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Mathematical Modelling of Tumour Acidity: Regulation of Intracellular pH

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Measurements of extracellular pH (pH_e) *in vivo* have shown that the microenvironment in tumours is more acidic than in normal tissue. However, both human and animal tumour cells have been shown to have an intracellular pH (pH_i) on the alkaline side of neutrality (pH 7.1–7.2). This gives rise to a reversed pH gradient between tumours and normal tissue which implies that cells within solid tumours are capable of maintaining their level of pH_i at physiological levels, despite lower than normal levels of pH_e. In this paper the authors describe a mathematical model that provides a possible explanation for the altered pH gradient observed in tumours. The authors examine the influence of changes in the microenvironment on the activity of several membrane based ion transport systems. Using qualitative analysis the authors show that the pH_i of tumour cells is less sensitive to external pH than for normal cells, because of their increased reliance on the inefficient glycolytic pathway for energy production. It is shown that under aerobic conditions the lactate$^-$/H^+ symporter could be the most active exchanger in the regulation of pH_i in tumour cells. However, under more hypoxic conditions lactate extrusion is reduced, and so this exchanger has little effect on resting pH_i in these regions. The authors also consider an extended model which incorporates the transfer of acids from the cytosol into acidic organelles. The model demonstrates that one of the major factors involved in the maintenance of cytosolic pH to physiological levels, despite an acidic extracellular pH in hypoxic areas of tumour tissue (median, 6.9–7.0), is enhanced sequestration of cytosolic protons into acidic cellular vesicles such as endoplasmic reticulum, golgi, endosomes, and lysosomes.

1. Introduction

Measurement of pH *in vivo* has shown that the microenvironment in tumours is generally more acidic than in normal tissue, with median pH values of about 7.0 in tumours and 7.5 in normal tissue (Tannock & Rotin, 1989; Warburg, 1930; Adam and Bellomo, 1997). The studies of Warburg in the early part of the century (Warburg, 1930) showed that tumour cells preferentially convert glucose and other substrates to lactic acid, even under aerobic conditions (Griffiths, 1991). Since then, Positron Emission Tomography (PET) and magnetic resonance spectroscopy (MRS) have consistently demonstrated the increased use of glycolysis in tumours (Kallinowski, 1988; Hoffman, 1989;

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Flier *et al*., 1987; Hawkins *et al*., 1992). Some normal tissues (for example, exercising muscle) also form large amounts of lactic acid which is rapidly removed (Stubbs *et al*., 1994). However, the increased use of the glycolytic pathway matched with the compromised vasculature of a tumour results in the poor removal of lactic acid and is believed to be the main cause of acidity within solid tumours (Vaupel *et al*., 1989).

Traditionally, most estimates of pH in tissue were obtained by insertion of pH electrodes (Tannock & Rotin, 1989). These were usually quite large in comparison to a tumour cell (Griffiths, 1991), and primarily measured interstitial or extracellular tissue pH. For most purposes, the parameter of interest is intracellular pH (pH_i), and since lactic acid production can lead to intracellular acidosis, tumours were thought for many years to have a more acidic pH_i (Stubbs *et al*., 1994). However, with the advent of ^{31}P-MRS imaging for non-invasive measurements of pH_i (Griffiths, 1991), both human and animal tumour cells have been shown to have intracellular pH, similar to normal cells, near neutrality (pH 7.0) (Rotin *et al*., 1989; Boyer & Tannock, 1992; Vaupel *et al*., 1989), or even slightly alkaline (pH 7.1–7.2) (Stubbs *et al*., 1994; Gerweck & Seetharaman, 1996). This gives rise to a reversed pH gradient across the cell membrane between normal tissues and tumours.

Despite the more acidic tumour microenvironment, most *in vitro* experiments are still performed at the relatively alkaline extracellular pH (pH_e) of 7.4. This is significant because slight changes in pH_e can have profound effects on cell phenotype (Martinez-Zaguilan *et al*., 1996). It has been shown that the metastatic potential of tumour cells depends directly on the degree of acidification (Abakarova, 1995). More specifically studies by Martinez-Zaguilan and coworkers (1996), have shown that the incubation of human melanoma cells under conditions of low pH_e causes them to become more invasive and migratory. Furthermore, tumour angiogenesis may be regulated by pH. The switch to the angiogenic phenotype depends on a net balance of positive and negative angiogenic factors released by the tumour. Recent evidence from several laboratories (Griffiths *et al*., 1997; Xie *et al*., 1998; Xu and Fidler, 1998) support a role for pH in this process, and indicate that tumour cells at low pH_e increase the expression of positive angiogenic factors, such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8).

The reversed cellular pH gradient in tumour cells has also been proposed as a potential anticancer strategy (Boyer & Tannock, 1992). Multidrug resistant tumour cells were shown to be able to regulate their level of pH_i, despite the lower than normal levels of pH_e (Keizer & Joenje, 1989). Also, the low pH_e observed in some areas of tumour tissue may not only influence the cytotoxicity of a number of clinically used anticancer drugs (Vukovic & Tannock, 1997) but suppress radiation-induced apoptosis (Park *et al*., 1997), and alter the effects of hyperthermia (Hofer & Mivechi, 1980).

Regulation of pH_i within a narrow range is crucial for the maintenance of normal metabolic activity (Wadsworth & VanRossum, 1994). Chronic intracellular acid loads result from cellular metabolism and the passive fluxes of weak acids across the cell membrane. In addition to the short-term homeostasis of physiochemical buffering, several membrane-based transport systems have been shown to be involved in the regulation of pH_i (Roos & Boron, 1981). Most cells have one or more types of Na^+-driven antiporters that regulate pH_i using the energy stored in the Na^+ gradient. 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 (Tannock & Rotin, 1989). In addition, protons can be transferred from the cytosol into intracellular organelles such as golgi, endosomes and lysosomes (Rudnick, 1986).

Mathematical modelling of tumour growth and metastasis has a long history. Models typically focus on specific aspects of tumour dynamics such as tumour-immune interactions (Sherratt & Nowak, 1992; Grossman & Berke, 1980), angiogenesis (Chaplain & Sleeman, 1990; Byrne & Chaplain, 1995) and tumour invasion (Perumpanani *et al*., 1996); for a general review, see (Adam & Bellomo, 1997). However, this previous modelling has almost entirely neglected

the effects of pH differences within the tumour. The exceptions are the work of Gatenby *et al*. (Gatenby, 1995, 1996; Gatenby & Gawlinski, 1996), and Kraus & Wolf (1996). Gatenby has studied the potential role of pH in the invasive tumour phenotype. His modelling predicts that a simple but sufficient mechanism of tumour invasion is the altered metabolic properties which are consistently seen in tumour cells. The work by Kraus & Wolf (1996) has focussed on the implications of an acidic tumour environment on tumour-immune system dynamics, and the implications for immunotherapies. Both of these modelling approaches represent pH differences between tumour and normal cells in a simply generic way, in particular, without considering intracellular and extracellular pH separately. In the present study, we develop mathematical models concentrating on the combined activities of cell membrane ion-transporters in the regulation of pH_i, and include representations of disorganized vasculature, hypoxia and high glycolytic metabolism, which are the hallmarks of most solid tumours.

