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A MODEL OF CELL GROWTH AND POSSIBILITIES OF TUMOUR TREATMENT BY SELECTIVE PROTEIN DEPLETION

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A simple model of a self-enlarging synthesizing unit with the growth-limiting assumptions on precursor transport losses and product turnover is used as a prototype dynamic system for a cell growth model. The model successfully describes the main features of cell growth and allows for a rational discussion of the cell cycle. The cell is viewed as a non-linear capacitance damped by transport losses. If transformation results in a fundamental dynamic difference in growth parameters of the cell, an intriguing possibility exists that a mixed pool of normal and transformed cells connected to a common source of nutrients may constitute a point-wise controllable system. Most of the cell mass is protein, the building blocks are amino acids and the systemic concentration of one of the essential acids is proposed as the control input. A rather simple strategy of control capitalizes on the presumed difference in the cycle dynamics and suggests a possibility for a selective protein depletion of transformed cells based on what underscores the very danger of tumours — their propensity to grow and proliferate under conditions in which normal cells would not do so.

1. Introduction

Cancer is the second (after cardio-vascular diseases) leading cause of death in the developed world. An enormous research effort of the last decades has produced dramatic advances in understanding mechanisms of transformation, i.e. of the process by which a normal cell becomes cancerous. The pace of discovery has quickened in the last several years with new tools of molecular biology coming to aid, many of which have actually been developed in the effort to understand cancer. Unfortunately, the treatment of cancers has not seen anything of the sort, and with several notable exceptions, the five-year survival rate has remained about the same throughout this period — some 50% overall.

In multicellular organisms division of an individual cell is an event controlled by the needs of the whole organism. While most cells are capable of dividing, or mitosis, they rarely do so if not stimulated to by the conditions of the tissues they form. If an injury is inflicted, for example, the local, as well as the infiltrating cells, may respond by mitosis and tissue regeneration in order to repair the damage. Once the repair is done the cells return to their quiet existence without proliferation. In some cases

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the division is a rule rather than exception — in the bone marrow cell proliferation continuously provides for blood cells replenishment; the intestinal lining cells do the same in order to make up for the loss of the outermost layers where in the harsh environment cells do not last very long. In a healthy individual the steady state is well controlled by local conditions of blood supply, geometrical inter-cellular relationships, territorial integrity, as well as the systemic factors such as growth factors production, nutrient availability, etc. The imbalance between cell proliferation and cell death caused by the loss of normal mitotic cycle controls leads to a tumour or neoplasm. If the growth remains local, the tumour is said to be benign, and a complete surgical resection leads to cure. Some tumours, however, possess mechanisms needed for the spread into, and proliferation in other tissues — those are characterized as malignant, and are referred to as cancers. The spread involves cell separation from the local tumour mass, entry into the blood or lymphatic circulation, transport to another site, exit into and continued growth at inappropriate sites. Treatment of cancers which have spread to various locations, and have formed the secondary tumours, or metastases, is very difficult — in order to succeed, the attack must be selective. Finding selective strategies is the main topic of clinical cancer research efforts. Indeed, the possibility of discovering a successful cancer treatment must be the main motivation of all research on cancer and related aspects of cell biology.

In general, tumours appear to be monoclonal, i.e. all of the tumour cells have descended from a single progenitor cell. Transformation which has made the progenitor cell cancerous is a slow, multiple stage process, requiring in most known cases a number of specific genetic defects. The genes affected are called oncogenes and the products they encode oncoproteins. The changes in DNA sequence may be produced by chemical carcinogens, ionizing radiation or viral infection, but many other factors play a role in the process. The end effect by which the cell is recognized as tumourous is the apparent lack of proliferation control. To decide whether a cell is transformed or not one can make two functional tests: i) if it divides in suspension, i.e. without "anchorage" ; or ii) if it grows into a tumour in a nude mouse (a mouse with no immune system), it most likely is transformed. The discovery of the first oncogen inspired a great deal of optimism based on the hope that perhaps only that single defect needed to be somehow corrected to cure cancer. But tens of oncogens were identified very quickly and it became clear that cancer was what it has been taken for — a multitude of diseases. Nevertheless, they all do lead to very similar manifestations and ultimate common path in the death of the patient, keeping the hope alive that there might be a single cure yet.

