Clinical & Experimental Metastasis **17**: 397–407, 1999.
© 1999 *Kluwer Academic Publishers. Printed in the Netherlands.*

Alterations in proteolytic activity at low pH and its association with invasion: A theoretical model

Steven D. Webb[1], Jonathan A. Sherratt[1] & Reginald G. Fish[2]
[1]*Department of Mathematics, Heriot-Watt University, Edinburgh EH14 4AS, UK;* [2]*Velindre Hospital, Whitchurch, Cardiff, CF4 7XL, UK*

Received 23 April 1999; accepted in revised form 16 July 1999

Key words: cysteine proteinases, invasion, metalloproteinases, modelling

Abstract

The extracellular pH (pH_e) of solid tumours is often lower than in normal tissues, with median pH values of about 7.0 in tumours and 7.5 in normal tissue. Despite this more acidic tumour microenvironment, non-invasive measurements of intracellular pH (pH_i) have shown that the pH_i of solid tumours is neutral or slightly alkaline compared to normal tissue (pH_i 7.0–7.4). This gives rise to a reversed cellular pH gradient between tumours and normal tissue, which has been implicated in many aspects of tumour progression. One such area is tumour invasion: the incubation of tumour cells at low pH has been shown to induce more aggressive invasive behaviour *in vitro*. In this paper the authors use mathematical models to investigate whether altered proteolytic activity at low pH is responsible for the stimulation of a more metastatic phenotype. The authors examined the effect of culture pH on the secretion and activity of two different classes of proteinases: the metalloproteinases (MMPs), and the cysteine proteinases (such as cathepsin B). The modelling suggests that changes in MMP activity at low pH do not have significant effects on invasive behaviour. However, the model predicts that the levels of active-cathepsin B are significantly altered by acidic pH. This result suggests a critical role for the cysteine proteinases in tumour progression.

Introduction

The extracellular pH (pH_e) of tumours is generally more acidic than that of normal tissue, with median pH values of about 7.0 in tumours and 7.5 in normal tissue [1, 2]. This is thought to be a consequence of a compromised tumour vasculature together with an increased use of the glycolytic pathway for energy production, whereby tumour cells preferentially convert glucose and other substrates into lactic acid [3–5]. In contrast, with the advent of ^{31}P-MRS imaging for the non-invasive measurements of pH, both human and animal tumour cells have been shown to have a neutral or slightly alkaline intracellular pH (pH_i 7.1–7.2) [6–9]. This gives rise to a cellular pH gradient difference between tumours and normal tissue, which provides the basis for pH-dependent selective treatment of cancer [10].

A number of cellular processes are affected by changes in microenvironmental pH, e.g. glycolysis, protein synthesis, DNA synthesis, exocytosis, and secretion [11]. It has been shown that the maintenance of intracellular pH within the range pH 7.0–7.2 appears to be necessary for normal cellular proliferation [12]. More specifically Martinez-Zaguilan et al. [13] observed that culturing of two human melanoma cell lines at acidic pH (6.8) caused significant increases in both migration and invasion. This suggests that low pH_e may be an important factor involved in the invasive behaviour of tumours.

Tumour invasion and metastasis are the major causes of treatment failure for cancer patients [14]. These processes can be subdivided into several steps, such as degradation of the basement membrane, intravasation, circulation, extravasation and secondary growth at the target organ [15, 16], and it is likely that each of these steps are individually pH sensitive [13]. For instance, proteolytic enzymes play an important role in the metastatic process by degrading the extracellular matrix (ECM), thus allowing escape from the primary tumour, and each class of proteinases is pH-sensitive [17, 18]. The ECM-degrading proteinases produced by most tumour cells can be subdivided into three classes: serine, metallo- and cysteine proteinases [19, 20]. In general, the enzymes are secreted as latent forms which require extracellular activation, and tumour spread is correlated with increased levels of the activated enzyme [15, 20].

Many studies have shown the importance of matrix metalloproteinases in tumour invasion [21, 22], as well as many normal morphogenetic processes such as embryonic development, and bone remodelling [17, 23]. Although metalloproteinase (MMP) activity is optimal in the physiological pH range [15, 24], experimental work of Martinez-Zaguilan [13] has shown that while there was a decrease in the overall amount of MMP activity in cells grown at low pH (6.8), there

Correspondence to: Dr Steven D. Webb, Department of Mathematics, Heriot-Watt University, Edinburgh EH14 4AS, UK. Tel: +44-131-4513719; Fax: +44-131-4513249; E-mail: S.D.Webb@ma.hw.ac.uk

was a significant increase in the relative amount of active MMP. Also, it has been shown that a reduction of the extracellular pH to 6.5 resulted in a redistribution of cathepsin B, a cysteine proteinase, toward the surface of tumour cells [25]. Furthermore, this pH-induced periphery redistribution of cathepsin B was accompanied by an enhanced secretion of active cathepsin B. The objective of the present study was to test the significance of these alterations in proteolytic activity at low pH.

Mathematical modelling of progression and growth in cancer has a rich history. Models typically focus on specific aspects of tumour dynamics such as avascular tumour growth [26–28], angiogenesis [29, 30], and tumour-immune interactions [31–33]; for a general review, see Adam and Bellomo [34]. By contrast, cancer invasion is a relatively new area for mathematical modelling. Recently, Perumpanani and co-workers have modelled cell invasiveness as a function of tumour cell interactions with the normal host cells, noninvasive tumour cells, and extracellular matrix [35]. Their studies show that the speed of invasiveness can be computed as a function of the phenotypic properties of the cell and the matrix. Furthermore, the potential role of pH in the invasive tumour phenotype has been studied mathematically, predicting a pH gradient extending from the tumour-host interface [36, 37]. However, this modelling approach represents the pH differences between tumour and normal cells in a simple generic way, in particular, without considering intracellular and extracellular pH separately.

In this paper, we present a mathematical model, building on our previous work on pH_i regulation [38], for tumour cell-ECM interactions during cancer invasion, focussing on the degradation of ECM components via active proteolytic enzymes. In the second section, we show that the effects of pH_e on the invasive behaviour of tumour cells are not mediated via changes in MMP activity at low pH. In the third section, we then extend our model to reflect the altered distribution and increased secretion of cathepsin B in malignant cells at low pH_e.

Role of pH in ECM proteolysis: Metalloproteinases

We propose an ordinary differential equation model, focussing on the role of the matrix metalloproteinases (MMPs) in the process of matrix degradation. We denote the concentrations of the tumour cells, ECM, pro-MMP and active-MMP at time t by $m(t)$, $c(t)$, $p(t)$ and $a(t)$ respectively. To study the effect of pH on the tumour cell dynamics and metastatic potential, we introduce two variables: intracellular pH (pH_i) and extracellular pH (pH_e). For convenience we represent these in the model via the corresponding hydrogen ion concentrations $I(t)$ and $E(t)$, respectively (recall that $pH = \log[H^+]$).

Steady-state aerobic energy metabolism consumes the same number of H^+-ions as are produced by the hydrolysis of ATP [39, 40], and as a result has very little effect on pH_i. However, in the absence of oxygen, cells rely on the more inefficient process of anaerobic glycolysis to obtain energy [7, 37]. During glycolysis hydrogen ions are formed which are actively transported outside the cell via membrane-based ion transport mechanisms.

Cells have developed several membrane transport mechanisms for regulating pH_i (see Roos and Boron [41] for review). The level of intra- and extracellular pH influences the operation of these exchangers which directly transport either H^+ out of the cell, or HCO_3 into the cell to neutralize H^+ in the cytosol [2]. Major transport mechanisms which have been implicated in pH_i regulation include the Na^+/H^+ exchanger, the lactate $/H^+$ symporter, the Na^+-dependent and cation-independent Cl $/HCO_3$ exchangers, and a vacuolar type proton ATPase. In addition, H^+-ions can be transfered from the cytosol into intracellular organelles such as golgi, endosomes and lysosomes. In our model, we use $u(I, E)$ to denote the combined activity of these transporting mechanisms (see Appendix for full details).