In Section 2, we derive a very simple mathematical model for the regulation of pH_i. We show that there is one stable steady state, with pH_i in the range 7.0–7.2. Straightforward analysis of this model shows that the intracellular pH of tumour cells is less sensitive to external pH than for normal cells, because of their high glycolytic activity. In Section 3, we consider an extension of the basic model of Section 2, here our focus is on the coupled movement of lactate$^-$ and H^+ out of the cell in a 1:1 symport. The model predicts that under aerobic conditions this exchanger may be a potential mechanism responsible for the reversed cellular pH gradient seen in many tumour cells. However, this amended model does not explain this reversal under anaerobic conditions. In Section 4, we consider another amended model which incorporates the transfer of H^+-ions into cellular organelles. The solutions of this improved model give a good fit with the experimental data, namely the more alkaline pH_i of cells within solid tumours. Our model predicts that the pH_i differences between tumours and normal tissue could be due to an enhanced H^+ transfer into acidic cellular vesicles in tumour cells.

2. Modelling pH_i Regulation

We begin by deriving a basic ordinary differential equation model for the regulation of pH_i. In particular, we concentrate on the high rates of aerobic and anaerobic production of lactic acid which are widely believed to be the cause of acidity within many solid tumours. The model consists of two variables: intracellular pH (pH_i) and extracellular pH (pH_e). For convenience we represent these in the models via the corresponding hydrogen ion concentrations I and E, respectively (recall that $pH = -\log[H^+]$).

The vasculature of many tumours is often compromised and unable to supply the nutritional needs of the expanding population of tumour cells, leading to the existence of hypoxic regions within solid tumours. A strong correlation between hypoxia and low pH has been observed in some rodent tumours (Helmlinger *et al*., 1997). To represent the functional vasculature in our modelling we introduce the parameter V, which represents the extent of vascularity. We assume that once hydrogen ions are transported outside the cell, the rate at which they are removed from the interstitial space is directly proportional to V (Fig. 1). We use the term $S_2 = S_2(V)$ to represent this removal.

Steady-state aerobic energy metabolism consumes the same number of H^+-ions as are produced by the hydrolysis of ATP (Hochachka & Mommsen, 1983; Busa & Nucatelli, 1984), and as a result has no net effect on pH_i. However, in the absence of oxygen, cells rely on the more inefficient process of anaerobic glycolysis to obtain energy. The glycolytic pathway causes a net production of H^+, leading to changes in pH_i. The rate of glycolysis is dependent in environmental conditions, and imaging has shown that human tumours consistently consume two to 15 times more glucose than normal tissue, with up to 80% of total body glucose metabolized by the tumour (Gatenby, 1996).

In this section the focus of our modelling is on the increased use of the glycolytic pathway by tumour cells, which occurs even under aerobic conditions. 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 Fig. 1, the form of $S_1(V)$ for tumour cells is more gently sloping which represents their high glycolytic rate and consequent net production of H^+-ions, even in the presence of oxygen (i.e. high V).

Two types of membrane transport mechanisms have been implicated in pH_i regulation in mammalian cells: the Na^+/H^+ antiporter and the Cl^-/HCO_3^- exchanger (Rotin *et al*., 1989). The Na^+/H^+ exchanger has been found to be important for tumour growth, as well as being involved in a variety of cellular processes. However, the many studies that have shown the importance of the Na^+/H^+ exchanger in pH_i regulation have been performed in the absence of HCO_3^- (Frelin *et al*., 1988). In short term experiments, the Na^+-dependent Cl^-/HCO_3^- exchanger has been found to promote pH_i recovery following an acid load under conditions in which the Na^+/H^+ exchanger is inactive. Indeed, at pH levels close to neutrality, the Na^+-driven Cl^-/HCO_3^- exchanger has been found to be more active than the Na^+/H^+ exchange in some cell lines (Boyer & Tannock, 1992).

We assume that there is a continual recruitment of glucose and other substrates to the interstitial space, even in hypoxic conditions (i.e. low V). Thus, hypoxic tumour cells, far from the supporting vasculature, can still cleave glucose for anaerobic energy metabolism. This is biologically reasonable since the diffusion distance for glucose is larger than that for O_2 (Vaupel *et al*., 1989). In an acidic environment, chronic acid loading can occur 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.

We use P_i $(i = 1, 2, 3)$ to denote the activities of the Na^+-driven Cl^-/HCO_3^- exchanger, the Na^+/H^+ antiporter and the Na^+-independent Cl^-/HCO_3^- exchanger, respectively. Using this notation the general conservation equations for I and E are

$$\frac{\partial I}{\partial t} = -P_1(I, E) - P_2(I, E) + P_3(I) + S_1(V) + \beta E \quad (1a)$$

$$\frac{\partial E}{\partial t} = P_1(I, E) + P_2(I, E) - P_3(I) - S_2(V)E - \beta E. \quad (1b)$$

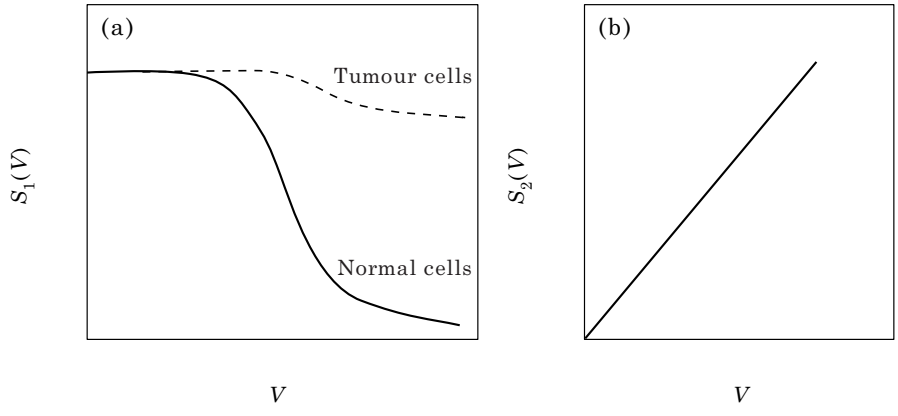

FIG. 1. (a) Schematic representation of the net production of H^+-ions from cellular metabolism of normal cells (—), and tumour cells (– – –). We represent the extent of functional vasculature in our modelling with the parameter V. We expect $S_1(V)$ to be a monotone decreasing function of V. As V increases $S_1(V)$ for normal cells 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 $S_1(V)$ 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^{-1}, comparable with the flux terms illustrated in Fig. 2. However, the parameter V has no quantitative interpretation; increasing V corresponds to greater vascularity.