As of now, the surgical treatment, whenever possible, is still the most efficient — if the cancer has not spread from its primary site, the complete removal leads to cure. If surgery is not possible, or the spread has occurred prior to surgery, chemotherapy may kill some cancers. Not all types are susceptible, however, and the treatment is in any case a balance game — killing as much of the cancer without killing the patient. The toxic chemicals used for chemotherapy are specific to different phases of the cell cycle, and only a number of cells will be killed by any single dose — some of them cancerous, some of them normal cells that proliferate continuously (most importantly cells in the bone marrow and intestines). Treatment protocols have been developed

over years of experimentation and clinical use aimed at combining different drugs in ways to maximize the chances of cancer elimination. Radiation treatment is another possibility, used mostly in conjunction with surgery. In this case, again, the problem is differentiating sufficiently between the normal and cancerous tissue. Even when the cancer is spatially distinct, the methods of radiation delivery available today are not precise enough. Asynchronous cell proliferation is a major drawback here as well — cells are not equally susceptible to radiation in different parts of the cycle.

Other physical treatment approaches have been tried and have to a great extent remained experimental — local hyperthermia (produced by ultrasound), for example, has been employed as an adjunct to chemotherapy.

Most promising of the new approaches are those based on using either naturally occurring, or engineered, substances that can interfere with cancer growth and spread: Tumour Necrosis Factor has been identified and tested in native and modified forms; Lymphokine Activated Killer cells have been prepared and used in conjunction with interleukine-2; vaccination against melanoma, which appears to have characteristic surface markers, has been under development; "magic bullet" drugs, i.e. cytotoxic drugs targeted by the aid of specific antibodies, show a great promise against cancers that do display antigens not found on the normal cells, etc. As the details of transformation fill in, new possibilities will certainly open up. Fifty plus oncogenes have been identified — the proteins they encode are found at different locations within the cell, and a troubling possibility exists that many cancer cells may not be identified as such by their surface antigens. Entering the cell in order to intervene, while not impossible, is going to be a lot more difficult than to exert the action on the surface. And nothing very efficient has been done even for those types of cancer that do possess strong surface antigens.

The unique approach presented here is based on the most universal of the features of all tumour cells — the property that in fact defines them as tumourous — their propensity to grow and proliferate under conditions in which normal cells would not.

2. Cell Growth Model

Let S_A denote the region of synthesis of the product A. Precursor of A is supplied by the source S_P, as shown in Fig. 1.

The following assumptions are made:

i) product A of synthesis at S_A goes to enlarge S_A (i.e. S_A is made of A);

ii) A undergoes turnover;

iii) precursor P_A is supplied via resistive path ts — path length is proportional to the square root of the size of S_A (see assumption iv)), i.e. system is uniformly expanding;

iv) S_A is a 2D structure, i.e. mass of A at S_A is proportional to the surface of S_A.

Note: Assumption iv) is not of crucial importance — it merely changes an exponent in the final equation. If bio-mass (macro-molecular substances) is concentrated on membranes it would be proportional to δ^2, where δ is a linear measure of the cell size. If the bio-mass was concentrated in volumes, mass would be proportional to δ^3. In reality m is proportional to δ^x, where $2 < x < 3$ (and probably closer to 2).

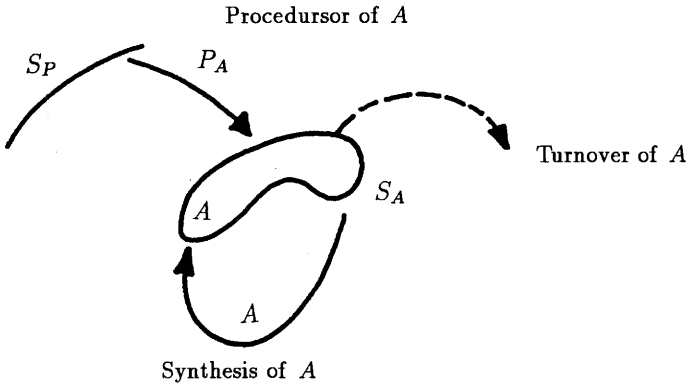


Fig. 1.

In the subsequent equations of growth the variables are defined as follows:

t – time,

m_A – mass of A at S_A,

m_P – mass of precursor P_A at S_A,

c_{P_0} – concentration of precursor P_A at S_P,

c_{P_A} – concentration of precursor P_A at S_A,

m_{A_S} – mass of A synthesized at S_A.

Flow of precursor between S_P and S_A and its mass balance at S_A can be expressed as

$$\frac{\mathrm{d}m_P}{\mathrm{d}t} = \frac{c_{P_0} - c_{P_A}}{R} - \frac{\mathrm{d}m_{A_S}}{\mathrm{d}t} \tag{1}$$

where R is the total resistance from S_P to S_A.

Rate of synthesis of A at S_A takes the form

$$\frac{\mathrm{d}m_{A_S}}{\mathrm{d}t} = \alpha c_{P_A} m_A \tag{2}$$

where α is a constant, c_{P_A} is the concentration of precursor at S_A, and m_A is the mass of A at S_A (size of synthesizing region). We assume that concentration c_{A_A} of A at S_A remains constant as S_A grows.