Once hydrogen ions are transported outside the cell they are removed from the tissue by the supporting vasculature [7]. The vasculature of many tumours is often compromised and unable to supply the nutritional needs of an expanding population of tumour cells, leading to the existence of hypoxic regions within solid tumours [42]. To represent the functional vasculature in our modelling we introduce the parameter V, which represents the extent of vascularity. For simplicity, we assume that the rate at which H^+-ions are removed from the extracellular space is directly proportional to V. We use the term $s_2 = s_2(V)$ to represent this removal (see Figure 1).

The focus of our modelling is on the increased use of the glycolytic pathway by tumour cells, which occurs even under aerobic conditions [37]. In the absence of quantitative data we describe the rate at which cellular metabolism can cause H^+-ions to accumulate intracellularly as a function $s_1(V)$. We would expect $s_1(V)$ to be a monotone decreasing function of V: in normal cells $s_1(V)$ falls to very low values at large V, representing that under physiological pH, aerobic metabolism has no net effect on pH_i. As illustrated in Figure 1, the form of $s_1(V)$ for tumour cells is more gently sloping which represents their high glycolytic activity and consequent production of H^+-ions, even in the presence of oxygen (i.e. high V). In our model, we denote $s_1(V)$ for normal and tumour cells by $s_{1n}(V)$ and $s_{1m}(V)$, respectively. For computational purposes, we have chosen simple specific functional forms possible for $s_1(V)$ and $s_2(V)$, and these are given in the Appendix.

Several studies have shown that tumour cells are able to survive and even proliferate in low pH_e environments which are ordinarily lethal to corresponding normal cells [6, 36, 43]. Under these conditions the pH_i of normal cells drops to values which are no longer permissive for cellular proliferation [12], whereas the pH_i in tumour cells is less sensitive to external pH and is maintained within physiological levels. In general, a slightly alkaline pH_i of about 7.2–7.25 appears to be necessary but not sufficient for normal proliferation [12]. In our model, we use the term $r(I)$ to represent the effect of low pH_i on the proliferation rate of the cells. It is assumed that proliferation can only take place

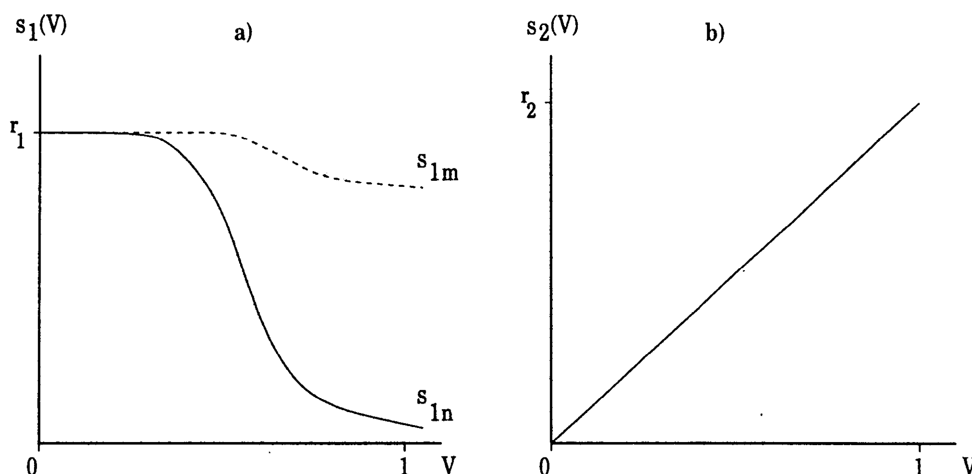

Figure 1. (a) Schematic representation of the net production of H^+-ions from cellular metabolism of normal cells (s_{1n}), and tumour cells (s_{1m}). We represent the extent of functional vasculature in our modelling by the parameter V. Here, $V = 0$ represents a poorly organised functional vasculature, leading to hypoxia. Increasing V corresponds to an increase in oxygen availability. In our model, we take $0 \leq V \leq 1$. We expect $s_1(V)$ to be a monotone decreasing function of V. As V increases $s_{1n}(V)$ falls to very low values, representing that under physiological pH, aerobic metabolism has no net effect on pH_i. In tumour cells, with their high glycolytic rate and consequent net production of H^+-ions, even in the presence of oxygen (i.e. high V), we expect $s_{1m}(V)$ to be significantly greater than zero. (b) The rate of removal of H^+-ions from the interstitial space by convective and/or diffusive transport. We assume that once hydrogen ions are transported outside the cell then the rate at which they are removed from the interstitial space is directly proportional to V. The quantitative values of $s_1(V)$ and $s_2(V)$ are of the order of mM/min, comparable with the flux terms illustrated in Figure 4.

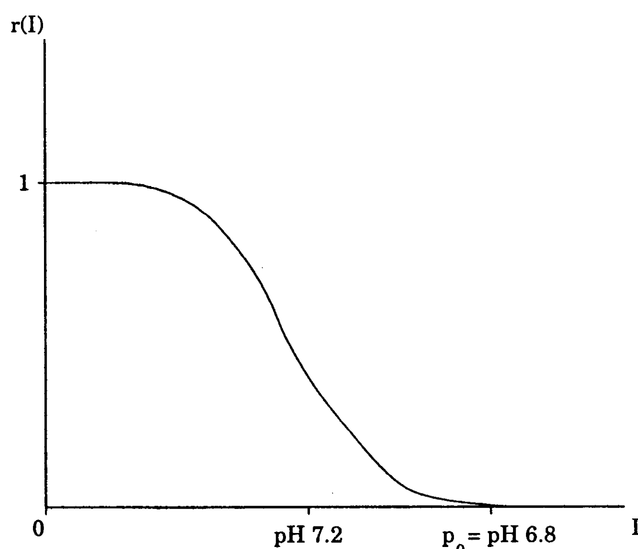

Figure 2. The qualitative form of the function $r(I)$, which represents the effect of low pH_i on the proliferation rate of the cells. The maintenance of intracellular pH within the physiological pH range (pH 7.2–7.25) is necessary for normal proliferation. If the intracellular pH is lowered below pH 7.2, growth is inhibited. We assume that proliferation can only take place if the pH_i is above a threshold level p_0, which is permissive for normal cellular growth. The value of p_0 varies between cell types but is typically in the pH range 6.6–7.0. We choose $p_0 = pH_i$ 6.8. Note that it is intracellular H^+-ion concentration, rather than pH_i, that is plotted on the horizontal axis; some corresponding pH values are indicated.

if the pH_i is above a threshold level p_0 which is permissive for normal cellular growth (see Figure 2).

In most cases, metalloproteinases are secreted as latent pro-enzymes that require extracellular activation to exert their proteolytic activity [17]. The *in vivo* mechanism of normal metalloproteinase activation is unknown, but may involve the action of other MMPs as well as other enzymes, such as plasmin and membrane-type MMP [44]. It is likely that the activation of latent metalloproteinases is pH sensitive [16]. For instance, Davis et al. [45] have observed that 94-kDa progelatinase, a latent pro-MMP, is activated by acid treatment. In our model, we neglect the activations by other enzymes to focus on the effects of pH. We use the term $f_1(E)$ to represent the effect of low pH_e on the autolytic activation of pro-MMP. Once activated, these enzymes can degrade extracellular matrix components. MMP activity is tightly regulated; typically, a metalloproteinase has a bell-shaped pH profile with a maximum activity in the physiological pH range [46, 47], and maintains $\approx$ 80 % of the maximum activity even at pH 6.8. In our model, we use a function $f_2(E)$ to reflect the effect of pH on the proteolysis of ECM.