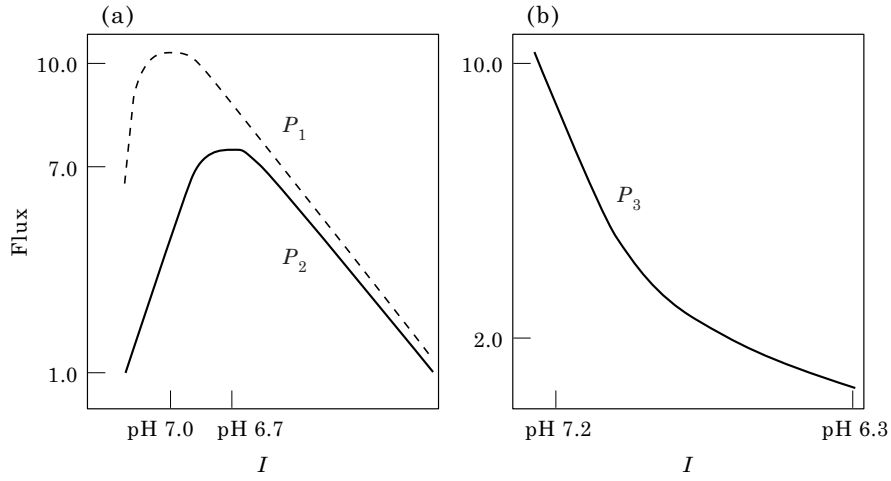

FIG. 2. An illustration of typical pH_i dependencies of the Na^+/H^+-antiporter and the Cl^-/HCO_3^- exchanger. We plot the rate of H^+-flux (mM min^{-1}) due to the activity of (a) the Na^+/H^+ exchanger (—), and the Na^+-dependent Cl^-/HCO_3^- exchanger (– – –), and (b) the Na^+-independent Cl^-/HCO_3^- exchanger, as a function of intracellular H^+-ion concentration (I). The curves for P_1 and P_2 are based on data in Boyer & Tannock (1992). We use $P_i (i = 1, 2, 3)$ to denote the activities of the Na^+-driven Cl^-/HCO_3^- exchanger, the Na^+/H^+ antiporter and the Na^+-independent Cl^-/HCO_3^- exchanger, respectively. The Na^+/H^+ antiporter and the Na^+-dependent Cl^-/HCO_3^- exchanger function as acid extruders, with maximum rates of operation in the range pH 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. Note that if it is intracellular hydrogen ion concentration, rather than pH_i that is plotted on the horizontal axis; some corresponding pH values are indicated.

Numerous experimental studies have characterized several membrane based ion-transport mechanisms in the regulation of pH_i, examining the influence of microenvironmental factors on the operation of these exchangers. A comprehensive summary of the mechanisms of pH_i regulation is given in the review by Roos & Boron (1981).

In their study of the regulation of intracellular pH in tumour cells, Boyer & Tannock (1992) examine the importance of the Na^+-driven exchangers in conditions that may exist in solid tumours. Experimental data from this study was used to estimate the qualitative functional forms of the P_i ($i = 1, 2, 3$). Figures 2 and 3 show the pH dependencies of these exchangers.

Biologically, it is the steady states of (1) that are of interest; analysis of these is described in the Appendix. This shows that the level of vasculature V plays a central role in pH_i regulation. For both cell types our model predicts an inverse relationship between V and the steady-state value of E. This implies that regions of hypoxia correspond to low extracellular pH. Intuitively, this is because hypoxic cells have a high rate of anaerobic glycolysis, resulting in the production of large amounts of lactic acid and consequent lower pH_e. The steady-state analysis also shows that as pH_e is reduced, there is a much greater reduction in the pH_i of normal cells than that of tumour cells. This is because of

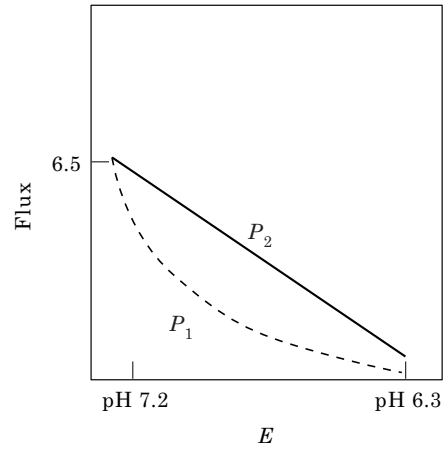

FIG. 3. An illustration of pH_i regulation by Na^+-driven transport systems. We plot the rate of H^+-flux (mM min^{-1}) due to the activity of the Na^+/H^+ exchanger (—), and the Na^+-dependent Cl^-/HCO_3^- exchanger (– – –), as a function of extracellular H^+-ion concentration (E) [based on data in Boyer & Tannack (1992)]. The level of extracellular pH also influences the operation of these exchangers. Exchanger activity is inversely related to E. Note that it is extracellular hydrogen ion concentration, rather than pH_e, that is plotted on the horizontal axis; some corresponding pH values are indicated.

their high glycolytic activity. However, values of pH_i predicted for tumour cells are very similar to the normal cell case. Thus, the solutions lack the well-documented pH_i difference between tumours and normal tissue. We now consider an amended model, in an attempt to recover this pH reversal.

3. Lactic Acid in Tumours

The basic cause of tumour acidity is the production of lactic acid from glycolysis (Griffiths, 1991). Lactic acid is a fairly strong acid, and under normal physiological conditions it is dissociated into lactate anions and H^+ (Hochachka & Mommsen, 1983). Studies have shown that most tumours release lactate in an amount linearly related to glucose consumption (i.e. rate of glycolysis) (Vaupel *et al*., 1989). However, to obtain electroneutrality during lactate export from the cell, a cation, usually H^+, would accompany this movement in a 1:1 symport (Stubbs *et al*., 1994). We now consider extending the model to reflect this coupled lactate and H^+-ion movement, by introducing a function to represent the variation in the rate of lactate$^-$/H^+ extrusion with variables, I and E, and with the parameter V.