Mass balance of A at S_A can be written as

$$\mathrm{d}m_A = \mathrm{d}m_{A_S} - \gamma m_A \mathrm{d}t \tag{3}$$

where γ is the turnover constant.

Assuming for now no accumulation of the precursor at S_A, i.e. setting $\mathrm{d}m_P/\mathrm{d}t = 0$, equation (1) gives

$$\frac{\mathrm{d}m_{A_S}}{\mathrm{d}t} = \frac{c_{P_0} - c_{P_A}}{R} \tag{4}$$

and from (2) and (4) we obtain

$$\frac{\mathrm{d}m_{A_S}}{\mathrm{d}t} = \frac{c_{P_0}}{R} - \frac{1}{\alpha R m_A}\frac{\mathrm{d}m_{A_S}}{\mathrm{d}t}$$

which gives

$$\frac{\mathrm{d}m_{A_S}}{\mathrm{d}t} = \frac{c_{P_0}\alpha m_A}{\alpha R m_A + 1} \tag{5}$$

Now equations (3), (5) yield

$$\frac{\mathrm{d}m_A}{\mathrm{d}t} = \frac{c_{P_0}\alpha m_A}{\alpha R m_A + 1} - \gamma m_A \tag{6}$$

With the assumption iv), i.e. m_A is proportional to δ^2, we can state $R = \rho\sqrt{m_A}$ and rewrite (6) as

$$\frac{\mathrm{d}m_A}{\mathrm{d}t} = \frac{c_{P_0}\alpha m_A}{\alpha\rho m_A^{3/2} + 1} - \gamma m_A \tag{7}$$

Equation (7) is a non-linear differential equation that describes the growth of the bio-synthesizing region S_A modelled with assumptions i) to iv). Parameters in this equation are

- c_{P_0} – concentration of the precursor at its source S_P,
- α – synthesis constant,
- γ – turnover constant,
- ρ – resistance for precursor transport.

Equation (7) has no closed-form solution. So we can look at some basic properties of the solution, and then solve the equation numerically.

Leaving the subscripts out for simplicity we can rewrite

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{c\alpha m}{\alpha\rho m^{3/2} + 1} - \gamma m \tag{8}$$

Firstly, note that $\mathrm{d}m/\mathrm{d}t$ is a function of m, but not of t. Let us assume $\mathrm{d}m/\mathrm{d}t = 0$ to find extrema

$$m_{e_1} = 0, \qquad m_{e_2} = \left[\frac{1}{\rho}\left(\frac{c}{\gamma} - \frac{1}{\alpha}\right)\right]^{2/3}$$

For physical relevance we assume $\rho, \gamma, \alpha > 0$.

We can enumerate several special features of the solution to equation (8):

- If $\alpha = 0$, then (8) reduces to $\mathrm{d}m/\mathrm{d}t = -\gamma m$, which leads to $m = m_0 e^{-\gamma t}$, i.e. m will exponentially decay from its initial value m_0 to 0;
- If $\alpha > 0$ and either $\rho = 0$, or $\gamma = 0$, then m will grow without bounds;
- For $\alpha > 0$, $\rho > 0$, and $\gamma > 0$, the quantity m will grow (or shrink, depending on m_0) asymptotically to m_e.

Figure 2 shows solutions to (8) found numerically using Runge-Kutta method. Note the influence of the initial condition $m(0) = m_0$ on the solution:

- If $m_0 = 0$, then $m = 0$;
- If $0 < m_0 < m_e$, then m asymptotically grows to $m_e = \left[\frac{1}{\rho}\left(\frac{c}{\gamma} - \frac{1}{\alpha}\right)\right]^{2/3}$;
- If $m_0 = m_e$, then $m = m_0$;
- If $m_0 > m_e$, then m asymptotically shrinks to m_e.

The initial condition m_0 determines the shape of the growth curve at $t = 0$, namely

- For $0 < m_0 < m_i$ (inflexion point) curve starts as concave (curve 1, Fig. 2);
- For $m_0 = m_i$ curve starts as linear (curve 2, Fig. 2):
- For $m_i < m_0 < m_e$ curve starts as convex (curve 3, Fig. 2).