Microenvironmental pH may also effect MMP secretion [48]. For instance, Kato et al. [16] have reported that two human cell lines (A549 and HT1080) secreted a higher level of 90-kDa gelatinase at pH 6.8 compared with pH 7.3. We use the term $f_3(I)$ to represent the effect of pH on the secretion of pro-MMP by the tumour cells.

With these definitions and assumptions, the model system is as follows:

m = tumour cells

$$\frac{dm}{dt} = \overbrace{k_1 r(I) m \left(1 - \frac{m}{k_2}\right)}^{\text{cell division}} \tag{1a}$$

c = ECM density

$$\frac{dc}{dt} = \overbrace{- k_3 f_2(E) a c}^{\text{proteolysis}} \tag{1b}$$

p = *pro-MMP*

$$\frac{dp}{dt} = \overbrace{k_4 f_3(I)m}^{\text{MMP secretion}} - \underbrace{k_5 p}_{\text{MMP degradation}} - \overbrace{k_6 f_1(E)p}^{\text{MMP activation}} \quad (1c)$$

a = *active-MMP*

$$\frac{da}{dt} = \overbrace{k_6 f_1(E)p}^{\text{MMP activation}} - \overbrace{k_7 a}^{\text{MMP degradation}} \quad (1d)$$

I = *intracellular* H^+

$$\frac{dI}{dt} = \underbrace{-u(I,E)}_{\text{ion transport}} + \overbrace{s_{1m}(V) + \beta E}^{\text{acid loading}} \quad (1e)$$

E = *extracellular* H^+

$$\frac{dE}{dt} = m \cdot [\overbrace{u(I,E)}^{\text{ion transport}} - \underbrace{s_2(V)E}_{H^+ \text{ removal by vasculature}} - \beta E]\,. \quad (1f)$$

Here the k_i's are positive constants. The term βE represents the passive movement of H^+-ions into the cell due to the internally negative membrane potential, we assume β is constant. To describe the evolution of the tumour cell population we use a logistic-type growth, with growth rate k_1 and carrying capacity k_2.

It is well known that the proteolytic activities of metalloproteinases are regulated by a family of secreted proteins known as tissue inhibitors of metalloproteinases (TIMPs) [17, 20]. The TIMPs are expressed in most tissues and act to inhibit MMP activity by forming a complex with active-MMPs [15]. No complex formation occurs between the TIMPs and the latent form of these enzymes [20]. Kato et al. [16] showed that an acidic culture medium hardly affected the secretion of TIMPs from a metastatic mouse B16 melanoma cell line. Thus, for simplicity we represent the inhibition of MMPs by TIMPs and active-MMP decay as a single term in the model: we use the simple linear term $k_7 a$. The decay of latent pro-MMP is assumed to be linear; with rate constant k_5.

Wherever possible, experimental data was used to estimate the functional forms and parameter values. Data is available for the rate of H^+ efflux due to the combined activity of both Na^+-driven and Cl /HCO_3 exchangers [8], and we have based the forms of f_1 and f_3 on data in Martinez-Zaguilan et al. [13]. We also know the time scale of the pH change during an *in vitro* cell cultivation [16]. However, there are still a number of parameter values for which we cannot get good estimates. For instance, we are not aware of experimental data that would enable H^+ production ($s_1(V)$) and removal ($s_2(V)$) rates to be estimated quantitatively. In this case, the parameters and functions were chosen in order to give the best fit with the experimental data (see Appendix for full details).

We solved Equation (1) numerically and compared the numerical solutions with data from Martinez-Zaguilan et al. [13], an experimental study which examined the effect of pH on the invasive potential of tumour cells. In this study the culturing of tumour cells at acidic pH (6.8) was found to increase both migratory and invasive behaviour. In their experiments, cells were grown for three weeks at either pH 6.8 or 7.4. Subsequently, cells were transferred to a membrane invasion culture system, and the invasive capabilities of these cells were evaluated in either acidic (pH 6.8) or normal (pH 7.4) conditions. They observed that cells exposed to low pH conditions for three weeks migrate and invade faster, regardless of the pH at which they were tested.

To simulate the incubation of cells in their respective media, we solve Equation (1) with the medium fixed at either acidic (6.8) or normal (7.4) pH_e, starting with a small number of tumour cells. The levels of pH_i, tumour cells and proteolytic enzymes rapidly attain their steady state values. Once these values have been obtained, we then use them as initial conditions in a simulation where we test the proteolytic capabilities of these cells. Here, we allow pH_e to vary over time, starting with an initial pH_e of either 6.8 or 7.4 for the culture medium. Our simulations show that during this testing period the ECM is gradually degraded to zero. The numerically calculated ECM profiles in the above cases are illustrated in Figure 3a.

Our results indicate that culturing cells at low pH (6.8) causes a change of less than 1% in ECM decay rates. This suggests that incubating cells under midly acidic conditions has very little effect on their invasive behaviour. Further numerical simulations of this model for a wide range of parameter values showed a decrease in the ability of acid cultured cells to degrade ECM, regardless of the pH at which the cells were tested. The reduced ability of cells to degrade ECM in our model solutions is due to a decrease in the overall amount of extracellular MMP activity from cells grown at acidic pH_e (6.8) (relative to pH_e 7.4). Thus, our model predicts that the effects of pH on the invasive potential of tumour cells are not mediated via changes in MMP activity at low pH.

The cysteine proteinases, such as cathepsins B, D and L, are lysosomal proteinases [49]. The predominant forms secreted are latent pro-forms. Nevertheless, it has been shown that the incubation of cells at a slightly acidic pH (6.5) induces the redistribution and release of active cathepsin B from a series of metastatic human cell lines [25]. We now consider an amended model to reflect this altered distribution and increased secretion of cathepsin B in malignant cells, in an attempt to explain the effect of low pH on the invasive machinery.

Role of pH in ECM proteolysis: Cysteine proteinases

In this section, we consider an alternative model in which we neglect the proteolytic activity of the metalloproteinases and focus on the action of the cysteine proteinases. The differences between the two models is that in the MMP model there is an overall decrease in the amount of extracellular