Since in normal steady-state conditions aerobic metabolism has no net effect on pH_i, the function $S_1(V)$ must reflect the net production of H^+-ions, coupled with the production of lactate, via the glycolytic pathway. *In vivo* experiments performed on isotransplanted rat tumours have shown that glucose uptake is directly proportional to its availability (Vaupel *et al*., 1989), and so we take the activity of the lactate$^-$/H^+ symporter (i.e. the rate at which cells release lactate) as proportional to $S_1(V)$. Finally, transport of lactate out of the cell is also inhibited at low extracellular pH. We model this term as $\alpha S_1(V)G(E)$, where $G(E)$ reflects the effect of low pH_e on this pathway, illustrated in Fig. 4, and α is a positive constant. In Section 2 the various pH regulating mechanisms were all controlled by the intracellular pH. In this improved model, however, the new term, which represents the lactate$^-$/H^+ symporter, is entirely dependent on changes in the extracellular

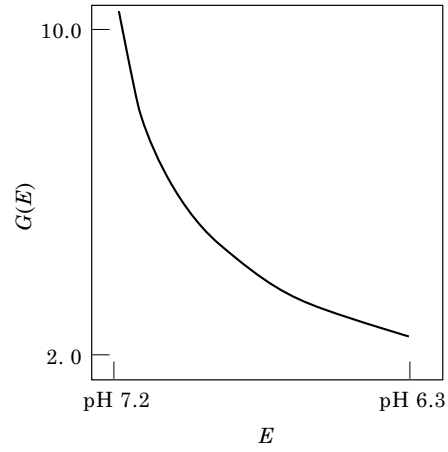

FIG. 4. The qualitative form of the function $G(E)$, which represents the effects of low pH_e on the activity of the lactate$^-$/H^+ symporter. If pH_e becomes too acidic then lactic acid extrusion will be reduced and lactate ions will accumulate intracellularly (Griffiths, 1991). The quantitative value of $G(E)$ is of the order of mM min^{-1}. Note that it is extracellular hydrogen ion concentration, rather than pH_e, that is plotted on the horizontal axis; some corresponding pH values are indicated.

environment. We add the new term $\alpha S_1(V)G(E)$ to the right-hand side of eqns (1a) and (1b), giving

$$\frac{\partial I}{\partial t} = -P_1(I, E) - P_2(I, E) + P_3(I) + S_1(V) + \beta E - \underbrace{\text{lactate}^-/H^+}_{\alpha S_1(V)G(E)} \quad (2a)$$

$$\frac{\partial E}{\partial t} = P_1(I, E) + P_2(I, E) - P_3(I) - S_2(V)E - \beta E + \alpha S_1(V)G(E). \quad (2b)$$

The steady-state solution of this system is of the same form as the original model described in Section 2, and with the same analysis as before we see that the lactate$^-$/H^+ symporter accounts for the effect of very little H^+-ion extrusion in normal cells provided V is sufficiently high, because $S_1(V)$ is then small. However, if α is fairly large, there is much more activity in this exchanger in tumour cells at higher values of V, and so increasing V may have more significant influence on the dynamics of the system. Figure 5 illustrates this important property.

In summary, our model predicts that the impact of lactate on the regulation of pH_i is likely to be small in hypoxic regions (low V).

However, if oxygen is available (higher V) then the rate of increase in this exchanger activity is large when compared with the rate of operation of the Na^+-driven exchangers (Figs 2 and 3). Our analysis shows that under aerobic conditions this exchanger could be the most active carrier protein in the regulation of pH_i in tumour cells. In terms of the model we associate high values of V with the rapid removal of H^+-ions by the supporting vasculature, and so we expect the greater proton extrusion to have little effect on pH_e. Therefore this amended model predicts that under aerobic conditions the lactate$^-$/H^+ symporter may be a potential mechanism responsible for the reversed cellular pH gradient seen in many tumour cells. However, the model does not explain this reversal under anaerobic conditions.

Short-term homeostasis of pH_i in cells involves the recruitment of rapid H^+-consuming mechanisms, which include the transfer of acids from the cytosol into organelles. Due to the large surface/volume ratio of organelles this is a rapid response in comparison to the slower mechanism of transmembrane ion transport (Roos & Boron, 1981). Since the lactate$^-$/H^+ symporter contributes relatively little to pH_i regulation under conditions of low V, we remove this term from the model and consider another amended model which incorporates the transfer of acids into organelles in these regions, in an attempt to explain pH reversal in avascular conditions.

4. Acidic Vesicles and their Association with Tumour Acidity

Lysosomes are membranous bags of hydrolytic enzymes involved in intracellular digestion of macromolecules. The lysosome maintains an interior pH of about 5 via an H^+-pump in the lysosomal membrane, and contains about 40 types of hydrolytic enzymes, including proteases, that are activated at low pH. Studies of Montcourrier *et al*. (1994) have characterized intracellular large acidic vesicles (LAVs, $\geqslant 5\,\mu$m in diameter), in which endocytosed extracellular matrix can be digested. These large compartments were found more frequently in breast cancer cells than in normal cells, and have a very high acidification potential with values of pH less than 4, compared with the typical lysosomal value of around pH 5 (Montcourrier *et al*., 1994). The presence of these LAVs has been shown to be relatively specific to cancer cells. We introduce the term $H(I)$ into our model, reflecting a cellular response to an acid load by

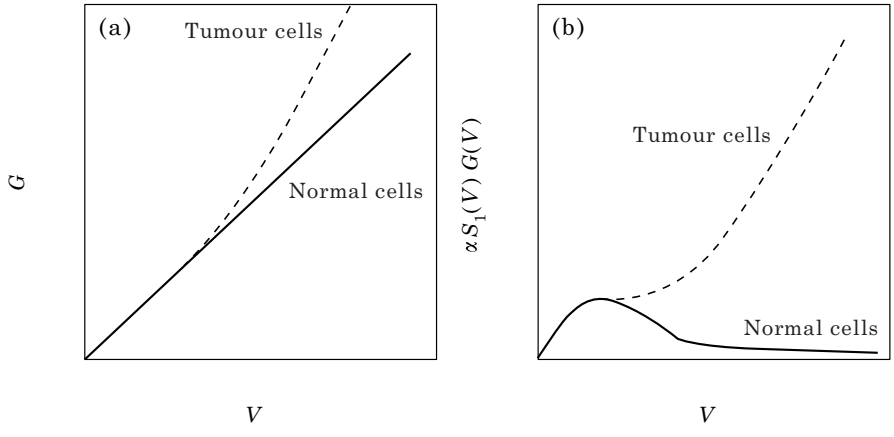

FIG. 5. (a) The qualitative form of G for normal cells (—), and tumour cells (– – –), as a function of the degree of vasculature (V) at steady state; (b) pH_i regulation by the lactate$^-$/H^+ symporter. We plot the activity of this exchanger for normal cells (—) and tumour cells (– – –) as a function of V at steady state. Note that the exchanger becomes quiescent in normal cells at high V, corresponding to the reversion of normal cells to the more efficient aerobic metabolism, resulting in little lactate production. Comparing the lactate$^-$/H^+ exchanger activity in tumour cells to P_1 and P_2 (see Figs 2 and 3), this pathway could be more active under aerobic conditions, depending on the value of α. The quantitative value of $\alpha S_1(V)G(V)$ is of the order of mM min^{-1}, comparable with the flux terms illustrated in Fig. 2. However, the parameter V has no quantitative interpretation; increasing V corresponds to greater vascularity.

