimentally. In addition to this, the model also offers a solution to an important, unanswered question—a possible explanation for a crucial event which occurs during angiogenesis (*anastomosis*) arises naturally from the results of the model. Although this is a well-known and well-documented event in angiogenesis, little research has been carried out into what stimulus causes it and the reason why it always occurs at a definite distance from the parent vessels (e.g. limbal vessels) is still unknown. As we have seen, the results of the model suggest that a possible explanation could be to do with *changing TAF concentration gradients* and the formation of a second steady state for the TAF concentration profile, occurring at a *definite distance* from the parent vessels.

One weakness of the model is that it does not consider explicitly any equation for

the endothelial cell density or tip density, for example. However, as we have noted, information concerning these variables can be obtained *indirectly* from the concentration profile of the TAF in the external host tissue, while the underlying *relative simplicity* of the model is still retained. Encouraged by the results of the present model, a more comprehensive model is currently being developed which includes a population balance term for the endothelial cells. When coupled with the present model for the TAF concentration, this will give a better overall view of the complicated process of angiogenesis. We also hope that our (*theoretical*) results may stimulate and encourage experiments to investigate our hypothesis concerning anastomosis.

Glossary

Angiogenesis the formation of blood vessels arising during processes such as embryonic development and solid tumour growth.

Neoangiogenesis the process whereby blood vessels arise where vessels have previously existed, e.g. during solid tumour growth and wound healing.

Vascularization the formation of all types of vessel within a tissue which has never previously developed vessels.

Neovascularization the formation of new vessels in tissues in which previously existing vessels have stopped functioning completely or are no longer sufficient.

Appendix 1

Steady States from Phase 1

The steady-state solution in circular and spherical geometries, with radial symmetry, requires the solution of the following equation:

$$\frac{1}{r^n} \frac{d}{dr} \left(r^n \frac{dc}{dr} \right) = \frac{m}{D} \quad (n = 2, 3),$$

with boundary conditions

$$c = c_b \quad \text{on } r = a, \quad c = \frac{dc}{dr} = 0 \quad \text{on } r = R(t),$$

where $r = a$ is the tumour radius and $r = R(t)$ is the extent of the boundary of the TAF into the external tissue (here $R(t)$ corresponds to $s(t)$ in the one-dimensional problem).

For $n = 2$, this has the solution

$$c = \frac{m}{4D} [(r - R^2) + R^2 \ln(R^2/r^2)],$$

with

$$4Dc_b/m = (a^2 - R^2) + R^2 \ln(R^2/a^2).$$

For $n = 3$, we have

$$c = \frac{m}{6D} \left(r^2 + \frac{2R^3}{r} - 3R^2 \right),$$

with

$$\frac{6Dc_b}{m} = a^2 + \frac{2R^3}{a} - 3R^2.$$

Nondimensionalization

When nondimensionalized, the three functions given in equations (2.10)–(2.12) reduce to the following forms:

(i) Michaelis–Menten kinetics:

$$\alpha/(\beta + c), \quad \text{where } \alpha = K_{\max}/m, \quad \beta = K_n/2c_b.$$

(ii) Logarithmic law:

$$\gamma/(\delta + c), \quad \text{where } \gamma = \chi_0/2mc_b, \quad \delta = 1/2c_b.$$

(iii) Receptor kinetic law:

$$\varepsilon/(\zeta + c)^2, \quad \text{where } \varepsilon = \chi_0 k/4mc_b^2, \quad \zeta = k/2c_b.$$

The function $q(X)$ in the sink term in equation (2.13) is unaffected by the nondimensionalization since it involves only the ratio of x and s which are both scaled in the same way. In the numerical calculations, the value for d was chosen to be 250, while the parameters γ and δ were taken to be 3 and 1 respectively.

Appendix 2

In this appendix, we briefly describe the numerical method employed for the solution of the implicit free boundary problem (2.14)–(2.16). Similar methods are employed for the solution of the equations (2.17)–(2.19) and (2.20)–(2.22). The problem is termed implicit since there is no explicit equation for the evolution of $s(t)$ —see, for example, Crank & Gupta (1972) and Crank (1984). We can state the free boundary problem as follows: find $c(x, t) \in C^{2,1}((0, s) \times (0, \tau))$ and $s(t) \in C^1(0, \tau)$ satisfying

$$c_t = c_{xx} - m - f(x/s, c), \quad (x, t) \in (0, s(t)) \times (0, \tau), \quad (\text{A.1})$$

$$c(0, t) = c_b, \quad c(s(t), t) = c_x(s(t), t) = 0 \quad (0 < t < \tau), \quad (\text{A.2})$$

together with an initial condition on $c(x, 0)$, which determines $s(0)$.