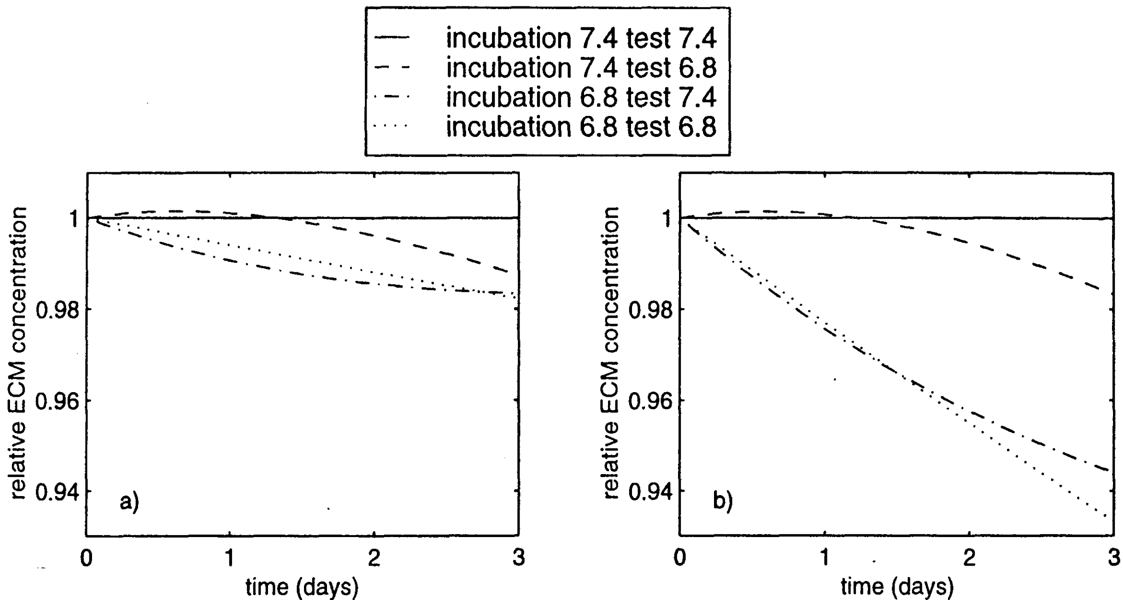

Figure 3. Numerically calculated decrease in ECM with time from (a) MMP activity and (b) cathepsin activty. We incubate the cells for three weeks with pH_e of the culture medium fixed at either 6.8 or 7.4, with initial conditions corresponding to a small level of tumour cells with an initial pH_i of 7.2. The pH_i, tumour cells, and proteolytic enzymes rapidly attain their steady state values (not shown), which we denote by pH_{i0}, m_0, p_0 and a_0, respectively. Once these values have been obtained we then use them as initial conditions in a simulation where we test the proteolytic capabilities of these cells. Here, we allow the pH_e to vary over time, starting with an initial pH_e of either 6.8 or 7.4. The curves shown represent the ECM decay profiles for the cells in the four cases; (i) cells grown and tested at normal pH (pH_e 7.4) (——), (ii) cells grown at pH 7.4 and tested with an initial pH_e of 6.8 (– – –), (iii) cells grown at pH_e 6.8 and tested with an initial pH_e of 7.4 (—·—), and (iv) cells grown and tested at acidic pH (pH_e 6.8) (· · ·). We plot the decrease in ECM relative to the case where the cells are incubated and tested at normal pH (case (i)). The ECM is gradually degraded to zero. With the metalloproteinases, there is very little quantitative difference between the different cases. In particular, the decrease in ECM when cells are cultured at pH_e 6.8 is very similar to that observed at pH_e 7.4. Thus, the modelling suggests that the effects of pH on the invasive potential of tumour cells are not mediated via changes in MMP activity at low pH. However, our results indicate an increased ability of acid cultured cells to degrade ECM in the cathepsin model. Here, there is a much more rapid decrease in ECM density from acid treated cells compared to when cells are cultured at normal pH (pH_e 7.4). The initial values of the incubation media are $m(0) = 0.1$, $a(0) = p(0) = c(0) = 0$, $I_m(0) =$ pH 7.2. At the end of the incubation time, the invasion capabilities of these cells are tested with the initial conditions $m(0) = m_0$, $a(0) = a_0$, $p(0) = p_0$, $c(0) = 1$, $I_m(0) = pH_{i0}$, with $E(0) = pH_e$ 6.8 or 7.4. The parameter values used in this simulation are $k_3k_4k_2/k_1^2 = 15$, $k_5/k_1 = 1$, $k_6/k_1 = 3.43$, $k_7/k_1 = 5 \times 10^5$ for (a), and $A_3A_4A_2/A_1^2 = 15$, and $A_5/A_1 = 5 \times 10^4$ for (b), with $r_1 = 1$, $r_2 = 14$, $r_3 = 0.01$, $r_4 = 10^7$, $\beta = 1$, $V = 0.5$, and $\alpha_m = 0.4$ (see Appendix for full details).

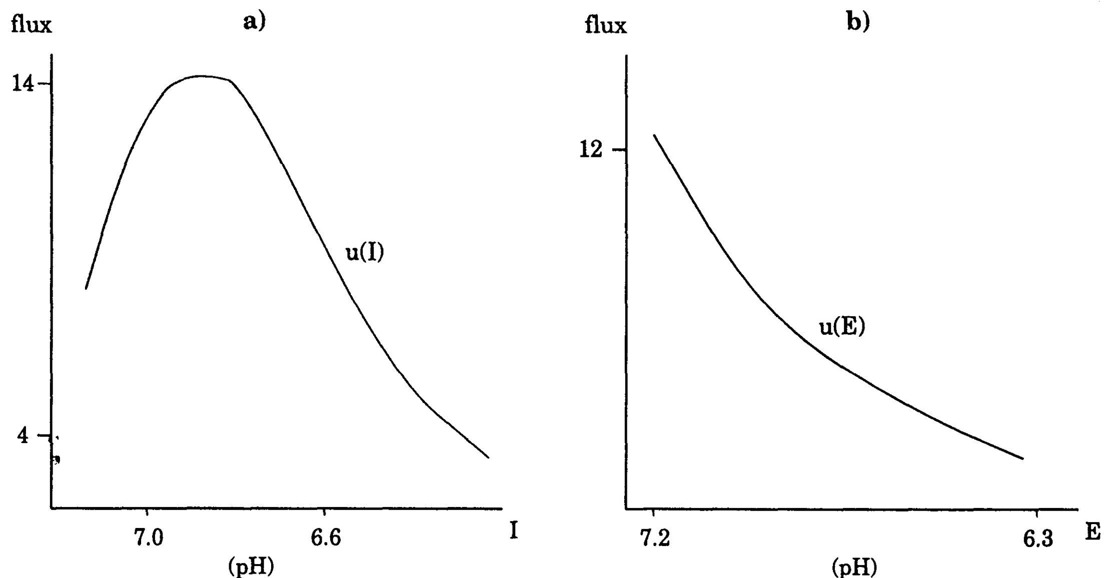

Figure 4. The effect of microenvironmental factors on the regulation of intracellular pH (pH_i). We plot the rate of H^+-flux (mM/min) due to the combined activity of the Na^+/H^+ antiporter and anion transport systems as a function of (a) intracellular H^+-ion concentration (I), and (b) extracellular H^+-ion concentration (E). We use u to denote the combined activity of these exchangers. The curves for u are based on data in Boyer and Tannock [8]. The Na^+/H^+ antiporter and the Na^+-dependent Cl /HCO_3 exchanger are involved primarily with the regulation of pH_i in acid loaded cells, with maximum rates of operation in the pH_i range 6.6–7.0. The activity of the Na^+-independent Cl /HCO_3 exchanger increases as pH_i rises, thereby decreasing pH_i whenever the cytosol becomes too alkaline. The level of extracellular pH also influences the operation of these exchangers. Exchanger activity is inversely related to E. Note that it is hydrogen ion concentration, rather than pH, that is plotted on the horizontal axes; some corresponding pH values are indicated.

proteinase activity from cells grown at pH 6.8 (relative to pH 7.4), whereas in the cathepsin model there is a substantial increase (e.g., 10-fold) in the secretion of active proteinase from acid cultured cells [25]. Also, the optimal pH for the proteolytic activity of the cysteine proteinases is very acidic, in the pH range of 2.8 to 5.0 [49, 50], compared to more physiological pH levels for the maximal MMP activity.

A number of studies have implicated the cysteine proteinases in malignant progression [50, 51]. For example, substantial increases in the secretion of procathepsin B have been reported in a series of B16 melanoma cell lines [52]. For the secreted procathepsins to participate in tumour cell invasion, they must be activated. As with the metalloproteinases, we expect the extracellular activation of the secretion of pro-cathepsins to be pH sensitive. However, we are not aware of experimental data that would enable these rates to be estimated quantitatively. For this reason, and in order to focus on the pH-induced secretion of active cathepsin-B (based on data in Rozhin et al. [25]), we remove the latent form from the model. As with the previous model system, we do not include a separate term in the cathepsin model to represent endogenous inhibitors of cathepsins (e.g. stefins and cystatins). Little is known about these inhibitors, and it is not known whether pH affects the secretion of these inhibitors in a similar manner to TIMPs and metalloproteinases. Thus, in this absence of experimental data we represent the inhibition of cathepsins and active-cathepsin decay with the single term $A_5 a$.