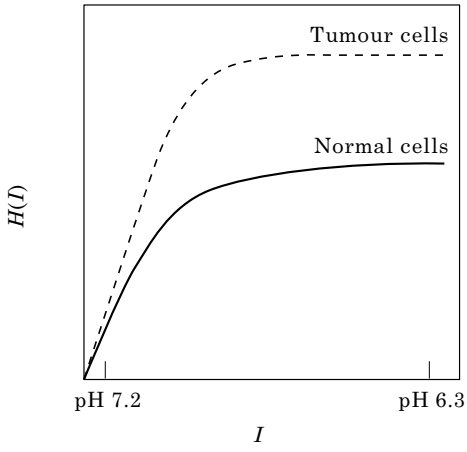

FIG. 6. Typical functional form of $H(I)$, which reflects the transfer of H^+-ions from the cytosol into organelles in response to an acid load. We expect this reaction to saturate for large enough I. In the absence of quantitative data we plot the curves for normal cells (—) and tumour cells (– – –). The results of Montcourrier *et al*. (1994) suggest that tumour cells are able to utilize the same short-term mechanisms for pH_i regulation as normal cells, but in addition are able to transfer larger amounts of H^+-ions into organelles of the form of LAVs. The quantitative value of $H(I)$ is of the order of mM min^{-1}, comparable with the flux terms illustrated in Fig. 2. Note that it is intracellular hydrogen ion concentration, rather than pH_i, that is plotted on the horizontal axis; some corresponding pH values are indicated.

the transfer of H^+-ions across organellar membranes. Therefore, we have

$$\frac{\partial I}{\partial t} = -P_1(I, E) - P_2(I, E) + P_3(I) + S_1(V) + \beta E - \overbrace{\lambda H(I)}^{\text{Organelles}} \quad (3a)$$

$$\frac{\partial E}{\partial t} = P_1(I, E) + P_2(I, E) - P_3(I) - S_2(V)E - \beta E. \quad (3b)$$

Note that with $H(I) = 0$ this reduces to the basic model (1a) and (1b) in Section 2.

The sequestration of H^+-ions by a number of intracellular organelles is dependent on pH_i. In response to an acid load, organellar acidification is rapid in comparison to ion transport across the cell membrane (Roos & Boron, 1981). This mechanism is part of the short-term regulation of pH_i, acting to minimize the initial pH_i decrease. In the absence of quantitative data we plot the qualitative form of the function $H(I)$ in the two cases, illustrated in Fig. 6. We expect the reaction to saturate for large enough I. The results of Montcourrier *et al*. (1994) suggest that tumour cells are able to utilize the same short-term mechanisms for pH_i regulation as normal cells, but are able to transfer larger amounts of H^+-ions into acidic organelles particularly in the form of LAVs.

At a steady state we have

$$E = \frac{S_1(V)}{S_2(V)} - \frac{\lambda H(I)}{S_2(V)}.$$

Substituting for E in (3b), we consider the variation of intracellular H^+-ion concentration as a function of V and λ. Qualitatively, we may understand the solutions of the amended model by examining the intersection of the two curves

$$y_1 = P_3(I) + (S_2(V) + \beta)\left(\frac{S_1(V)}{S_2(V)} - \frac{\lambda H(I)}{S_2(V)}\right)$$

and

$$y_2 = P_1(I; V, \lambda) + P_2(I; V, \lambda).$$

These curves are illustrated in Fig. 7(a) and (b) for various values of V and λ. The effect of increasing λ is a significant increase in the maximum value of y_2. Also, as I increases then y_1 decreases. However, this decrease is more gradual at lower values of V. In the case of the tumour cell we are concerned with high values of λ, and since regions of tumours which have reduced pH_e are hypoxic (i.e. low V), we decrease V to examine the influence of microenvironmental conditions such as might exist within solid tumours. Conversely, we associate the normal cell with high V, and low values of λ.

By examining the intersection of the two curves y_1 and y_2 in both cases, we can estimate the value of the resting pH_i at steady state for tumour and normal tissue. In both cases there are two points of intersection. The stability of these critical points remains the same as in Section 3, and so there is only one stable steady state in each case, at pH_i between 7.2 and 7.0. The qualitative difference between the two cases is clearly illustrated in Fig. 7(c) and (d). In the tumour cell case there is a greater increase in y_2 in the pH range 7.2–7.0 compared with normal cells. This results in a more alkaline resting pH_i

at steady state in the tumour cell case compared with normal cells. The difference is about 0.5–1 pH units, which compares favourably with observed experimental data (Tannock & Rotin, 1989; Stubbs *et al*., 1994).

In our model, we have used a very simple representation of the sequestering of H^+-ions by organelles, which is a rapid response due to changes in the intracellular pH. The solutions of this improved model show that there is a slightly more alkaline pH_i of cells within solid tumours (i.e. low V). Our model predicts that the pH_i differences between tumours and normal tissue could be due to the enhanced H^+ transfer which is associated to the existence of large acidic vesicles that are found more frequently in tumour cells. In summary, our results show that in regions of reduced pH_e, in which cell membrane transporters are relatively inactive, a potential mechanism of pH_i regulation within tumour cells could be the removal of H^+-ions into acidic organelles leading to further acidification of these various vacuoles.

