Aspects of Angiogenesis

by

Howard A. Levine
Department of Mathematics
Iowa State University

with the collaboration of

Serdal Pamuk
Michael W. Smiley
Department of Mathematics
Iowa State University

Marit Nilsen-Hamilton
Department of Biochemistry and Biophysics
Iowa State University

Brian D. Sleeman
Department of Applied Mathematics
University of Leeds

and the guidance of

Vaclov Ourednik
Department ofDepartment of Biomedical Sciences
Iowa State University

and

Donald Sakaguchi
Department of Zoology and Genetics
Iowa State University

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Outline and Overview

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   B. Retinal development.

   C. Development of the optic nerve.

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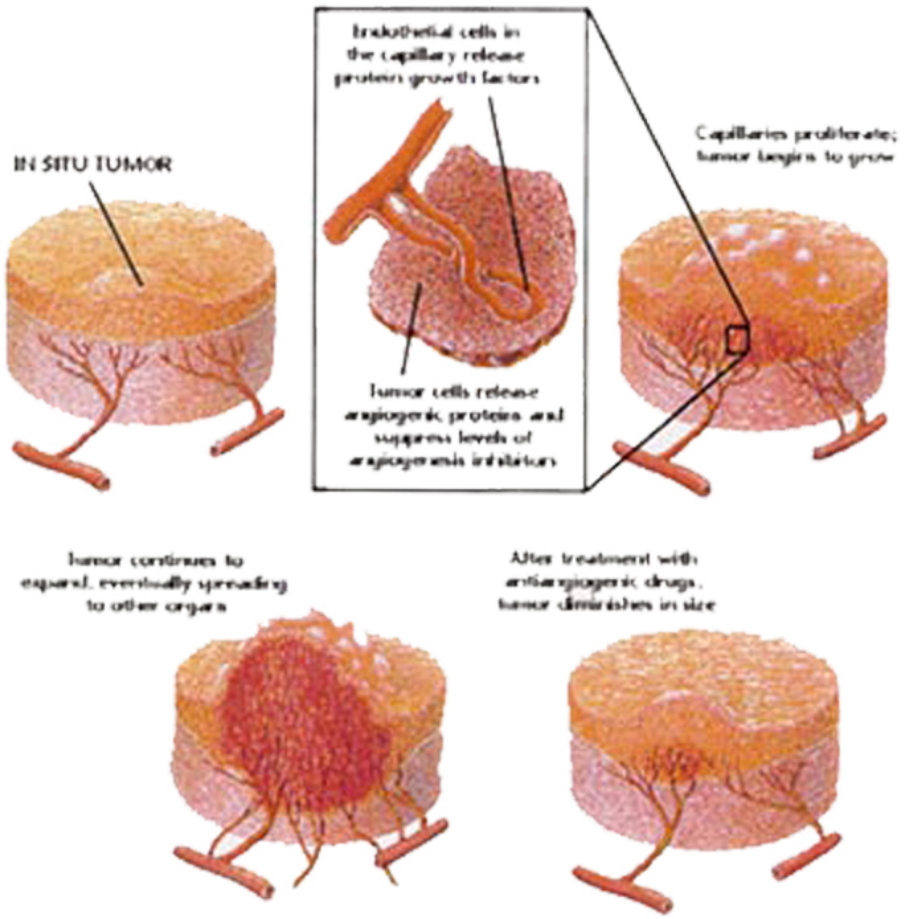
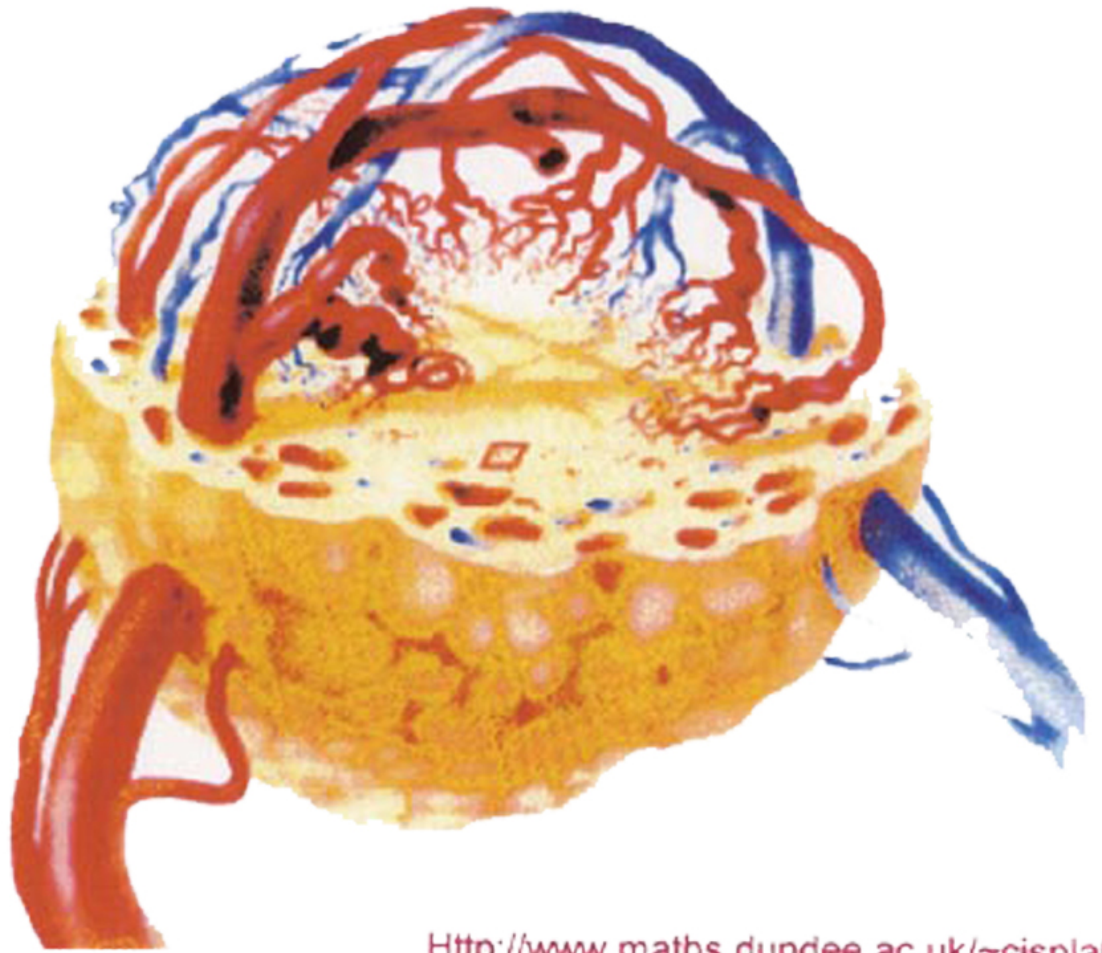
I. Cancer and the tumor growth model of J. Folkman.

Folkman proposed that tumors must be vascularized to grow



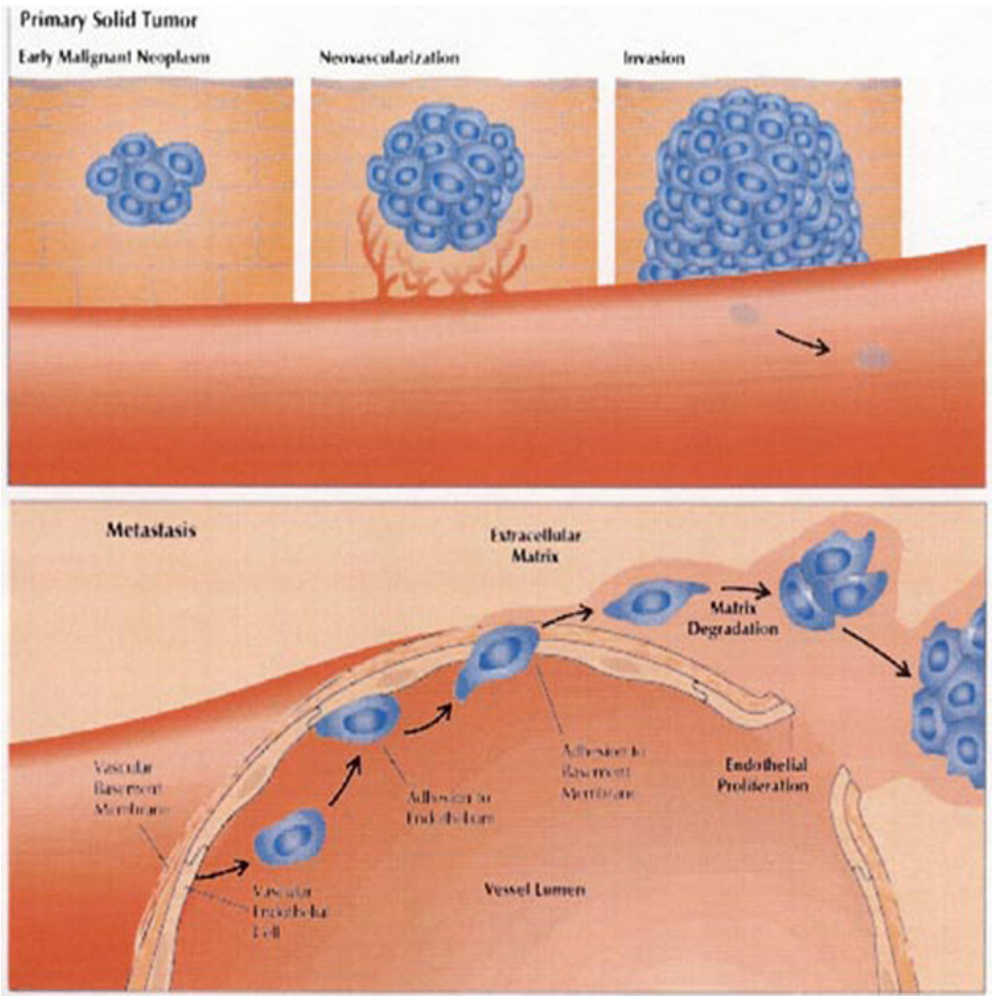
http://kzoo.edu/~k97kb01/pfolio/cancer.html

A rapidly growing tumor is highly vasculatorized



Http://www.maths.dundee.ac.uk/~cisplatin/angio.htm

Metastasis



From Eckhart, Hosp. Pract. 1999

I. J. Folkman's Model

(From: Angiogenesis:Key Principles-Science-Technology-Medicine)

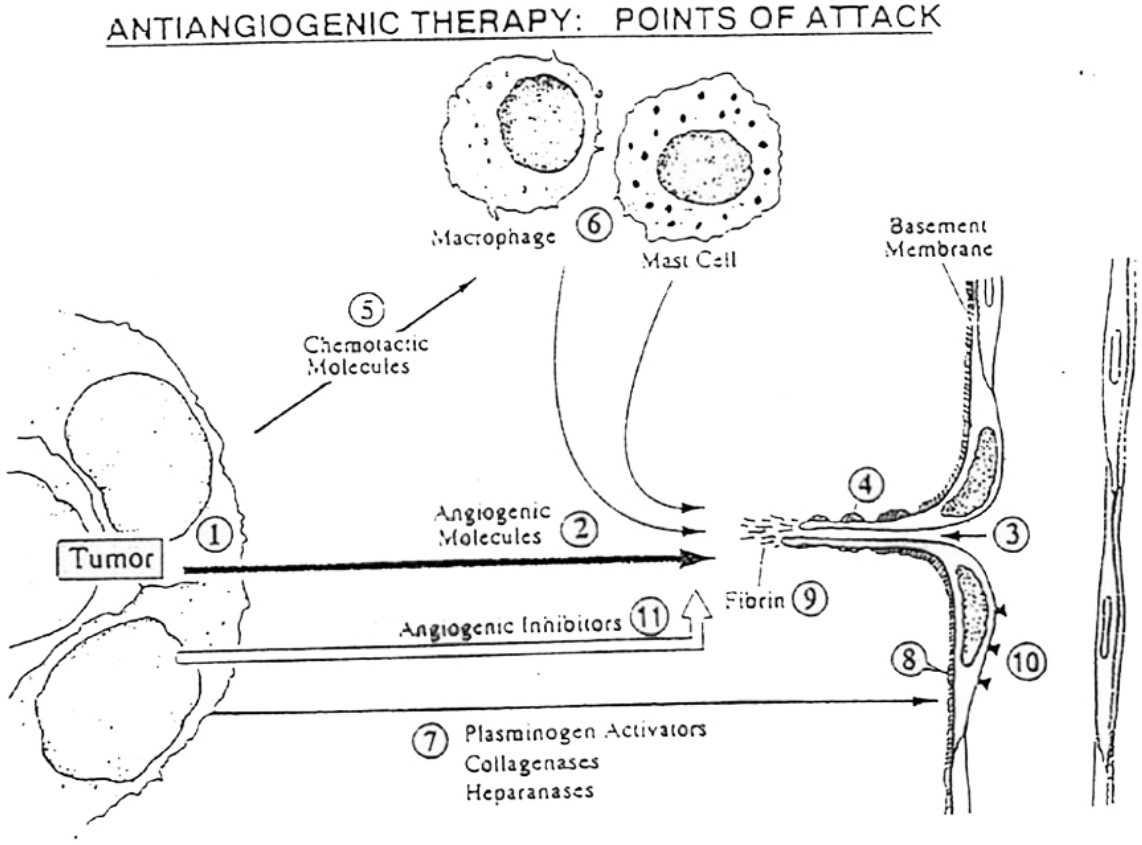
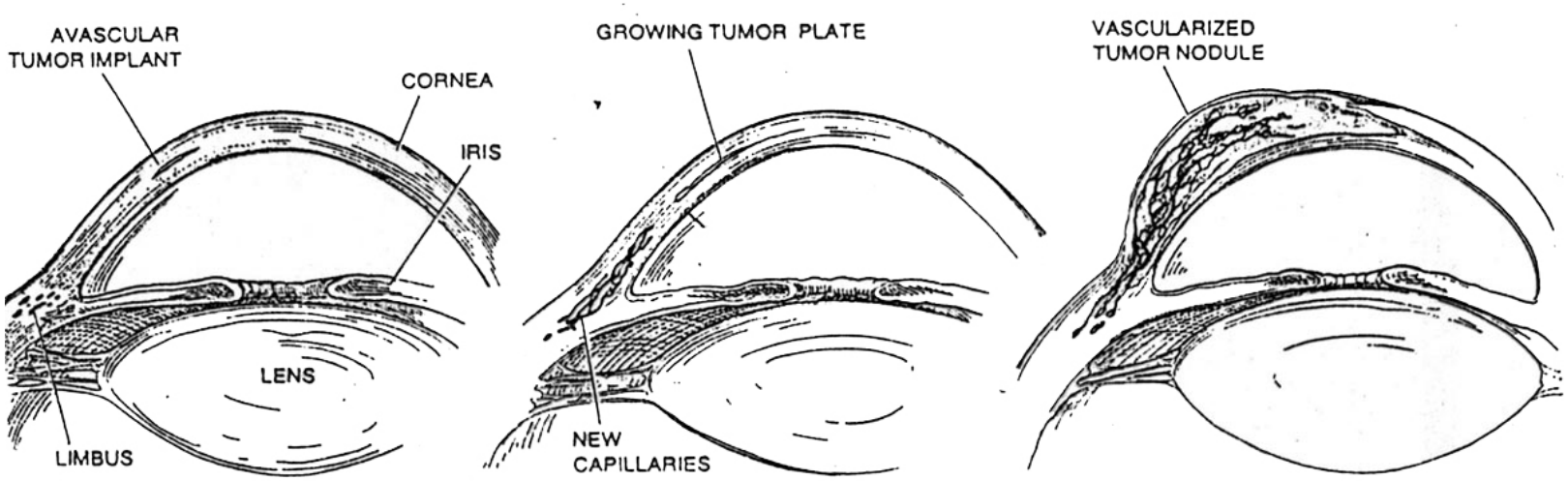


Diagram of different targets for antiangiogenic therapy.