The numerical method for the solution of (A.1, A.2) is based on a coordinate transformation. This idea was introduced for the Stefan problem (which is an explicit free boundary problem) by Landau (1950); its application to a problem involving implicit free boundaries is described in Stuart & Floater (1990). We introduce a

coordinate change which maps $0 < x < s(t)$ onto $0 < y < 1$. The use of a fixed spatial grid in the variable y corresponds to a moving mesh in the variable x . We define

$$y = x/s(t), \quad v(y, t) = c(x, t).$$

Under this transformation the problem becomes: find $v(y, t) \in C^{2,1}((0, 1) \times (0, \tau))$, $s(t) \in C^1(0, \tau)$ satisfying, for $\dot{s} = ds/dt$,

$$v_t = \frac{1}{s^2} v_{yy} + \frac{y\dot{s}}{s} v_y - m - f(y, v), \quad (y, t) \in (0, 1) \times (0, \tau), \tag{A.3}$$

$$v(0, t) = c_b, \quad v(1, t) = v_y(1, t) = 0, \tag{A.4}$$

together with initial conditions in $v(y, 0)$ and $s(0)$.

The numerical method is as follows. First, assume that $s(t)$ is a known function of time. Equations (A.3, A.4) are discretized by a finite-difference scheme which is centred in space and employs backward Euler time-stepping on the differential operator, together with explicit treatment of the source term. The two boundary conditions enforced are $v(0, t) = c_b$ and $v_y(1, t) = 0$. Using this algorithm, we may advance from $t = n\Delta t$ to $t = (n + 1)\Delta t$ given $s(n\Delta t)$ and $s((n + 1)\Delta t)$. However, in general, such a solution will not satisfy $v(1, (n + 1)\Delta t) = 0$. Thus, the problem reduces to a single nonlinear equation for the determination of $s((n + 1)\Delta t)$ at each time-step to enforce the third boundary condition in space. We solve this one-dimensional shooting problem at each time-step. This problem is solved by a secant iteration to obtain rapid convergence and avoid the calculation of Jacobians. The value of s at $n\Delta t$ is taken as the initial guess for the value at $(n + 1)\Delta t$. In the simulations contained in this paper, a maximum of three iterations is required per time-step.

Appendix 3

Here, we prove the existence of a solution to the free boundary problem

$$c_{xx} = m + f(x/s, c) \quad (0 < x < s), \tag{A.5}$$

$$c(0) = c_b, \quad c(s) = c_x(s) = 0. \tag{A.6}$$

We seek a solution with $(s, c(x)) \in \mathbb{R} \times C^2([0, s])$. Recall that $m, c_b > 0$. It is assumed that

- (i) $f(y, c) \in C^1(\mathbb{R}^2, \mathbb{R}) \forall y \in [0, 1]$ and $\forall c \geq 0$;
- (ii) $0 < f(y, c) \leq \kappa$ uniformly for $y \in [0, 1]$ and $\forall c > 0$.

We note that the hypotheses of Theorem A.2 are satisfied by a function f defined by (2.9) together with any of the functions (2.10)–(2.12). The hypotheses of Theorem A.3 are satisfied by a function f defined by (2.9) together with the function (2.10).

We employ a shooting method. Let $y = x/s$ and define $v(y) = c(x)$. Then $v(y)$

satisfies the equations

$$\begin{aligned} v_{yy} &= s^2[m + f(y, v)] \quad (0 < y < 1), \\ v(0) &= c_b, \quad v(1) = v_y(1) = 0. \end{aligned}$$

The shooting method is to introduce the function $u(y; s)$ satisfying

$$u_{yy} = s^2[m + f(y, u)] \quad (y < 1)$$

subject to

$$u(1; s) = u_y(1; s) = 0.$$

It is then necessary to prove the existence of zeros of the function

$$G(s) := u(0; s) - c_b.$$

To do this, we show that $G(s)$ is well defined for $s > 0$ and demonstrate that it takes the value 0 for some positive s at least once. Under an additional assumption on $f(y, c)$, we show that the solution is unique. The results involve comparison with solutions of the problem

$$\begin{aligned} w_{yy} &= \bar{s}^2 m, \\ w(0) &= c_b, \quad w(1) = w_y(1) = 0, \end{aligned}$$

where $\bar{s} = (2c_b/m)^{1/2}$. In the coordinate x , this solution represents the steady solution from phase 1 of the evolution, before the sink term modelling the action of the proliferating endothelial cells is introduced.