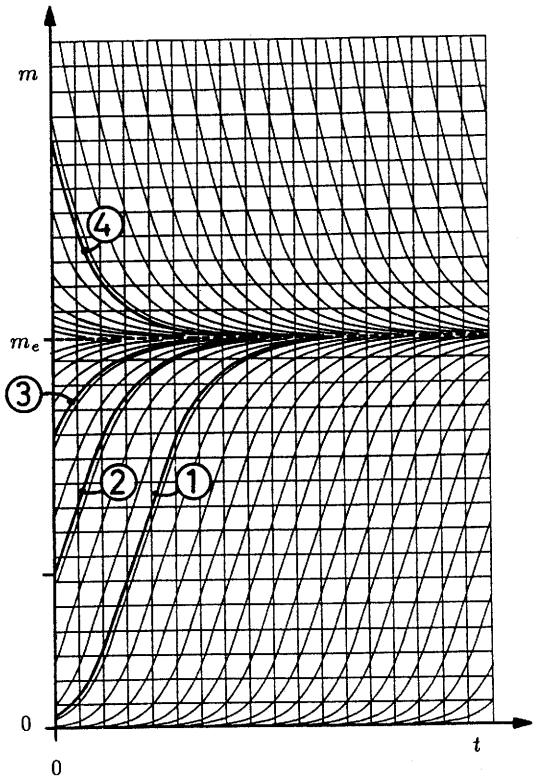
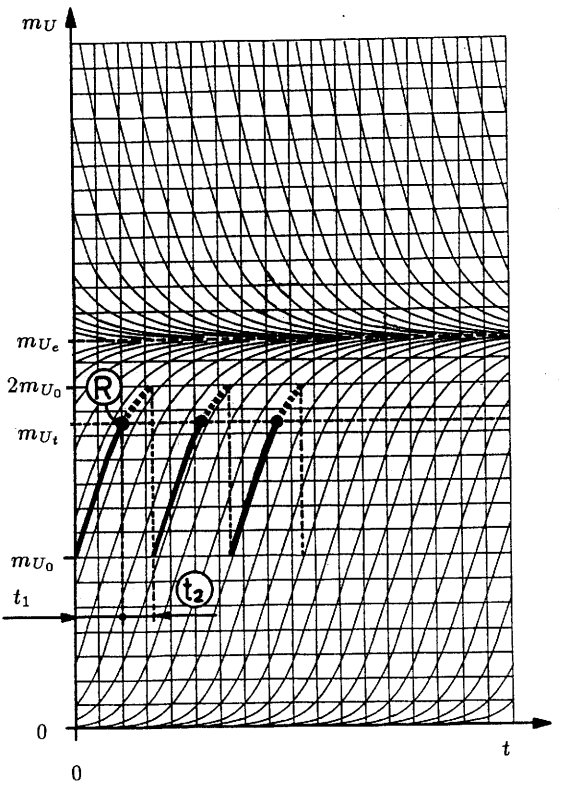


Fig. 2.

Let us now turn our attention to cell growth by assuming that its global bio-synthesis can be described by a multitude of well synchronized processes described by equation (8), so that the cell mass (at least its bio-synthesizing component) will also be represented by equation (8). Further, let us assume that the cell commitment to division is governed by a (still hypothetical, with some candidates being currently

investigated) protein U. Protein U is synthesized on a membrane of the size proportional to the total cell mass. Protein U undergoes rapid turnover. It binds to a nucleus-localized receptor. Once the nucleus receptor is saturated, excess U accumulates outside the nucleus and triggers the cell for the next cycle (crossing over the restriction point into S-phase).

With above assumptions we can state that the quantity m_U of U present in the cell will be determined by equation (8) (assumption of rapid turnover leads to quantity of U being simply proportional to the size of the synthesizing region). Commitment to division is then described by the condition that m_U exceeds a threshold level m_{U_t} (see Fig. 3) The crossing would correspond to passing the restriction point R, i.e. entry into S-phase. The growth function past the point R might be different — in Figure 3, and all those that follow, it is shown as a simple extension (dashed line).



R – restriction point

t_1 – G_1

t_2 – $S + G_2 + M$

Fig. 3.

The shape of the growth curve will depend on the relation between m_{U_e} and m_{U_t}. Figures 4(a) to 4(c) show three possibilities:

i) If the cell cycle portion of the growth curve is centered on the inflection point m_{U_i}, i.e. $m_{U_i} = (m_{U_0} + 2m_{U_0})/2$, or $m_{U_0} = 2/3m_{U_i}$, growth will be linear for all practical considerations (Fig. 4(a)). The best-fit line through that section of the curve gives coefficient of determination of $R^2 = 0.99993$! That explains why the measurements of amino acid incorporation, done with full growth support, show constant rate throughout the cycle (and suggests that under those conditions the cycle is nearly centred on the inflection point).

ii) Figure 4(b) shows a convex shape of the cell cycle portion of the growth curve with $m_{U_0} > m_{U_i}$. The convexity is more pronounced as m_{U_e} approaches m_{U_t} (a slowly expanding culture).

iii) Figure 4(c) shows a cell cycle on the concave part of the growth curve with $2m_{U_0} < m_{U_i}$. This requires a high value of m_{U_e} with respect to m_{U_t}. The limit on m_{U_e} may make it difficult to establish this condition.