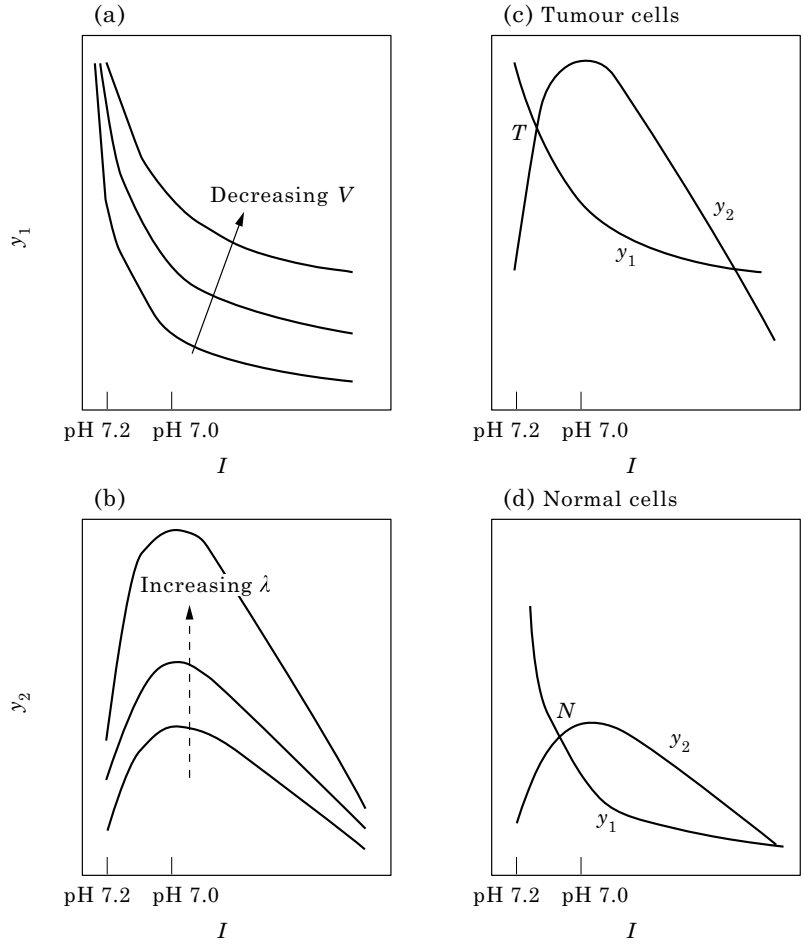

FIG. 7. The sequestering of H^+-ions by cellular organelles in response to an acid load. We plot (a) y_1, and (b) y_2 as a function of intracellular H^+-ion concentration (I); the arrows indicate the way in which the solution changes as V increases (solid arrow), and as the rate at which H^+-ions are sequestered by organelles (λ) increases (dashed arrow). For the tumour cell case we are concerned with high values of λ, and since regions of tumours which have reduced pH_e are hypoxic (i.e. low V), we decrease V to examine the influence of microenvironmental conditions such as might exist within solid tumours. Conversely, we associate the normal cell case with high V, and low values of λ. Qualitatively we may understand the solutions of the amended model by examining the intersection of the two curves, y_1 and y_2, in both cases. We plot (c) the tumour, and (d) the normal cell cases. In the tumour cell case, at $I = T$, our model predicts a more alkaline resting pH_i than for normal cells, at $I = N$ (i.e. around 0.5–1 pH units more alkaline). Note that it is intracellular hydrogen ion concentration, rather than pH_i, that is plotted on the horizontal axis; some corresponding pH values are indicated.

5. Discussion

The cellular pH gradient is reversed in tumours compared with normal cells; in normal cells, the pH_i is lower than the pH_e, whereas the opposite is true for tumours (Griffiths, 1991). This difference provides an exploitable avenue for the treatment of cancer (Gerweck & Seetharaman, 1996). In this paper we have described a mathematical model that provides explanation for the altered pH gradient observed in tumours. In the model the basic differences between tumour and normal cells are the inadequate development of tumour vasculature, and the increased rate of glycolysis and production of H^+-ions that is observed in many tumours. In Section 2, we focussed on the influence of the glycolytic tumour cell metabolism on resting pH_i. Analysis of the model showed that the pH_i of tumour cells is less sensitive to external pH than for normal cells, because of their increased reliance on the inefficient glycolytic pathway for energy production. This implies that tumour cells may be able to maintain their level of pH_i to physiological levels in low pH_e environments. However, there was very little difference between the pH_i of tumours and normal tissue, suggesting that mechanisms other than those considered here may play a more active role in the regulation of pH_i in tumour cells.

The anaerobic and aerobic production of lactic acid together with the disorganised tumour vasculature is generally believed to be the cause of tumour acidity (Griffiths, 1991; Newell *et al*., 1993; Vaupel *et al*., 1989). Under normal conditions lactic acid is dissociated into lactate anions and H^+ (Hochachka & Mommsen, 1983). During lactate export from the cell, a cation, usually H^+, would accompany this movement in a 1:1 symport. The inclusion of a lactate$^-$/H^+ symporter in our model has shown that under aerobic conditions, when tumour cells exhibit high rates of aerobic glycolysis, this exchanger could be the most active carrier protein in the regulation of pH_i in tumour cells. The model predicts that under such conditions, this enhanced H^+ extrusion may be responsible for the reversed cellular pH gradient seen in many tumours. Lactic acid extrusion is reduced in regions of low pH_e (Griffiths, 1991). Subsequent analysis of this model has shown that this exchanger has little effect on resting pH_i under hypoxic conditions, and so the inclusion of this symporter does not explain the reversed cellular pH gradient under these conditions.

Short-term homeostasis of pH_i in cells involves the recruitment of rapid H^+-consuming mechanisms, which include the transfer of acids from the cytosol into organelles. Studies of Montcourrier *et al*. (1994) have characterized intracellular large acidic vesicles (LAVs, $\geqslant 5\ \mu$m in diameter), which were found more frequently in breast cancer cells than in normal cells. These large compartments have a very high acidification potential with values of pH less than 4, compared with typical lysosomal values of about pH 5 (Montcourrier *et al*., 1994). Since the lactate$^-$/H^+ symporter contributes relatively little to pH_i regulation under hypoxic conditions, we removed this term from the model and considered an improved system which incorporates the transfer of acids into organelles in these regions. Here, the difference between tumour and normal cells is that tumour cells are able to utilize the same short-term mechanisms for pH_i regulation as normal cells, but in addition are able to transfer larger amounts of H^+-ions into organelles, particularly in the form of LAVs. The solutions of this amended model give a good fit with the experimental data, namely a more alkaline pH_i of cells within solid tumours (pH 7.1–7.2). This difference in pH_i is about 0.5–1 units, which compares favourably with experimental data. Our model thus predicts that the most active mechanism of pH_i regulation within hypoxic tumour cells is the transfer of H^+-ions from the cytosol into acidic organelles. This result suggests that one of the major factors involved in the maintenance of cytosolic pH to physiological levels, despite lower than normal levels of pH_e in hypoxic areas of tumour tissue, is enhanced sequestration of cytosolic protons into acidic cellular vesicles such as endoplasmic reticulum, golgi, endosomes and lysosomes. The predicted enhanced vacuolar acidification in tumour cells can be tested experimentally.

