Mathematical models for explaining the Warburg effect: a review focussed on ATP and biomass production

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Abstract

For producing ATP, tumour cells rely on glycolysis leading to lactate to about the same extent as on respiration. Thus, the ATP synthesis flux from glycolysis is considerably higher than in the corresponding healthy cells. This is known as the Warburg effect (named after German biochemist Otto H. Warburg) and also applies to striated muscle cells, activated lymphocytes, microglia, endothelial cells and several other cell types. For similar phenomena in several yeasts and many bacteria, the terms Crabtree effect and overflow metabolism respectively, are used. The Warburg effect is paradoxical at first sight because the molar ATP yield of glycolysis is much lower than that of respiration. Although a straightforward explanation is that glycolysis allows a higher ATP production rate, the question arises why cells do not re-allocate protein to the high-yield pathway of respiration. Mathematical modelling can help explain this phenomenon. Here, we review several models at various scales proposed in the literature for explaining the Warburg effect. These models support the hypothesis that glycolysis allows for a higher proliferation rate due to increased ATP production and precursor supply rates.

Introduction

For producing ATP, tumour cells in mammalian tissues rely much more on glycolysis leading to lactate (in comparison with respiration) than the healthy cells from which the tumour cells originated. This is known as the Warburg effect, named after German biochemist Otto H. Warburg [1–3]. He published these observations in several German papers in the 1920s [4] and in 1956 in English [5]. Warburg himself explained the effect by impaired function of mitochondria in tumour cells [4,5].

Similar phenomena are observed in many other cell types. Examples are provided by *Saccharomyces cerevisiae* and several other yeasts (Crabtree effect) [6], and also by *Escherichia coli* and many other bacteria (overflow metabolism) [7,8]. An example of mammalian cells that have immediate access to oxygen in the blood but are, nevertheless, highly glycolytic, is provided by endothelial cells. These cells generate up to 85 % of their ATP via glycolysis [9]. Lymphocytes show a metabolic shift upon activation. Although they mainly use respiration (oxidative phosphorylation) in the quiescent state, glycolysis is up-regulated during activation, even in the presence of oxygen [10,11]. In the case of lymphocytes, the term Warburg effect is explicitly used as well [10]. Kupffer cells are ontogenetically related to lymphocytes and are resident macrophages in the liver. They also enhance glucose utilization after activation (e.g. by endotoxins) [12]. Microglia cells are the resident macrophages in the brain [13]. These cells suppress their mitochondrial function and up-regulate glycolysis upon activation, for example, by lipopolysaccharides [14].

The major consumers of glucose in the body are the muscular system and brain. Skeletal muscles consist of two types of fibres: slow- and fast-twitch fibres [15]. Fast-twitch fibres are predominant in muscles capable of short bursts of fast movement and only contain a few mitochondria. These fibres obtain most ATP by glycolysis and increase their glycolytic rate at heavy exercise [16]. Slow-twitch fibres, in contrast, predominate in muscles contracting slowly and steadily. They contain many mitochondria. Astrocytes are a special type of glial cells. They show an interesting metabolic interaction with neurons in the brain. Although both cell types are capable of respiring, astrocytes tend to convert glucose into lactate whereas activated neurons can take up the resulting lactate and degrade it to carbon dioxide and water [17].

The term glycolysis is used in the literature with slightly different meanings; it may refer to the conversion of glucose into pyruvate, being a pre-requisite of respiration. Alternatively, it may denote the conversion of glucose into

Key words: cancer metabolism, metabolic modelling, rate vs. yield, respirofermentation, Warburg effect.

Abbreviations: FBA, flux balance analysis; FBAwmc, FBA with macromolecular crowding; LP, linear programme; TCA, tricarboxylic acid.

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lactate (or in micro-organisms, ethanol, acetate etc.), which in microbiology is often called fermentation. A related term is 'substrate-level phosphorylation' [15]. It comprises both the ATP synthesis in glycolysis and the direct phosphorylation in the tricarboxylic acid (TCA) cycle. The Warburg effect usually refers to fermentation.