Folkman's experimental evidence, (Sci. Am.,1976)

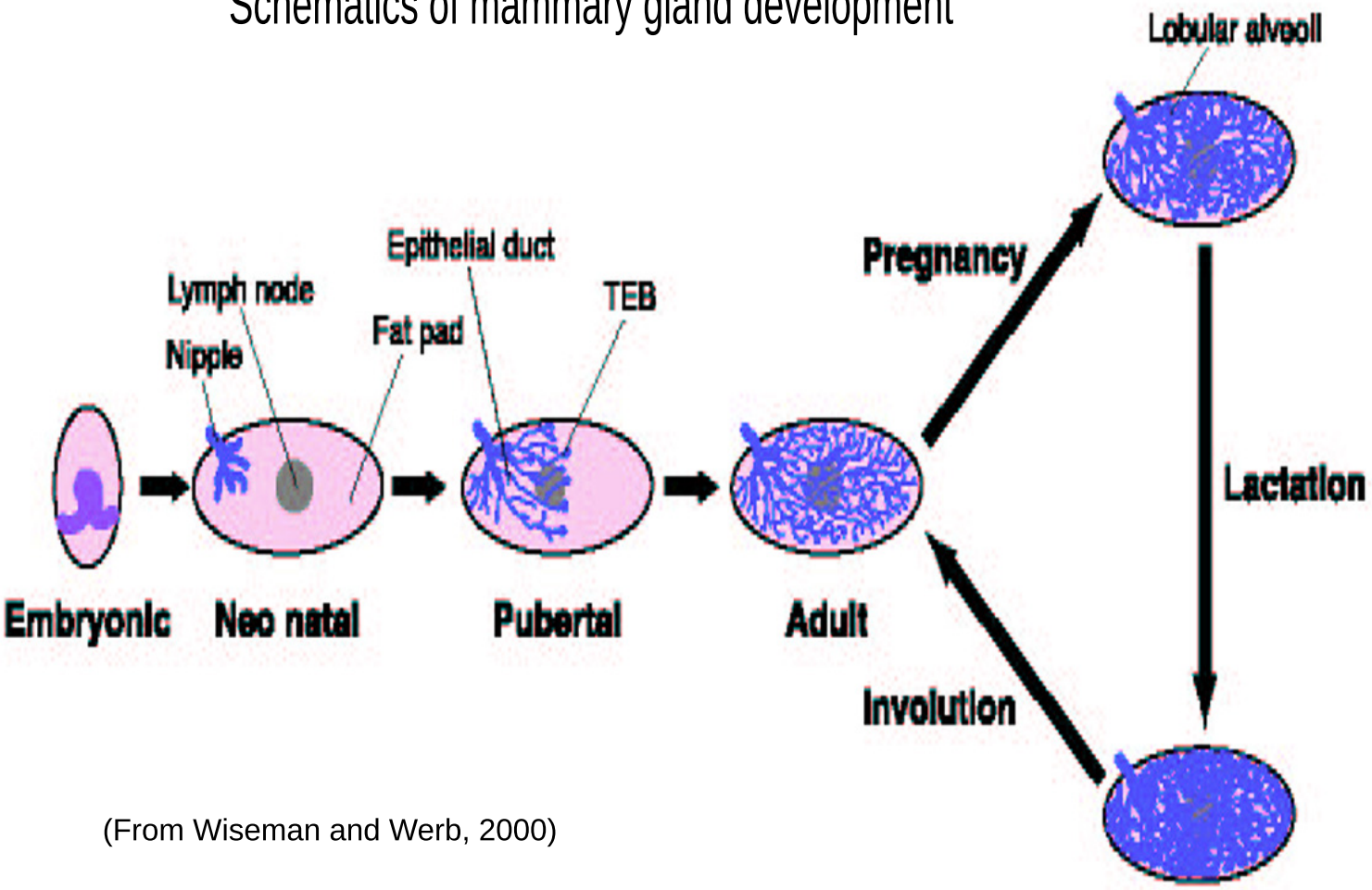


TUMOR IMPLANTED IN CORNEA of a rabbit grows slowly as a thin plate during the avascular phase (*1*). After a week or so, in response to the tumor angiogenesis factor, capillaries begin growing toward the tumor from the limbus of the cornea, which is some 2.5 millimeters away (*2*). Within two or three weeks the capillaries penetrate the tumor, and it begins to grow rapidly in three dimensions (*3*).

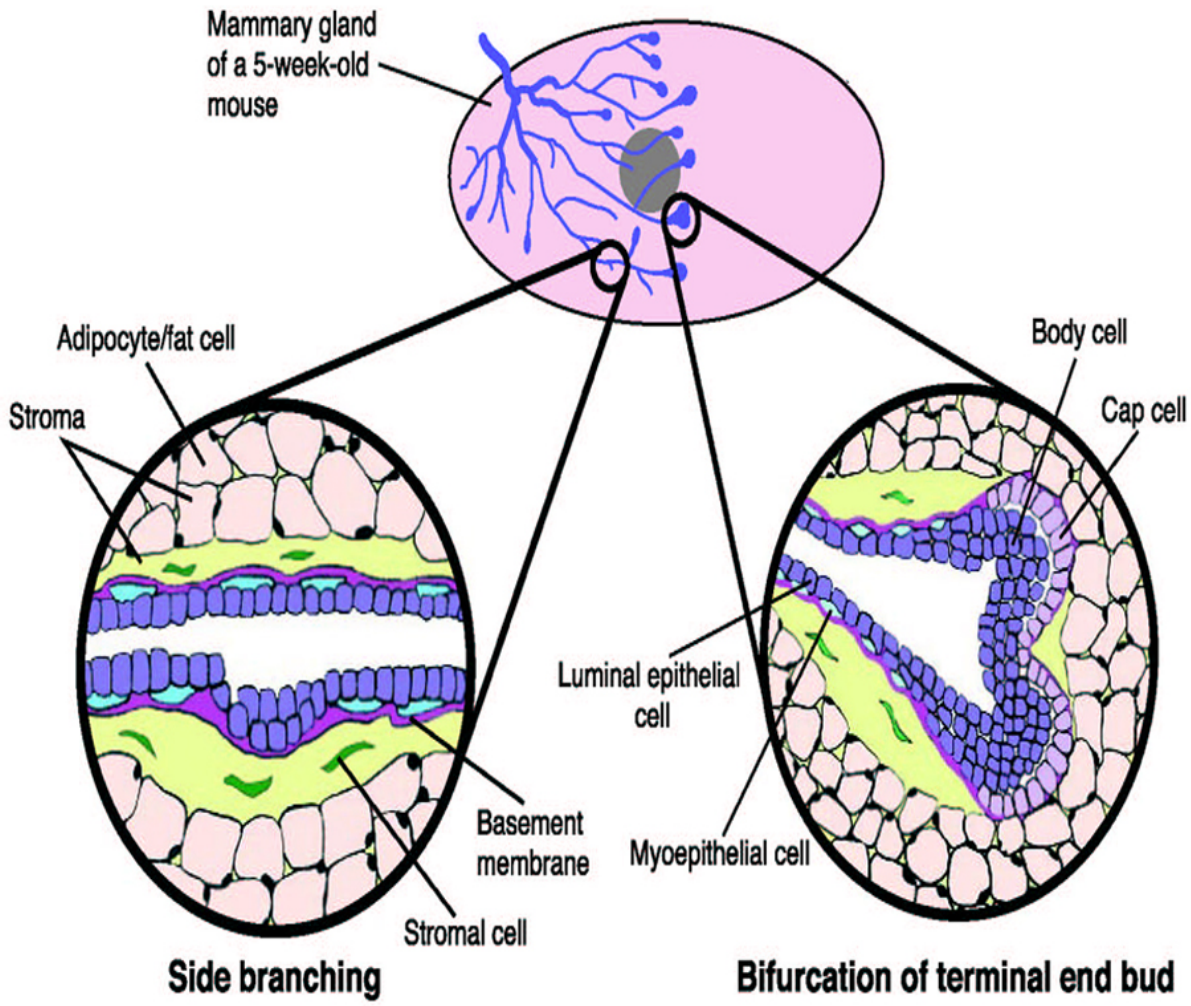
II. Other "developmental" phenomena.

A. Mammary gland development.

Schematics of mammary gland development



(From Wiseman and Werb, 2000)



. Fine structure of tree branching. (From Wiseman and Werb, 2002.)

From Silberstein (2001)

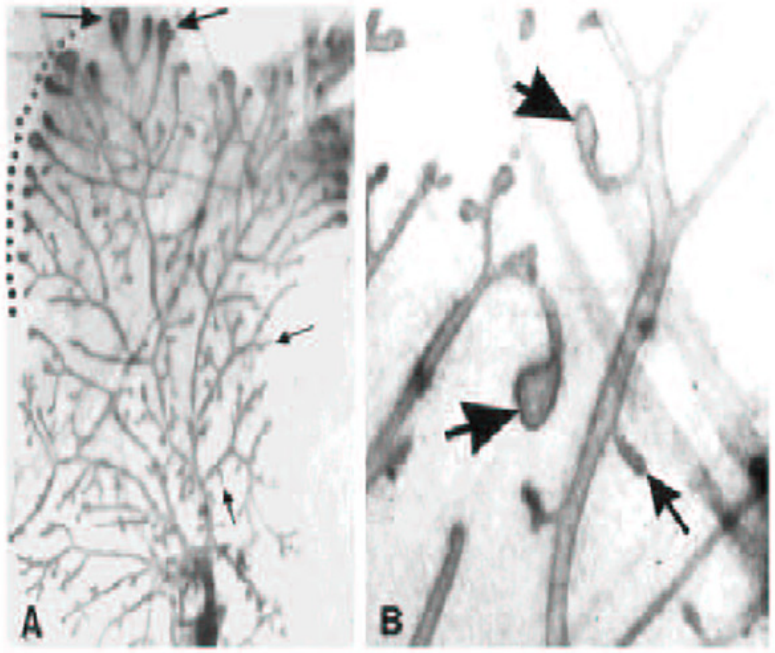


Fig. 1. **A:** Photomicrograph illustrating the mammary ductal system in a 5-week-old nulliparous mouse. Note the "open" ductal architecture, which leaves 80% or more of the gland parenchyma-free. End buds (large arrows), which identify growing ducts, are arrayed along the left-hand side of the gland in varying stages of regression (darkest spots indicate the most regressed). Lateral branches (small arrows) mark growth-quiescent ducts and dark object at base of image is the nipple. Magnification approximately ×15. **B:** Open architecture is achieved by local regulation when buds turn to avoid collision with nearby ducts (large arrows) and lateral branches abort extension (small arrow). Magnification approximately ×70.

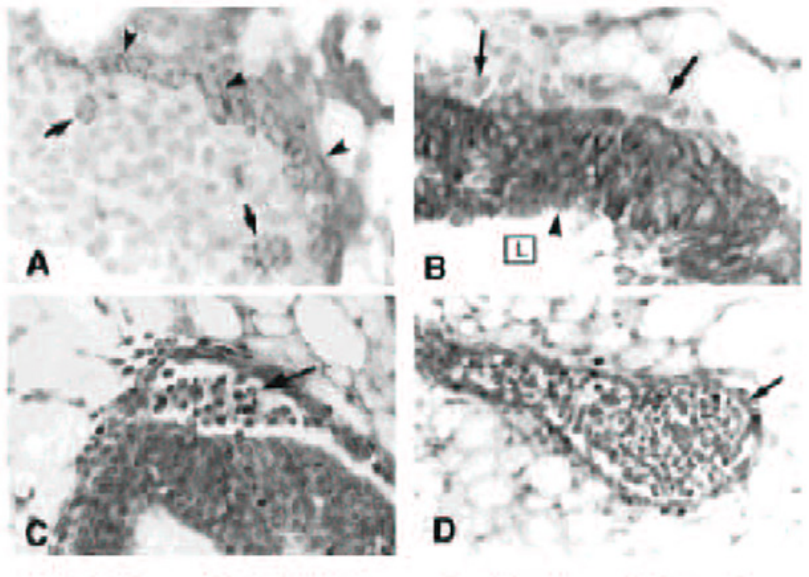


Fig. 8. E- and P-cadherin immunolocalization and effects of cognate blocking antibodies on end bud organization (from Daniel et al., 1995). **A:** P-cadherin staining on cap cell membranes (arrowheads), as well as on small numbers of cells in the lumenal compartment (arrows). Magnification ×400. **B:** E-cadherin staining on lumenal cell membranes (arrowhead). Note absence of stain on cap cells (arrows). Magnification ×400. **C:** Effect of anti-P cadherin on cap cell organization. Note focal disorganization (arrow), but otherwise intact cap cell layer. Magnification ×400. **D:** Effect of anti-E cadherin on lumenal cell organization. Note intact cap cell layer (arrow). Magnification ×800.

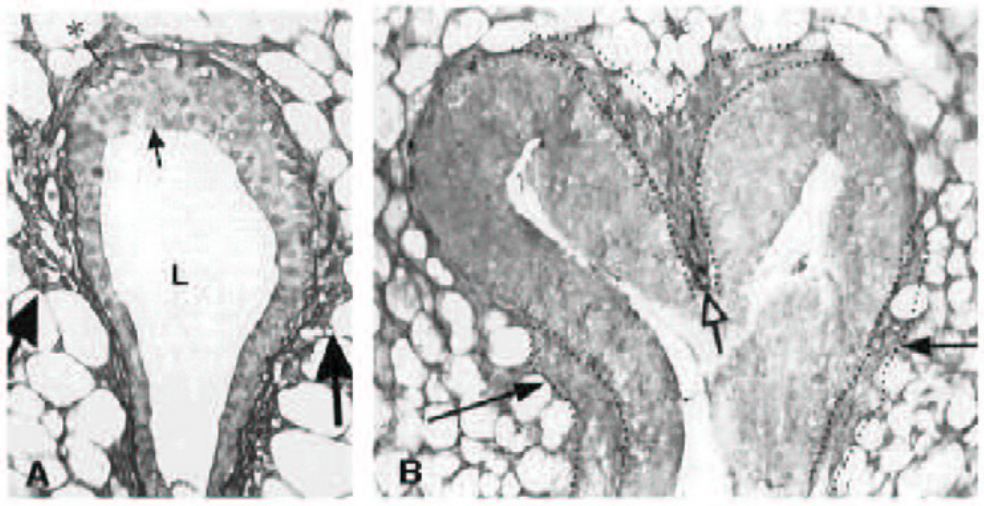


Fig. 2. **A:** Photomicrograph of a longitudinal section through an end bud illustrating the cap cell layer (asterisk) with prominent subcapsular spaces separating it from the underlying pre-lumenal body cells (small arrow). Fibrous connective tissue (large arrows) invests the end bud at points where constriction to ductal dimensions begins. Magnification ×280. **B:** Photomicrograph illustrating a bifurcating end bud. Two foci of fibrotic induction are apparent (bounded by dotted lines). Fibrous tissue within the cleft (open arrow) has extended partially over the new end buds, apparently directing growth of the left-hand lobe off at an angle. Solid arrows, fibrous induction on the flanks. Magnification ×250.

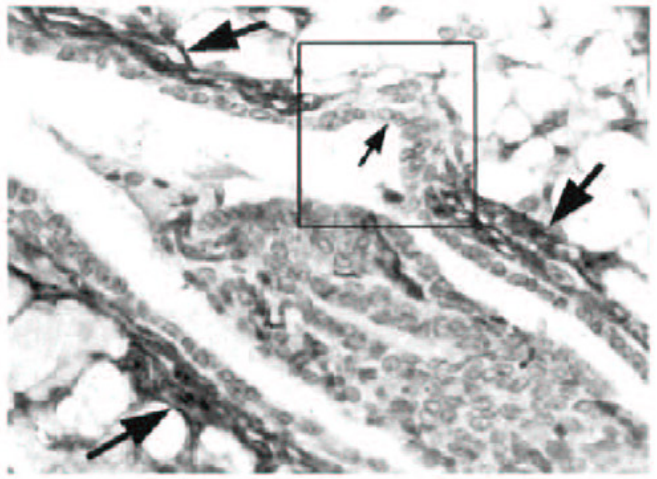
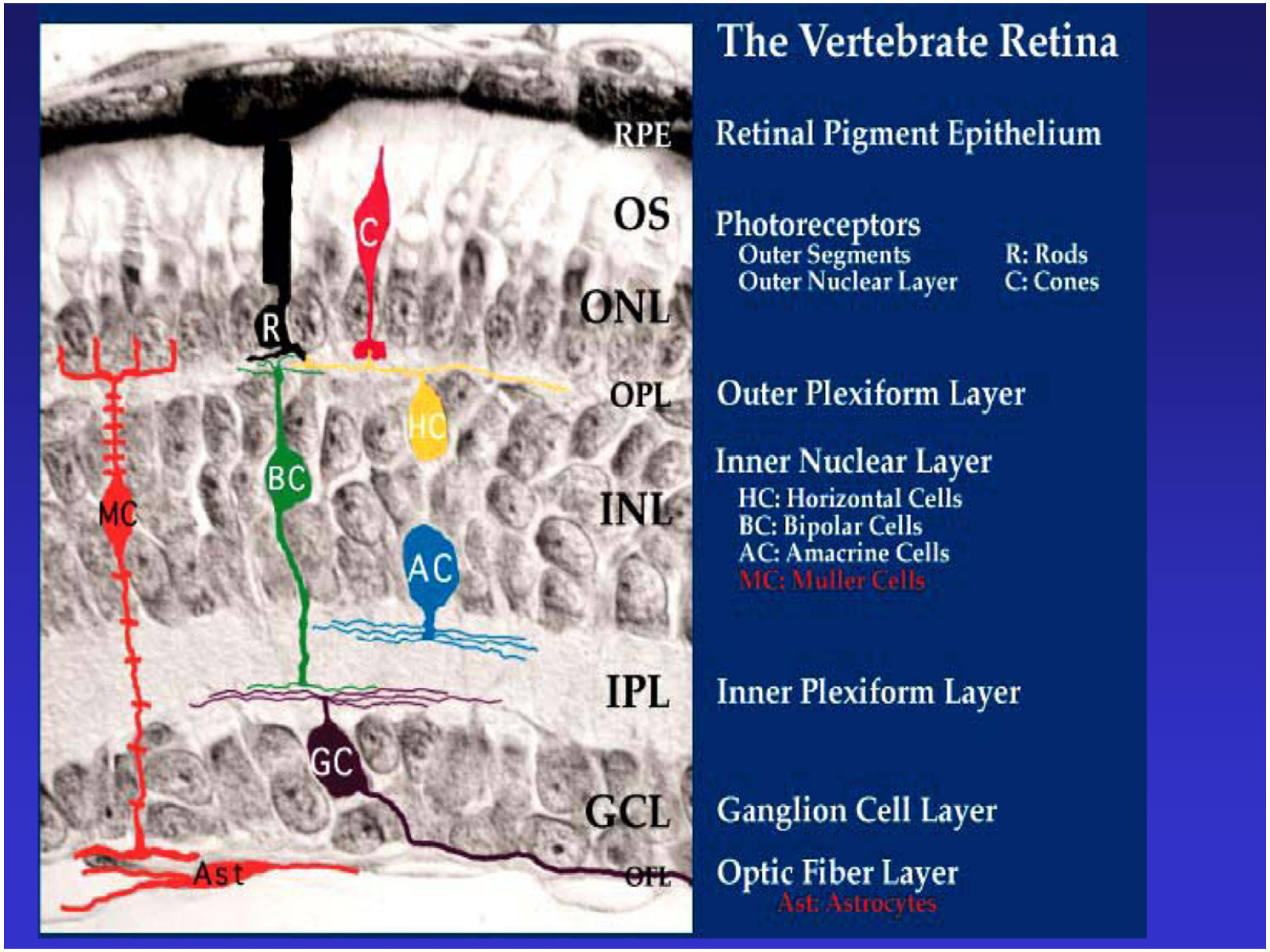


