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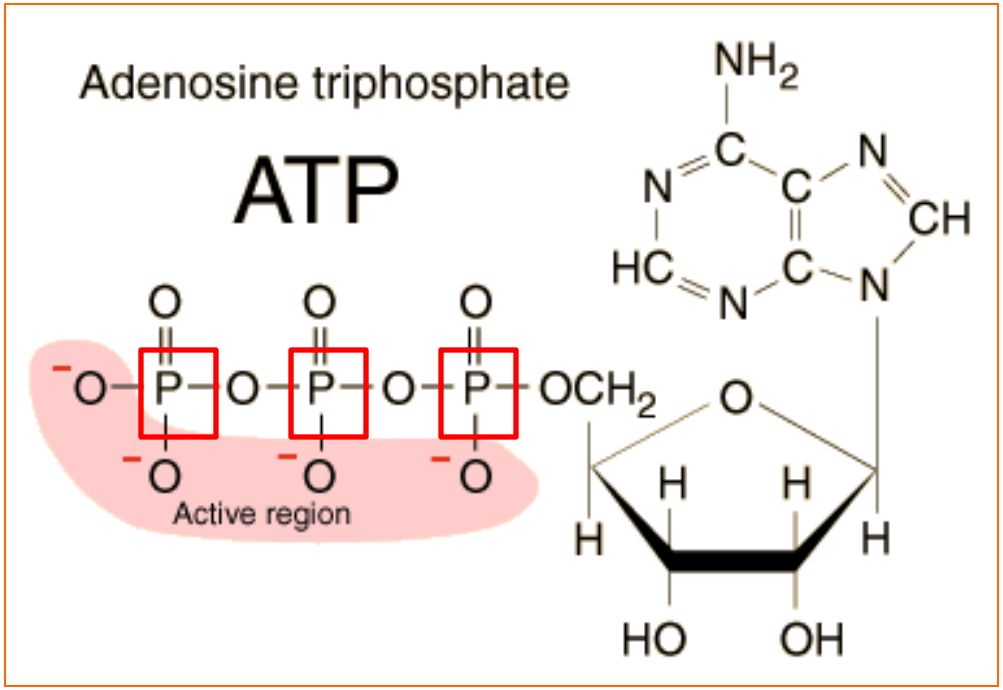
Modeling liquid and cells flow in tumor growth

2. Glucose metabolism and ATP production

PRAGUE, August 2011

Adenosine-triphosphate is obtained by the *phosphorylation* of ADP or AMP or by direct synthesis. It is converted back to its precursors by metabolic processes transferring chemical energy to the cells.

ATP production rate can be considered as a **viability index** of cells



J.J. Casciari, S.V. Sotirchos, R.M. Sutherland. *Variations in tumor cell growth rates and metabolism with oxygen concentration, glucose concentration, and extracellular pH.* J. Cell.Physiol., 151 (1992), 386–394.

J.J. Casciari, S.V. Sotirchos, R.M. Sutherland. *Mathematical modelling of microenvironment and growth in EMT6/Ro multicellular tumour spheroids.* Cell. Prolif., 25 (1992), 1–22.

J.P. Freyer, R.M. Sutherland. *A reduction in the in situ rates of oxygen and glucose consumptionof cells in EMT6/Ro spheroids during growth*. J. Cell. Physiol., 124 (1985), 516–524.

M. Guppy, P. Leedman, X.L. Zu, V. Russel. *Contribution by different fuels and metabolic pathways to the total ATP turnover of proliferating MCF-7 breast cancer cells.* Biochem. J., 364 (2002), 309–315.

Some experimental papers

first mathematical models

K. Smallbone, D.J. Gavaghan, R.A. Gatenby, P.K. Maini. *The role of acidity in solid tumour growth and invasion.* J. Theor. Biol., 235 (2005), 476–484.

R. Venkatasubramanian, M.A. Henson, N.S. Forbes. *Incorporating energy metabolism into a growth model of multicellular tumor spheroids.* J. Theor. Biol., 242 (2006), 440–453.

Travelling waves

R.A. Gatenby, E.T. Gawlinski. *The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models.* Cancer Res., 63 (2003), 3847–3854.

Steady states in spheroids

A. BERTUZZI, A. F., A. GANDOLFI, C. SINISGALLI. *ATP production and necrosis formation in a tumour spheroid model*. Mathematical Modelling of Natural Phenomena 2 (2007) 30-46.

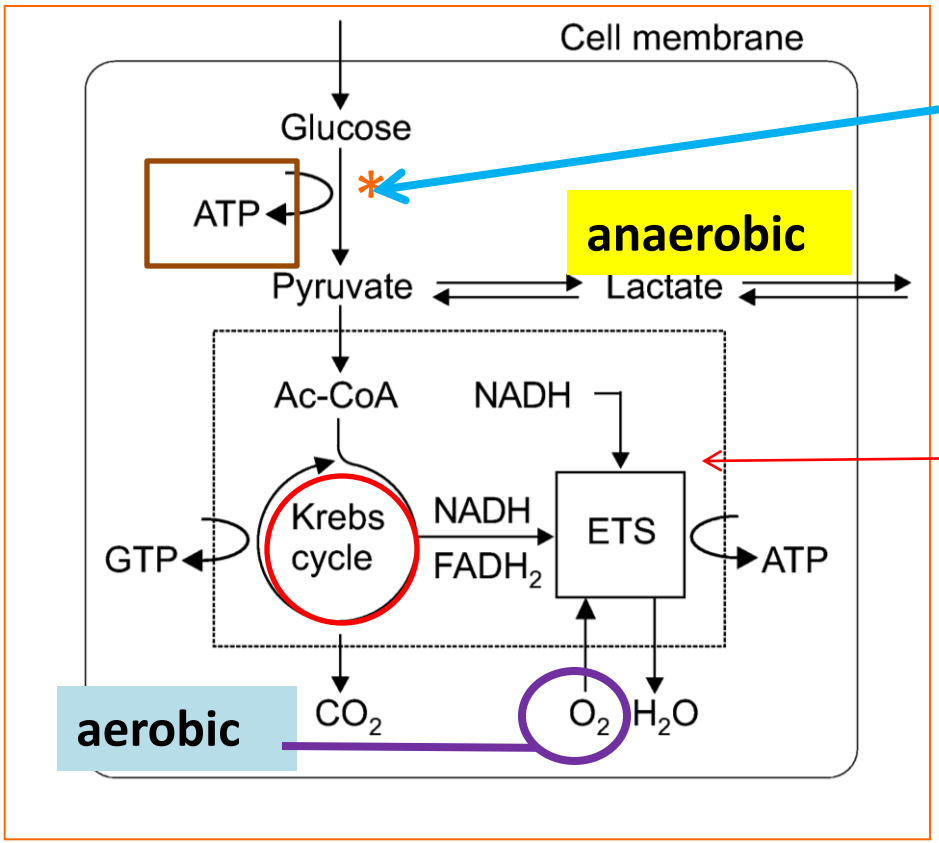
A. BERTUZZI, A. F., A. GANDOLFI, C. SINISGALLI. *Necrotic core in EMT6/Ro tumour spheroids: is it caused by an ATP deficit?* J. Theor. Biol. 262 (2010) 142-150

L. BIANCHINI, A. F. *A model combining acid-mediated tumour invasion and nutrient dynamics*. Nonlinear Analysis Real World Applications 10 (2009) 1955-1975.