Metabolic pathways are characterized both by their rate and by their molar yield [18–20]. While the rate quantifies the moles of product built per unit time, the yield quantifies the moles of product per mole of the substrate. The Warburg effect is paradoxical at first sight because the ATP-versus-glucose yield of glycolysis equals two and is, thus, much lower than that of respiration. The ATP yield of respiration depends on the biological species and partly on conditions. Typical values are near 30 [21]. The ATP yield in *E. coli* is lower than in animals, notably ∼26 [22]. *S. cerevisiae* lacks complex 1 of the respiratory chain and, therefore, only achieves a yield of 16 [23].

On the other hand, glycolysis can reach much higher rates than respiration. Warburg [5] wrote that cancer cells can obtain about the same amount of energy from fermentation as from respiration. In striated muscle cells, glycolysis is up to 100 times faster than respiration [15]. Mechanistic explanations are that the respiratory pathway is much longer and that the enzymes of the respiratory chain are located in the membrane and thus operate in a 2D environment in contrast with the 3D cytoplasm, which harbours the glycolytic enzymes. In line with these considerations, the issues of macromolecular crowding [3,7] and membrane occupancy [24] have been suggested as further explanations for the low rate of oxidative phosphorylation.

The question arises why cells do not re-allocate protein to the high-yield pathway (respiration) [25,26]. One may assume that investing it into respiration would always imply a higher ATP formation than investing it into glycolysis. The question of the physiological advantage of the Warburg effect has been tackled by mathematical modelling [2,3,19,20,26 – 30]. Here, we review and briefly discuss these papers. In that context, we do not restrict the discussion to tumour cells.

Causes of the Warburg effect

When discussing the various explanations for the Warburg effect, some philosophical reasoning is helpful. The Greek philosopher Aristotle made a distinction between four different causes for the structure of things: *Causa finalis*, *causa materialis*, *causa efficiens* and *causa formalis* [31]. For example, the question 'Why is glycolysis up-regulated?' could be answered by saying that this is useful for a certain purpose (Aristotle's *causa finalis*) or because certain transcription factors up-regulate certain genes (*causa efficiens*). The *causa formalis* would here be the encoding of glycolytic enzymes in the genome.

The following explanations for the Warburg effect have been proposed in the literature, where the boundary between material and efficient causes is not always clear-cut:

Material causes:

1. Compromised mitochondrial function [5]
2. Anaerobic conditions [32]

Efficient cause:

3. Regulatory effects by glycolytic enzymes [10]

Final causes:

4. Increased ATP production rate [20]
5. Supply of precursors [9]
6. Poisoning of competitors by end products [33]
7. Avoidance of harmful effects by, for example, reactive oxygen species [31]

Formal cause:

8. A genome duplication in an ancestor of *S. cerevisiae* led to an increased gene dosage of glycolytic enzymes [34].

The contribution of these factors has been intensely and controversially discussed in the literature [10,11,26]. For example, lack of oxygen in the interior of tumours is a natural explanation of the Warburg effect, as long as they are not yet vascularized. However, tumours usually show the Warburg effect even in the presence of oxygen [2].

Here, we focus on the final causes and, in particular, on the explanations in terms of higher ATP production rate and the supply of metabolic precursors because most models proposed in the literature are based on these explanations. Note that both factors lead to a higher biomass production rate.

Overview of models

We here discuss various models for explaining the Warburg effect focussed on ATP and biomass production [causes (4) and (5) above]. We do so essentially in chronological order of their publication.

Several earlier models use evolutionary game theory [35]. A pioneering publication on game-theoretical approaches in biochemistry is that by Pfeiffer et al. [20], dealing with co-operation and competition in ATP-producing pathways. It was focused on the interplay between fermentation and respiration in *S. cerevisiae*, but also mentioned other organisms and the Warburg effect in tumour cells. A game-theoretical situation arises when several cells (or organisms) feed on glucose or another common resource and can use two (or more) different strategies, for example fermentation and respiration. The outcome for each cell depends not only on its own strategy but also on that of the other 'players' (i.e., cells). It would be more economical for all cells to use respiration because the resource would then last longer. However, as soon as one cell switches to fermentation, it has a short-term advantage due to the higher ATP production rate and can out-compete the others. In the models, the trade-off between rate (ATP per time) and yield (ATP per mole of glucose) is analysed. Pfeiffer et al. [20] used a differential equation system, as in population dynamics, to describe this game-

theoretical situation. This leads to a 'tragedy of the commons', where all cells use the shared resource inefficiently by additionally fermenting sugars, corresponding to a defector strategy in game theory. Thus, tumour cells can be considered as defectors using a selfish strategy [27]. Alternative game-theoretical models with a different interpretation of the term 'defector strategy' have been proposed by Kareva [36] and Archetti [37]. A population dynamics model for cancer cells had been proposed earlier [30].