LEMMA A.1 *Under assumptions (i) and (ii), the function $G(s)$ exists for $s \geq 0$ and satisfies $G(s) \in C^1([0, \infty))$.*

Proof. Let $s \geq 0$. If $u(y; s)$ is defined on $y \in [0, 1]$ it must be nonnegative since any solution of the initial value problem cannot have a positive maximum in $[0, 1]$ by (ii). Thus, in $(0, 1)$, we obtain the inequality

$$0 \leq u_{yy} \leq s^2(m + \kappa),$$

and integration yields

$$0 \leq u(y) \leq \frac{1}{2}s^2(m + \kappa)(y - 1)^2.$$

Thus $G(s)$ exists and is defined for $s \geq 0$. Applying Theorem 7.5 in Coddington & Levinson (1955: Chap. 1), we deduce that $G \in C^1([0, \infty))$. \square

THEOREM A.2 *Under assumptions (i) and (ii), there exists a solution of (A.5, A.6) with free boundary s satisfying $0 < s < \bar{s}$.*

Proof. Since $u(y; 0) = 0$, we deduce that $G(0) = -c_b < 0$. Now consider $G(\bar{s})$. We have, for $s = \bar{s}$,

$$u_{yy}(y; \bar{s}) - w_{yy}(y) = \bar{s}^2 f(y, u(y; \bar{s})).$$

Integrating twice we obtain

$$w_y(y) - u_y(y; \bar{s}) = \bar{s}^2 \int_y^1 f(\xi, u(\xi; \bar{s})) d\xi,$$

$$\Rightarrow u(0; \bar{s}) - w(0) = \bar{s}^2 \int_0^1 \int_y^1 f(\xi, u(\xi; \bar{s})) d\xi dy.$$

By the positivity of u , and hence of f , we deduce that $u(0; \bar{s}) > w(0)$ and thus $G(\bar{s}) > 0$. Hence continuity of $G(s)$ implies that $G(s) = 0$ for at least one $s \in (0, \bar{s})$. \square

THEOREM A.3 *In addition to (i) and (ii), assume that $f_c(y, c) \geq 0$ for $y \in [0, 1]$ and $c \geq 0$. Then the solution constructed in Theorem A.2 is unique.*

Proof. We show that $G(s)$ is monotone increasing in $s > 0$. Let $\varepsilon = s^2$ and define $z(y; s) = u_\varepsilon(y; s)$. Then z satisfies the equation

$$z_{yy} = m + f(y, u) + s^2 f_u(y, u)z \quad (y < 1)$$

subject to

$$z(1; s) = z_y(1; s) = 0.$$

From the defining equation, we see that $z_{yy}(1; s) > 0$. Hence there exists $\delta > 0$ such that z is positive for some interval $(1 - \delta, 1)$. Furthermore, $z(y; s)$ can have no positive maxima for $y \in [0, 1]$ since $z_{yy} > 0$ for $z \geq 0$ and $y \in [0, 1]$. Hence $z(0; s) > 0$. From this, it is clear that $G(s)$ is monotone increasing for $s > 0$ and uniqueness follows. \square