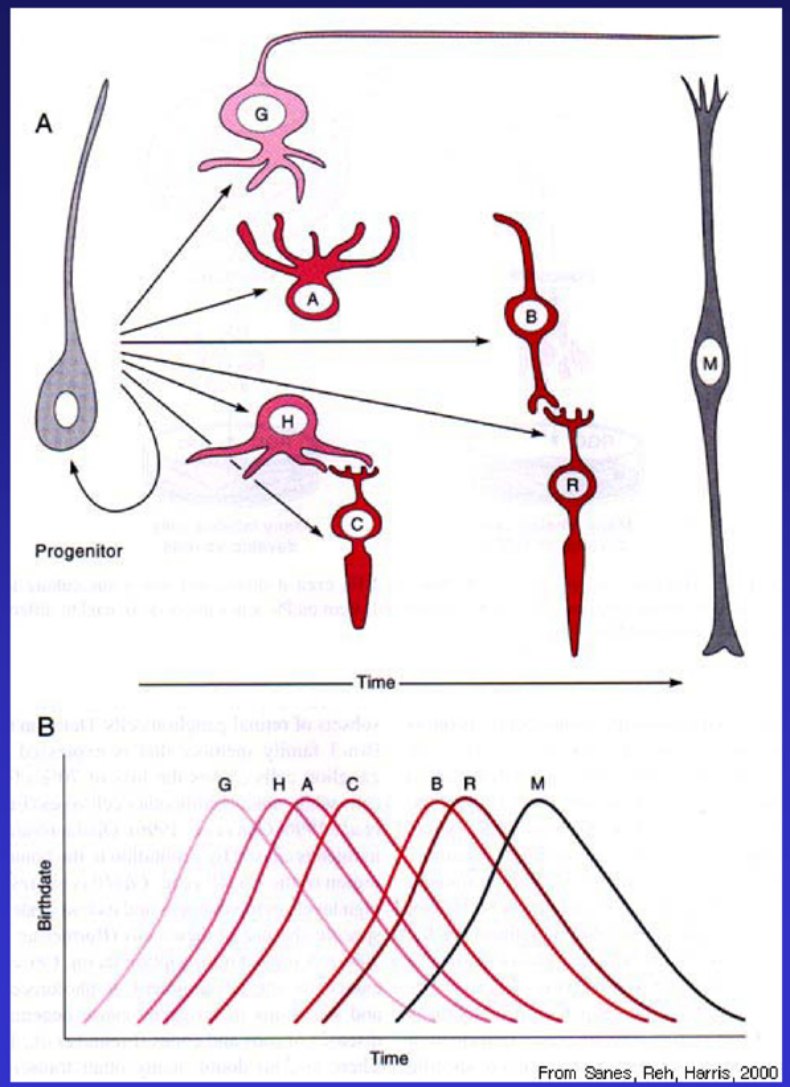
Fig. 4. Photomicrograph illustrating TGF-β1 flux over incipient, lateral bud with heavy TGF-β1 immunostaining associated with the collagen fibers in the periductal ECM (large arrows). Lateral bud (box) has just begun pushing (small arrow) on ECM, which lacks immunodetectable TGF-β1. Note the abrupt loss of staining beginning on the lateral margins of the bud. Magnification ×300 (from Silberstein et al., 1992).

(From Silberstein, 2001)


The Vertebrate Retina
RPE
Retinal Pigment Epithelium
OS
ONL
Photoreceptors
Outer Segments
Outer Nuclear Layer
R: Rods
C: Cones
R
C
OPL
Outer Plexiform Layer
HC
BC
MC
INL
Inner Nuclear Layer
HC: Horizontal Cells
BC: Bipolar Cells
AC: Amacrine Cells
MC: Muller Cells
AC
IPL
Inner Plexiform Layer
GC
GCL
Ganglion Cell Layer
Ast
OFL
Optic Fiber Layer
Ast: Astrocytes

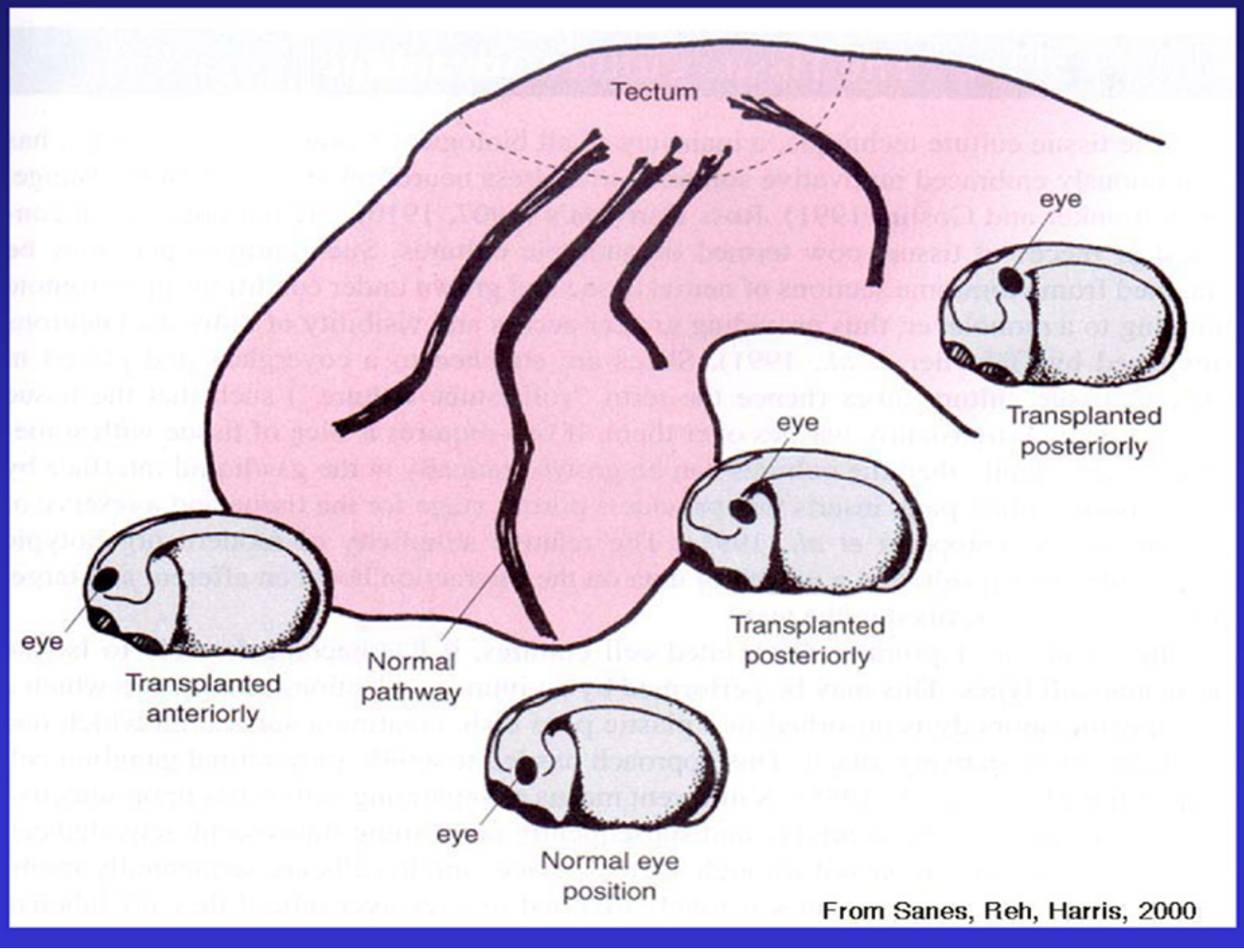
Cellular determination in a vertebrate retina:

RGCs, HCs, ACs, Cones
BPs, Rods, MCs



C. Development of the optic nerve.

Axons can find their appropriate targets when transplanted to novel locations.

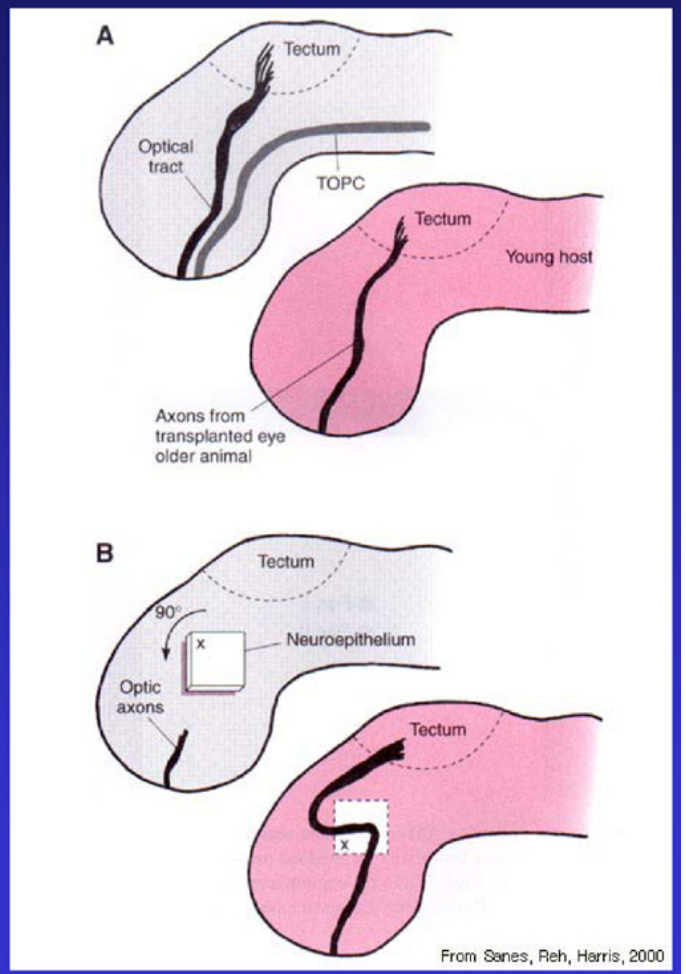


Retinal axons follow local guidance cues in the neuroepithelium

- When the retinal axons first grow into the brain they may grow alongside the previously formed TPOC.
  - When an eye from an older animal is transplanted to a young host in which the TPOC is not yet formed, the axons still grow correctly to the tectum, showing that they do not need TPOC fibers to guide them.

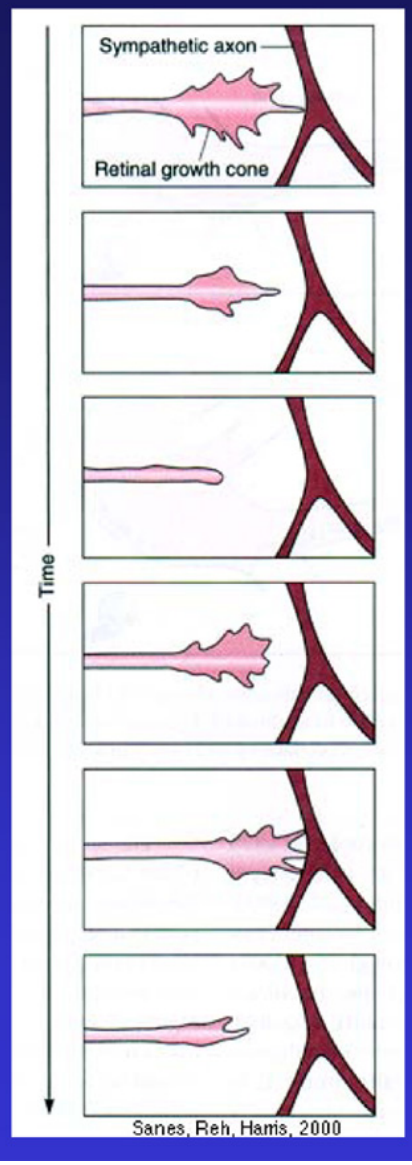
- A piece of neuroepithelium in front of the retinal axons is rotated 90 degrees.
  - When the retinal axons enter the rotated piece, they are deflected in the direction of the rotation, but correct their trajectories when they exit the rotated piece, showing that these axons pay attention to localized cues within the neuroepithelium

tract of the postoptic commissure (TPOC)



Repulsive factors: Growth cone collapse

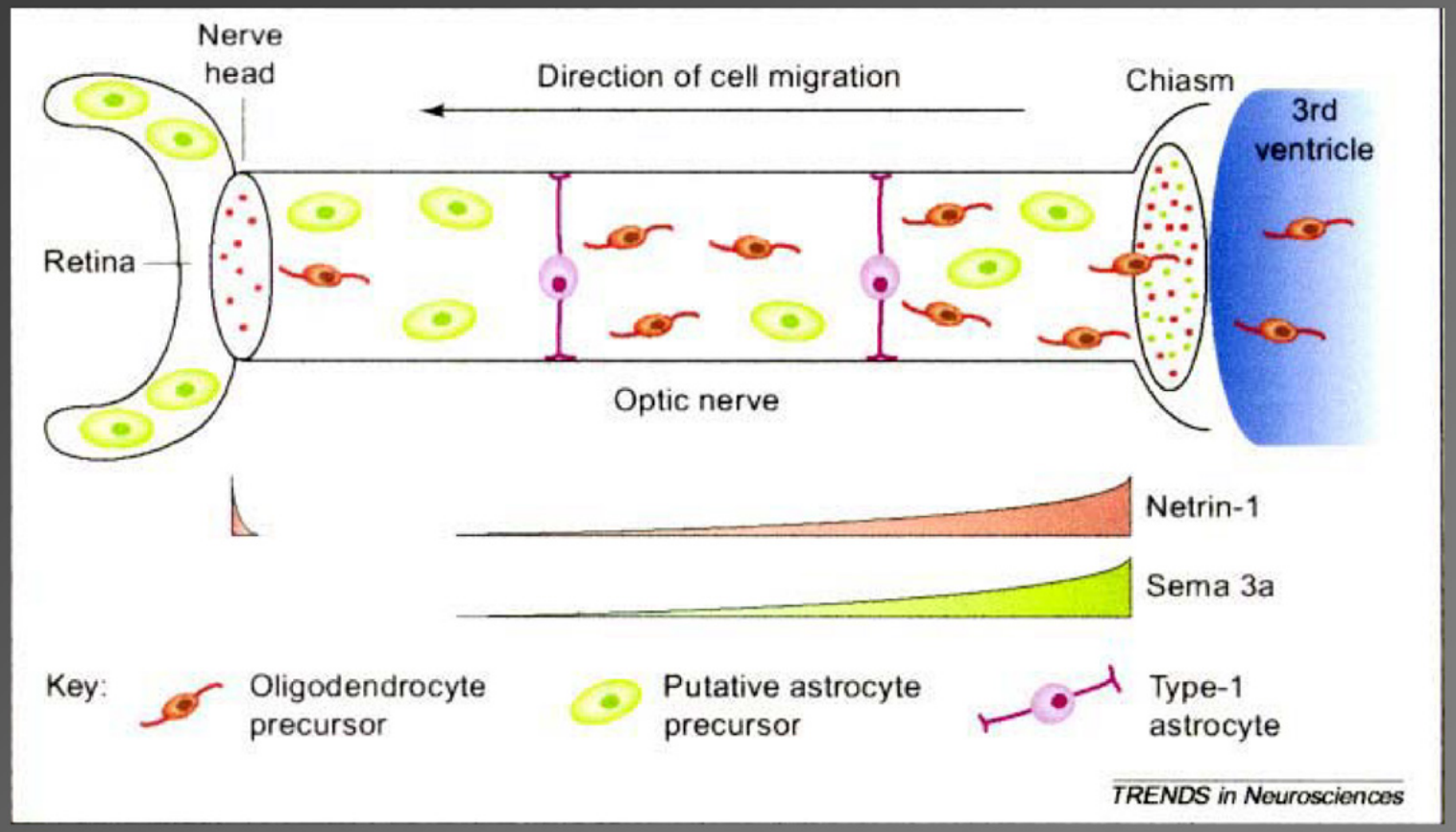
- Growth cone collapse. A time-lapse series of a growth cone from a retinal ganglion cell encountering an axon of a sympathetic neuron in culture.
- Upon first contact the growth cone retracts and collapses. After several minutes, the growth cone reforms, but when it encounters the sympathetic fiber again, it collapses once more.



Soluble (secreted) guidance cues

- They create gradients in the developing nervous system, providing positional information.
- Many have pleiotropic effects: distinct effects on a specific cell type at various developmental stages, or different effects on different cell types at the same stage (BDNF: promotes migration of granule cells and later promotes their survival (Borghesani et al., 2002).
- Factors that have previously been identified as trophins, mitogens, or inducing differentiation also contribute to cell migration (BDNF, PDNF).