Another game-theoretical approach based on pay-off matrices and the concept of Nash equilibrium was presented in [28]. The resulting game is a Prisoner's Dilemma, in which the defector strategy is again the only stable outcome. However, this depends on enzyme costs. When costs for the high-yield pathway are low, a Harmony Game results, leading to pure respiration [26]. Further papers on game-theoretical approaches are reviewed in the study by Hummert et al. [38].

One group of models for explaining the Warburg effect is based on the maximization of biomass production. Since biomass production requires both metabolic precursors and energy, this corresponds to both causes (4) and (5) given above. It is worth noting that the supply of precursors and that of energy are intertwined. Whenever the glycolytic rate is high while respiration is limited, much pyruvate can be used for building biomass, for example, after transamination into alanine.

A kinetic model rather than constraint-based model was proposed by Molenaar et al. [19]. It is a medium-scale model, in which the Warburg effect is explained by the balance of the protein costs between glucose uptake and catabolism. The model includes the synthesis of precursors, ribosomes and membrane lipids as well as substrate transporters. It was shown how the shift in metabolic efficiency originated from a trade-off between investments in enzyme synthesis and metabolic yields for alternative catabolic pathways. When glucose is expensive (low concentration), it is completely utilized, otherwise (high concentration) it can be 'squandered'. Moreover, the model explains the increase in ribosomal content with increasing growth rate and other growth-rate related physiological characteristics of bacteria.

Several models belong to a methodological framework called flux balance analysis (FBA) [39–41]. In that analysis, a steady-state condition is used because most metabolic networks operate at steady state. Moreover, sign restrictions for irreversible reactions and (in some cases) upper limits for the rates of some reactions are applied. The reaction rates (fluxes) are calculated by a linear maximization criterion, expressing that a linear combination of rates attains a maximum value. It has been controversially discussed in the literature whether rate or yield is maximized in FBA [42,43]. The interpretation of the optimization criterion depends on whether the uptake rate is normalized.

The laboratory headed by Zoltán Oltvai proposed a model in which ATP production rate was maximized [3]. It is related to an earlier model by the same group by which the overflow metabolism in *E. coli* had been explained [7,8]. The model [3] essentially consists of four reactions, with one of them corresponding to precursor synthesis. They did not consider that three of these, glycolysis, respiration and precursor synthesis, partly overlap with each other. A constraint on a linear combination of reaction rates was included. The coefficients were given by the volumetric requirements of enzymes, so that the constraint expressed the volume of the cell that can be occupied by enzymes. That analysis has been named FBA with macromolecular crowding (FBAwmc) [7,8]. Moreover, the model constrains the glucose uptake rate. It predicts that, at low glucose uptake rates, mitochondrial respiration is the most efficient pathway for ATP generation. Above a critical glucose uptake rate however, a gradual activation of aerobic glycolysis and slight decrease in mitochondrial respiration results in the highest rate of ATP production.

A model for explaining the Warburg effect based on the maximization of biomass production and using FBA was published by Shlomi et al. [2]. They used a genome-scale model involving more than 3700 reactions. Constraints for both the glucose uptake and the total enzyme solvent capacity were included. In the latter, a linear combination of reaction rates was considered to be bounded from above. The coefficients were defined by the quotient of the molecular masses and turnover numbers of the enzymes involved. The predicted metabolic behaviour depends on the bound on the glucose uptake rate. At low glucose uptake and, thus, low growth rate, pure respiration is used. As the glucose uptake is increased, the respiration rate first increases up to a critical point, then decreases comcomitantly with an increase in the glycolytic rate. This is nicely seen in a plot in the phase plane spanned by glycolytic rate and respiration rate, where the admissible region is a polygon [2]. Interestingly, the model predicts a preference of cancer cells for glutamine uptake over other amino acids, in agreement with the observed behaviour. The question arises whether, for the purpose of explaining the Warburg effect, such a large network needs to be analysed.