An interesting situation takes place when $m_0 > m_e$. The cell should shrink to m_e. Now, it is reasonable to expect that for the vital functions the cell needs a minimum amount of mass (protein) — should the m_e be pulled bellow this minimum, the cell will die on its way to m_e.

3. Controllability

In this section a brief introduction to the concept of controllability is given by way of an illustration. Figure 5 shows a simple linear dynamic system comprising three fluid tanks connected by piping and a single pump to a larger fluid reservoir.

Each of the tanks as seen from the pump has a time constant which is the product of the resistance to flow and the capacitance of the tank. If the three time constants are different, one can show that the system is point-wise controllable. That means that it can be transferred from any initial state, defined in this case by the three levels of fluid in the tanks, to any final (desired) state in a finite time by some control action, in this case by the work of that single pump. In fact, any number of tanks connected to the pump in the way shown, provided the time constants are all different, is also controllable. Formally, this can be checked by a controllability criterion: a linear system

$$\frac{\mathrm{d}x}{\mathrm{d}t} = Ax + Bu \tag{9}$$

$$y = Cx \tag{10}$$

where x is the state vector of length n, u – the control vector of length r, and y – the output vector of length m, of the system defined by the matrices A, B and C, is controllable if and only if the $n \times nr$ composite matrix $[B, AB, A^2B, \ldots, A^{n-1}B]$ is of rank n.

Any number of tanks that would have the same time constant would behave in the same way, as the pump is used to pump the fluid in and out of the reservoir

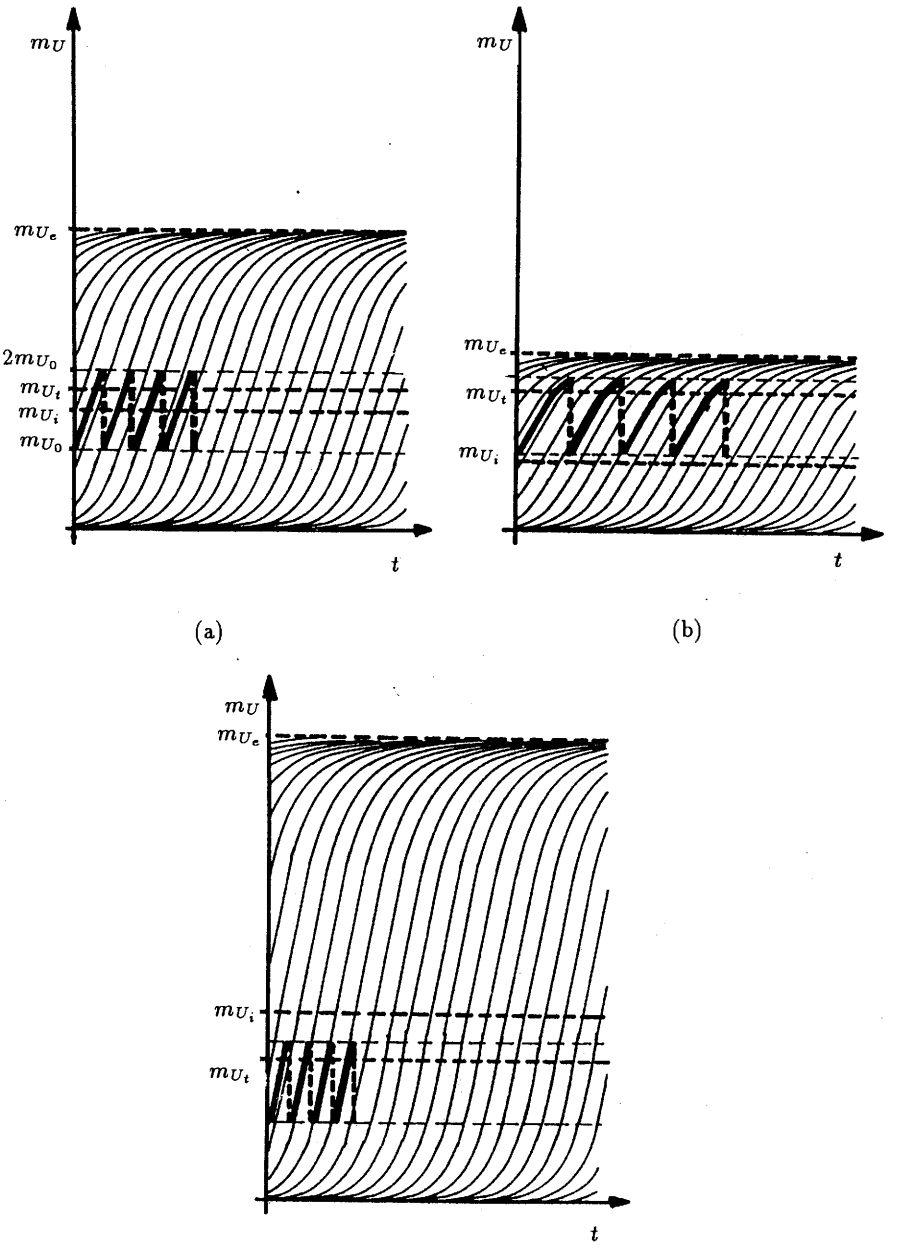


Fig. 4.