Tumour invasion and metastasis are the major causes of treatment failure for cancer patients (Liotta & Stetler-Stevenson, 1993). These processes can be subdivided into several steps, and

it is likely that these steps are individually pH sensitive (Martinez-Zaguilan *et al*., 1996). A natural application of our modelling would be to study the effect of variations in pH on each of these processes. For instance, invasion involves the degradation of the extracellular matrix (ECM) by tumour cells, via the production of proteolytic enzymes (Liotta & Stetler-Stevenson, 1993). The proteases appearing in the extracellular fluid are secreted by the tumour cells via acidic organelles in the form of an inactive pro-enzyme, and an acidic pH is required for the activation of some of these proteases. The interactions between invasive cells, ECM and proteases has been modelled by Perumpanani & co-workers (1996). However, this previous modelling has not examined the significance of the pH differences within the tumour on the tumour cell proteolysis of ECM. Thus, a possible extension of this previous work would be to study the way in which tumour pH can act in combination with this mechanism to enhance tumour cell invasion. Another important aspect of metastasis is a process called angiogenesis. Angiogenesis is the development of new blood vessels, and is an essential stage in the growth and metastasis of solid tumours. This process transforms a small, usually harmless, *in situ* tumour into large a mass that can spread to other organs (Holmgren *et al*., 1995). Tumour angiogenesis is initiated by the release of tumour angiogenesis factors by the tumour. This process has been modelled extensively by Chaplain & co-workers (1990, 1995). Recently, it has been shown that tumour microenvironmental factors can result in the specific up-regulation of some of these angiogenic growth factors (Griffiths *et al*., 1997; Xie *et al*., 1998; Xu & Fidler, 1998). Our results could be used, in combination with previous modelling on angiogenesis, to investigate the role of pH in this process with particular applications to anti-angiogenesis strategies.

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APPENDIX

Steady States

In this Appendix, we look for equilibrium points for eqns (1a) and (1b). These are solutions of $\partial I/\partial t = \partial E/\partial t = 0$, namely

$$0 = -P_1(I, E) - P_2(I, E) + P_3(I) + S_1(V) + \beta E \quad \text{(A.1a)}$$

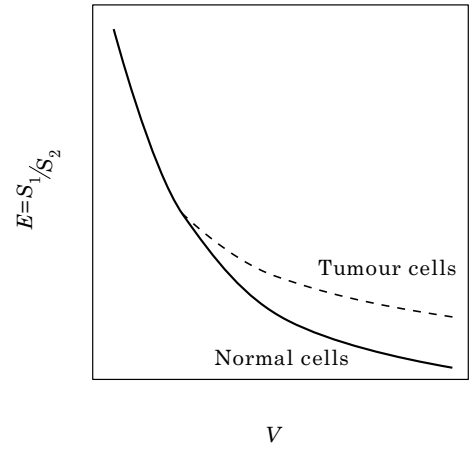

FIG. A1. A comparison of steady-state solutions for tumour and normal cells. At a steady state our model predicts an inverse relationship between extracellular H^+-ion concentration (E) and degree of vasculature (V). Curves shown represent normal cell (—) and tumour cell (– – –) states. Since S_1 and S_2 are, respectively, increasing and decreasing, this predicts that regions with low V correspond to an acidic pH_e. The normal and tumour cell cases are very similar in hypoxic conditions (low V), but differ as V increases, with the decrease in E in the tumour cell case being more gradual than for normal cells. This makes sense intuitively since studies have shown that tumour cells have a high rate of aerobic glycolysis, resulting in the production of large amounts of lactic acid and lower pH_e.

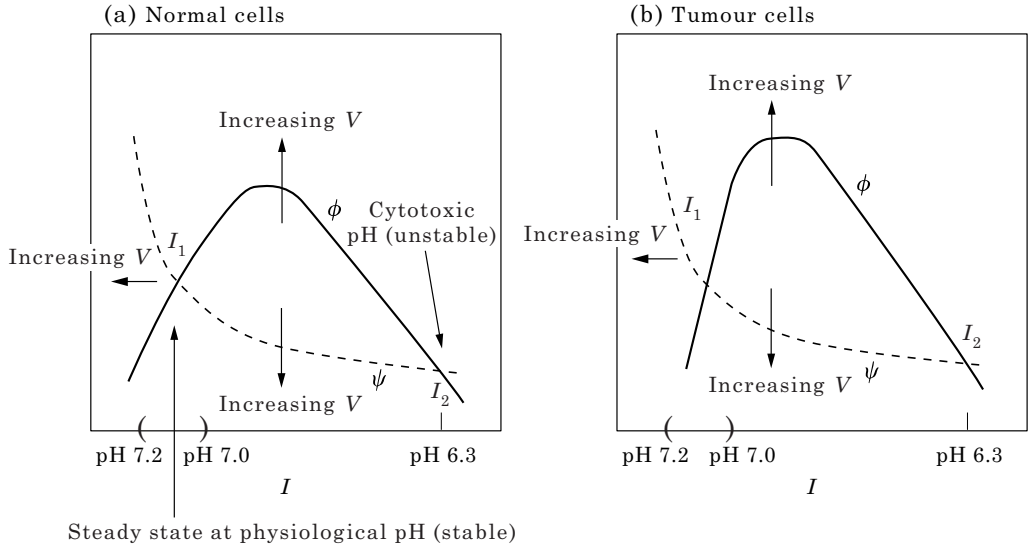

FIG. A2. (a), (b) The functions $\phi(I)$, $\psi(I)$ for various values of V: the intersections define the equilibrium points of the system. We plot the two curves for (a) normal cells and (b) tumours cells. The arrows indicate the way in which the solution changes as V increases. There are two steady states corresponding to two different resting pH_is. Clearly the pH value of the steady state at $I = I_2$, in the pH_i range 6.2–6.3, is cytotoxic and far below normal physiological range. Stability analysis (see the Appendix) shows that $I = I_2$ is unstable, while the steady state at $I = I_1$ is stable. At $I = I_1$, increasing V has the effect of increasing the steady state resting pH_i for both the normal and tumour cells. It is clear that there is very little qualitative difference between the tumour and normal cell cases. However, the magnitude of the increase in ϕ over the range pH 7.0–7.2 is significantly larger in tumour cells than in the normal cell case, and so decreasing V at the same rate in both cases will account for a decrease in resting pH_i at a slower rate for tumour cells than for normal cells. The parameter V has no quantitative interpretation; increasing V corresponds to greater vascularity. Note that it is intracellular hydrogen ion concentration, rather than pH_i, that is plotted on the horizontal axis; some corresponding pH values are indicated.