Guided by the principle of Ockham's razor, a minimal model was established [26]. That principle says that for understanding a process, all unimportant details should be neglected. Thus, it should be possible to explain the Warburg effect on the basis of an extremely reduced model. As glycolysis and oxidative phosphorylation are compared and a fermentation product must be produced and excreted, three (overall) reactions should be sufficient [26] (Figure 1).

Glucose is consumed by reaction 1 to produce 2 moles of pyruvate and m_1 moles of ATP. Only pyruvate is considered as an internal metabolite, whereas the concentrations of all other substances are fixed. Fermentation is modelled by the pathway leading from glucose to P_2. Respiration leads to P_3 and produces $m_1 + m_3$ moles of ATP. The typical values $m_1 = 2$ and $m_3 = 30$ are used. In case that also reaction 2 produces ATP, also m_2 would be positive, such as in acetate fermentation by *E. coli*, where $m_2 = 1$. The conversion of NAD^+ into NADH in the TCA cycle is included implicitly because the ATP produced on that basis is considered. In

contrast, the NADH consumed in the second reaction is neglected.

The optimization and side constraints applied to the network are based on ideas put forward earlier by the works in the Ruppin and Oltvai laboratories [2,3,7]. Thus, the network was analysed by a linear programming approach related to FBAwmc. The enzyme concentrations in the pathways of glycolysis and respiration are regarded to be variable because they can change both during evolution and during development of a given organism. Thus, re-allocation of protein between enzymes and pathways is allowed for. To describe the Warburg effect, the following linear optimization problem is phrased:

Maximize

$$J_{\mathrm{ATP}}\ (v_2, v_3) = m_1\ v_2 + (m_1 + m_3)\, v_3 \tag{1a}$$

subject to

$$(\alpha_1 + \alpha_2)\, v_2 + (\alpha_1 + \alpha_3)\, v_3 \leqslant C \tag{1b}$$

$$v_2 \geqslant 0, v_3 \geqslant 0 \tag{1c}$$

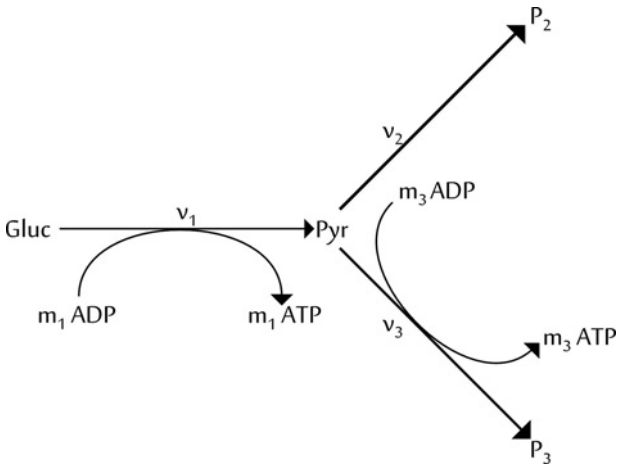
As the metabolic system is considered to be at steady state, v_1 can be eliminated and written in terms of v_2 and v_3. The constraint (eqn 1b) reflects the organism's resource limits in creating enzymes for the reactions [25]. Note that the maximal velocities of enzymes are given by the products of enzyme concentrations and turnover numbers. The coefficients α_i express the different turnover numbers as well as the different synthesis costs of enzymes, which are largely determined by the molar masses of the enzymes and the different synthesis costs of amino acids [2,3,7,25,44]. Written in terms of enzyme concentrations (to which the maximal velocities are proportional), such a side constraint has been used in metabolic modelling earlier [45,46]. Furthermore, relationship (eqn 1b) can reflect macromolecular crowding [3], i.e., volume limitations in the cell.