This model consists of conservation equations for tumour cells, ECM, intracellular and extracellular pH, and active-cathepsins. Here, we represent active-cathepsins via the variable $a(t)$. The model system is as follows:

m= tumour cells

$$\frac{dm}{dt} = A_1 r(I) m \left(1 - \frac{m}{A_2}\right) \tag{2a}$$

c= ECM density

$$\frac{dc}{dt} = \overbrace{-A_3 h_1(E) a c}^{\text{proteolysis}} \tag{2b}$$

a= active-cathepsin

$$\frac{da}{dt} = \overbrace{A_4 h_2(I) m}^{\text{cathepsin secretion}} - \overbrace{A_5 a}^{\text{cathepsin decay}} \tag{2c}$$

I= intracellular H^+

$$\frac{dI}{dt} = -u(I, E) + s_{1m}(V) + \beta E \tag{2d}$$

E= extracellular H^+

$$\frac{dE}{dt} = m \cdot [u(I, E) - s_2(V) E - \beta E]. \tag{2e}$$

Here the A_i's are positive constants. The function $h_1(E)$ represents the effect of pH on the proteolysis of ECM via active-cathepsins. Here, we take h_1 to have a bell shaped pH profile with a maximum near pH 4.0. The acidic pH-induced secretion of active cathepsin B is represented by the term $h_2(I)$, and we determined its functional form from data in Rozhin et al. [25]. In this study, the incubation of a highly metastatic B16a cell line at pH 6.5 resulted in a 10-fold increase in the secretion of active cathepsin B. In the pH range 6.2 to 7.6, the function $h_2(I)$ is strictly increasing. For simplicity, we take h_2 to be linear. The functions we use for h_1 and h_2 are given in the Appendix. As in the previous model, we use the term $r(I)$ to reflect the effect of pH on the proliferation rate of the cells.

We solved this model numerically for a wide range of parameter values. Again we incubated and tested the cells at either acidic (pH_e 6.8) or normal (pH_e 7.4) conditions. As before, the rate of ECM decay was determined by the ability of cells to degrade ECM material. In Figure 3b we plot the numerically calculated ECM profiles for the different cases. Our simulations show that culturing cells at pH 6.8 causes an increase of about 5% in ECM decay rates compared to when cells are grown under normal conditions (pH 7.4). In this case, there is a much more rapid decrease in the ECM density.

The important features in the cathepsin model are the more acidic pH value for the optimum proteolytic activity of the cathepsins compared to the MMP case and the increased secretion of active-enzyme from acid cultured cells. These are represented by the functional forms $h_1(E)$ and $h_2(I)$, in the ECM and active-cathepsin equations. To investigate the importance of these terms, we varied these functions in turn, and compared the relative change in the model solutions. Our results showed that varying $h_1(E)$ has a negligible effect on the ability of cells to degrade ECM at either acidic or normal pH, indicating that the effect of pH on the proteolytic activity of the cells is not the crucial term in the model. Hence, we can conclude that $h_2(I)$, the function representing the effect of low pH_i on the secretion of active-cathepsins, is crucial, and is the key term which accounts for the differences between the two enzyme models.

In our model, we have used a very simple representation of the pH-induced peripheral redistribution of cathepsin B, which is accompanied by the secretion of the active form of this enzyme. With this alternative model, there is a good agreement with the experimental data in Martinez-Zaguilan et al. [13], namely that the numerical solutions show a significant increase in the ability of acid cultured cells to degrade ECM, regardless of the pH at which the cells are tested. Our model predicts that the acidic pH-induced redistribution of active cathepsins to the cell surface of malignant cells could be a major factor in the acquisition of a more metastatic phenotype at low pH.

Discussion

Solid tumours have been observed to develop an acidic extracellular environment (pH_e 6.8–7.0). In contrast, ^{31}P NMR measurements have shown that the intracellular pH (pH_i) within tumours is slightly alkaline (pH_i 7.1–7.2). This gives rise to a reversed cellular pH gradient between tumours and normal tissue. This gradient difference may be permissive

for the tumour phenotype and aid tumour growth and invasion [37]. In this paper we have developed basic ordinary differential equation models to examine whether an altered proteolytic activity at low pH is responsible for the stimulation of a more metastatic phenotype. Our study examined one aspect of tumour invasion, i.e. the effect of pH on the generation and regulation of extracellular proteolysis. Our modelling suggests that changes in MMP activity at low pH do not have significant effects on invasive behaviour. However, our model shows that the levels of active cathepsin B are significantly increased by acidic pH, and suggests a critical role for at least one cysteine proteinase in tumour invasion.

In the second section, we focussed on one class of molecules, the matrix metalloproteinases (MMPs). Here, the model is based on experimental data from Martinez-Zaguilan et al. [13]. In this study, cells were cultured at either pH 6.8 or 7.4 for 28 days and then analysed by gelatin zymography, where the extracellular MMP activity was examined. Although their results did not show an increase in the overall amount of MMP activity from cells grown at low pH, there was a significant increase in the relative amount of active-MMP. Numerical solutions of our model show that culturing cells at acidic pH (6.8) had very little effect on their invasive behaviour. There was very little difference in the proteolytic activity from acid cultured cells and cells grown at normal pH. To test this further, we studied numerical simulations of this model for a wide range of parameter values. However, our simulations show that, in cells cultured at low pH, invasive behaviour is less than or the same as that observed under normal conditions. Thus, it seems unlikely that changes in MMP activity at low pH could be responsible for the induction of a more aggressive invasive behaviour.

Cathepsin B is a lysosomal cysteine proteinase involved in both intracellular and extracellular protein degradation. The amount of cathepsin B secreted varies considerably according to cell type and culture conditions. Rozhin et al. [25] have shown that a reduction of pH_e to 6.5 resulted in a redistribution of cathepsin B containing vesicles to the surface of tumour cells. Moreover, this resulted in an enhanced secretion of active cathepsin B. Since our modelling suggests that changes in MMP activity at low pH do not have significant effects on invasive behaviour, we considered an alternative system in which we neglected the proteolytic activity of the metalloproteinases and focussed on the acidic pH induced secretion of active cathepsin B. The main difference between the two models is the effect of pH on the secretion of the two different classes of enzymes; in the MMP model there is an overall decrease in the amount of extracellular proteinase activity from cells grown at pH 6.8 (relative to pH 7.4), whereas in the cathepsin model there is a substantial increase (e.g., 10-fold) in the secretion of active proteinase from acid cultured cells [25]. From numerical solutions of this alternative model we were able to reproduce the quantitative features of the experimental data in Martinez-Zaguilan et al. [13]. In particular, we were able to show an increased ability of acid cultured cells to degrade ECM. Furthermore, this result is independent of the pH at which the cells are tested. Our model thus predicts that the pH-induced peripheral redistribution of cathepsin B could be a major factor in the acquisition of a more metastatic phenotype in malignant cells at low pH_e.

Human and animal tumours *in vivo* often contain both hypoxic and well oxygenated areas [53] and approximately one unit variations in pH_e between tumour capillaries have been observed in animal model systems [42]. The present theoretical model suggests that, in addition to metalloproteinase activity, the cysteine proteinases play a critical role in tumour progression, a prediction with important implications for the design of anti-invasive therapies.

Appendix: Functional forms and parameter values

Ion-transporter activity u(I,E)

In our model system, we use $u(I, E)$ to denote the combined activity of the membrane based ion-transport exchangers and the sequestion of cytosolic protons into acidic cellular vesicles. Several experimental studies have examined the effect of microenvironmental factors on the operation of these transporting mechanisms [54, 55]. In their study of the regulation of intracellular pH in tumour cells, Boyer and Tannock [8] examine the importance of the Na^+-driven exchangers in conditions that may exist in solid tumours. We use experimental data from this study to estimate the functional form of $u(I, E)$. There is some evidence that a vacuolar type H^+-ATPase is expressed in the plasma membrane of some tumour cell lines [56]; this is an established mechanism in other highly invasive cells, such as osteoclasts [57] and macrophages [58]. However, in this paper, we neglect such factors since the inclusion of proton fluxes due to ATPase pumps will have little effect on the form on the function $u(I, E)$. Under normal conditions, the rate of H^+-flux due to the combined activity of the Na^+/H^+ antiporter and anion-transport systems is estimated to be in the range 4mM/min to 14mM/min, with maximum rates of operation in the pH_i range 6.6–7.0 [8]. In Figure 4 we illustrate the effect of pH on $u(I, E)$.