$$0 = P_1(I, E) + P_2(I, E) - P_3(I) - S_2(V)E - \beta E. \quad \text{(A.1b)}$$

Adding eqns (A.1a) and (A.1b) we obtain

$$0 = S_1(V) - S_2(V)E. \quad \text{(A.2)}$$

This gives us a relationship between E and V. Since $S_1(V)$ and $S_2(V)$ are, respectively, decreasing and increasing functions, this relationship predicts that regions with low V (i.e. poor removal of H^+-ions), in which cells are dependent on anaerobic glycolysis for the production of energy, correspond to a low pH_e (Fig. A1). The normal and tumour cell cases are very similar in hypoxic conditions (low V), when both cell types exhibit primarily anaerobic metabolism, but differ under aerobic conditions (higher V). This can be seen schematically in Fig. A1. For both cell types our model predicts an inverse relationship between E and V, but for tumour cells the decrease in E, which is associated with an increase in V, is more gradual. This makes sense intuitively since tumour cells have a high rate of aerobic glycolysis, resulting in the production of large amounts of lactic acid and lower pH_e.

If we now substitute E as given by (A.2) into (A.1a), we have

$$\phi(I; V) \equiv P_1(I, f(V)) + P_2(I, f(V)) = P_3(I) + g(V) \equiv \psi(I; V) \quad \text{(A.3)}$$

with $f(V) = S_1(V)/S_2(V)$, $g(V) = S_1(V) + \beta f(V)$. Using this formula, and the qualitative forms of $f(V)$ and $g(V)$, we consider the variation of intracellular H^+-ion concentration, as a function of V. We may understand the solutions of (A.3) by examining the intersection of the graphs $\phi = P_1(I, f(V)) + P_2(I, f(V))$ and $\psi = P_3(I) + g(V)$. In Fig. A2 we plot these two curves as a function of I, illustrating the effects of increasing V; the points of intersection define the equilibrium points of the system. Here, there are two steady states corresponding to two different resting pH_is. Clearly the pH value of the steady

state at $I = I_2$ which is in the range 6.2–6.3, is cytotoxic and far below normal physiological levels. However, the steady state with the larger pH_i does occur at reasonable values; this predicted pH_i value increases with increasing V for both the normal and tumour cells.

To investigate the stability of the steady states we define $U = I + E$. Substitution into (1a) and (1b) leads to an equivalent set of equations, one for the total H^+ concentration (U) and one for the extracellular H^+ concentration (E), namely

$$\frac{\partial U}{\partial t} = S_1(V) - S_2(V)E, \qquad \text{(A.4a)}$$

$$\frac{\partial E}{\partial t} = P_1(U, E) + P_2(U, E) - P_3(U, E) - S_2(V)E - \beta E = h(U, E). \qquad \text{(A.4b)}$$

The stability matrix is given by

$$A = \begin{pmatrix} 0 & -S_2(V) \\ \dfrac{\partial h}{\partial U} & \dfrac{\partial h}{\partial E} \end{pmatrix}_{U^*, E^*}$$

where U^*, E^* are the equilibrium points for eqns (A.4a) and (A.4b). For stability we require

$$\text{Trace } A < 0 \Rightarrow 0 > \frac{\partial h}{\partial E} = \frac{\partial P_1}{\partial E} + \frac{\partial P_2}{\partial E} - \frac{\partial P_3}{\partial E} - S_2(V) - \beta \qquad \text{(A.5a)}$$

and

$$\text{Det } A > 0 \Rightarrow S_2(V)\frac{\partial h}{\partial U} > 0$$

$$\Rightarrow 0 < \frac{\partial h}{\partial U} = \frac{\partial P_1}{\partial U} + \frac{\partial P_2}{\partial U} - \frac{\partial P_3}{\partial U}, \qquad \text{(A.5b)}$$

since $S_2(V)$ is positive for all $V > 0$. With the P_i ($i = 1, 2, 3$) as in Figs 2 and 3, the signs of $\partial P_i/\partial E$ are such that $\partial h/\partial E < 0$, (A.5a) is always satisfied. Thus, we are only concerned with the sign of $\partial h/\partial U$. The equilibrium points are $I = I_1$ for $7.2 > pH_i > 7.0$ and $I = I_2$ for $6.3 > pH_i > 6.2$. The first of these gives $\partial h/\partial U|_{I = I_1} > 0$ and so for $7.2 > pH_i > 7.0$ the stability condition is satisfied. That is, the steady state at $I = I_1$ is stable. On the other hand if $6.3 > pH_i > 6.2$ then $\partial P_1/\partial U < 0$ and $\partial P_2/\partial U < 0$. From Figs 2 and 3 we see in fact that

$$\left|\frac{\partial P_1}{\partial U} + \frac{\partial P_2}{\partial U}\right| > \left|\frac{\partial P_3}{\partial U}\right| \quad \text{for } 6.3 > pH_i > 6.2.$$

We thus have

$$\left.\frac{\partial h}{\partial U}\right|_{I = I_2} = \left.\frac{\partial P_1}{\partial U} + \frac{\partial P_2}{\partial U} - \frac{\partial P_3}{\partial U}\right|_{I = I_2} < 0,$$

and so (A.5b) is not satisfied. This in turn implies that the steady state at $I = I_2$ is unstable.

A decrease in V causes a decrease in pH_e (Fig. A1), and the steady-state analysis, illustrated in Fig. A2, shows that this implies a decrease in resting pH_i, as expected intuitively. The results show that there is very little difference between the tumour and normal cell cases; in particular, the predicted value for tumour resting pH_i does not have the slightly more alkaline value than normal that is observed experimentally. The solutions in the two cases differ only in quantitative details, that would vary between cell types; this is inconsistent with experimental observations of the reversed pH gradient in a wide range of tumour cell types.

The main difference between the overall form of the solutions in the normal and tumour cell cases is the magnitude of the increase in ϕ over the range pH 7.0–7.2 which is larger in tumour cells than in the normal cell case. Thus, decreasing V at the same rate in both cases will result in a decrease in resting pH_i at a slower rate for tumour cells than for normal cells, because of this steeper gradient of ϕ. In effect, if V is low, tumour cells may be able to maintain their level of pH_i within the narrow range that is compatible with normal cellular functions, despite a more acidic extracellular environment. Thus, the metabolic changes and consequent acidic extracellular environment observed in many solid tumours appears to be a potential disadvantage to normal cells which are less able to adapt to changes in pH_e.