Moreover, it can be assumed that the first and third overall reactions are irreversible because they include at least one irreversible partial reaction. For example, the hexokinase and phosphofructokinase reactions involved in glycolysis are irreversible. The second reaction may be reversible (e.g. lactate dehydrogenase or alanine aminotransferase). However, for simplicity's sake, all reactions are considered to be irreversible, so that their rates are non-negative, as expressed by inequality (eqn 1c) [26]. In future studies, the model could be extended by allowing the second reaction to be reversible, so that cells such as neurons can be described that take up lactate and respire it.

System (1) is a linear programme (LP) and thus can be solved easily. For the system under consideration (Figure 1), it only involves three variables. By eliminating v_1, an LP in two variables (see above) is left, for which it is easy to analyse the feasible region graphically. Figures 2 and 3 show the feasible region for eqn (1) [47].

Figure 1 | Minimal reaction scheme for analysing the Warburg effect

The minimal model includes glycolysis from glucose (Gluc) up to pyruvate (Pyr), conversion of pyruvate into a fermentation product (P_2) such as lactate or into biomass and the TCA cycle together with oxidative phosphorylation, leading to respiration products (P_3), such as CO_2 and H_2O. v_i, reaction rates, m_i, stoichiometric coefficients of ATP.



Two cases can be distinguished depending on whether the costs for the high-yield pathway (quantified by the coefficient α_3) are low or high:

Case (i): 'cheap' high-yield pathway

Figure 2 shows the situation when respiration costs are low, with suitably chosen parameter values in arbirtrary units. Solving the LP led to a flux distribution corresponding to pure respiration. Note that the solution must always be situated at a vertex (corner point) of the admissible region (except in degenerate cases) because the optimization problem is linear.

Case (ii): 'costly' high-yield pathway

Figure 3 shows the situation when respiration is costly. The maximum feasible value for ATP production is achieved when using pure fermentation. This explains why fermentation can be advantageous even though its yield is lower.

A re-allocation of protein to the high-yield pathway only pays if the synthesis costs for that pathway are low enough. If these costs are above a certain threshold, it is better to concentrate protein on high-rate/low-efficiency pathways such as glycolysis. The condition for the latter case reads [26]:

$$\frac{\alpha_1 + \alpha_3}{\alpha_1 + \alpha_2} > \frac{(m_1 + m_3)}{m_1} \tag{2}$$

From above, it can be seen that, depending on the costs for the pathways, either pure respiration or pure fermentation results from the LP. However, as observed already by Warburg [4], usually a mixture of the two pathways is used,

Figure 2 | **Pure respiration**

Feasible region for eqn (1) with low cost respiration: $\alpha_1 = 1$, $\alpha_2 = 1$, $\alpha_3 = 10$, $C = 200$ (coloured region). Also shown are the level contours for the objective function J_{ATP} with stoichiometric coefficients $m_1 = 2$, $m_3 = 30$. The optimal solution (•) is $(v_2{:}v_3) = (0{:}18.18)$, corresponding to $v_1 = 18.18$, $J_{ATP} = 581.8$ and a yield ratio of $J_{ATP}/v_1 = 32$. This situation corresponds to pure respiration if respiratory enzymes are cheap.