REFERENCES

- ADAM, J. A. 1991a Self-activation and inhibition: A simple nonlinear model. *Appl. Math. Lett.* **4**(2), 85–7.
- ADAM, J. A. 1991b Self-activation and inhibition: The effect of a zero-flux boundary. *Appl. Math. Lett.* **4**(3), 45–7.
- ADAM, J. A., & MAGGELAKIS, S. A. 1990 Diffusion regulated growth characteristics of a spherical prevascular carcinoma. *Bull. Math. Biol.* **52**, 549–82.
- AUSPRUNK, D. H., & FOLKMAN, J. 1977 Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumour angiogenesis. *Microvasc. Res.* **14**, 53–65.
- AUSPRUNK, D. H., KNIGHTON, D. R., & FOLKMAN, J. 1974 Differentiation of vascular endothelium in the chick chorioallantois: A structural and autoradiographic study. *Dev. Biol.* **38**, 237.
- BAIRD, A., & WALICKE, P. A. 1989 Fibroblast growth factors. *Br. Med. Bull.* **45**, 438–52.
- BALDING, D., & McELWAIN, D. L. S. 1985 A mathematical model of tumour-induced capillary growth. *J. Theor. Biol.* **114**, 53–73.
- BARD, R. H., MYELLO, J. H., & FREED, S. Z. 1986 Detection of tumour angiogenesis factor in adenocarcinoma of kidney. *Urology* **27**, 447–50.
- BIRDWELL, C. R., GOSPODAROWICZ, G. L., & NICOLSON, G. L. 1977 Factors from 3T3 cells stimulate proliferation of cultured vascular endothelial cells. *Nature (Lond.)* **268**, 528–31.
- CHAPLAIN, M. A. J. 1990 Mathematical models for the growth of solid tumours and the tip morphogenesis of *Acetabularia*. Ph.D. Thesis, University of Dundee.

- CHAPLAIN, M. A. J., & SLEEMAN, B. D. 1990 A mathematical model for the production and secretion of tumour angiogenesis factor in tumours. *IMA J. Math. Appl. Med. Biol.* **7**, 93–108.
- CODDINGTON, E. A., & LEVINSON, N. 1955 *Theory of Ordinary Differential Equations*. New York: McGraw-Hill.
- CRANK, J. 1984 *Free and Moving Boundary Problems*. Oxford: Clarendon Press.
- CRANK, J., & GUPTA, R. S. 1972 A moving boundary problem arising from the diffusion of oxygen in absorbing tissue. *J. Inst. Math. Applic.* **10**, 19–33.
- DESHPANDE, R. G., & SHETNA, Y. I. 1989 Isolation and characterization of tumour angiogenesis factor from solid tumours and body fluids from cancer patients. *Indian J. Med. Res.* **90**, 241–7.
- EDDY, H. A., & CASARETT, G. W. 1973 Development of the vascular system in the hamster malignant neurolemmoma. *Microvasc. Res.* **6**, 63–82.
- EDELSTEIN, L. 1982 The propagation of fungal colonies: A model for tissue growth. *J. Theor. Biol.* **98**, 679–701.
- ERROI, A. L., KUMAR, P. M., & KUMAR, S. 1986 Effects of a purified low molecular weight tumour angiogenesis factor on cell morphology of bovine brain capillary endothelial cells growing on a native collagen substratum. *Anticancer Res.* **6**, 1045–51.
- FOLKMAN, J. 1972 Anti-angiogenesis: New concept for therapy of solid tumors. *Ann. Surg.* **175**, 409–16.
- FOLKMAN, J. 1976 The vascularization of tumors. *Sci. Am.* **234**, 58–73.
- FOLKMAN, J., AND KLAGSBRUN, M. 1987 Angiogenic factors. *Science* **235**, 442–7.
- FOLKMAN, J., MERLER, E., ABERNETHY, C., & WILLIAMS, G. 1971 Isolation of a tumor factor responsible for angiogenesis. *J. Exp. Med.* **133**, 275–88.
- GALIB, T. A., BRUCH, J. C., & SLOSS, J. M. 1981 Solution of an oxygen diffusion-absorption problem. *Int. J. Biomed. Comp.* **12**, 157–80.
- GIMBRONE, M. A., COTRAN, R. S., LEAPMAN, S. B., & FOLKMAN, J. 1974 Tumor growth and neovascularization: An experimental model using the rabbit cornea. *J. Natl. Cancer Inst.* **52**, 413–27.
- GREENSPAN, H. P. 1976 On the growth and stability of cell cultures and solid tumours. *J. Theor. Biol.* **56**, 229–42.
- GROSS, J., AZIZKHAN, R. G., BISWAS, C., BRUNS, R. R., HSIEH, D. S. T., & FOLKMAN, J. 1981 Inhibition of tumour growth, vascularization and collagenolysis in the rabbit cornea by medroxyprogesterone. *Proc. Natl. Acad. Sci. USA* **78**, 1176–80.
- HILTMAN, P., & LORY, P. 1983 On oxygen diffusion in a spherical cell with Michaelis–Menten oxygen uptake kinetics. *Bull. Math. Biol.* **45**, 661–4.
- ISHIWATA, I., ISHIWATA, C., SOMA, M., & ISHIKAWA, H. 1987 Establishment of HUOCA-II, a human ovarian clear cell adenocarcinoma cell line, and its angiogenic activity. *J. Natl. Cancer Inst.* **78**, 667–71.
- ISHIWATA, I., ISHIWATA, C., SOMA, M., ONO, I., NAKAGUCHI, T., & ISHIKAWA, H. 1988 Tumour angiogenic activity of gynecologic tumour cell lines on the chorioallantoic membrane. *Gynecol. Oncol.* **29**, 87–93.
- JAFFE, E. A., NACHMAN, R. L., BECKER, C. G., & MINICK, C. R. 1973 Culture of human endothelial cells derived from umbilical veins. *J. Clin. Invest.* **52**, 2745.
- KLAGSBRUN, M., KNIGHTON, D., & FOLKMAN, J. 1976 Tumour angiogenesis activity in cells grown in tissue culture. *Cancer Res.* **36**, 110–14.
- KUMAR, P., ERROI, A., SATTAR, A., & KUMAR, S. 1985 Weibel–Palade bodies as a marker for neovascularization induced by tumour and rheumatoid angiogenesis factors. *Cancer Res.* **45**, 4339–48.