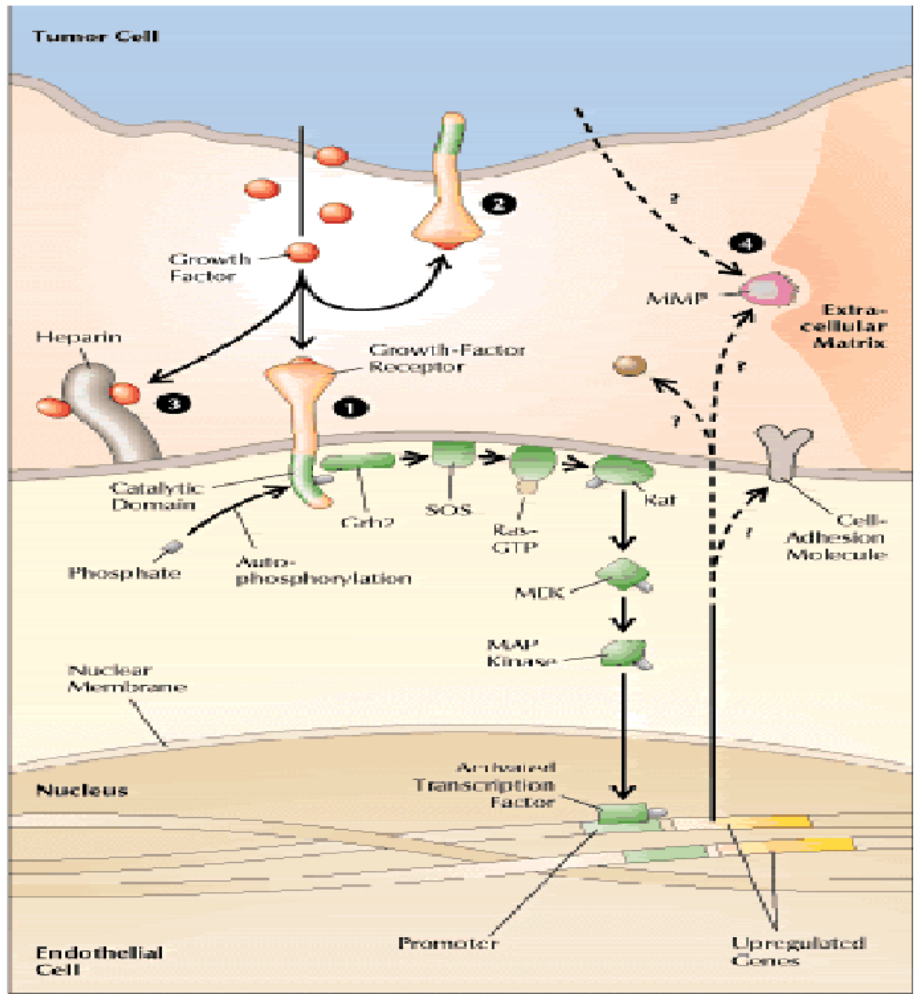
Glial cell migration respects axonal guidance cues



Tsai and Miller, 2002

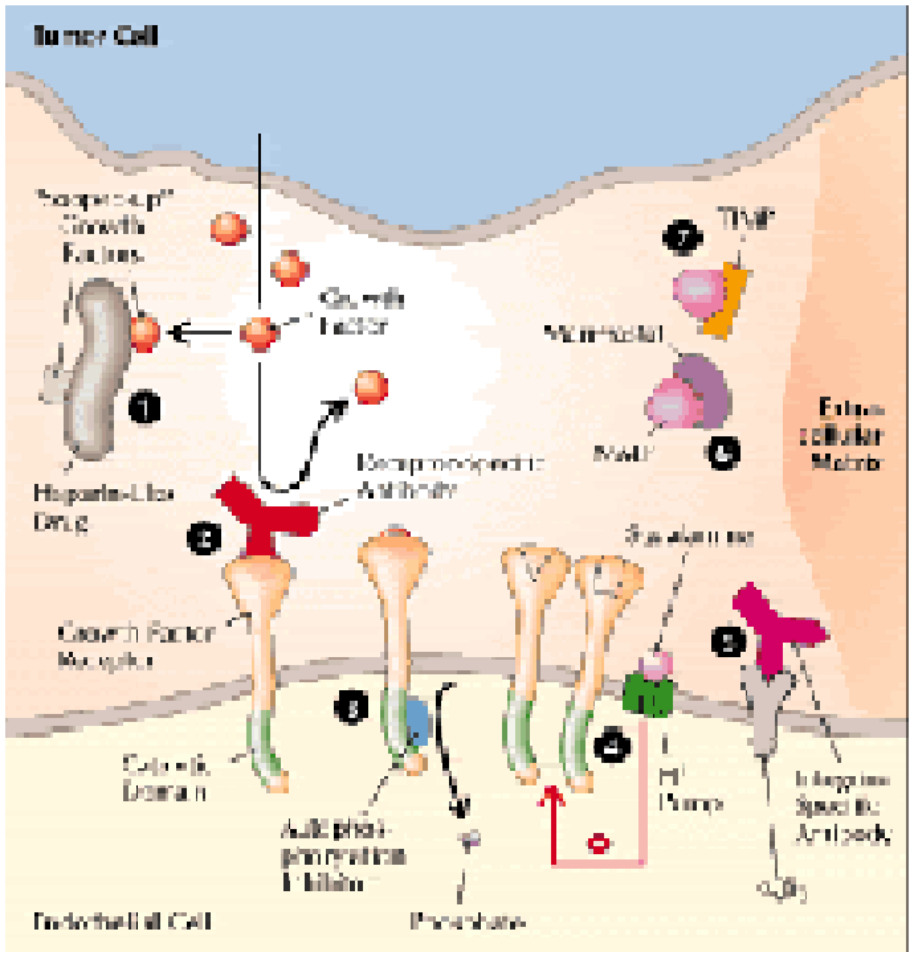
III. The biochemistry of the onset of angiogenesis

Cell to cell signaling



From Eckhart, Hosp. Pract. 1999

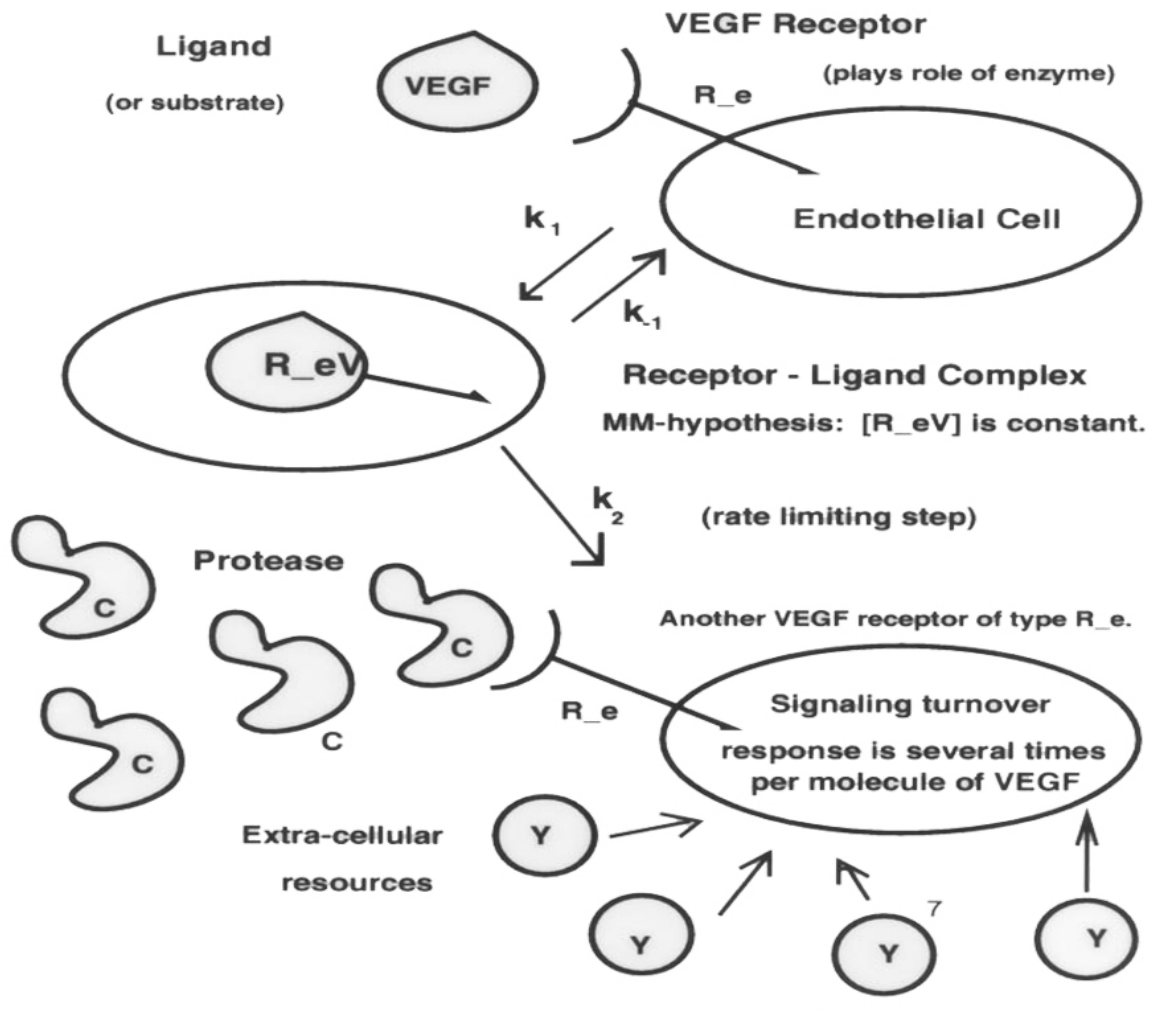
Inhibition pathways



From Eckhart, Hosp. Pract. 1999

IV. The role of chemical kinetics

Growth factor-protease exchange via endothelial cell signaling. Modeled via Michaelis-Menten enzyme kinetics.



1. VEGF signals EC expression of protease:

$$V + R_e \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \{R_e V\}$$

$$Y + \{R_e V\} \xrightarrow{k_2} mC + R_e$$

2. Angiostatin acts as a direct protease inhibitor:

$$C_{act} + A \overset{\nu_e}{\rightleftarrows} C_I$$

3. Proteolysis of fibronectin by active protease:

$$C_{act} + F \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} \{C_{act} F\}$$

$$\{C_{act} F\} \xrightarrow{k_4} C_{act} + F'$$

1. Protease mass balance:

$$[C] = [C_{act}] + [C_I] + [\{C_{act}F\}].$$

2. MM hypothesis for VEGF signaling and for fibronecin proteolysis:

$$[\{R_eV\}] = [R_e][V]/K_m^1,$$
$$[\{C_{act}F\}] = [C_{act}][F]/K_m^2.$$

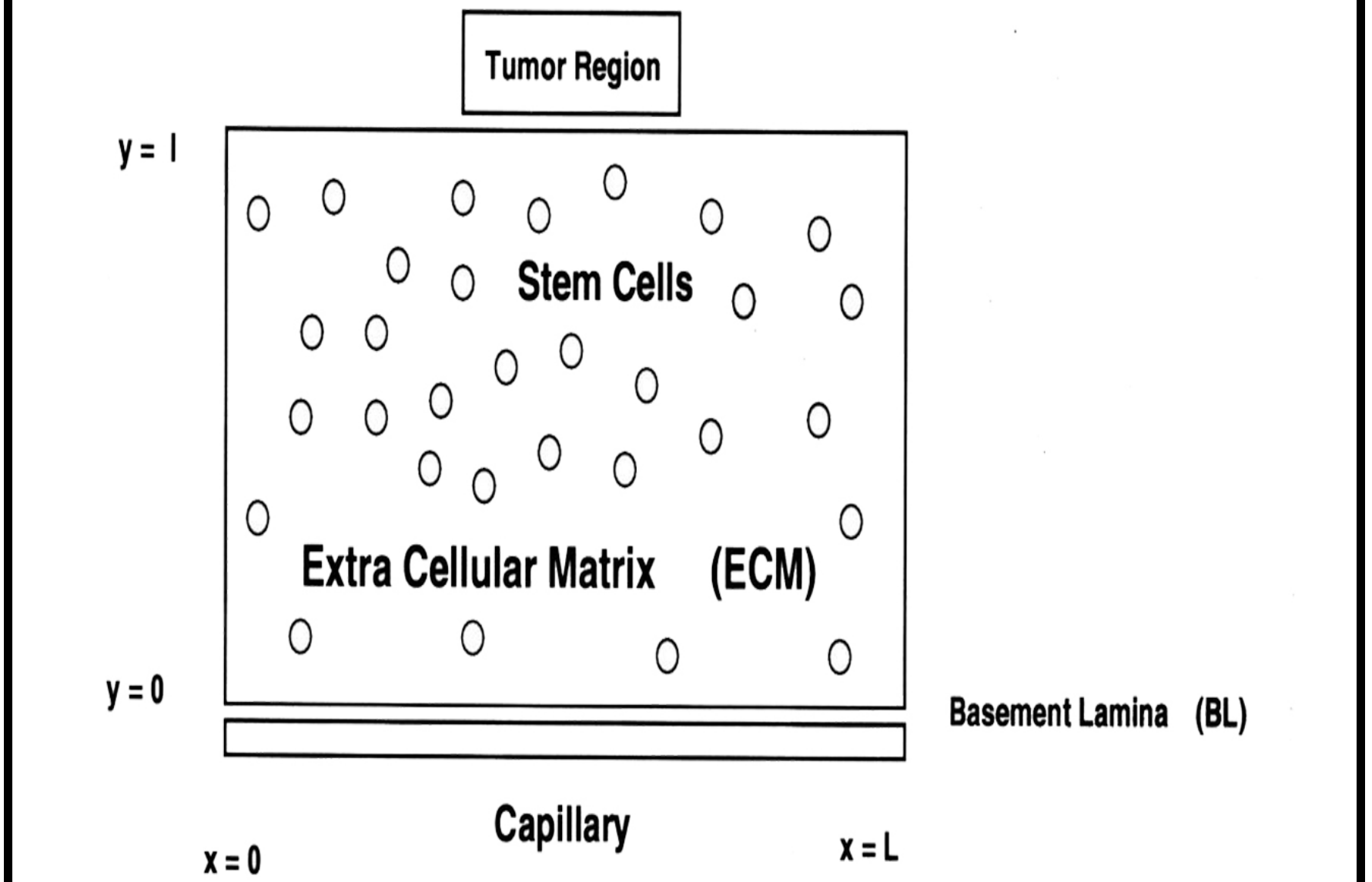
Note: One molecule of VEGF yields m molecules of C per cell cycle (amplification). In fact; m=m([V])!!)

Notation to follow: For the EC reaction: $K_m^1 = (k_2[Y] + k_{-1})/k_1$ and $K_{cat}^1 = k_2[Y]\delta$ where δ is the number of receptors per endothelial cell.

This makes EC density proportional to receptor density.

For the last reaction pair,$K_m^2 = (k_4 + k_{-3})/k_3$ and $K_{cat}^2 = k_4$.

A segment of capillary wall of fixed length L on the x axis at $y = 0$.
A tumor is located at $y = \ell$.


Tumor Region
y = l
Stem Cells
Extra Cellular Matrix (ECM)
y = 0
Basement Lamina (BL)
Capillary
x = 0
x = L

Notation for the capillary wall:

$$
\begin{aligned}
v(x,t) &= \text{angiogenic factor, } V, \\
c(x,t) &= \text{total available proteolytic enzyme, } C, \\
c_a(x,t) &= \text{free active proteolytic enzyme, } C_{act}, \\
c_i(x,t) &= \text{inhibited proteolytic enzyme, } C_I, \\
\tilde{c_a}(x,t) &= \text{total available active enzyme, } \tilde{C}_{act}, \\
f(x,t) &= \text{fibronectin, } F, \\
a(x,t) &= \text{angiostatin as inhibitor, } A, \\
\eta(x,t) &= \text{endothelial cell density.}
\end{aligned}
$$

In the ECM, the notation becomes:

$$
\begin{aligned}
V(x,y,t) &= \text{angiogenic factor, } V, \\
C(x,y,t) &= \text{proteolytic enzyme, } C, \\
C_a(x,y,t) &= \text{free active proteolytic enzyme, } C_{act}, \\
C_i(x,y,t) &= \text{inhibited proteolytic enzyme, } C_I, \\
\tilde{C}_a(x,y,t) &= \text{total available active enzyme, } \tilde{C}_{act}, \\
F(x,y,t) &= \text{fibronectin, } F, \\
A(x,y,t) &= \text{angiostatin as inhibitor, } A, \\
N(x,y,t) &= \text{endothelial cell density.}
\end{aligned}
$$

A. Kinetic equations in the capillary

Mass conservation yields:

$$c_i = \nu_e \iota_a c_a, \quad \tilde{c_a} = c - c_i = c_a + c_a f / K_m^3.$$

With $V_{max}^1 = K_{cat}^1 \eta_0$, mass action gives:

$$\begin{aligned}
\frac{\partial v}{\partial t} &= -\frac{V_{max}^1 v}{K_m^1 + v} \frac{\eta}{\eta_0} + \sigma_1 \frac{\eta}{\eta_0} + \nu_1 \frac{\partial V}{\partial y}(x, 0, t) - \mu_1 v \\
\frac{\partial c}{\partial t} &= \frac{m V_{max}^1 \, v}{K_m^1 + v} \frac{\eta}{\eta_0} - \mu_2 c \\
\frac{\partial a}{\partial t} &= a_r(x,t) + \sigma_2 \frac{\eta}{\eta_0} - \mu_2 a \\
\frac{\partial f}{\partial t} &= \frac{4}{T_f} \left(1 - \frac{f}{f_0}\right) f \frac{\eta}{\eta_0} - \frac{K_{cat}^2 \tilde{c_a} f}{K_m^2 + f}
\end{aligned}$$

If $v(x,0) = c(x,0) = a(x,0) = a_r = 0$, $f(x,0) = f_0$, then the dynamics is driven by the flux term in v_t, namely $\nu_1 V_y(x,0,t)$.

Here $a_r(x,t) =$ angiostatin source term. This is assumed constant and non-zero for the "treatment" phase of the simulations.

B. Kinetic equations in the ECM

Mass conservation again:

$$C_i = \nu_e I_a C_a, \quad \tilde{C}_a = C - C_i = C_a + C_a F/K_m^2.$$

Again mass action considerations yield:

$$\frac{\partial V}{\partial t} = \nabla \cdot (D_V \nabla V) - \frac{V_{max}^1 \, V}{K_m^1 + V} \frac{N}{\eta_0} + \sigma_1 \frac{N}{\eta_0} - \mu_1 V + V_r(x, y, t)$$

$$\frac{\partial C}{\partial t} = \frac{m V_{max}^1 \, V}{K_m^1 + V} \frac{N}{\eta_0} - \mu_2 C$$

$$\frac{\partial A}{\partial t} = \nabla \cdot (D_A \nabla A) + \sigma_2 \frac{N}{\eta_0} - \mu_3 A + a_r(x, t)\left(1 - \frac{F}{F_0}\right)$$

$$\frac{\partial F}{\partial t} = D_F \Delta F + \frac{4}{T_F}\left(1 - \frac{F}{F_0}\right) F - \frac{K_{cat}^2 \tilde{C}_a F}{K_m^2 + F}$$

($V_r(x, y, t)$ is a source term for other potential ECM sources of growth factor such as stem cells, fibroblasts, macrophages etc.)