Travelling waves

A. F., M.A. HERRERO, M. ROCHA RODRIGO. *Slow and fast invasion waves in a model of acid-mediated tumour growth*. Math. Biosci. 220 (2009) 45-56

CELL ENERGY METABOLISM



$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{Lactate} + \text{NAD}^+$$

$$* \ \text{Glucose} + 2\text{NAD}^+ + 2\text{P}_\text{i} + 2\text{ADP} \rightarrow 2\text{Pyruvate} + 2(\text{NADH} + \text{H}^+) + 2\text{ATP} + 2\text{H}_2\text{O}$$

$$\text{Pyruvate} + 4\text{NAD}^+ + \text{FAD} + 3\text{H}_2\text{O} + \text{P}_\text{i} + \text{GDP} \rightarrow 3\text{CO}_2 + 4(\text{NADH} + \text{H}^+) + \text{FADH}_2 + \text{GTP}$$

$$10(\text{NADH} + \text{H}^+) + 2\text{FADH}_2 + 6\text{O}_2 \rightarrow 10\text{NAD}^+ + 2\text{FAD} + 12\text{H}_2\text{O}$$

Abbreviations:

ATP, adenosine triphosphate;
ADP, adenosine diphosphate;
P_i, inorganic orthophosphate;
GTP, guanosine triphosphate;
GDP, guanosine diphosphate;
NAD+, nicotinamide adenine dinucleotide (oxidized form);
NADH, nicotinamide adenine dinucleotide (reduced form);
FAD, flavin adenine dinucleotide (oxidized form);
FADH2, flavin adenine dinucleotide (reduced form);
Ac-CoA, acetyl-CoA.

The transformation of **1 NADH molecule** in NAD+ provides energy for the formation, in average, of about ***2,5* ATP molecules** ,
1 FADH2 molecule generates about ***1,5* ATP molecules**

Conclusions:

1. anaerobic metabolism is far less efficient in ATP production
2. it leads to the production of LACTATE and eventually to lactic acid (H^+ ions)

It is typical of quiescence

Otto Heinrich Warburg (Nobel laureate, 1924) postulated (1966) that cancer cells choose **anaerobic pathway** (Warburg hypothesis)

Warburg effect was later attributed simply to **hypoxia.**

Today Warburg hypothesis is being reconsidered because the shift to glicolytic pathway interferes with the mechanism of apoptosis

ATP PRODUCTION MODEL

Concentrations are denoted by **σ**, with subscript **G, O, L, P** for glucose, oxygen, lactate and pyruvate.

f denotes **consumption rate**.

Overbar denotes **intracellular** concentration,and ***V*** is the cell volume.

Pyruvate and lactate

$$v\frac{d\bar{\sigma}_P}{dt} = 2f_G(\sigma_G) - \psi(\bar{\sigma}_P, \bar{\sigma}_L) - \phi_P(\bar{\sigma}_P, \sigma_O)\,,$$
$$v\frac{d\bar{\sigma}_L}{dt} = \psi(\bar{\sigma}_P, \bar{\sigma}_L) + F_L\frac{\sigma_L}{K_L + \sigma_L} + \bar{h}v(\sigma_L - \bar{\sigma}_L)\,,$$

with

$$f_G(\sigma_G) = F_G\frac{\sigma_G}{K_G + \sigma_G}\,,$$

glucose uptake (mol/cell·sec)
(**no Pasteur effect** = uptake increase in hypoxic state, for the moment)

$$\psi(\bar{\sigma}_P, \bar{\sigma}_L) = (k_+\bar{\sigma}_P - k_-\bar{\sigma}_L)v\,,$$

pyruvate→lactate flux

$$\phi_P(\bar{\sigma}_P, \sigma_O) = F_P\frac{\bar{\sigma}_P}{K_P + \bar{\sigma}_P}\frac{\sigma_O}{K_O + \sigma_O}\,.$$

pyruvate flux to Krebs cycle

Glycolytic phenotype: high k_+ and/or low F_P

At the **steady state**, the **intracellular** concentrations of pyruvate and lactate can be derived in terms of the **extracellular** concentrations.

The **consumption rates f_O and f_L** can be computed simply on the basis of **stoichiometry** :

$$f_O = 6f_G + 3f_L\,,$$

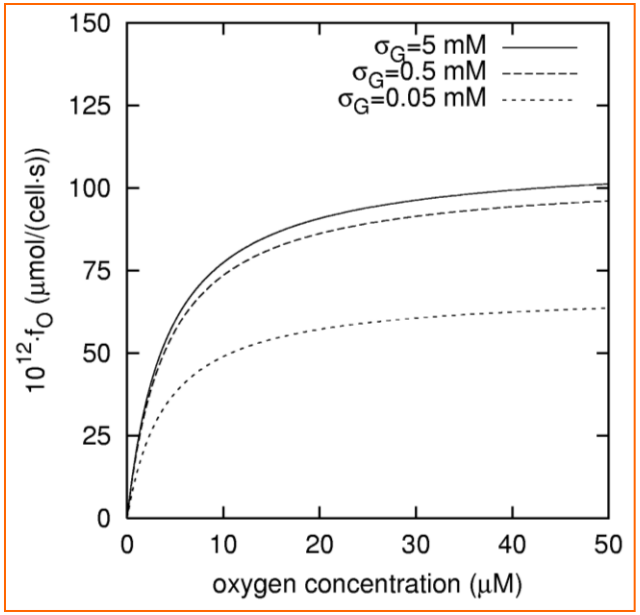
$$f_O = 3\phi_P\,.$$

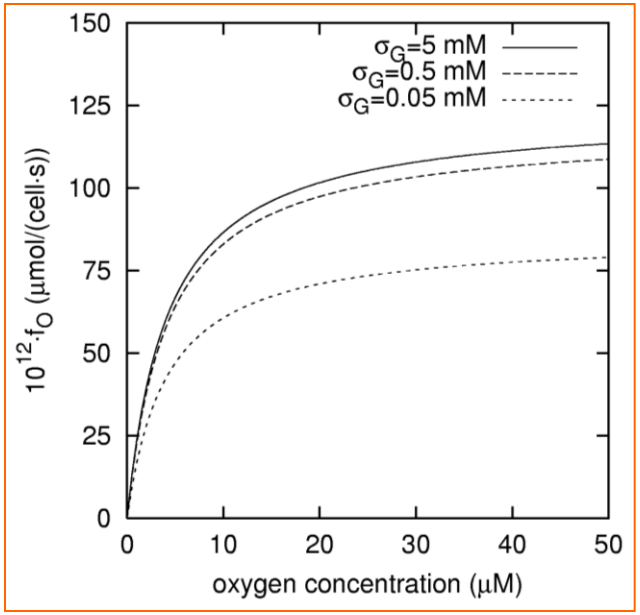
- f_O and f_L are functions of σ_G, σ_O and σ_L
- f_O increases and eventually saturates with σ_O, σ_G and σ_L (**no Crabtree effect** = increase of f_O when σ_G decreases)
- f_L **can be negative**, meaning lactate production.
- f_G, f_O and f_L depend on eight parameters or parameter combinations.