Acid loading $s_1(V)$, βE and hydrogen removal $s_2(V)$

In our model, we use $s_{1_n}(V)$ and $s_{1_m}(V)$ to denote the rate at which cellular metabolism can cause H^+ to accumulate intracellularly for normal and tumour cells, respectively. We assume that the rate at which H^+-ions are removed from the interstitial space is directly proportional to V. We use the term $s_2(V)$ to represent this linear removal from the interstitial space.

For computational purposes, we have chosen simple functional forms for $s_1(V)$ and $s_2(V)$, namely

$$s_{1_i}(V) = r_1\left(1 - \frac{\alpha_i V^{r_2}}{r_3+V^{r_2}}\right)$$

where $i = n, m$ and $s_2(V) = r_4 V$.

Here the r_i's are positive constants. We take $1 > \alpha_n > \alpha_m$, so that $s_{1_n}(V)$ and $s_{1_m}(V)$ have the appropriate qualitative forms (see Figure 1).

In an acidic environment, chronic acid loading occurs in cells due to their internally negative membrane potential. To represent this passive movement of H^+-ions into the cell we use a term of the form βE; for simplicity, we assume that β is constant.

A number of studies on blood flow, oxygen and nutrient supply in tumours have been reported since the early part of the century [1, 7]. In spite of this available data, we are not aware of experimental data that would enable the functions $s_1(V)$ and $s_2(V)$ to be estimated quantitatively. In the absence of such data, we have used numerical simulations to perform a parameter sensitivity analysis. Here, we perturb each parameter in turn and determine the effect on the model solutions. The analysis indicates that the qualitative nature of the solutions does not depend on the parameters r_3 and β. We choose $r_3 = 0.01$, $\beta = 1$. The remaining parameters were chosen to give the best fit with data from Vaupel et al. [7]. In this review article, data for the pH distribution in tumours is compared with the data for normal tissues. We choose $r_1 = 1$, $\alpha_n = 0.8$, $\alpha_m = 0.4$ and $r_4 = 10^7$. This choice of parameters gives a value of pH_i in the range 7.2–7.25 in tumours at pH_e 6.8, and in normal tissues at pH_e 7.4. We take $r_2 = 14$, so that $s_1(V)$ has the appropriate qualitative form (see Figure 1).

Estimation of the pH change time scale

Kato et al. [16] have examined the pH change in a culture medium during an *in vitro* cell cultivation. In this study, B16-F10 melanoma cells were incubated in a serum-free culture with various initial pH values for four different time periods. From this data, we can estimate the time scale of the pH change in our model solution. We reduce the model [Equation (1)] to a separate submodel in which only pH_i and pH_e are considered, namely

$$\frac{dI}{dt} = - u(I, E) + s_{1m}(V) + \beta E, \tag{A.1a}$$

$$\frac{dE}{dt} = u(I, E) - s_2(V)E - \beta E . \tag{A.1b}$$

We have solved this submodel numerically, with various initial pH values, and compared the numerical solutions with the experimental data from Kato et al. [16]. From this, we can obtain order of magnitude estimates for the parameters in the equations (A.1a) and (A.1b).

The proliferation rate r(I)

A slightly alkaline intracellular pH (pH_i) of about 7.2–7.4 appears to be a necessary, but not sufficient, criterion for normal cellular proliferation [59, 60]. If the intracellular pH is lowered below pH 7.2, growth is inhibited [12]. We assume that proliferation can only take place if the pH_i is above a threshold level p_0, which is permissive for a proliferative response. The value of p_0 varies between cell types, but is typically in the pH_i range 6.6–7.0. We choose $p_0 = 6.8$. The qualitative form of the function $r(I)$ is shown in Figure 2.

Decay of ECM $f_2(E)$ and $h_1(E)$

The proteolytic effect of both the metalloproteinases and cysteine proteinases are pH dependent; typically, these enzymes exhibit bell-shaped dependencies on pH_e [46, 47]. The optimal pH for the proteolytic activity of the cysteine proteinases is very acidic, i.e., in the pH range 2.8 to 5.0 [49]. In contrast, the metalloproteinases have a more alkaline optima (pH_e 7.0–8.2) [47]. The qualitative forms of $f_2(E)$ and $h_1(E)$ are illustrated in Figure 5. The pH profile for the metalloproteinases is determined from data in Kato et al. [16]. From this, we can estimate that the optimum pH for MMP activity is pH 7.0. The pH-profile for the cysteine proteinases was estimated from experimental data in Briozzo et al. [49]. Here, the maximum enzymatic activity occurs at pH_e 4.0.

Matrix metalloproteinases

Martinez-Zaguilan et al. [13] found that although there was less extracellular MMP activity from cells grown at low pH (6.8), there was a significant increase in the relative amount of active MMP in these cells. In this study the ratio of active to pro-MMP (86 and 97KDa gelatinase, respectively) was estimated to be 0.78 for cells grown at pH_e 7.4, and 1.2 for cells grown at pH_e 6.8. By comparing these results with the final tumour levels of pro and active-MMP, we can estimate several of the parameter values and determine the nature of the functional forms f_1 and f_3. From this, we estimate $k_3k_4k_2/k_1^2 = 15$, $k_5/k_1 = 1$, $k_6/k_1 = 3.43$, and $k_7/k_1 = 5 \times 10^5$. Moreover, we can infer that in the pH range 6.2 to 7.6 both $f_1(E)$ and $f_3(E)$ are strictly increasing functions. For simplicity, we assume that they are linear (see Figure 6).

Cysteine proteinases

Rohzin et al. [25] have observed that the culturing of B16a melanoma cells at acidic pH_e (6.5) results in a significant increase in the secretion of cathepsin B. At pH_e 6.5, the secretion of cathepsin B was 10 times greater than at pH_e 7.4. The cathepsin B secreted into the culture media did not require proteolytic activation, suggesting that it was a mature form of this enzyme. From this data, we can determine the nature of the functional form $h_2(I)$, which represents the effect of pH on the secretion of active cathepsin B. We find that, in the pH range 6.2 to 7.6, the function $h_2(I)$ is strictly increasing. As before, we take h_2 to be linear. The qualitative form of the function $h_2(I)$ is shown in Figure 6. There is no experimental data from which the parameter values A_3 and A_4 can be determined. In this absence of data, we assume that these parameters are comparable with the production (k_6) and decay (k_7) terms in the MMP model. From this, we estimate $A_3A_4A_2/A_1^2 = 15$ and $A_5/A_1 = 5 \times 10^5$.