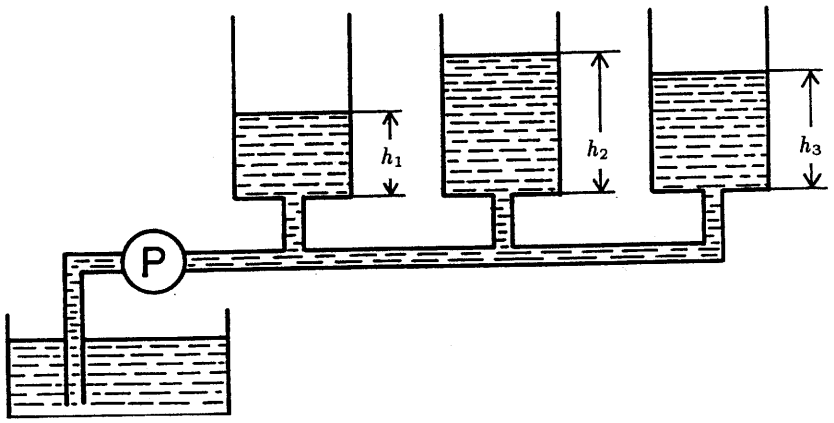


Fig. 5.

— they would not be independently controllable. For non-linear systems criteria of controllability are more complicated and will not be shown here, in spite of the fact that cells as modelled above do show strong non-linearity in the dynamics of growth. The example with tanks presented here is believed to serve as an important analogue to the problem at hand. It is rather counterintuitive to accept the possibility of controlling those tanks with a single pump. We shall make good use of the example in contemplating strategies for selective tumour depletion.

4. Cell as a Capacitance

In the growth model of the cell we have seen that the equilibrium size of the cell is a function of the precursor concentration, i.e. $m_e = \left[\frac{1}{\rho}\left(\frac{c}{\gamma} - \frac{1}{\alpha}\right)\right]^{2/3}$; the higher the concentration c, the higher the equilibrium size m_e. Influence of the other constants is easy to appreciate as well — the equilibrium size (ultimately the amount of the trigger protein U) is increased by: i) higher synthesis constant α; ii) lower turnover constant γ; and iii) lower transport resistances ρ. There are good indications that the process of transformation leads to some or all of the contributing factors to push the m_e higher. The enormous complexity of the bio-synthesis process, whereby tens of thousands of substances are produced in a fully coordinated fashion, should not scare one away from taking a global view in modelling the cell — equation (8) does very well in showing the basic features of total cell mass dynamics. Consider a non-dividing cell in equilibrium, whereby the amount of products it is able to synthesize under the conditions given is just enough to compensate for the breakdown of those products. If the concentration of the precursor, and we shall consider only one — the critical, or limiting one of all those going into the process of synthesis, is changed to a lower value c_{new}, the cell will reduce its mass to the new value determined by $m_e(c_{new})$. In doing so it will behave very much as a fluid tank changing its level in response to a change in pressure in the supply line. Non-linearity of the response, by which the cell response differs from that of a simple tank, will depend on the

step size. If the concentration of the critical precursor is now increased, the cell will respond by a mass increase. Most of the mass is protein and the building blocks are 20 amino acids. Ten of those (*arginine*, *threonine*, *methionine*, *lysine*,*valine*, *leucine*, *isoleucine*, *histidine*, *phenylalanine* and *tryptophan*) are essential for vertebrates, i.e. they cannot be synthesized from any other substances and thus must be taken through diet (*arginine* can be synthesized, but apparently not in sufficient amounts). While any of the amino acids could be selected as a control parameter for the cell growth — lack of any single one totally inhibits protein synthesis — taking a non-essential one may require stronger control to fight cells' ability to compensate by increased synthesis. This strongly suggests controlling the concentration of one of the essential amino acids. Amino acids cross the cell membrane and the internal pool, with some exceptions, is most of the time near equilibrium with the extracellular fluid, which in turn is near equilibrium with the blood levels. Thus controlling the level of at least one, and preferably of only one, essential amino acid in the blood circulation should give an effective control input to the cell protein synthesis process.