- LANGER, R., CONN, H., VACANTI, J., HAUDENSCHILD, C., & FOLKMAN, J. 1980 Control of tumor growth in animals by diffusion of an antiangiogenesis inhibitor. *Proc. Natl. Acad. Sci. USA* **77**, 4331–5.
- LANGER, R., & FOLKMAN, J. 1976 Polymers for the sustained release of proteins and other macromolecules. *Nature (Lond.)* **263**, 797–800.
- LANDAU, H. G. 1950 Heat conduction in a melting solid. *Q. Appl. Math.* **8**, 81–94.
- LIN, S. H. 1976 Oxygen diffusion in a spherical cell with nonlinear uptake kinetics. *J. Theor. Biol.* **60**, 449–57.
- LIOTTA, L. A., SAIDEL, G. M., & KLEINERMAN, J. 1977 Diffusion model of tumor vascularization and growth. *Bull. Math. Biol.* **39**, 117–29.
- LISNIAK, I. A., & SOPOTINSKAIA, E. B. 1989 The content of tumour angiogenesis factor in the blood serum of C57B1/6 mice during the growth and metastasis of transplanted tumours. *Eksp. Oncol.* **11**, 76–80.
- MC ELWAIN, D. L. S. 1978 A re-examination of oxygen diffusion in a spherical cell with Michaelis–Menten oxygen uptake kinetics. *J. Theor. Biol.* **71**, 255–63.
- MADRI, J. A., & PRATT, B. M. 1986 Endothelial cell–matrix interactions: *In vitro* models of angiogenesis. *J. Histochem. Cytochem.* **34**, 85.
- MILLS, G. B., MAY, C., HILL, M., CAMPBELL, S., SHAW, P., & MARKS, A. 1990 Ascitic fluid from ovarian cancer patients contains growth factors necessary for intraperitoneal growth of human ovarian adenocarcinoma cells. *J. Clin. Invest.* **86**, 851–5.
- MURRAY, J. D. 1989 *Mathematical Biology*. Berlin: Springer.
- MUTHUKKARUPPAN, V. R., KUBAI, L., & AUERBACH, R. 1982 Tumor-induced neovascularization in the mouse eye. *J. Natl. Cancer Inst.* **69**, 699–705.
- NAKAMURA, S., & TSUBOKAWA, T. 1989 Extraction of angiogenesis factor from chronic subdural haematomas. Significance in capsule formation and haematoma growth. *Brain Inj.* **3**, 129–36.
- OOSAKI, T., SEKIYA, S., & TAKAMIZAWA, H. 1987 Fundamental studies on tumour angiogenesis factors with a choriocarcinoma model. *Nippon Sanka Fujinka Gakkai Zasshi* **39**, 2137–43.
- PAWELETZ, N., & KNIERIM, M. 1990 Tumor-related angiogenesis. *Crit. Rev. Oncol. Hematol.* **9**, 197–242.
- PAWLIKOWSKI, M., CIESLAK, D., STEPIEN, H., BERNER, J., & RYBINSKI, K. 1989 Elevated blood serum levels of epidermal growth factor in some patients with gastric cancer. *Endokrynol. Pol.* **40**, 149–53.
- REILLY, W., & MCAUSLAN, B. R. 1988 Matrix control of tumour angiogenesis. *Adv. Exp. Med. Biol.* **42**, 221–7.
- ROMBERGER, C. F., WOLLMAN, R. L., & WAINER, B. H. 1990 Distant angiogenesis in a patient with glioblastoma multiforme. *Clin. Neuropathol.* **9**, 97–100.
- SEKIYA, S., OOSAKI, T., & TAKAMIZAWA, H. 1986 Tumour angiogenesis activity of human choriocarcinoma cells grown *in vitro*. *Gynecol. Oncol.* **25**, 271–80.
- SHAHABUDDIN, S., ARNOLD, F., COSTELLO, C. B., & KUMAR, S. 1984 Tumour angiogenesis factor in urological cancers. *Br. J. Urol.* **56**, 490–4.
- SHAHABUDDIN, S., & KUMAR, S. 1983 Quantitation of angiogenesis factor in bovine retina and tumour extracts by means of radioimmunoassay. *Br. J. Ophthalmol.* **67**, 286–91.
- SHAHABUDDIN, S., KUMAR, S., WEST, D., & ARNOLD, F. 1985 A study of angiogenesis factors from five different sources using a radioimmunoassay. *Int. J. Cancer* **35**, 87–91.
- SHERRATT, J. A., & MURRAY, J. D. 1990 Models of epidermal wound healing. *Proc. R. Soc. Lond. B* **241**, 29–36.
- SHOLLEY, M. M., FERGUSON, G. P., SEIBEL, H. R., MONTOUR, J. L., & WILSON, J. D. 1984 Mechanisms of neovascularization. Vascular sprouting can occur without proliferation of endothelial cells. *Lab. Invest.* **51**, 624.