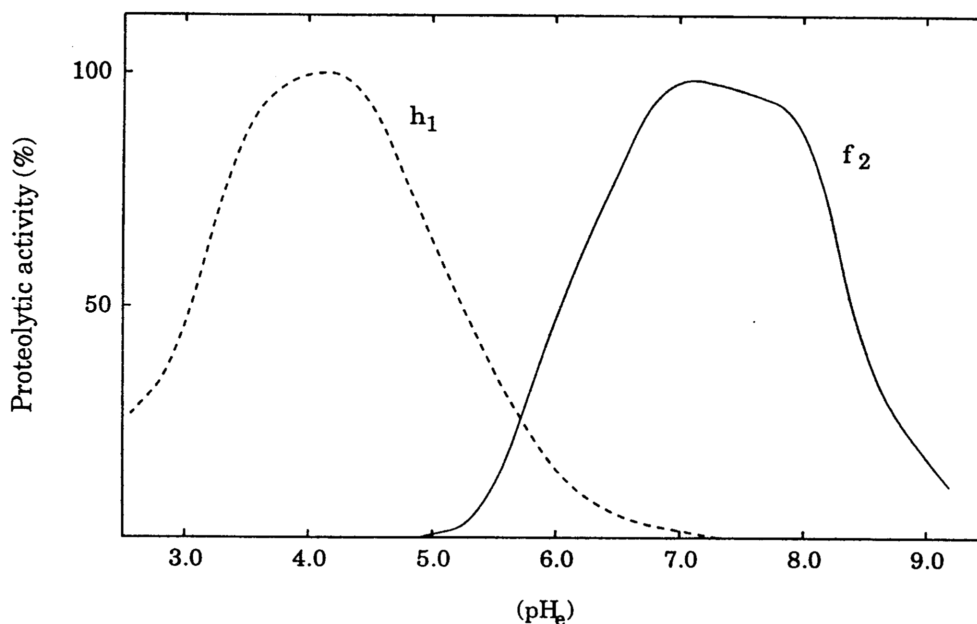

Figure 5. Effect of pH_e on proteinase activity. The curves illustrate the pH_e-dependencies of the metalloproteinases (redrawn from Kato et al. [16]) and the cysteine proteinases (based on data in Briozzo et al. [49]). We use h_1 and f_2 to denote the effect of pH on the proteolytic activity of the cysteine proteinases and metalloproteinases, respectively. The activities are expressed as a percentage of the maximum activity for each enzyme. Both these enzymes exhibit bell-shaped dependencies on pH_e. The optimum pH for proteolytic activity is 7.0 for the metalloproteinases, and 4.0 for the cysteine proteinases.

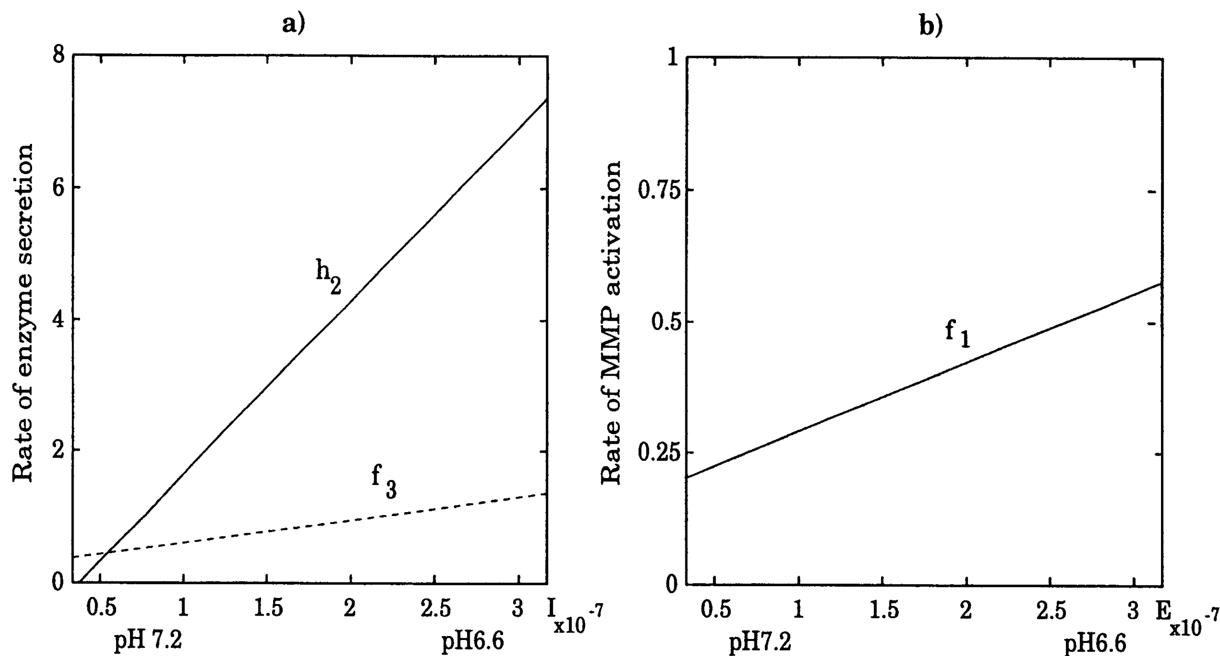

Figure 6. (a) Effect of pH_i on proteinase secretion. We plot the rate of enzyme secretion as a function of intracellular H^+-ion concentration (I). We use f_3 and h_2 to denote the effect of pH on the secretion of pro-MMP and active-cathepsins, respectively. The function h_2 is derived from data in Rozhin et al. [25]. In the pH range 6.2 to 7.6, both f_3 and h_2 are strictly increasing functions. Comparing the rate of secretion for the two classes of enzyme proteins, we see that the magnitude of the increase in the secretion of active-cathepsins B is significantly larger than in the MMP case. (b) The qualitative form of the function $f_1(E)$, which reflects the effect of pH on the autolytic activation of pro-MMP. In the pH range 6.2–7.6, the activation of latent enzymes increases as pH_e becomes more acidic. The functional forms of f_1 and f_3 are based on data in Martinez-Zaguilan et al. [13]. Note that it is hydrogen ion concentration, rather than pH, that is plotted on the horizontal axes; some corresponding pH values are indicated.

Acknowledgements

SDW was supported by an earmarked studentship from the Engineering and Physical Sciences Research Council.