C. Coupling between capillary and ECM equations

1. Cell movement into the ECM from the capillary:

$$N(x,0,t) \quad = \quad \psi_1 \eta(x,t) H(f_1 - f(x,t)).$$

2. Flux of VEGF and angiostatin at the capillary:

$$D_V(x,0,t)\frac{\partial V(x,0,t)}{\partial y} \quad = \quad \rho_1(V(x,0,t) - v(x,t)),$$

$$D_A(x,0,t)\frac{\partial A(x,0,t)}{\partial y} \quad = \quad \rho_2(A(x,0,t) - a(x,t)).$$

V. The role of reinforced random walks.

A. Random walk equations in the capillary

For the endothelial cell movement equation,

$$\frac{\partial \eta}{\partial t} = D_\eta \frac{\partial}{\partial x}\left(\eta \frac{\partial}{\partial x}\left(\ln \frac{\eta}{\tau_{cap}(\tilde{c_a}, f)}\right)\right).$$

Here $\tau_{cap}(\tilde{c_a}, f)$ is the probability transition function (PTF). EC is movement $\tilde{c_a}, f$ dependent and is (*to a point*) up the $\tilde{c_a}$ gradient and down the f gradient.

A simple PTF is $\tau_{cap}(\tilde{c_a}, f) = \tilde{c_a}^{\gamma_1} f^{-\gamma_2}$. We molify this a bit to avoid the zeros of (τ).

A better model is: $\tau_{cap}(\tilde{c_a}, f) = \tilde{c_a}^{\gamma_1} e^{-\gamma_3 \tilde{c_a}} (f(1 - f/f_0)^{\gamma_2}$.

B. Random walk equations in the ECM

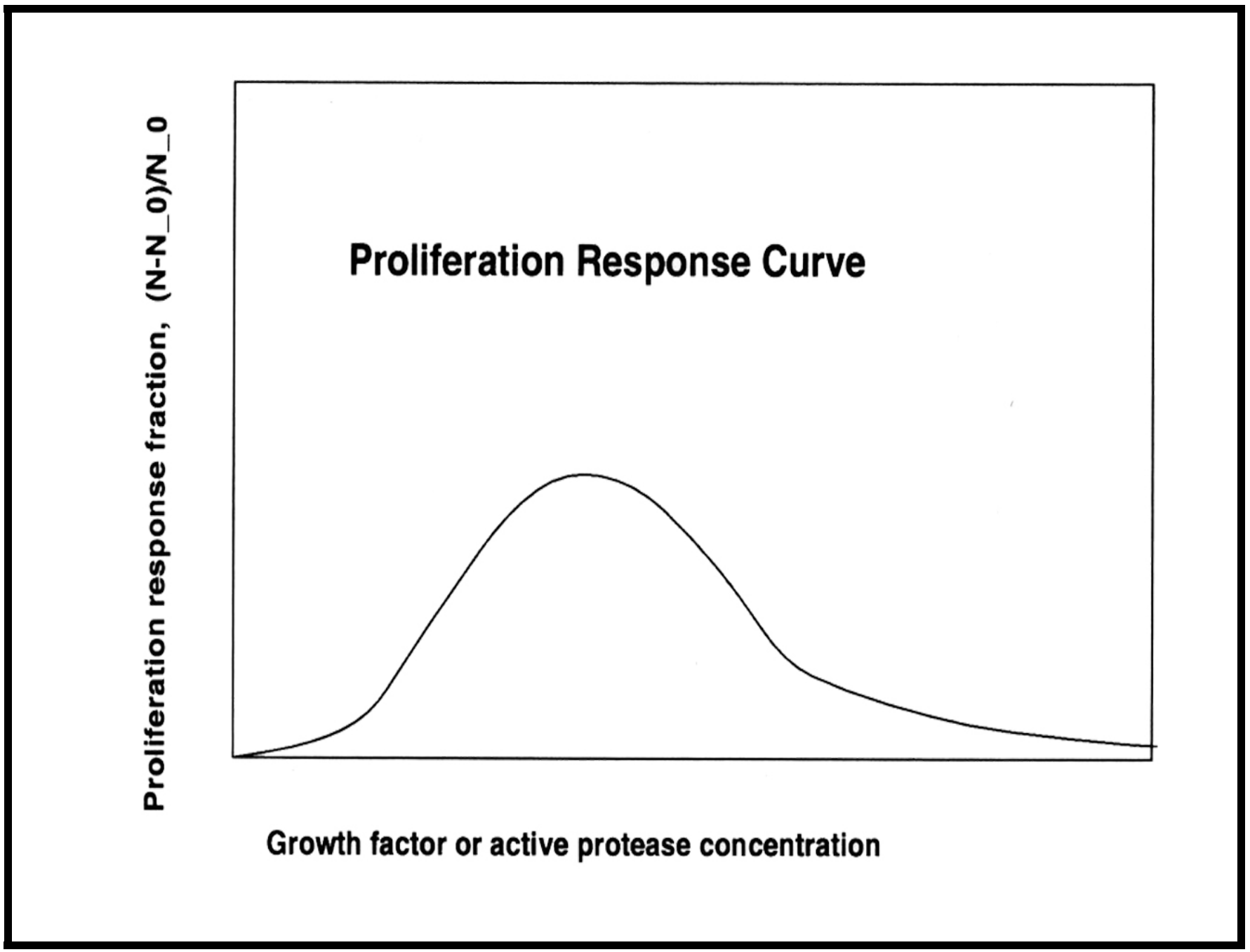
Cells will proliferate once they reach the ECM:

$$\frac{\partial N}{\partial t} = D_N \nabla \cdot \left[N \nabla \ln \left(\frac{N}{\tau_{ecm}(\tilde{C}_a, F)} \right) \right] + \left\{ N \left[\theta_1 \left(1 - \frac{N}{\eta_0} \right) + G_1(\tilde{C}_a) \frac{\partial \tilde{C}_a}{\partial t} \right] - \mu_1 N \right\}$$

Proliferation of EC occurs only near the tips of growing capillaries.

The PTFs, τ_{cap}, τ_{ecm}, are of the same form.

Cell proliferation responds to protease biphasically. That is, $G(X) = \frac{\Theta'(X)}{1+\Theta(X)}$ where $\Theta(z)$ is biphasic:


Proliferation Response Curve
Proliferation response fraction, (N-N_0)/N_0
Growth factor or active protease concentration

VI. Boundary and initial conditions.

Normalizations: $\eta_0 = f_0 = F_0 = 1$.

Initial conditions in the capillary:

$$\eta(x,0) = 1, \quad v(x,0) = 0, \quad c(x,0) = 0,$$
$$a(x,0) = 0, \quad c_a(x,0) = 0, \quad f(x,0) = 1.$$

Initial conditions in the ECM:

$$N(x,y,0) = 0, \quad V(x,y,0) = 0, \quad C(x,y,0) = 0,$$
$$A(x,y,0) = 0, \quad C_a(x,y,0) = 0, \quad F(x,y,0) = 1.$$

Boundary conditions away from the tumor surface:

At the capillary ends, $y = 0$, and $x = 0, L$:

$$\eta \frac{\partial}{\partial x}\left(\ln \frac{\eta}{\tau_{cap}}\right) = 0.$$

In the ECM at $x = 0, L$ with $0 < y \leq \ell$

$$N \frac{\partial}{\partial x}\left(\ln \frac{N}{\tau_{ecm}}\right) = 0.$$

No flux conditions at $x = 0, L$:

$$\begin{aligned} D_V \partial_x V(0,y,t) = D_V \partial_x V(L,y,t) &= 0, \\ D_F \partial_x F(0,y,t) = D_F \partial_x F(L,y,t) &= 0, \\ D_A \partial_x A(0,y,t) = D_A \partial_x A(L,y,t) &= 0. \end{aligned}$$

At the tumor, $y = \ell$, a proposed mechanism for EC penetration is:

$$-D_N N \frac{\partial}{\partial y}\left[\ln\left(\frac{N}{\tau_{ecm}}\right)\right] + \mu_1' N = 0.$$

For angiostatin and fibronectin, no flux conditions:

$$D_A \partial_y A(x,\ell,t) = 0, \quad D_F \partial_y F(x,\ell,t) = 0,$$

The growth factor to the ECM from the tumor is controlled by the flux:

$$D_V \frac{\partial V(x,\ell,t)}{\partial y} = V_0[1-\cos(2\pi x/L)]^{\beta_1} e^{-\Theta_1 t}.$$

$1/\Theta_1$ is the relaxation time for the avascular tumor's capacity to supply growth factor.

VII. Some illustrative computations.

Two cases for the angiostatin:

A. $a_r(x,t) = 0$, no therapeutic agent present.

B. $a_r(x,t) = A_0 > 0$ agent uniformly distributed in the circulatory system.

We took $\Theta_1 = 0$. That is, we assumed infinite tumor capacity to supply growth factor at a fixed rate.

The model involves 67 biological and empirical parameters. Some of them (enzyme constants, cell movement constants, protein diffusion constants) were found in the literature. Others were guesses or, in the absence of other information, set to zero or unity where appropriate.

The proliferation sensitivity constant was adjusted to give tip proliferation. (Changing this constant also changes the tip speeds. Low sensitivity slows the tip speed while very high sensitivity causes singularities in the EC density.)

Results and conclusions:

I. No angiostatin case:

A. The onset of sprouting time and the onset of vascularization time, when scaled up to one or two mm. agree with the experimental results of Folkman et. al. as well as with CAM assay experiments.

B. The channel widths (6-10 μmm) are the same order of magnetude as for capillary diameters.

C. EC proliferation is a maximum a little behind the moving tip.

D. Tip speed increases as the forming capillary approaches the tumor source.

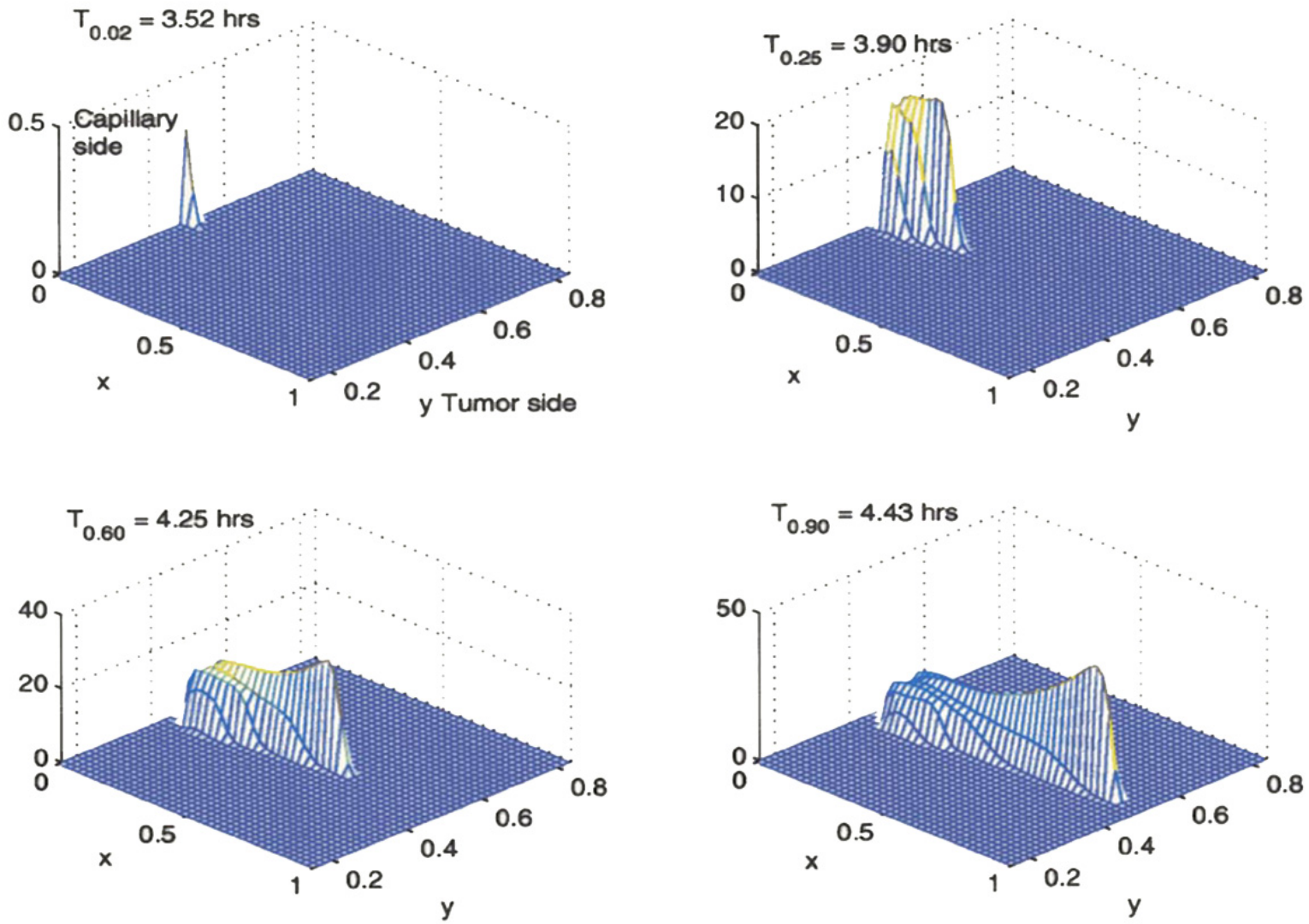
E. The model predicts the onset of sprouting without EC movement into the ECM when one allows for protease diffusion in the ECM.

Travel times and tip speeds

Distance from capillary to tumor in microns	Time in hours	Mean velocity (mm/day)	Extrap. times in days for 1 mm	Extrap. times in days for 2 mm
0.00	3.49		5.817	11.633
2.50	3.74	0.242	6.817	13.633
5.00	3.88	0.436	7.317	14.633
7.50	3.99	0.545	7.650	15.300
10.00	4.09	0.580	7.900	15.800
12.50	4.18	0.703	8.100	16.200
15.00	4.25	0.831	8.267	16.533
17.50	4.32	0.914	8.410	16.819
20.00	4.38	1.015	8.535	17.069
22.50	4.43	1.015	8.646	17.291

($\ell = 25\mu\text{mm}$, $L = 50\mu\text{mm}$)