- STRYDOM, D. J., FETT, J. W., LOBB, L. R., ALDERMAN, E. M., BETHUNE, J. L., RIORDAN, J. F., & VALLEE, B. L. 1985 Amino acid sequence of human tumour derived angiogenin. *Biochemistry* **24**, 5486–94.
- STUART, A. M., & FLOATER, M. F. 1990 On the computation of blow-up. *Eur. J. Appl. Math.* **1**, 47–71.
- WARREN, B. A., & SHUBIK, P. 1966 The growth of the blood supply to melanoma transplants in the hamster cheek pouch. *Lab. Invest.* **15**, 464.
- WILLMOTT, N., GOLDBERG, J., BESSENT, R., MCKILLOP, J., & MCARDLE, C. 1991 Abnormal vasculature of solid tumours: Significance for microsphere-based targeting strategies. *Int. J. Radiat. Biol.* **60**, 195–9).

Forthcoming Meetings

The Sixth IMA Conference on The Mathematical Theory of the Dynamics of Biological Systems 1–3 July, 1992, University of Oxford

This conference will include sessions on *Epidemiology and immunology of infectious diseases* (Organizers: A. McLean and R. M. Anderson F.R.S.); *Developmental biology, physiological and medical systems* (Organizers: P. L. Maini and J. D. Murray F.R.S.); *Evolutionary dynamics of biological systems* (Organizers: A. I. Houston and J. R. Krebs F.R.S.); and *Harvesting, control and regulation of biological populations and systems* (Organizers: J. W. Horwood and R. M. May F.R.S.).

Each session will include three or four invited papers and a larger number of shorter contributed papers. There will also be posters for those who wish to contribute in this way. Abstracts of contributed papers should be sent for consideration by the organizing committee by **29 February 1992 at the latest**.

Decision on acceptance will be made by 21 March 1992. All papers read at the meeting, whether invited or contributed, will be considered for publication in this journal and subject to normal refereeing: manuscripts should be available at the time of the meeting so that the proceedings may appear in the first issues of the journal in 1993.

Further particulars are available from the Conference Secretary, IMA, 16 Nelson Street, Southend-on-Sea, Essex SS1 1EF (Telephone 0702-354020, Fax 0702-354111) or from the Editor by e-mail: hiorns@uk.ac.ox.vax.