References

1. Warburg O. The metabolism of tumours. London: Constable 1930.
2. Tannock IF, Rotin D. Acid pH in tumours and its potential for therapeutic exploitation. Cancer Res 1989; 49: 4373–84.
3. Kallinowski F, Vaupel P, Runkel S. Glucose uptake, lactate release, ketone body turnover, metabolic micromilieu and pH distributions in human breast cancer xenografts in nude rats. Cancer Res 1988; 48: 7264–72.
4. Hoffman FA. Metabolic changes in malignancy. In Liotta LA (ed): Cancer Growth and Progression. Boston: Kluwer Academic Publishers 1989; 18–27.
5. Hawkins RA, Hoh C, Glaspy J et al. PET-FDG imaging in cancer. Appl Radiol 1992; 5: 51–7.
6. Stubbs M, Rodrigues L, Howe FA et al. Metabolic consequences of a reversed pH gradient in rat tumours. Cancer Res 1994; 54: 4011–16.
7. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: A review. Cancer Res 1989; 49: 6449–65.
8. Boyer MJ, Tannock IF. Regulation of intracellular pH in tumor cell lines: Influence of microenvironmental conditions. Cancer Res 1992; 52: 4441–47.
9. Rotin D, Steele-Norwood D, Grinstein S, Tannock I. Requirement of the Na^+/H^+ exchanger for tumor growth. Cancer Res 1989; 49: 205–11.
10. Gerweck LE, Seetharaman K. Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. Cancer Res 1996; 56: 1194–98.
11. Busa WB, 1986, Mechanisms and consequences of pH-mediated cell regulation. Ann Rev Physiol 1986; 48: 389–402.
12. Gillies RJ, Martinez-Zaguilan R, Peterson EP, Perona R. Role of intracellular pH in mammalian cell proliferation. Cell Physiol Biochem 1992; 2: 159–79.
13. Martinez-Zaguilan R, Seftor EA, Seftor REB et al. Acidic pH enhances the invasive behaviour of human melanoma cells. Clin Exp Metastasis 1996; 14: 176–86.
14. Liotta LA, Stetler-Stevenson WG. Principles of molecular cell biology of cancer: Cancer metastasis. Cancer: Principles & Practice of Oncology. Philadelphia: Lippincott Co. 1993; 134–49.
15. Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 1997; 89: 1260–70.
16. Kato Y, Nakayama Y, Umeda M, Miyazaki K. Induction of 103-kDa gelatinase/type IV collagenase by acidic culture conditions in mouse metastatic melanoma cell lines. J Biol Chem 1992; 267: 11424–30.
17. Powell WC, Matrisian LM. Complex roles of matrix metalloproteinases in tumour progression. Curr Topics Microbiol Immunol 1996; 213: 1–21.
18. Woessner JF. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991; 5: 1245–54.
19. Duffy MJ. The role of proteolytic enzymes in cancer invasion and metstasis. Clin Exp Metstasis 1992; 10: 145–55.
20. Mignatti P, Rifkin DB. Membrane and matrix localization of proteinases in tumour invasion. Physiol Rev 1993; 73: 161–95.
21. Stetler-Stevenson WG, Liotta LA, Kleiner Jr DE. Extracellular matrix: Role of matrix metalloproteinases in tumour invasion and metstasis. FASEB J 1993; 7: 1434–41.
22. Sato H, Takino T, Okada Y et al. A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 1994; 370: 61–5.
23. Birkedal-Hansen H. Proteolytic remodeling of extracellular matrix. Curr Opin Cell Biol 1995; 73: 728–35.
24. Ishihara A, Nabeshima K, Koono M. Partial purification and characterization of serum protease form tumor-bearing rats which cleaves type IV collagen. Invasion Metastasis 1986; 6: 225–45.
25. Rozhin J, Sameni M, Ziegler G, Sloane BF. Pericellular pH affects distribution and secretion of cathepsin B in malignant cells. Cancer Res 1994; 54: 6517–25.
26. Ward JP, King JR. Mathematical modelling of avascular tumour growth. IMA J Math Appl Med Biol 1997; 14: 39–69.
27. Adam JA. A mathematical model of tumour growth. 2. Effects of geometry and spatial non-uniformity on stability. Math. Biosci 1987; 86: 183–211.
28. Burton AC. Rate of growth of solid tumours as a problem of diffusion. Growth 1966; 30: 159–76.
29. Chaplain MAJ, Sleeman BD. A mathematical model for the production and secretion of tumour angiogenesis factor in tumours. IMA J Math Appl Med Biol 1990; 7: 93–108.
30. Byrne HM, Chaplain MAJ. Mathematical models for tumour angiogenesis – numerical simulations and non-linear wave solutions. Bull Math Biol 1995; 57: 461–86.
31. Sherratt JA, Nowak MA. Oncogenes, anti-oncogenes and the immune response to cancer: A mathematical model. Proc R Soc Lond 1992; B248: 261–71.
32. Grossman Z, Berke G. Tumour escape from immune elimination. J Theor Biol 1980; 51: 317–40.
33. Owen MR, Sherratt JA. Pattern formation and spatiotemporal irregularity in a model for macrophage-tumour interaction. J Theor Biol 1997; 189: 63–80.
34. Adam JA, Bellomo N. A Survey of Models for Tumour-Immune System Dynamics. Boston: Birkhauser 1997.
35. Perumpanani AJ, Sherratt JA, Norbury J, Byrne HM, 1996, Biological inferences from a mathematical model for malignant invasion. Invasion Metastasis 1996; 16: 209–21.
36. Gatenby RA, Gawlinski ET. A reaction-diffusion model of cancer invasion. Cancer Res 1996; 56: 5745–53.
37. Gatenby RA. Altered glucose metabolism and the invasive tumour phenotype: Insights provided through mathematical models (Review). Int J Oncol 1996; 8: 597–601.
38. Webb SD, Sherratt JA, Fish RG. Mathematical modelling of tumour acidity: regulation of intracellular pH. J Theor Biol 1999; 196: 237–50.
39. Hochachka PW, Mommsen TP. Protons and Anaerobiosis. Science 1983; 219: 1391–7.
40. Busa WB, Nucatelli R. Metabolic regulation via intracellular pH. Am J Physiol 1984; 246: R409–38.
41. Roos A, Boron WF. Intracellular pH. Physiol Rev 1981; 61: 296–434.
42. Helmlinger G, Yuan F, Dellian M, Jain RK. Interstitial pH and pO_2 gradients in solid tumours *in vivo*: High-resolution reveal a lack of correlation. Nature Med 1997; 3: 177–81.
43. Casciari JJ, Otirchos SV, Sutherland RM. Variations in tumour growth rates and metabolism with oxygen concentration, glucose concentration, and extracellular pH. J Cell Physiol 1992; 151: 386–94.
44. Vassalli J, Pepper S. Membrane proteases in focus. Nature 1994; 370: 14–5.
45. Davis GE, Martin BM. A latent MR 94,000 gelatin-degrading metalloproteinase induced during differentiation of HL-60 promyelocytic leukemia cells – a member of the collagenase family of enzymes. Cancer Res 1990; 50: 1113–20.
46. Kramer MD, Robinson P, Vlodavsky I et al. Characterization of an extracellular matrix-degrading protease derived from a highly metastatic tumour cell line. Eur J Cancer Clin Oncol 1985; 21: 307–16.
47. Turner GA. Increased release of tumour cells by collagenase at acid pH: a possible mechanism for metastasis. Experimentia 1979; 35: 1657–8.
48. Jang A, Hill RP. An examination of the effect of hypoxia acidosis, and glucose starvation on the expression of metastasis-associated genes in murine tumor cells. Clin Exp Metastasis 1997; 15: 469–83.
49. Briozzo P, Morisset M, Capony F et al. *In vitro* degradation of extracellular matrix with Mr 52,000 cathepsin D secreted by breast cancer cells. Cancer Res 1988; 48: 3688–92.
50. Van der Stappen JWJ, Williams AC, Maciewicz RA, Paraskeva C. Activation of cathepsin B, secreted by a colorectal cancer cell line requires low pH and is mediated by cathepsin D. Int J Cancer 1996; 67: 547–54.
51. Chambers AF, Colella R, Denhardt DT, Wilson SM. Increased expression of cathepsins L and B and decreased activity of their inhibitors

in metastatic, ras-transformed NIH 3T3 cells. Mol Carcin 1992; 5: 238–45.

52. Qian F, Bajkowski AS, Steiner DF et al. Expression of five cathepsins in murine melanomas of varying metastatic potential and normal tissues. Cancer Res 1989; 49: 4870–5.
53. Sartorelli AC. Therpeutic attack of hypoxic cells of solid tumors: Presidential address. Cancer Res 1988; 48: 775–8.
54. Frelin C, Vigne P, Ladoux A, Lazdunski M. The regulation of the intracellular pH in cells from vertebrates. Eur J Biochem 1988; 174: 3–14.
55. Strazzabosco M, Boyer MJ. Regulation of intracellular pH in the hepatocyte. J Hepatol 1996; 24: 631–4.
56. Martinez-Zaguilan R, Lynch RM, Martinez GM, Gillies RJ. Vacuolar-type H^+-ATPases are functionally expressed in plasma membranes of human tumor-cells. Am J Physiol 1993; 265: C1015–29.
57. Vaananen HK, Karhukorpi EK, Sundquist K et al. Evidence for the presence of a proton pump of the vacuolar H^+-ATPase type in the ruffled borders of osteoclasts. J Cell Biol 1990; 111: 1305–11.
58. Tapper H, Sundler R. Cytosolic pH regulation in mouse macrophages – proton extrusion by plasma-membrane-localized H^+-ATPase. Biochem 1992; 281: 245–50.
59. Grinstein S, Rotin D, Mason MJ. Na^+/H^+ exchange and growth factor induced cytosolic pH changes. Role in cellular proliferation. Biochim Biophys Acta 1989; 988: 73–97.
60. Madshus IH. Regulation of intracellular pH in eukaryotic cells. Biochem J 1988; 250: 1–8.