Figure 5.1 Time course for EC propagation in the ECM



x scale: 0.1 = 2.5 microns, y scale: 0.1 = 5 microns

Figure 5.2 Time course for EC propagation in the capillary

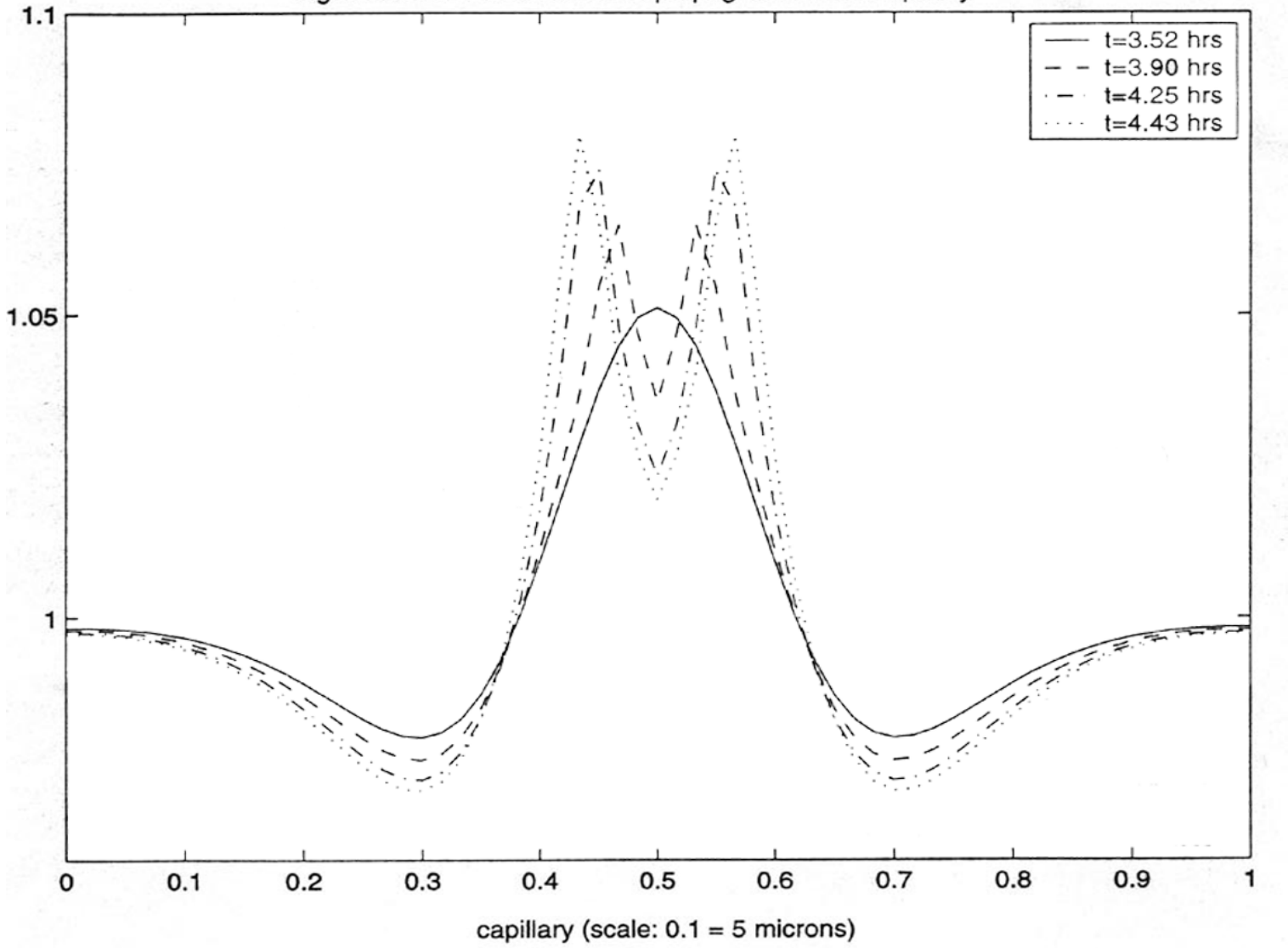
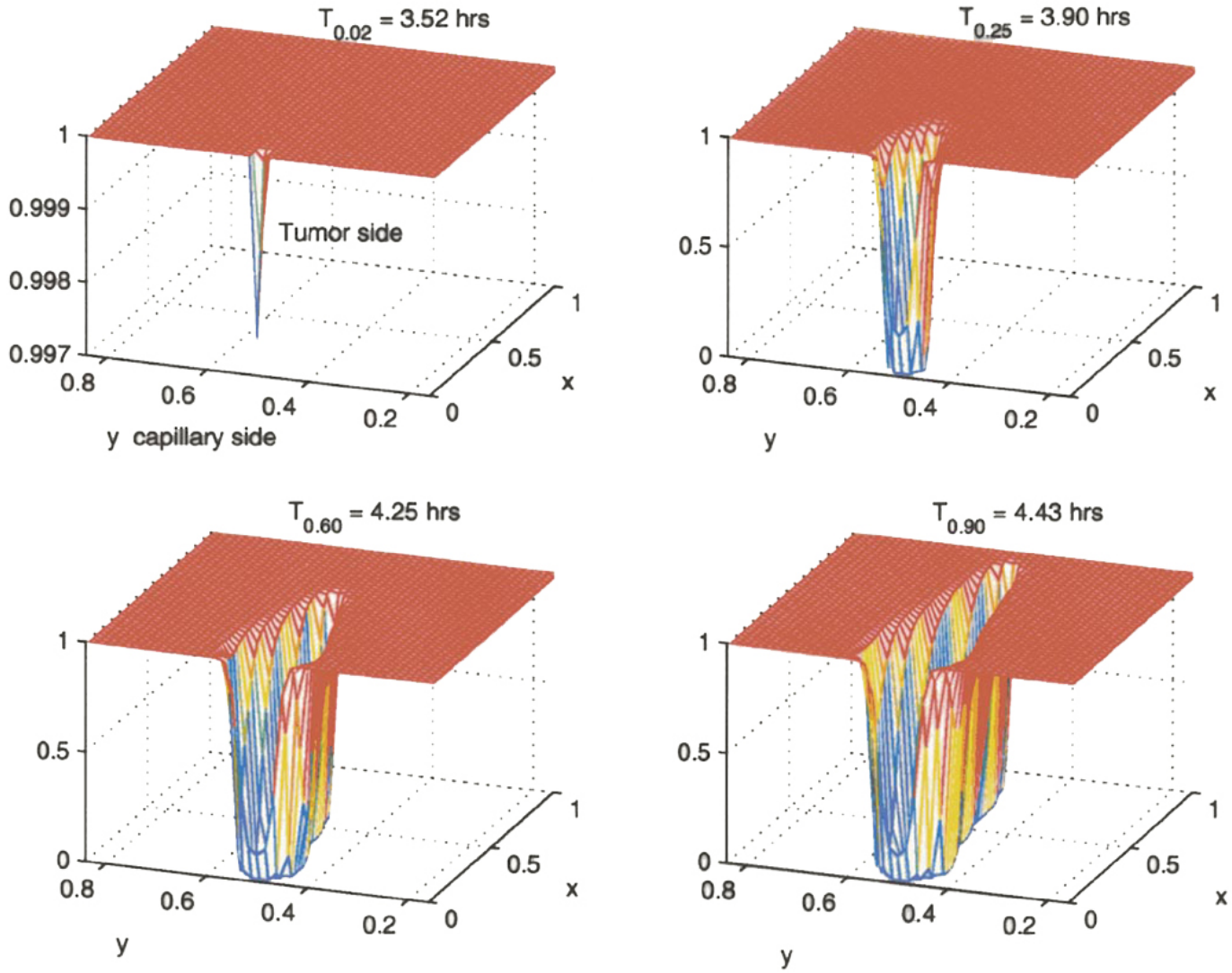


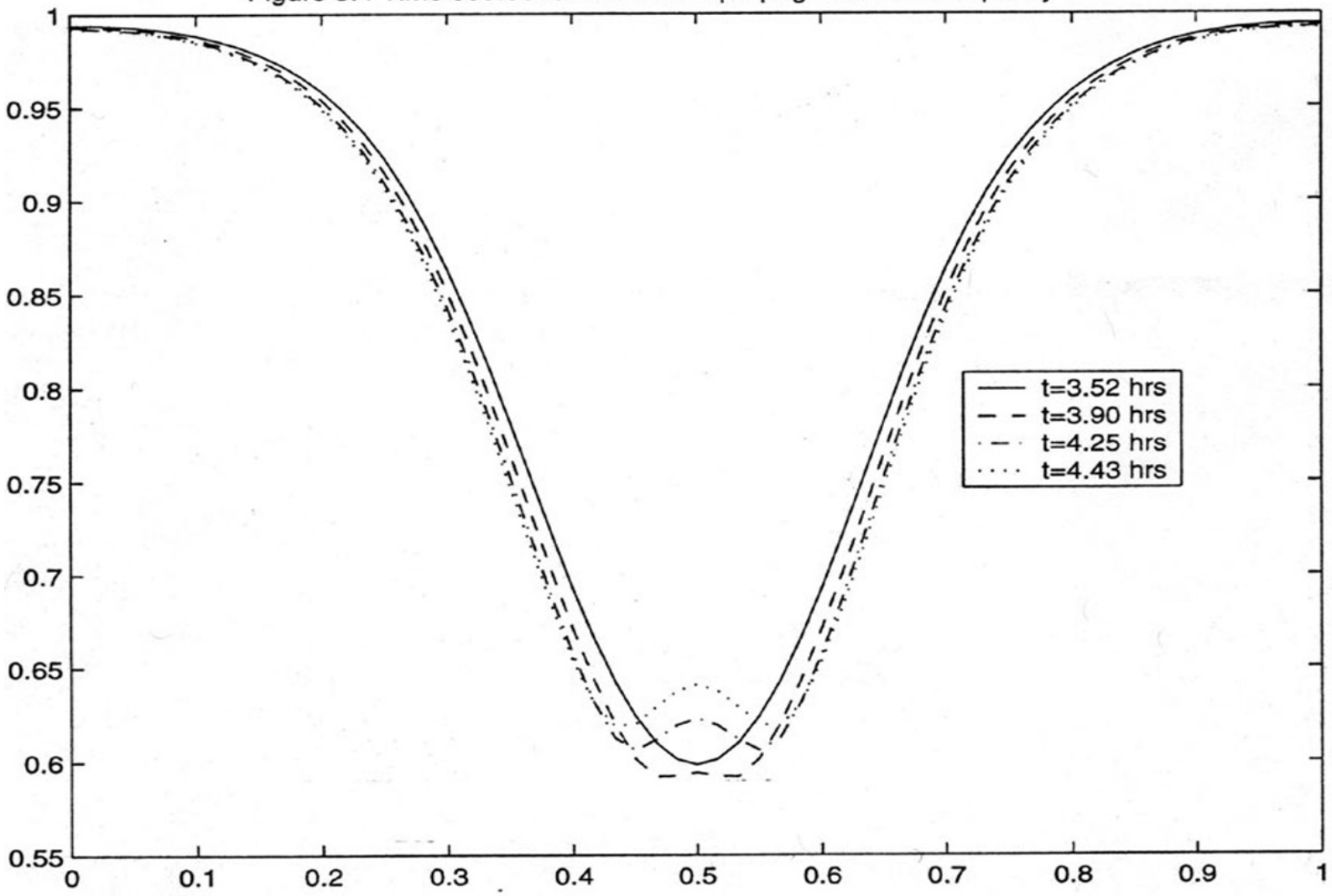
Figure 5.3 Time course for fibronectin propagation in the ECM



x scale: 0.1 = 2.5 microns, y scale: 0.1 = 5 microns

Fibronectin density in the capillary

Figure 5.4 Time course for fibronectin propagation in the capillary



II. Angiostatin case with a large equilibrium constant:

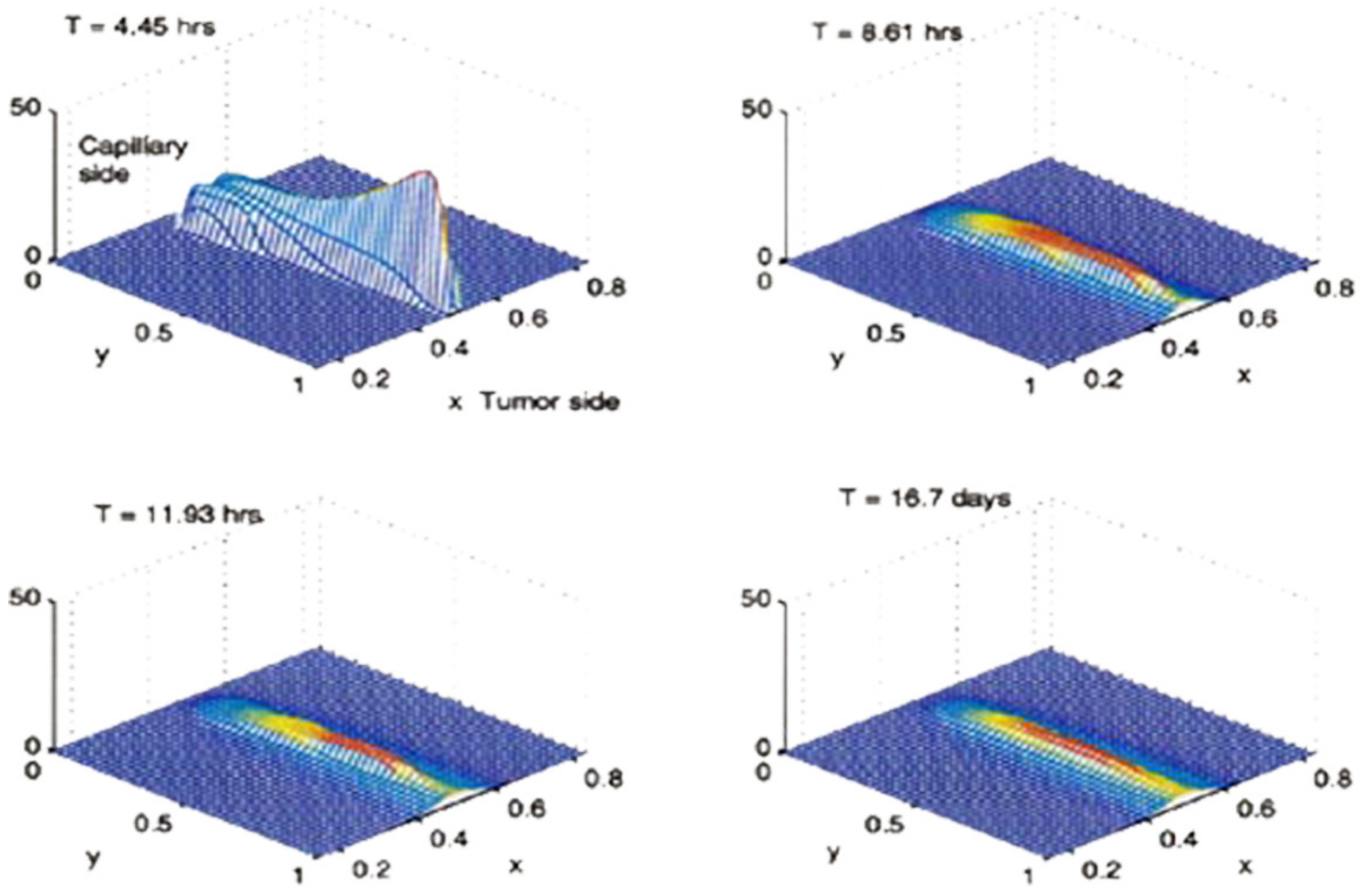
A. The opening from the mother capillary closes.

B. The EC density in the daughter capillary drops.

C. The tip retreats and the channel closes.

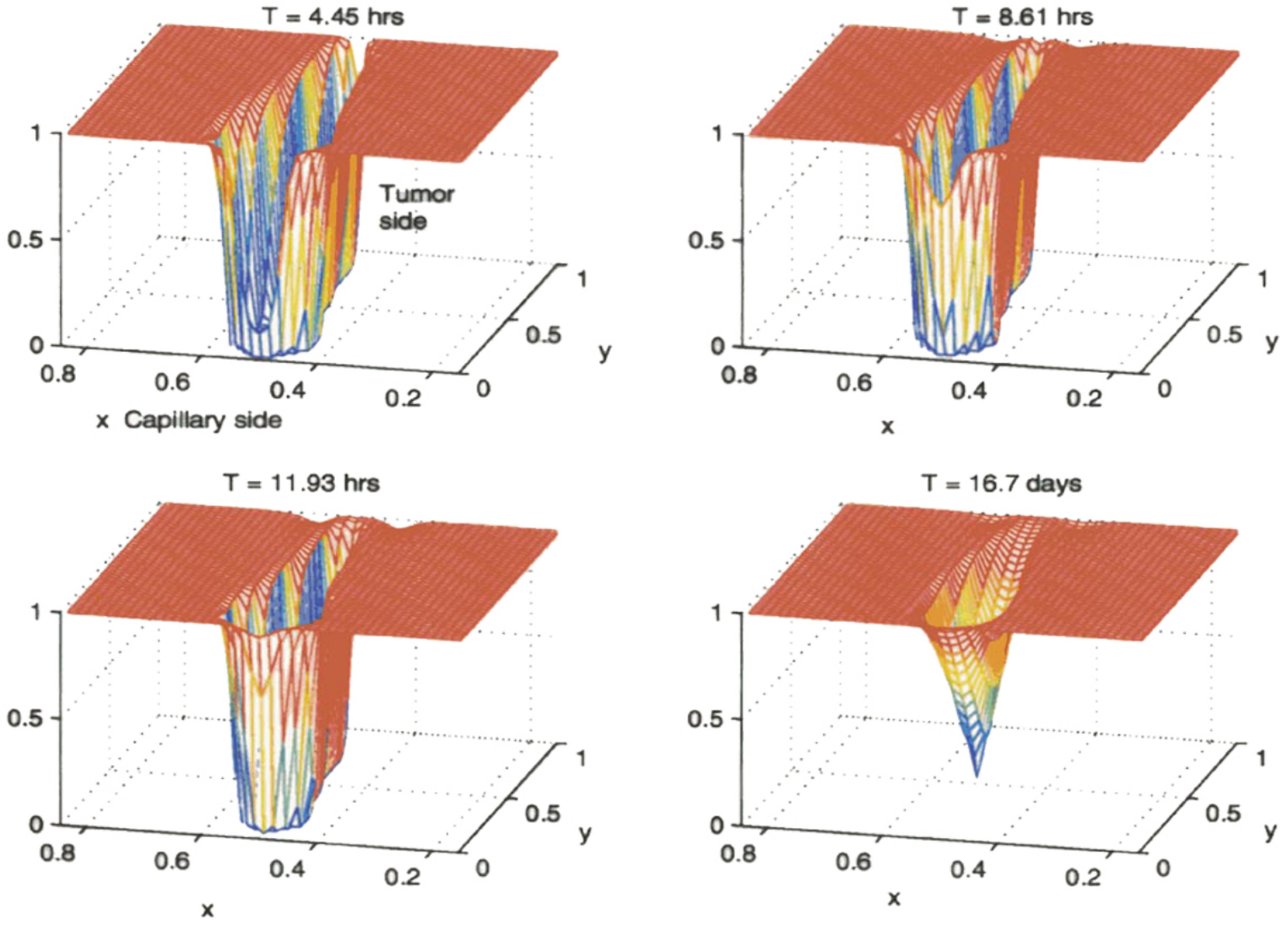
D. It takes much longer for the channel to close completely than for the EC density to fall to negligible values.