CELL CONSUMPTION RATES

σ_L= 0

f_O

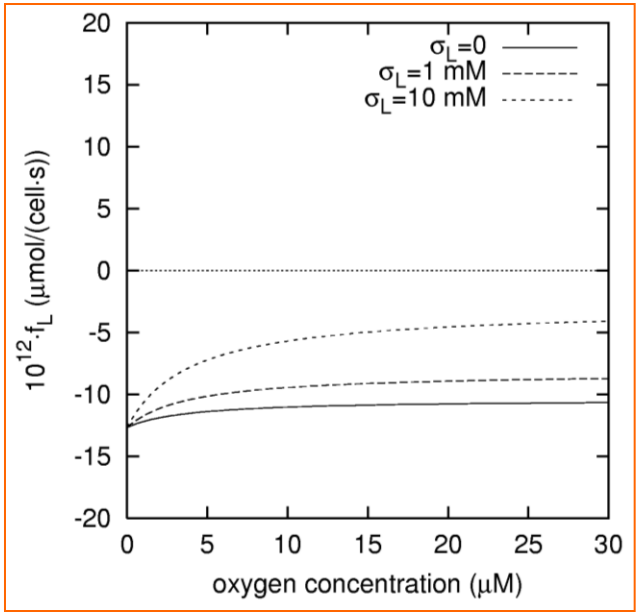


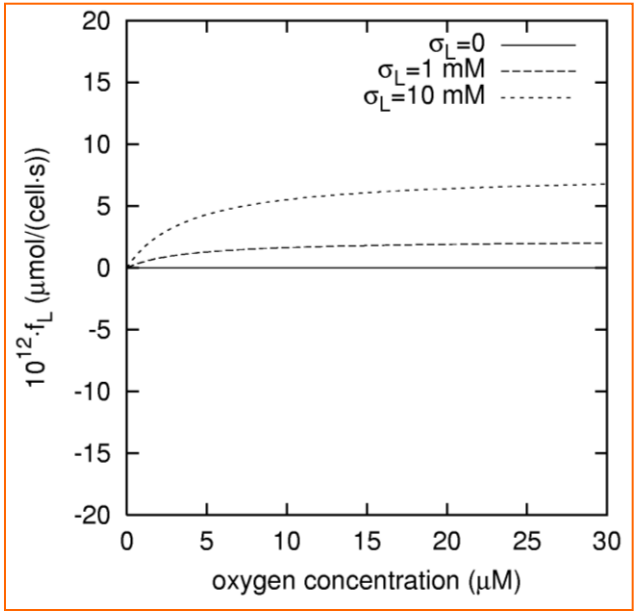


σ_L= 10mM

σ_G= 2µM

f_L





σ_G= 0

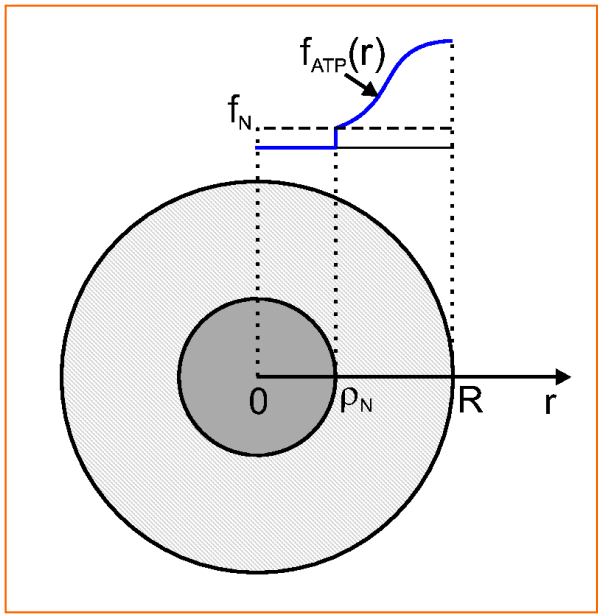
Denoting by η_1 and η_2 the efficiency of ADP phosphorylation into ATP, driven by the oxidation of NADH and $FADH_2$, the **ATP production rate f_{ATP}** can be expressed as:

$$f_{ATP} = 2f_G + (5\eta_1 + \eta_2 + 1)f_O/3\,.$$

$\eta_1 \approx 2.5$, $\eta_2 \approx 1.5$

NECROTIC CORE FORMATION

We assume that cells die when f_{ATP} reaches the **threshold f_N**, viable rim : $\rho_N < r < R$ defined by the inequality **$f_{ATP} > f_N$.**



nutrients diffusion can be considered quasi-steady

The extracellular concentrations σ_G, σ_O and σ_L are prescribed at r = R:

$$\sigma_i(R) = \sigma_i^* > 0\,, \quad i = G, O\,, \qquad \sigma_L(R) = \sigma_L^* \geq 0\,.$$

(*) denotes concentrations on the boundary of the spheroid

DEFINITION OF ρ_N

In the **viable region** the concentrations σ_G, σ_L, σ_O obey the equations

$$
\begin{aligned}
D_{e_G}\Delta\sigma_G &= \frac{\nu^*}{v} f_G(\sigma_G) \\
D_{e_L}\Delta\sigma_L &= \frac{\nu^*}{v} f_L(\sigma_G,\sigma_L,\sigma_O) \\
D_{e_O}\Delta\sigma_O &= \frac{\nu^*}{v} f_O(\sigma_G,\sigma_L,\sigma_O)\,,
\end{aligned}
$$

ν^* = cellular volume fraction, supposed constant
D_{ei} = effective diffusivities.