5. Strategy

Having presented a very simple controllable system which should serve only as a remote analogy to the problem at hand, we turn our attention to planning a strategy of attack on tumour cells. The goal is clear: to control the conditions of systemic nutrient availability during a finite time in such a way as to selectively deplete the pool of tumour cells of essential products. The control problem is complicated by the fact that cell populations undergoing division are not synchronized — that is true for both the normal cells (bone marrow, intestines) and the tumour cells. If one were to use standard approach to control, the initial conditions should be known. So the first goal is to synchronize — a long sought condition that should be of great benefit to conventional treatments (chemotherapy, radiation therapy) as well. According to the model presented above the cells which are dividing have the "equilibrium" (not in the true sense since they are prevented from reaching it by the constant cycling) value m_{U_e} of the trigger protein U higher than the threshold level m_{U_t}. Since m_{U_e} is determined by the equilibrium size m_e of the cell, which in turn is determined by the concentration of the (critical) precursor c, we can use the concentration of the precursor c to stop cell division. The most plausible explanation for the transformation is that, under the same conditions, including the precursor concentration c,

$$m_{U_e}/\text{tumour} > m_{U_e}/\text{normal}$$

So if c is lowered sufficiently for tumour cells to stop dividing, the normal ones will stop as well.

Thus, in the first phase of the treatment, as shown in Fig. 6, c is to be decreased until all cells enter the rest phase, or G_0. Once this is done we can initiate further control with the known initial conditions. Preferably, the task of killing the tumour will be completed by control of the concentration of the chosen amino acid alone, but one could take an alternative approach and use this first step as synchronization only, followed by a conventional treatment such as chemotherapy. In the next, and most

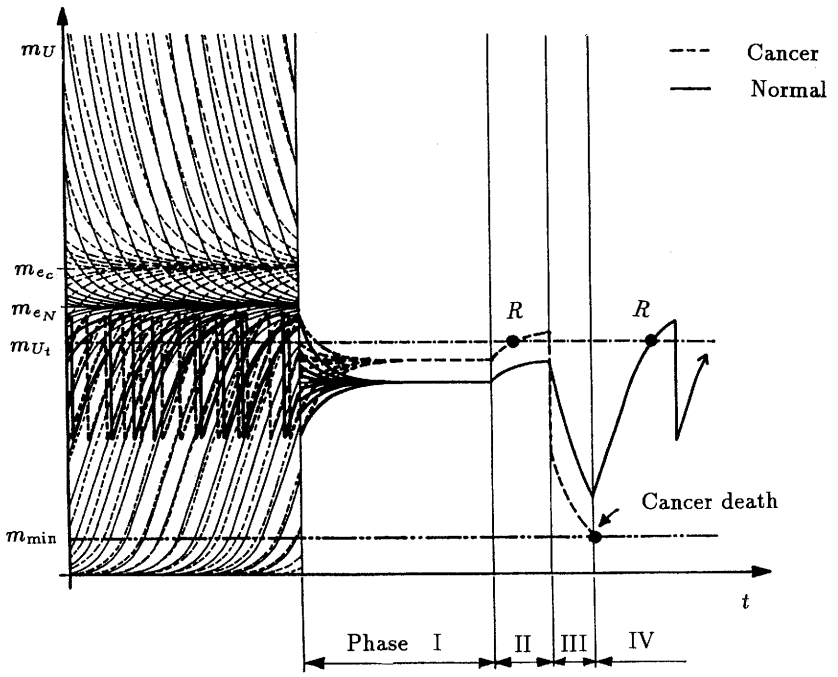


Fig. 6.

critically precise step, the concentration is increased to the level at which the tumour cells will cycle, but the normal ones will not (this is the condition which would be most suitable for conventional treatments — the normal cells are protected in G_0 and the tumour cells could be attacked as they cycle through mitosis). The task of controlling the system is now going to be much simpler. Tumour cells have been pushed over the restriction point and must complete the next division or otherwise die. So after a time of holding the concentration at this critical level, in the third phase it is decreased to the lowest possible level (caring to avoid systemic collapse due to lack of proteins). All of the cells are now loosing their mass, but the normal ones started much bigger — from the G_0 phase of the cycle — while the tumour ones have just completed a division (M phase) and have a mass of perhaps 2/3 of the normal ones! Controllability of the system has been used to a great advantage — beyond what could be done with a linear system. After several hours tumour cells will run out of the minimum amount of protein needed, and die, while the normal cells are still safely above that limit. At the end, in the fourth phase, the concentration of the controlled amino acid is brought back to normal.

In all of above we have considered only the intrinsic parameters of cells (i.e. α, γ, ρ), ignoring the external, additional influences past that of c. For example, it is clear that an external resistance on transport will influence the dynamics, by effectively increasing internal transport loses ρ. However, ignoring the external resistances can be justified in view of the micro-organization of tumours. They grow in globular structures with blood vessels attracted towards the globules and supplying

the outer layers of dividing cells. Below the outer layer, or two, tumour cells are in the rest phase. The core, if big enough, is necrotic. Thus, targeting only the outermost layer adjacent to blood supply should suffice — any cells sandwiched between necrotic layers will die as well.