Figure 6.1 Time course for EC propagation in the ECM



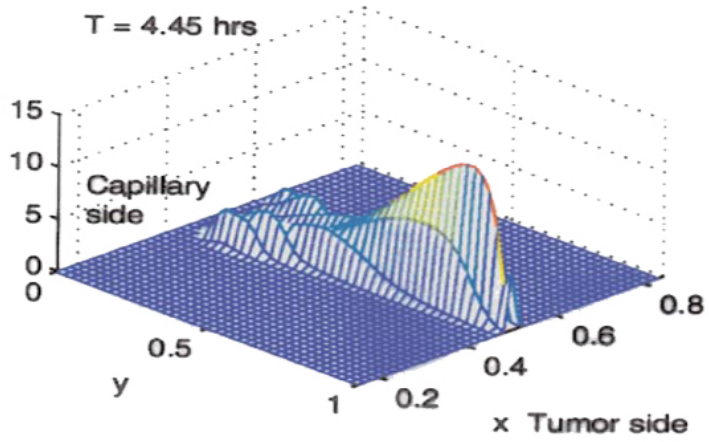
y axis scale: 0.1 = 2.5 microns, x axis scale: 0.1 = 5 microns

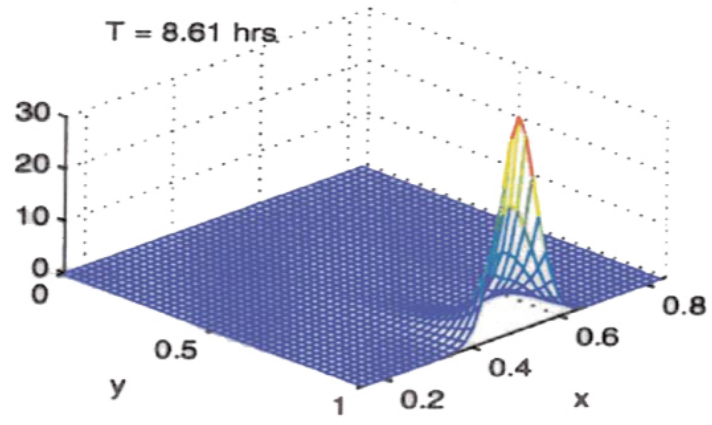
Figure 6.3 Time course for fibronectin propagation in the ECM

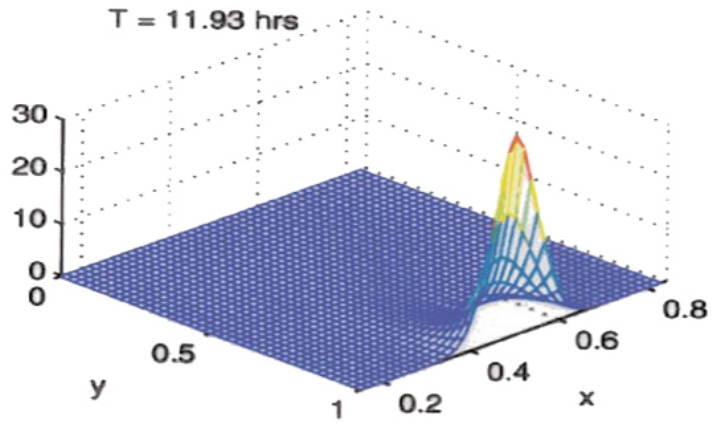


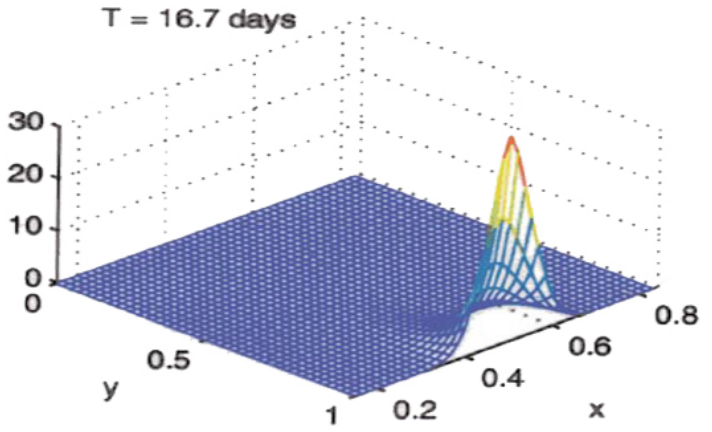
v axis scale: 0.1 = 2.5 hrs. x axis scale: 0.1 = 5 microns

Time course for protease in the ECM

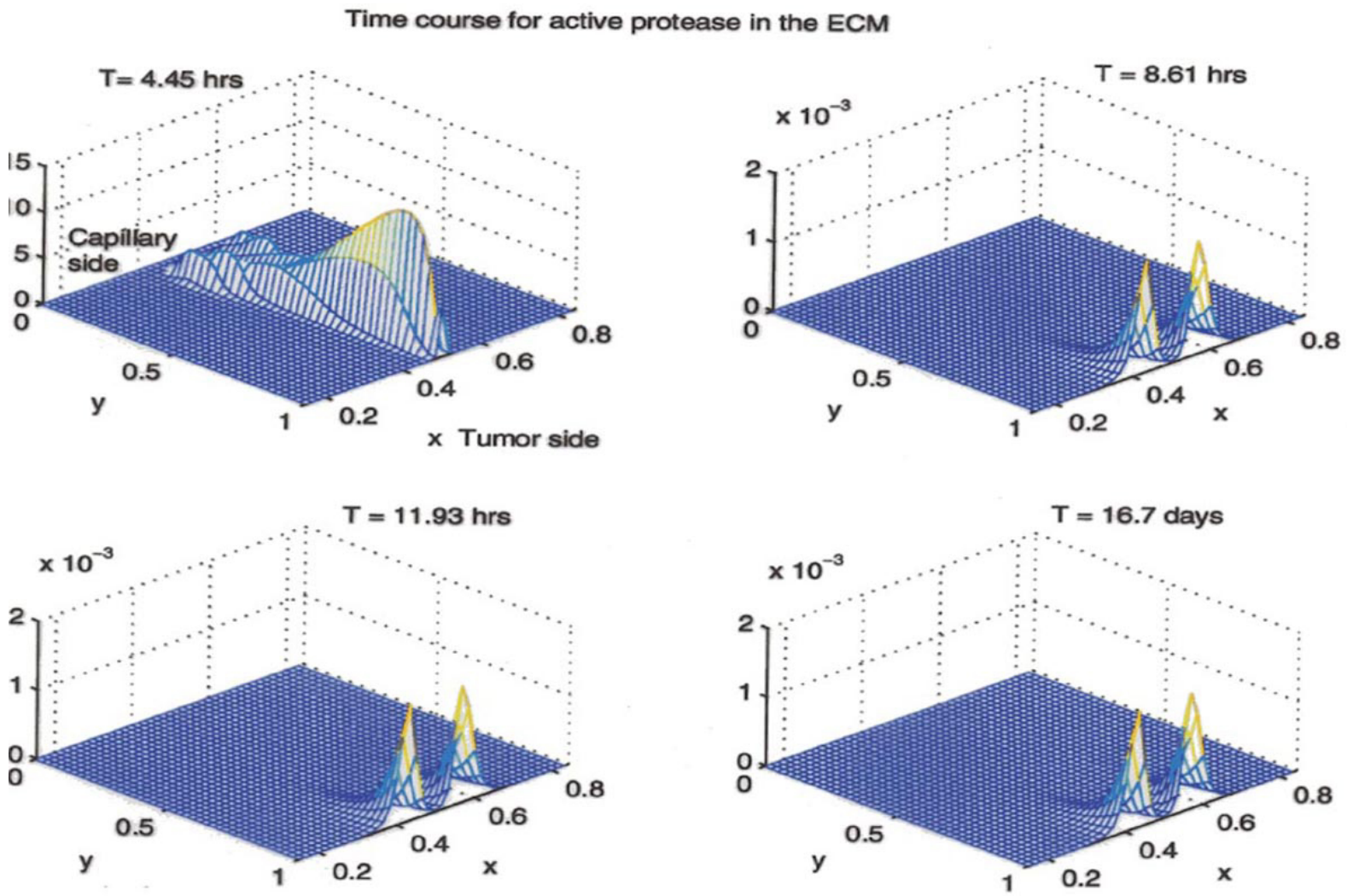


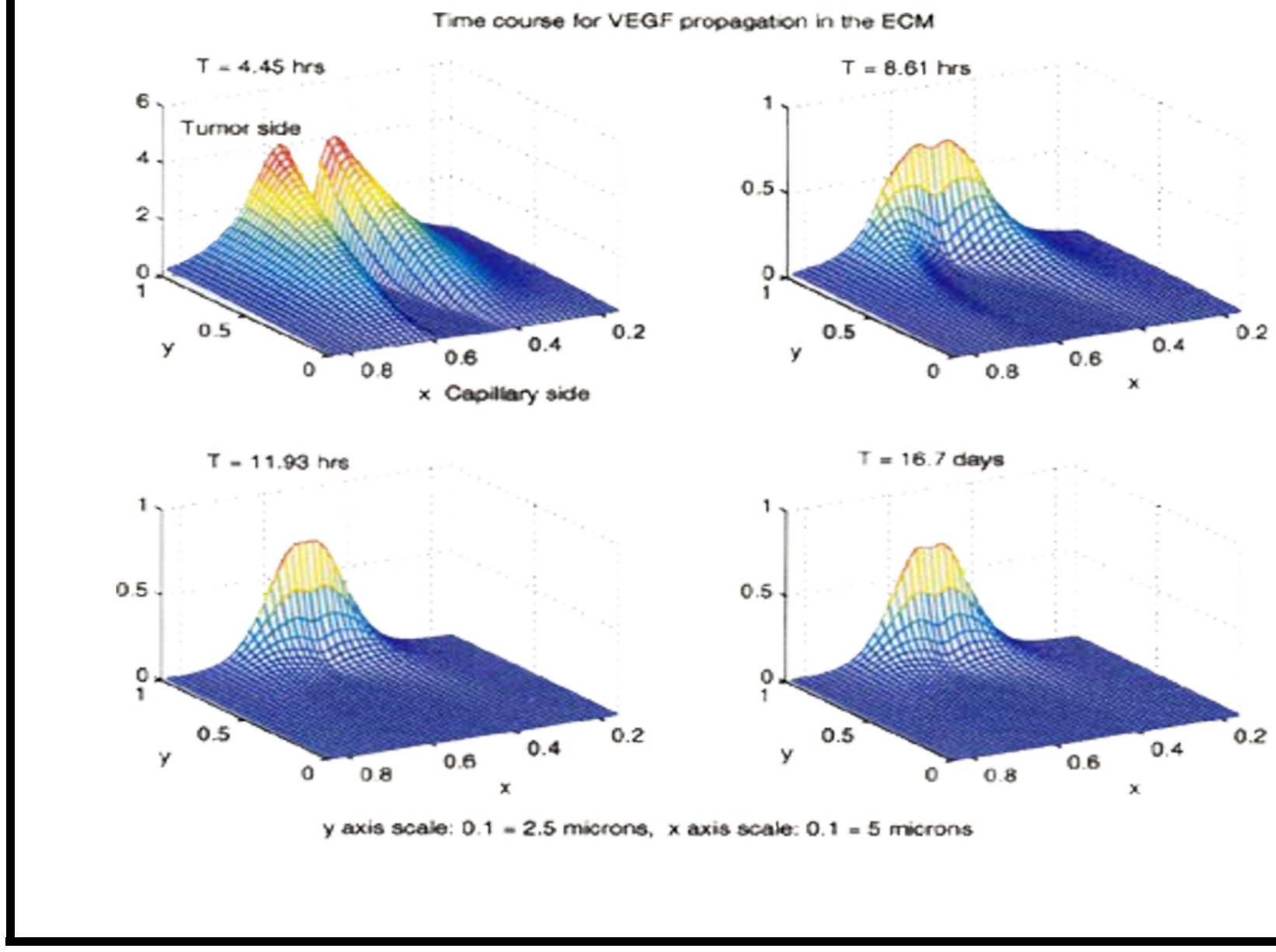




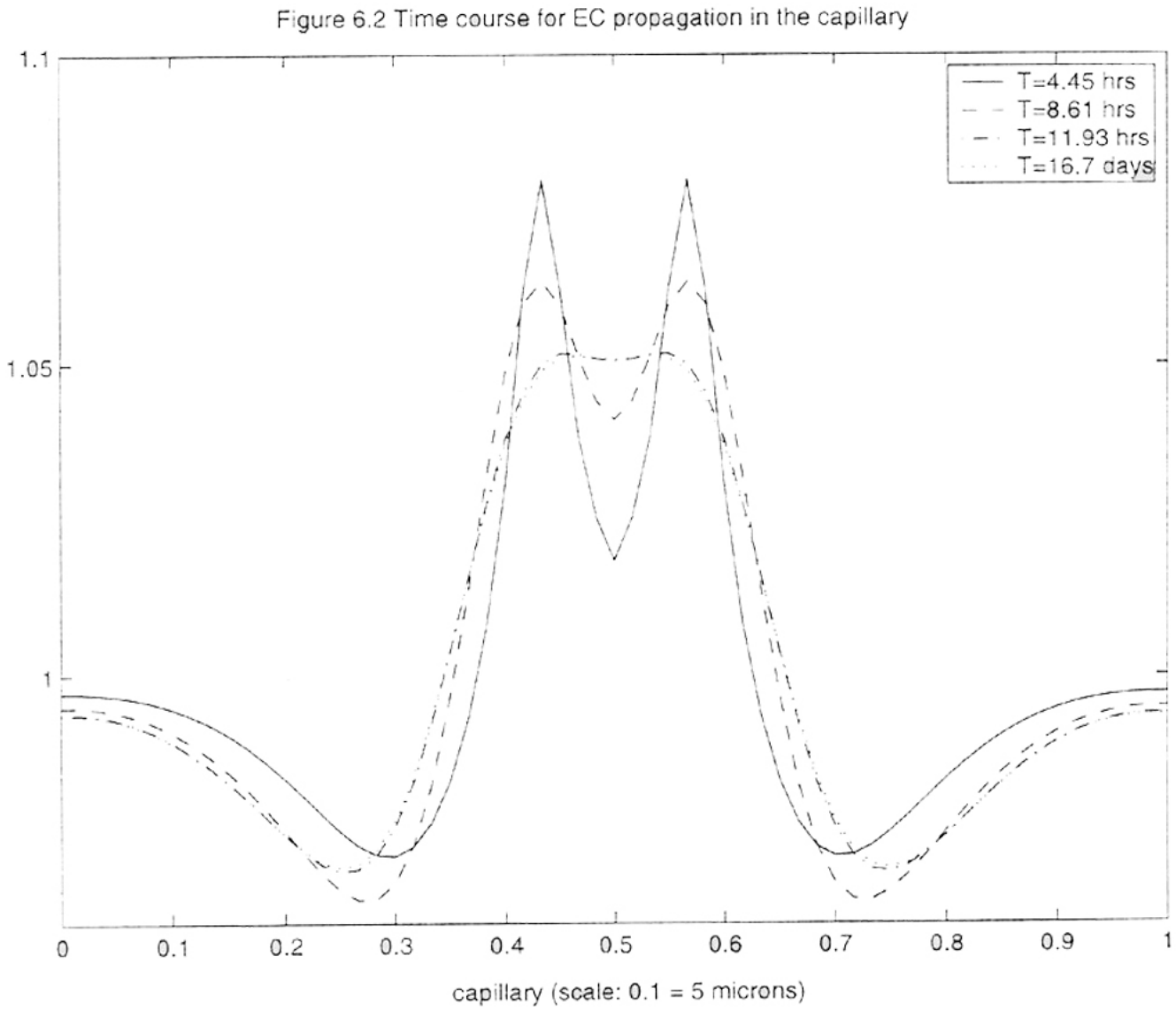


y axis scale: 0.1 = 2.5 microns, x axis scale: 0.1 = 5 microns


Time course for active protease in the ECM
T= 4.45 hrs
Capillary side
y
x Tumor side
T = 8.61 hrs
x 10^-3
T = 11.93 hrs
T = 16.7 days
y axis scale: 0.1 = 2.5 microns, x axis scale: 0.1 = 5 microns

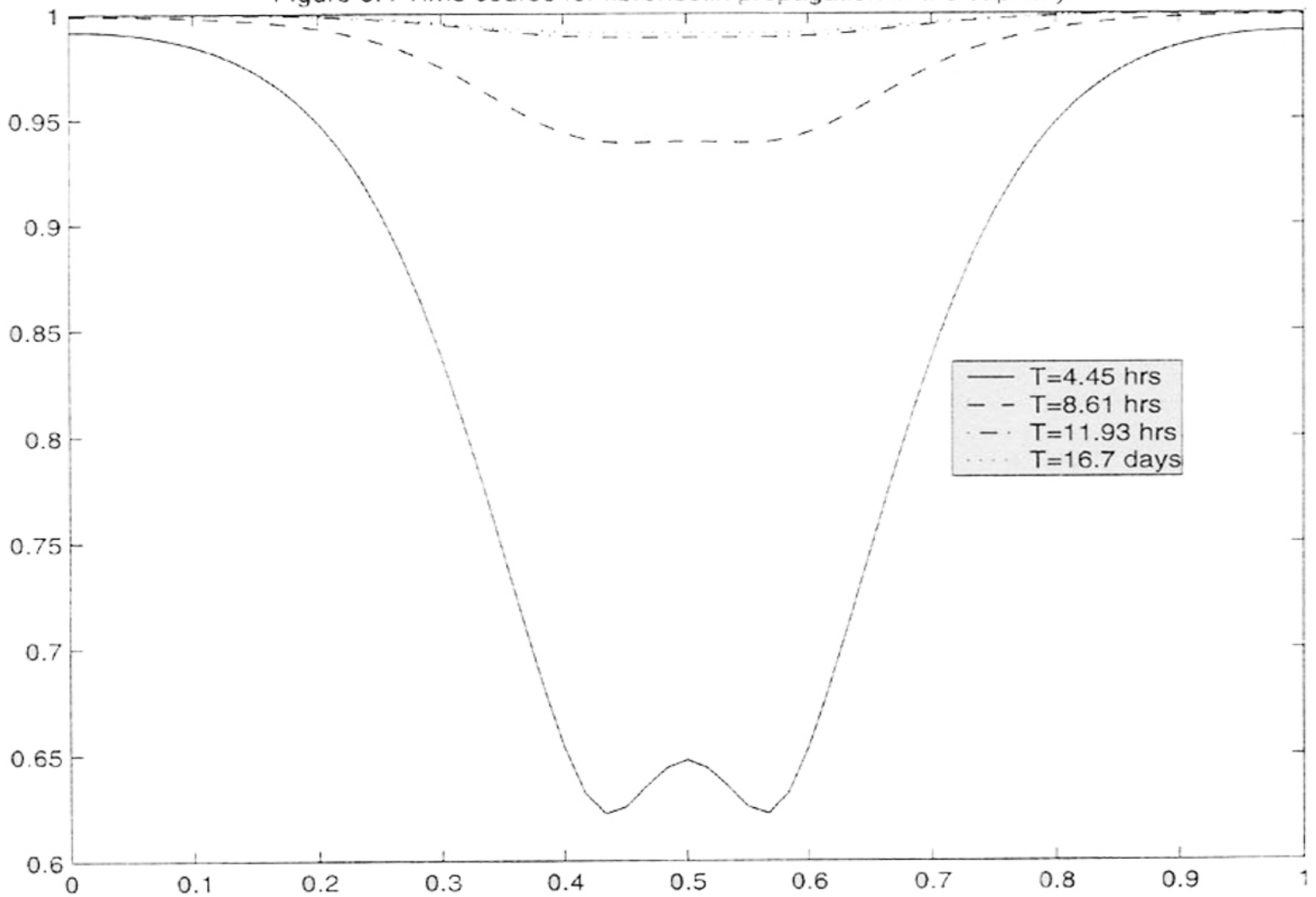

Time course for VEGF propagation in the ECM
T = 4.45 hrs
Tumor side
x Capillary side
T = 8.61 hrs
T = 11.93 hrs
T = 16.7 days
y axis scale: 0.1 = 2.5 microns, x axis scale: 0.1 = 5 microns