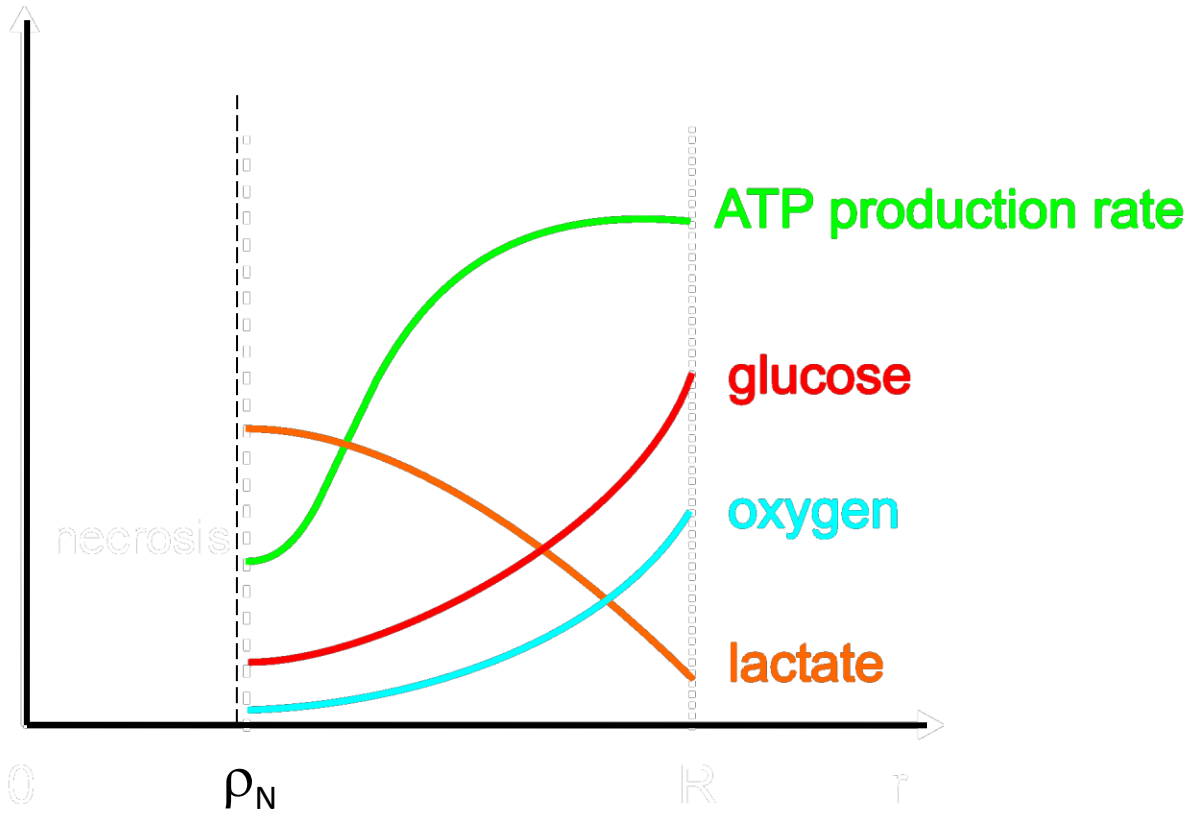
When a necrotic core is present, i.e. $\rho_N > 0$, the **necrotic boundary** bears the conditions

threshold →
no flux →

$$
\begin{aligned}
f_{ATP}(\rho_N) &= f_N\,, \\
\sigma_i'(\rho_N) &= 0\,, \qquad i = G, L, O\,.
\end{aligned}
$$

In the absence of necrosis, the no-flux conditions on σ_i's hold at r = 0.

Typical profiles



The lactate level could be the cause of necrosis

RESULTS

- For R sufficiently large, the inequality $f_{ATP}(r) > f_N$ cannot hold in the whole interval (0, R).

- For R sufficiently large, there exists one and only one solution σ_G, σ_O, σ_L, ρ_N of the free boundary problem.
- ρ_N is an increasing function of R

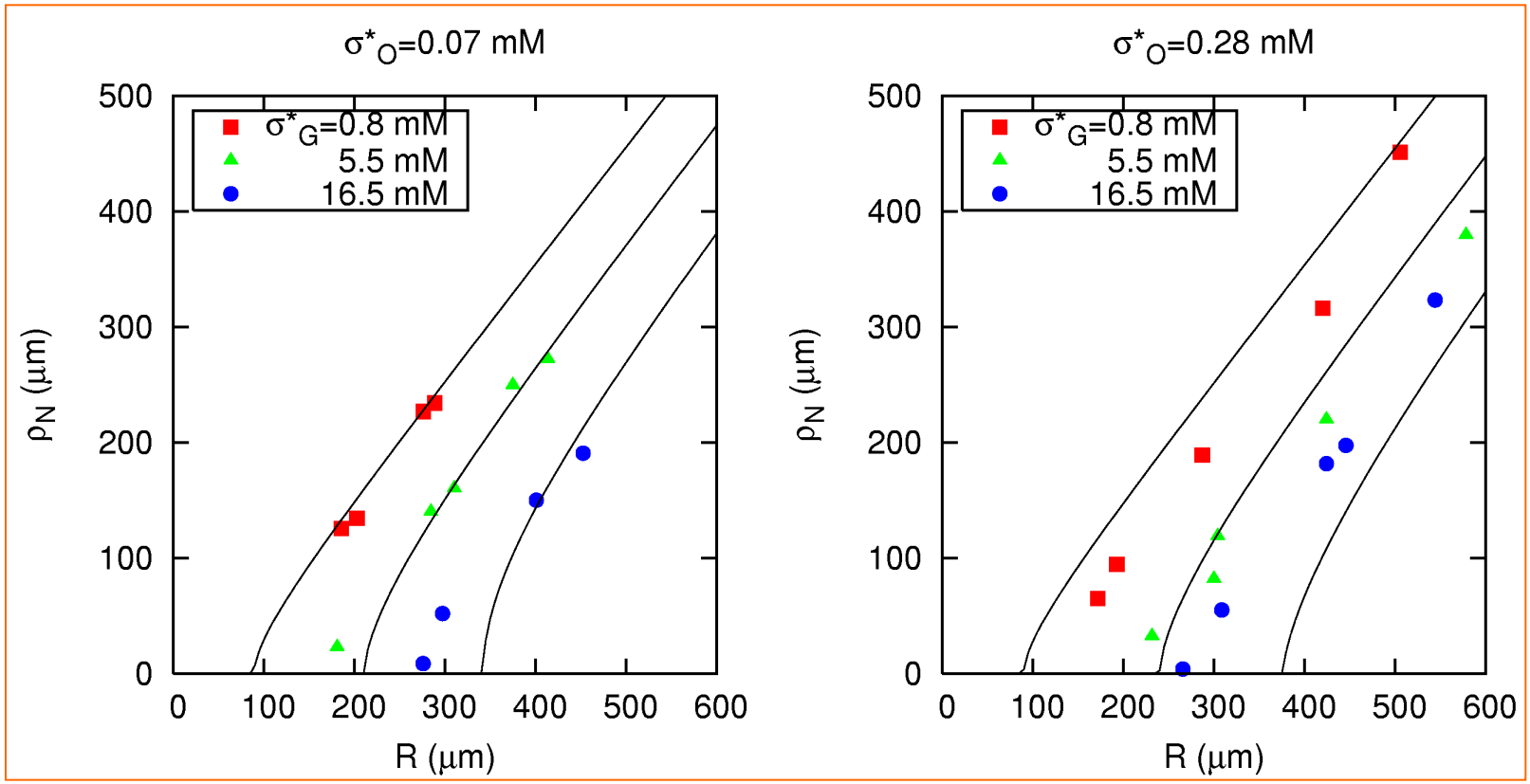
existence and uniqueness of (ρ_N , R)

The proof goes through a complicated shooting-type argument, which is also the basis of the numerical method

COMPARISON WITH EXPERIMENTS

(First attempt: not so good !!)