European Congress of Mathematics (CEM/ECM) 6–10 July, 1992, Paris

The first congress of the newly formed European Mathematical Society will include sessions on pure and applied mathematics as well as a round table on mathematics in biology and medicine.

Further particulars from CEM, College de France, 3 rue d'Ulm, Paris (5e) or e-mail: EUCM@FRMAP711.BITNET.

3rd International Conference on Mathematical Population Dynamics 1–5 June, 1992, University of Pau, France

The 3rd International Conference on Mathematical Population Dynamics will take place in Pau (France) from 1–5 June, 1992. It is intended to be an interdisciplinary meeting of biologists and mathematicians concerned with populations of biomolecules, genes and cells, as well as other topics of mathematical population biology and epidemiology. The meeting will be of interest to applied mathematicians, probabilists and statisticians, ecologists, epidemiologists and biomedical scientists. Mathematical theories and analysis of models will be included, together with quantitative data from

cell and molecular biology, epidemiology and cancer research. The Scientific Committee consists of: S. Busenberg, O. Diekmann, K. Hadeler, M. Iannelli, P. Tautu, and G. Webb. A small selection of topics covered by the conference is: *Structured populations* (Differential- and integral equations, semigroups of operators, dynamical systems, mathematical epidemiology,...); *Stochastic models* (Branching processes, random walks, spatial processes, cellular automata, biostatistical methods,...); *Molecular biology* (Genome instability, gene amplification, RNA splicing, oncogenes/anti-oncogenes, mutation, replication,...); *Cell biology* (Cell cycle control, cell kinetics, cell differentiation, malignant transformation, senescence, metabolic control, adaptive systems,...); *Biomedicine* (AIDS, long latency syndromes, cancer, stem cell dynamics, normal blood cell production and leukemia, pharmacokinetics,...).

Proceedings of reviewed and selected papers will be published. Previous conferences in this series were held in 1986 (University of Mississippi, USA, the proceedings were published as a special issue of 'Computers & Mathematics', Vol. 18, no. 10/11, 1989), and 1989 (Rutgers University, New Jersey, USA, proceedings published in the Marcel Dekker series 'Lecture Notes in Pure and Applied Mathematics').

Persons interested can contact: O. Arino, I.P.R.A. Mathématiques, Université de Pau, 64000 Pau France, (e-mail(bitnet): Arino@FRUPPA51, tel.: (33)59923058; telefax: (33)59841696.

IMA CONFERENCE INFORMATION

For further details and application forms for the following forthcoming IMA meetings please contact: Miss Pamela Irving, Conference Officer, The Institute of Mathematics and its Applications, 16 Nelson Street, Southend-on-Sea, Essex, SS1 1EF.

1991

September 17th	Workshop: An Introduction to Parallel Computing for Numerical Applications	Oxford
September 18th to 20th	Parallel Computation	Oxford
September 23rd to 25th	International Conference on Mathematical Modelling of Materials Processing	University of Bristol
September 25th to 27th	Credit Scoring and Credit Control II	University of Edinburgh
December 16th to 18th	Third IMA Conference on Cryptography and Coding	Royal Agricultural College, Cirencester

1992

March	Mathematics for Engineers and Scientists	Coventry Polytechnic
March 30th and 31st	Mathematics in Industrial Maintenance	University of Edinburgh
March/April	Business Modelling	Glasgow College
July 1st to 4th	Sixth IMA Conference on Mathematics and Biology	University of Oxford
September 1st to 4th	Sixth IMA Conference on Control Theory	Manchester
September 7th to 10th	Aerospace Vehicle Dynamics and Control	Cranfield Institute of Technology
September 21st to 23rd	Fourth IMA Conference on Stably Stratified Flow and Turbulence	University of Surrey
December 14th to 16th	Third IMA Conference on Mathematics in Signal Processing	University of Warwick

1993

April 14th to 16th	The Mathematics of Food Production and Preservation	Belfast
--------------------	---	---------

Conferences which the IMA are co-sponsoring

1991

November 26th and 27th	International Conference on Computation in Electromagnetics	IEE, London
------------------------	---	-------------

1992

June 16th to 19th	Third European Conference on the Mathematics of Oil Recovery	Delft University of Technology, The Netherlands
-------------------	--	---