Cell density in the capillary



Fibronectin density in the capillary

Figure 6.4 Time course for fibronectin propagation in the capillary



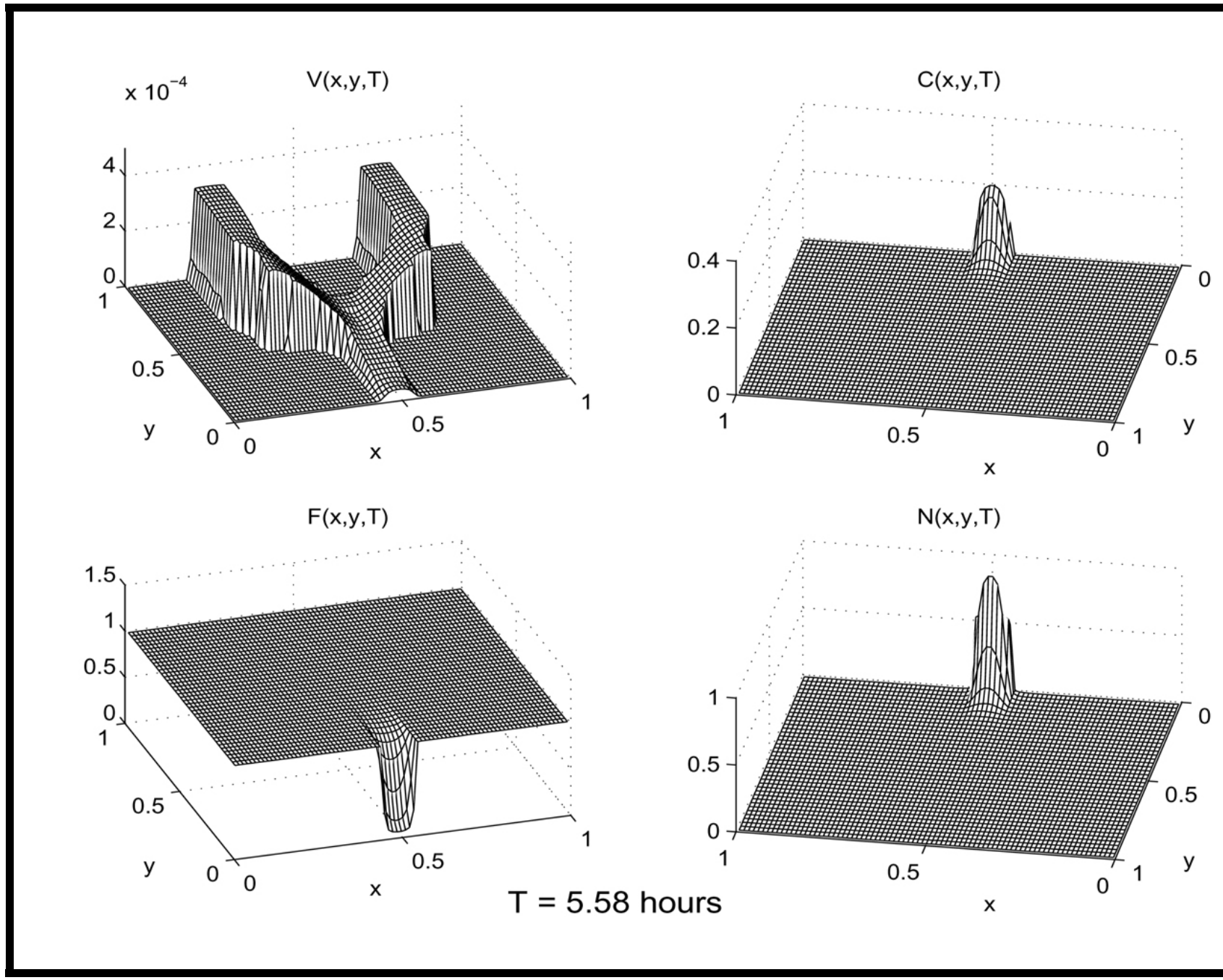
Smiley's simulations

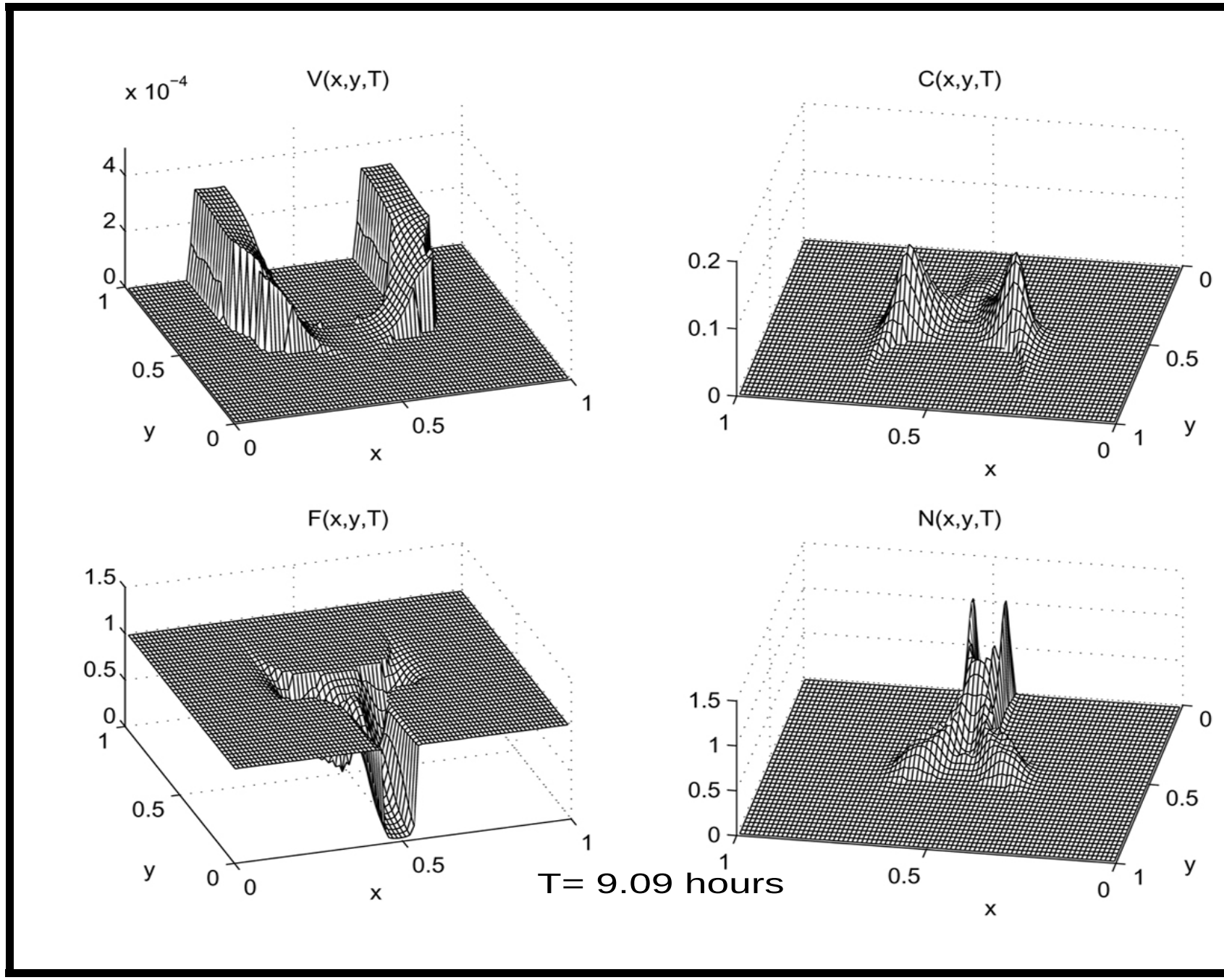
a. Two diffusible paths.

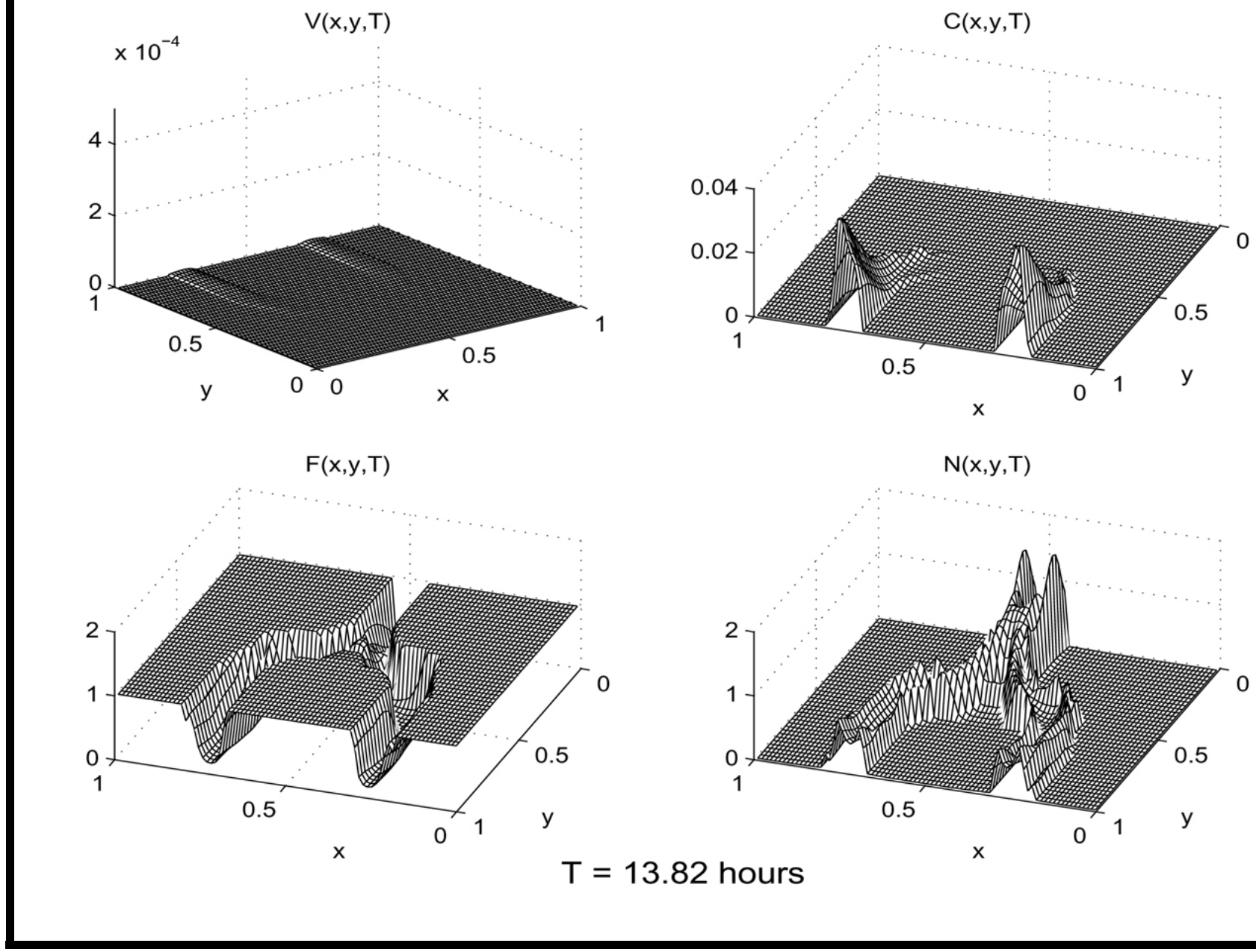
b. EC probability transition function isgrowth factor (not protease) dependent.

c. Variable EC/protease response to growth factor.

d. Fibronectin PTF has form $F(1-F)$ so that EC probability density will tend to aggregate along the channel wall.

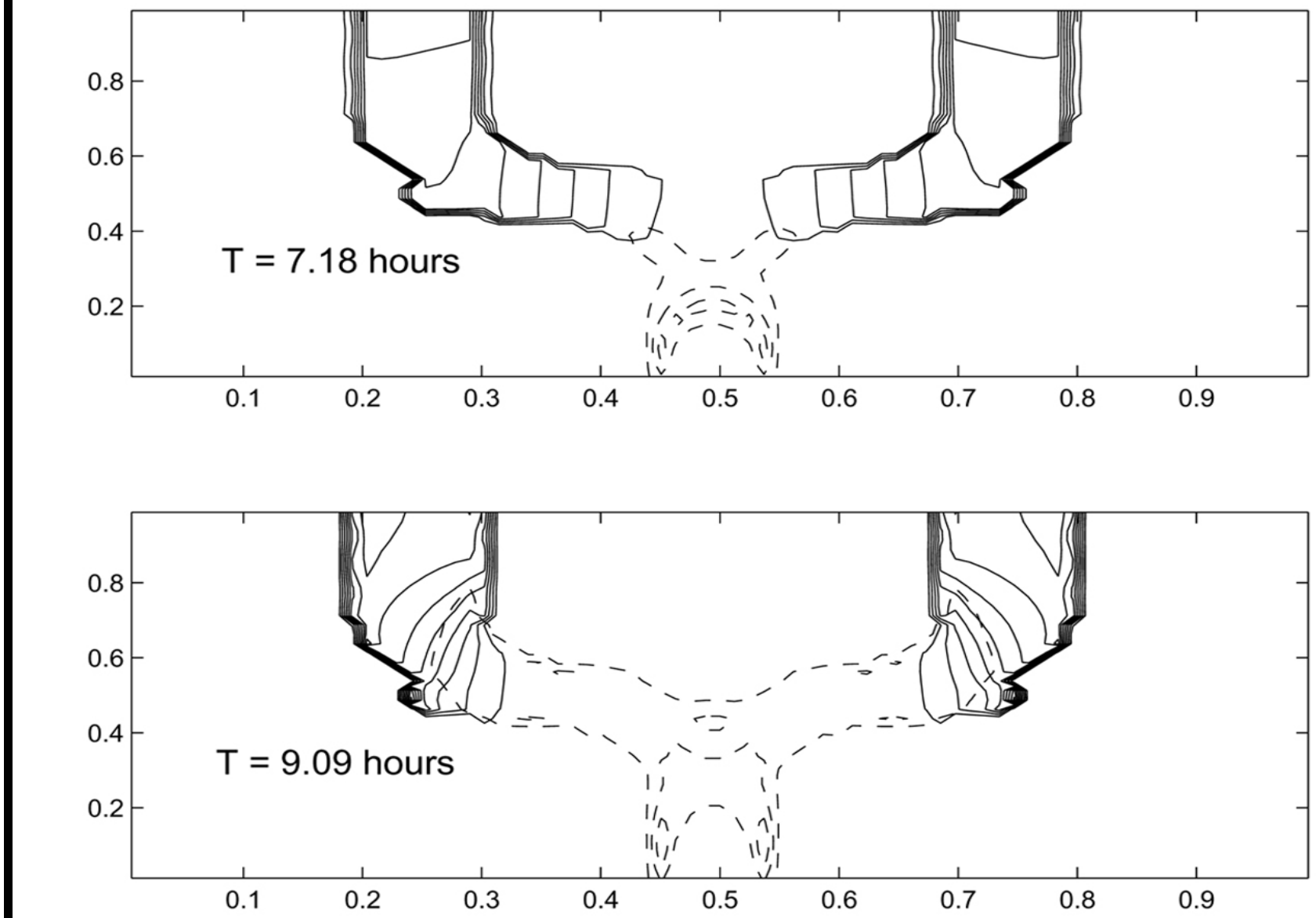

V(x,y,T)
x 10^-4
4
2
0
1
0.5
y
0
0
0.5
x
1
C(x,y,T)
0.4
0.2
0
1
0.5
0
0.5
y
1
x
F(x,y,T)
1.5
1
0.5
0
1
0.5
y
0
0
0.5
x
1
N(x,y,T)
1
0.5
0
1
0.5
0
0.5
y
1
x
T = 5.58 hours


V(x,y,T)
x 10^-4
4
2
0
1
0.5
y
0
0
0.5
x
1
C(x,y,T)
0.2
0.1
0
1
0.5
0
1
y
0.5
0
F(x,y,T)
1.5
1
0.5
0
1
0.5
y
0
0
0.5
x
1
N(x,y,T)
1.5
1
0.5
0
1
0.5
0
1
y
0.5
0
T= 9.09 hours


V(x,y,T)
x 10^-4
4
2
0
1
0.5
y
0
0
0.5
x
1
C(x,y,T)
0.04
0.02
0
1
0.5
0
0
0.5
y
1
x
F(x,y,T)
2
1
0
1
0.5
x
0
0
0.5
y
1
N(x,y,T)
2
1
0
1
0.5
x
0
0
0.5
y
1
T = 13.82 hours

EC and GF level sets

Solid lines correspond to GF level sets, dashed lines to EC level sets.


T = 7.18 hours
T = 9.09 hours
0.8
0.6
0.4
0.2
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9

VIII. Work in progress and future directions.

1. Model adapts other inhibitor pathways:
   a. Inhibitors which block capillary invasion of ECM.
   b. Inhibitors which limit EC proliferation (endostatins).
   c. Inhibitors which bind to VEGF or its receptors.
2. Modeling ideas can be used to model
   a. the role of plasminogin/plasmin in angiogenesis.
   b. the role of growth factor released by ECM stem cells in response to tumor necrotic factors (TNF's),

3. The model extends to other cell types such as:
   a. mast cells which respond to TNF's by expressing heparin, an FGF stabilizer.
   b. machrophages, transformers of hypoxic factors into growth factors.
   c. pericytes, regulators of the capillary wall thickness and tissue remodeling.
   d. fibroblasts which maintain ECM protein.
4. Test the model against laboratory experiments.
5. Develop software for a 2d-3d version of the model.