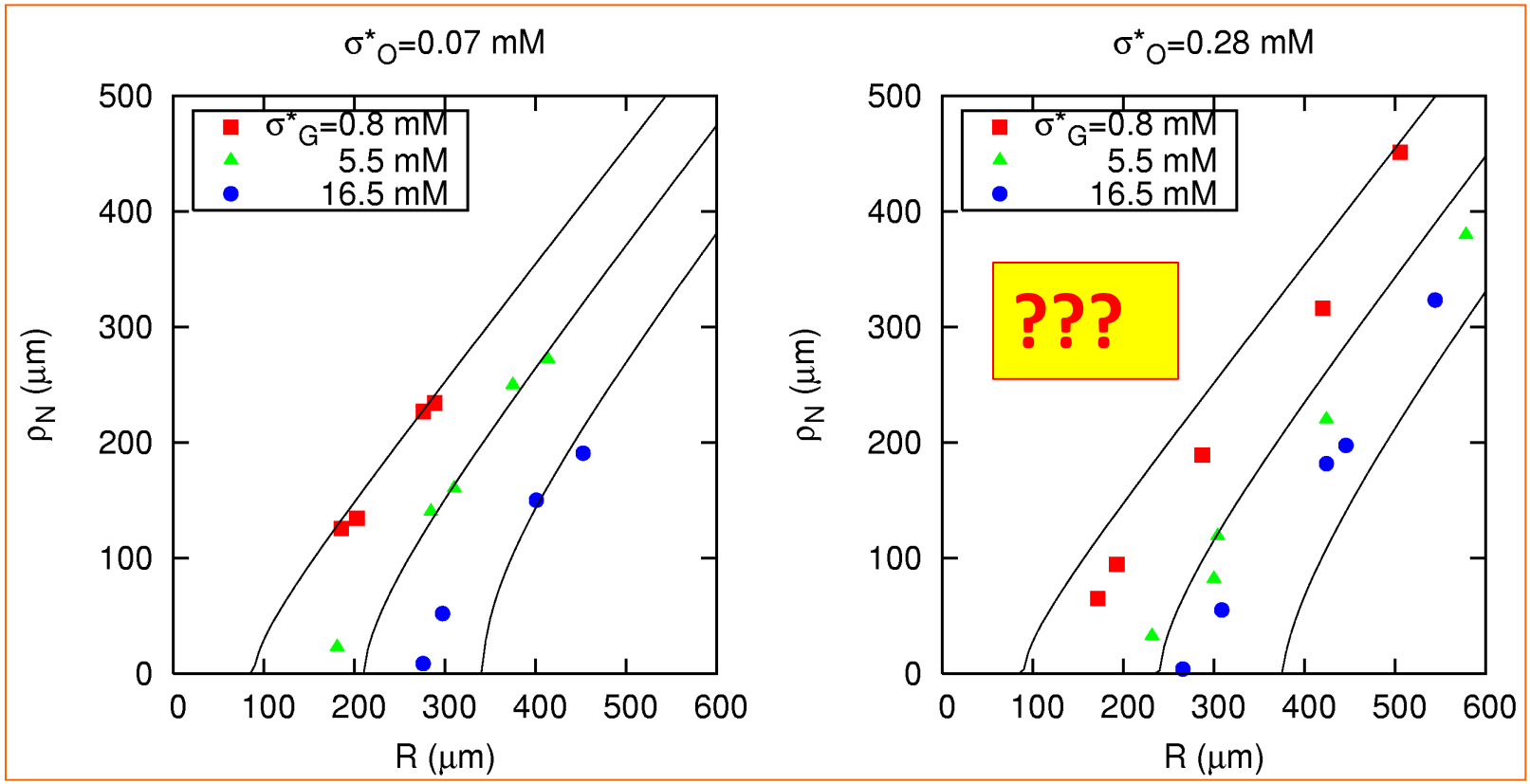
NECROTIC RADIUS *vs.* R (data from Freyer & Sutherland, 1986)



A single set of parameters (including the threshold f_N) for all the six cases.

The parameters were estimated by LS fitting under the constraints that the maximal glucose and oxygen consumptions in a spheroid with R=75 μm (σ_G=5.5 mM, σ_O=0.28 mM) match the measured values (Freyer & Sutherland, 1985)

NECROTIC RADIUS *vs.* R (data from Freyer & Sutherland, 1986)



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INCLUDING PASTEUR EFFECT

Data by Freyer & Sutherland (1985) on EMT6 cells evidence a remarkable **Pasteur effect** (i.e., the glucose consumption increases as the oxygen concentration decreases).

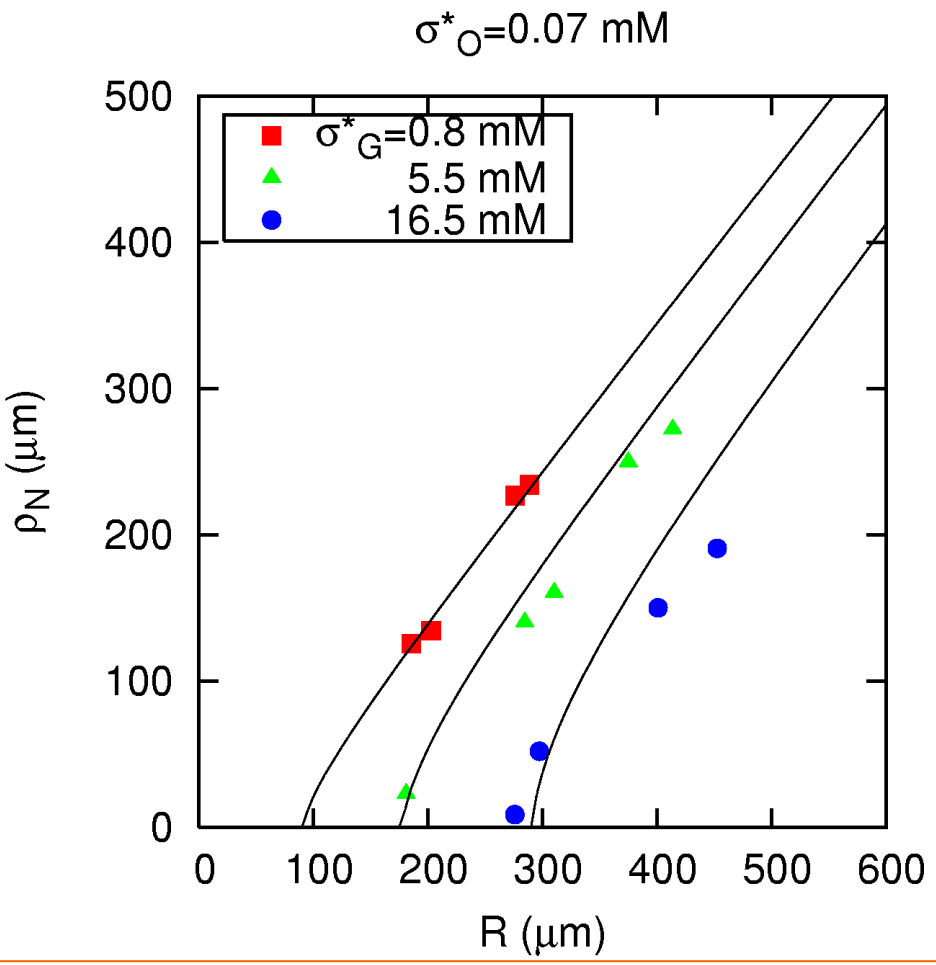
We simply represented this phenomenon making the maximal glucose consumption F_G dependent on the oxygen concentration.