The *IMA Journal of Mathematics Applied in Medicine and Biology* recognises that the progress of research in the broad fields of medicine and biology increasingly depends upon the uses of mathematical models. As well as the formulation of relevant models, importance is attached to rigorous theoretical development and to validation using observational data from real systems. The type of applied mathematics is often characteristically different from that which is found in more traditional applications, and thus presents new challenges for mathematicians. The journal seeks to stimulate research in which mathematicians are seen to be tackling problems which those in the medical and biological fraternities would like addressed. The broad aims of this journal are twofold: 1. To embrace the use of mathematics in medical and biological research with emphasis upon the special insights and enhanced understanding which arise from these uses; and 2. To encourage and stimulate mathematicians, particularly younger ones, by suggesting topics and situations which their skills may be applied to solve mathematical problems arising in medicine and biology. The journal will publish papers dealing with applications in any area of medicine or biology. Original papers of an expository nature will be a feature of the journal.

Editor: Dr R. W. Hiorns, Department of Statistics, University of Oxford, 1 South Parks Road, Oxford OX1 3TG, England.

INSTRUCTIONS TO AUTHORS

1. *Submission of manuscripts.* Four copies of each manuscript should be sent to the Editor, together with the originals and three photocopies of any illustrations. Only the originals of illustrations will be returned to authors if a paper is not accepted for publication.

The Editor will correspond directly with the authors on the acceptability of their papers, and if the Editor deems a submitted paper to be a specialized contribution more appropriate for publication in one of the other journals of the IMA he will forward it to the Editor of that journal for consideration.

Authors may not submit manuscripts that are under consideration for publication elsewhere.

2. *Manuscript layout.* Manuscripts should be typewritten on one side only with wide margins on high quality paper using double spacing throughout. Each page of the manuscript should be numbered. The front page should contain the article title, author's name and affiliation and a summary. The summary or abstract should not exceed 300 words and should be intelligible to general readers without recourse to the main text. Footnotes and a list of notation should be avoided.

Articles should, in general, begin with an introduction which adequately covers the literature and scope of the paper. Each section in the paper should be numbered and equation numbers should be related to their own section. Each article should have, in general, a conclusion or discussion section. Any appendices should follow the Reference section. Keywords or phrases should be typed at the bottom of the first page.

Equations should be typed wherever possible and punctuated to conform to their place in the syntax of the sentence.

The SI system of units should be used in all papers.

3. *Illustrations and tables.* Drawings should be in indian ink on white card, faintly blue- or green-lined graph paper or on tracing cloth or paper. Authors should letter their drawings neatly with upper and lower case lettering. In general it is unnecessary to supply diagrams more than twice the linear dimensions desired in the final reproduction. *Please note: it is most important that lines and symbols should be drawn boldly enough to bear reducing to one-half of the desired size of the original, and great care should be exercised to see that the lines are regular in thickness, especially where they meet.* Redrawing of submitted diagrams will delay publication. A list of captions and titles should be typed at the end of the manuscript. Any photographs should be high quality glossy prints. The author's name and the figure number should be indicated lightly on the back of the print in soft pencil. Tables should be presented in the same style as the other IMA journals; it is important to insert *tie-lines* under column headings where required.

4. *References.* References will be listed at the end of the main text. The preferred system is the Harvard system, whereby the surname of the author and year of publication of the reference are used in the text. The list of references should be in alphabetical order of first-cited names. References by the same author(s) should be in chronological order. The normal form of listed references is author's surname, initials; year in parenthesis; article title; journal name (abbreviated in accordance with the World List of Scientific Periodicals (4th edn)); volume number (underlined); inclusive page numbers.

5. *Copyright/offprints.* Authors submitting a paper do so on the understanding that, if it is accepted for publication, exclusive copyright in the paper shall be assigned to the publisher. In consideration for assignment of copyright, the publisher will supply 50 offprints without covers of each paper. Further offprints may be ordered at extra cost at the proof stage. The publisher will not put any limitation on the personal freedom of the author to use material contained in the paper in other works which may be published.

CONTENTS

THOMPSON, E. A. and GUO, S. W., Evaluation of Likelihood Ratios for Complex Genetic Models	149
GUO, S. W. and THOMPSON, E. A., Monte Carlo Estimation of Variance Component Models for Large Complex Pedigrees	171
CHAPLAIN, M. A. J. and STUART, A. M., A Mathematical Model for the Diffusion of Tumour Angiogenesis Factor into the Surrounding Host Tissue	191