6. Implementation

Two basic approaches to the treatment are possible: i) to exert control over the concentration of the selected amino acid in an extracorporal circulation apparatus resembling that used for dialysis; ii) to exert the control intracorporally, by decomposing or deactivating (masking) the selected amino acid by chemical means. Increasing the concentration in both cases is done by injecting the required amount of the amino acid. The first method appears more elegant, safer and probably allows for tighter control. The second one is more convenient to implement, but may cause stronger side effects.

Extracorporal control approach requires connections to an artery (femoral for example) and a vein and an interposed filter to remove the selected amino acid. The higher the single filter pass removal ratio, the higher the speed of control of the selected amino acid concentration on the whole body level. An estimate of the time constant for control can be made as the ratio of the "hold-up" (total amount of amino acids in the body) to "through-put" (amino acid mass flow through the extracorporal filter). If the efficiency of the filter was 100%, i.e. assuming total removal in a single filter pass, time constant with the femoral artery tap would be on the order of two to four hours — sufficiently fast for the control requirements against cell cycle duration of about 24 hours.

Pump and the filter must be designed to minimize damage of the blood cells. The technology used for dialysis and blood oxygenating machines can be readily applied. The specific addition in this case is the enzymatic system to remove the selected amino acid. This is to be done in a secondary, or external circulation separated from the blood stream by a molecular sieve. Low molecular substances, including amino acids, are separated from the main flow, reacted against the enzymes and returned, with one amino acid either degraded or adsorbed.

Amino acids which are not utilized for synthesis of proteins and other nitrogen-containing compounds enter various metabolic pathways whereby they get converted into mostly energy supplying substances. The liver and muscles, and to a lesser extent brain and kidneys are the main sites of amino acid degradation. While it might be possible to use plant, or microbial degradation enzymes, it appears that the best, and safest approach is to chose a pathway occurring naturally in mammals. In selecting one of a great number of possibilities consideration should be given to several issues: selectivity of the enzymatic action; possible toxicity of the byproducts; availability, purity and the cost of the enzyme; cofactors; energy requirements, etc. The following list is by no means comprehensive — it simply lists some possibilities considered acceptable in view of the criteria above. If *threonine* is selected for control, *threonine aldolase* can be used to degrade *threonine* into *glycine* and *acetaldehyde*. For *phenylalanine* the enzyme *phenylalanine hydroxylase* can be used to convert it into *tyrosine* — cofactor is *DL-6-methyl-5,6,7,8-tetra-hydropterine*. *Arginine* can be catabolized

to *urea* and *ornithine* by *arginase*. *Histidine* can be converted to *urocanic acid* by *histidine ammonia-lyase*. *Tryptophan* is degraded by *tryptophan 2,3-dioxygenase* into *N-formylkynurenine*.

In the natural process of protein assembly amino acids are first attached to a specific tRNA molecule. The process of attachment is highly specific, is well understood and suggests itself as a model for the adsorption filter design. Any one of the amino acids selected can be removed from the blood in this manner.

An amino acid attachment to its corresponding tRNA proceeds in two steps, both catalyzed by the same enzyme *aminoacyl-tRNA synthetase* and powered by *ATP* (*adenosine triphosphate*). In the first step the amino acid is activated by formation of an *aminoacyl-adenylate* (also called *aminoacyl-AMP*; *AMP* for adenosine monophosphate) from an amino acid and *ATP*. In the absence of the corresponding tRNA, the *aminoacyl-AMP* intermediate is a stable molecule and does not dissociate from the synthetase. This points to the first possibility for the removal of the selected amino acid — use of the corresponding aminoacyl-tRNA synthetase and *ATP* in the external circulation of the filter. In the external flow the enzyme may be used free in solution (molecular weight of these synthetases is on the order of 100 to 200 kDaltons and can be easily kept isolated from the blood flow behind the molecular sieve of the hollow fiber filter) or bound to a crosslinked gel. *ATP* is added to the external circulation as needed.

In the second step the aminoacyl group of the *aminoacyl-AMP* is transferred to a tRNA to form *aminoacyl-tRNA* which is an active intermediate in the protein synthesis. This is a further possibility of removal of the selected amino acid from the blood stream. If the corresponding tRNA is supplied the enzyme *aminoacyl-tRNA* synthetase can be used only to couple the amino acid to tRNA instead of binding it itself. Both the selected *aminoacyl-tRNA* synthetase and the corresponding tRNA can be supplied to the external circulation in solution. Alternatively, the enzyme can be gel-immobilized and tRNA supplied in solution, or tRNA can be gel-immobilized and the enzyme supplied in solution. All of the intermediates and the products are large enough to remain isolated from the blood flow behind the molecular sieve.