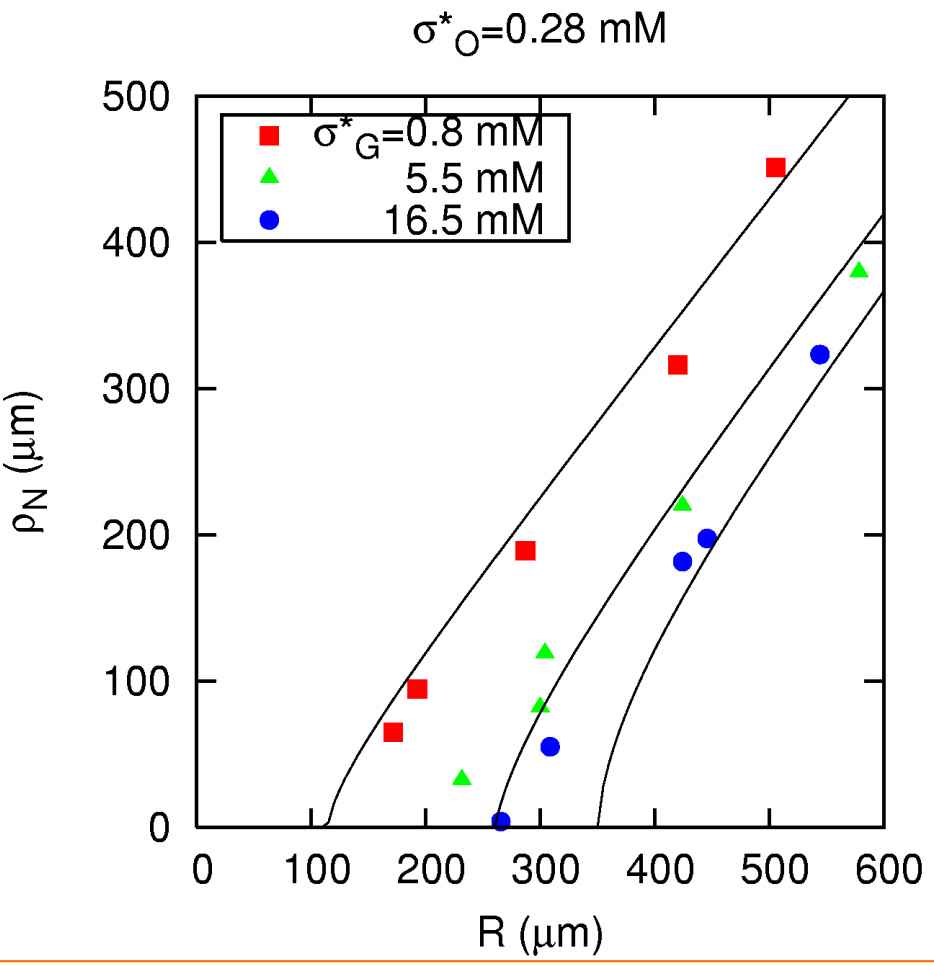
According to the measurements, we have chosen the empirical function:

$$F_G(\sigma_O) = 15 \frac{1}{\sigma_O^{0.5} + 0.28} \quad \text{[mM]}$$

NECROTIC RADIUS *vs.* R (data from Freyer & Sutherland, 1986)

Model with Pasteur effect





Further improvement: add a necrotic threshold for **acidity**

Combining the effects of APT threshold & acidity threshold

L. Bianchini, A. F. *A model combining acid-mediated tumour invasion and nutrient dynamics*, Nonlinear Analysis: Real World Appl. 10 (2009) 1955-1975.

Vascularization in the gap affected by acid, acid production controlled by the ***dynamics of glucose***

Many possible cases (with or without gap, necrotic core, etc.)

Theoretical results (existence and uniqueness)

Travelling waves

The level of ***lactate*** determines (through a complex mechanism) the local value of ***pH*** **:**

$$lactate^- + H^+$$

Cells in the glycolitic regime may increase their ***glucose uptake***, thus producing ***more lactate***

The prevailing phenotype is ***acid resistant*** thanks to *compensation mechanisms* keeping the internal pH at normal levels

S. D. WEBB*†, J. A. SHERRATT* AND R. G. FISH‡

Mathematical Modelling of Tumour Acidity: Regulation of Intracellular pH

J. theor. Biol. (1999) **196,** 237–250

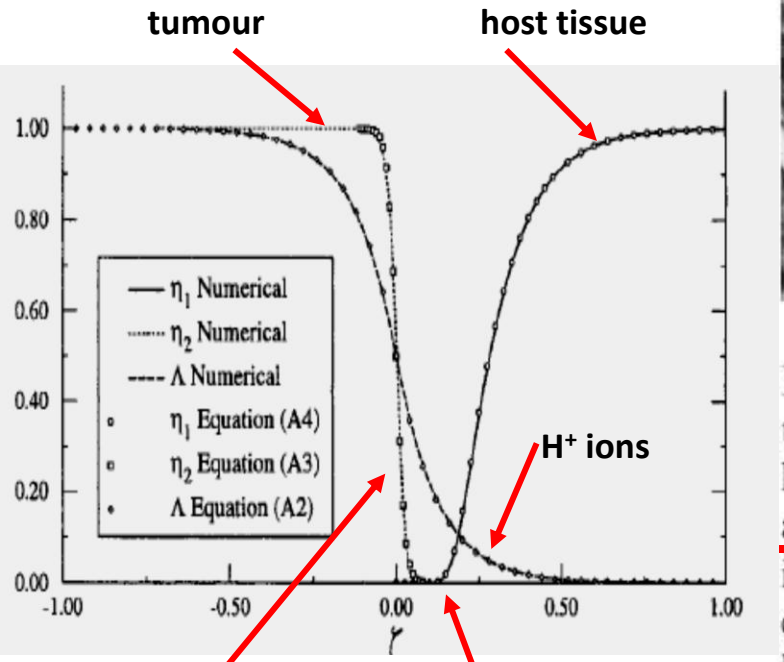
Apoptosis threshold

for normal cells: **pH=7.1** (Casciari et al., 1992)

for tumour cells: **pH=6.8** (Dairkee et al., 1995)

And the result is …

K. Smallbone, R.A.Gatenby, R.J.Gilles, Ph.K.Maini,D.J.Gavaghan. ***Metabolic changes during carcinogenesis: Potential impact on invasiveness.*** J. Theor. Biol, 244 (2007) 703-713.



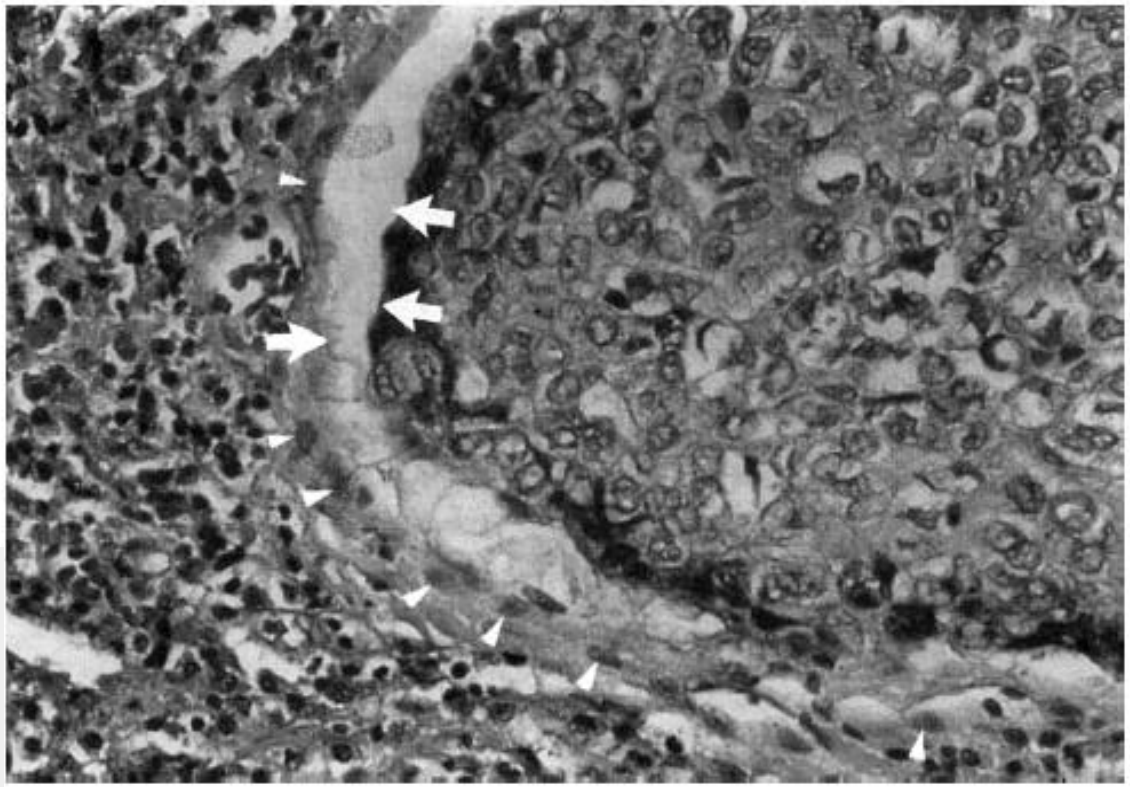


Figure 3. Heamotoxylin and eosin stained micrographs of the tumour-host interface of a formalin-fixed specimen from human squamous cell carcinoma of the head and neck. An acellular gap between the tumour and normal tissue edges is identified (arrows), consistent with the predictions of the mathematical model (*cf.* Figure 2). Note the dying normal cells just beyond this acellular gap (arrowheads) presumably due to acid-induced apoptosis.

(from R.A.Gatenby-E.T.Gawlinski, 1996)

R.A. Gatenby, E.T. Gawlinski. *The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models.* Cancer Res., 63 (2003), 3847–3854.

One space dimension

s = time

logistic damage

Host tissue

$$\frac{\partial N_1}{\partial s} = r_1 N_1 \left(1 - \frac{N_1}{K_1}\right) - d_1 L N_1,$$

tumour

$$\frac{\partial N_2}{\partial s} = r_2 N_2 \left(1 - \frac{N_2}{K_2}\right) + D_2 \frac{\partial}{\partial y}\left[\left(1 - \frac{N_1}{K_1}\right)\frac{\partial N_2}{\partial y}\right],$$

H^+ ions

$$\frac{\partial L}{\partial s} = r_3 N_2 - d_3 L + D_3 \frac{\partial^2 L}{\partial y^2}.$$

production **decay** **large** **small** **reduced diffusivity**

Defects: mass conservation? Damage on the tumour? Metabolism? Diffusion as main transport mechanism?…

Non-dimensional variables

prol. rate host tissue

$$u = \frac{N_1}{K_1}, \quad v = \frac{N_2}{K_2}, \quad w = \frac{d_3}{r_3 K_2} L, \quad t = r_1 s, \quad x = \sqrt{\frac{r_1}{D_3}}\, y.$$

carrying capacities

decay/production

ions diffusivity

damage rate

***b* > 1**

$$a = \frac{d_1 r_3 K_2}{d_3 r_1}, \quad b = \frac{r_2}{r_1}, \quad c = \frac{d_3}{r_1}, \quad d = \frac{D_2}{D_3}.$$

very small

Basic non-dimensional parameters

The normalized G.G. model

One space dimension (space coord.*x)*

Normalized non-dimensional variables:

all concentrations vary between 0 and 1

Normalized logistic growth rate

Acidic aggression

a>0 **Host tissue**

$$u_t = u(1-u) - auw,$$

d<<1, b>1 **tumour**

$$v_t = d[(1-u)v_x]_x + bv(1-v),$$

c>0 **H+ ions**

$$w_t = w_{xx} + c(v-w).$$

production - decay

Normalized diffusivity

Search for a *travelling wave*

Set

$$u(x,t) = u(z), \quad v(x,t) = v(z), \quad w(x,t) = w(z)$$

with $\boldsymbol{Z = x - \theta t}$

Solutions of this form, plotted vs. *x*, are graphs which, as time varies***, travel*** with the ***speed*** $\boldsymbol{\theta}$ to the right ($\theta>0$: **our case**), or to the left ($\theta<0$)

The system

$$u_t = u(1-u) - auw,$$

$$v_t = d[(1-u)v_x]_x + bv(1-v),$$

$$w_t = w_{xx} + c(v-w).$$

becomes

$$u_t(x-\theta t) \to -\theta u'(z)$$

$$u_x(x-\theta t) \to u'(z)$$

etc.

$$0 = \theta u' + u(1-u) - auw,$$

$$0 = d[(1-u)v'' - u'v'] + \theta v' + bv(1-v),$$

$$0 = w'' + \theta w' + c(v-w),$$

Asymptotic values corresponding to invasion

$$0 = \theta u' + u(1-u) - auw,$$

$$0 = d[(1-u)v'' - u'v'] + \theta v' + bv(1-v),$$

$$0 = w'' + \theta w' + c(v-w),$$

Normal cells: $max(0, 1-a) \to 1$

Tumour cells: $1 \to 0$

H+ ions : $1 \to 0$

For $a < 1$ a fraction of normal cells survives

A. Fasano, M.A. Herrero, M. Rocha Rodrigo: Math. Biosci. 220 (2009) 45-56
(study of all possible travelling waves)

$$0 = \theta u' + u(1-u) - auw,$$

$$0 = d[(1-u)v'' - u'v'] + \theta v' + bv(1-v),$$

$$0 = w'' + \theta w' + c(v-w),$$

Two classes of waves:

- ***slow waves:*** $\theta = \theta_0 d^{\alpha}$ ***(d<<1): singular perturbation !!!***
- ***fast waves:*** $\theta = O(1)$ *as* $d \to 0$

← **The interesting ones**

Slow waves:

Technique: matching *inner* and *outer* solutions

Take $\xi = z/d^{\alpha}$ *as a* ***fast variable***: looking at the ***front region*** with a ***magnifying lens***

Summary of the results

$$0 = \theta u' + u(1-u) - auw,$$

$$0 = d[(1-u)v'' - u'v'] + \theta v' + bv(1-v),$$

$$0 = w'' + \theta w' + c(v-w),$$

slow waves: $\theta = \theta_0 d^{\alpha} \quad 0 < \alpha \leq ½,$

No solutions for $\alpha > ½$

$$\theta_0 > 0 \text{ for } \alpha \in (0,1/2),$$

$$\theta_0 \geq \sqrt{b \min(a/2,1)} \text{ for } \alpha = 1/2$$

similar to Fisher's case

The parameter ***a*** decides whether the two cellular species ***overlap*** or are separated by a ***gap***

α > 2

$$u(z;d) \simeq \begin{cases} \sqrt{\dfrac{\theta_0\sqrt{c}}{2\pi}}\, d^{\alpha/2} e^{[\phi_-(z)-\phi_+(z_+)]/d^\alpha} & if\ z<0, \cong 0 \\ \sqrt{\dfrac{\theta_0\sqrt{c}}{2\pi}}\, d^{\alpha/2} e^{[\phi_+(z)-\phi_+(z_+)]/d^\alpha} & if\ 0<z<z_+, \\ 1-\dfrac{a}{2}e^{-\sqrt{c}z} & if\ z>z_+. \end{cases}$$

gap

$$\phi_+(z) = \frac{1}{\theta_0}\left[\frac{a}{2\sqrt{c}}(1-e^{-\sqrt{c}z}) - z\right]$$

$$z_+ = \frac{1}{\sqrt{c}}\log\frac{a}{2} > 0$$

GAP THICKNESS

Numerical simulations

α = ½ , minimal speed

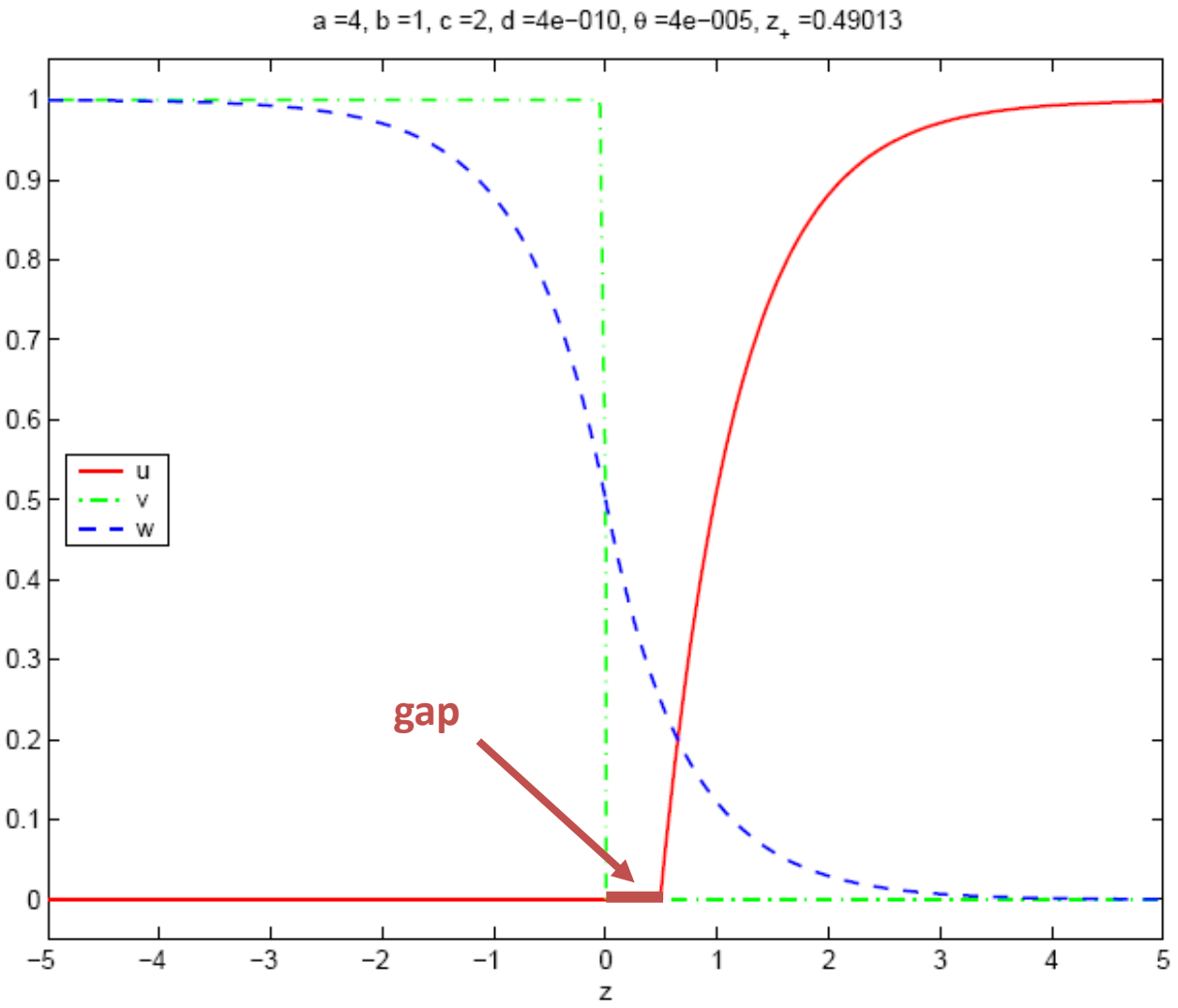
$$\theta = 2\sqrt{bDd}$$

The propagating front of the tumour is very steep

as a consequence of *d<<1*

(this is the case treated by G.G.)

$a > 2$



Other invasion models are based on a combined mechanism of ***ECM lysis*** and ***haptotaxis***

(still based on the analysis of travelling waves)

A two parameter family of travelling waves with a singular barrier arising from the modelling of extracellular matrix mediated cellular invasion

Abbey J. Perumpanani [a,b], Jonathan A. Sherratt [c,*], John Norbury [d], Helen M. Byrne [e]

Physica D 126 (1999) 145–159