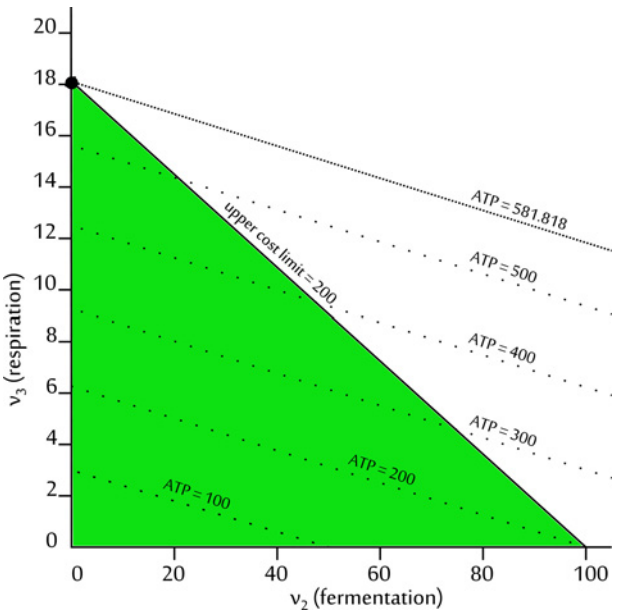


Figure 3 | **Pure fermentation**

Feasible region for eqn (1) with costly respiration: $\alpha_1 = 1$, $\alpha_2 = 1$, $\alpha_3 = 50$, $C = 200$ (coloured region). Also shown are the level contours for the objective function J_{ATP} with stoichiometric coefficients $m_1 = 2$, $m_3 = 30$. The optimal solution (•) is $(v_2{:}v_3) = (100{:}0)$, corresponding to $v_1 = 100$, $J_{ATP} = 200$ and a yield ratio of $J_{ATP}/v_1 = 2$. This situation corresponds to pure fermentation if respiratory enzymes are costly.

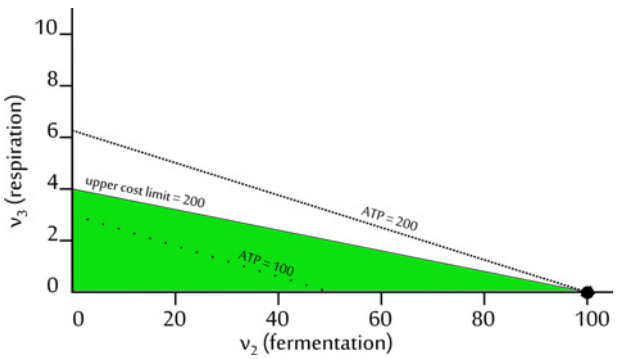
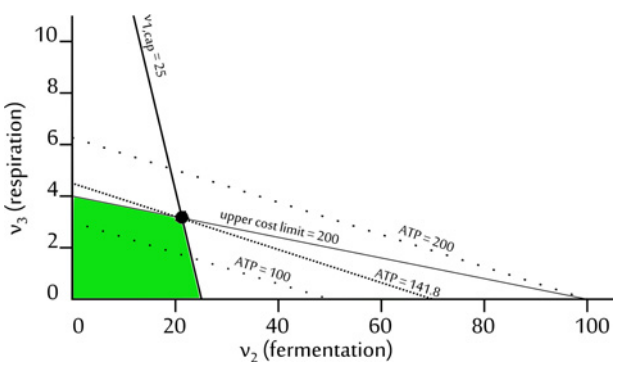


Figure 4 | **Respirofermentation**

Feasible region for eqn (1) with the same values as in Figure 3, but with a moderate limit on the uptake of glucose (Gluc): $v_1 = v_2 + v_3 \leqslant v_{1,cap} = 25$. Also shown are the level contours for the objective function J_{ATP}. The optimal solution (•) is $(v_2{:}v_3) = (21.94{:}3.06)$, corresponding to $v_1 = 25$, $J_{ATP} = 141.8$ and a yield ratio of $J_{ATP}/v_1 \approx 5.67$. This situation corresponds to respiro-fermentation.



the so-called respiro-fermentation. This metabolic mode can be obtained by imposing an additional constraint, notably on the substrate uptake or availability [47]. Similar constraints were also used in other studies [2,3,7]. Due to the low yield of fermentation, this pathway consumes glucose very fast. Thus, the limitation of glucose uptake due to its availability or the maximal capacity of glucose transporters becomes relevant. This can be written as the following additional constraint:

$$v_1 \leqslant v_{1,cap} \tag{3}$$

By using appropriate values for $v_{1,cap}$, the feasible region can be restricted as shown in Figure 4. It is now a quadrangle. Solving the LP shows that the solution leads to positive rate values for both v_2 and v_3 (• in Figure 4). This corresponds to respiro-fermentation. Due to the limited substrate uptake, the cell has left-over enzyme resources that can be used in respiration [47]. Thus, the observed metabolic mode in the Warburg effect is explained in an elegant way. The advantage of the model proposed in [47] in comparison with that of Shlomi et al. [2] is that the phase plane analysis can be performed with a much smaller model.

Besides a limitation of substrate availability, a constraint on oxygen is often relevant in living organisms. This is related to explanation 2 (anaerobic conditions) mentioned above. An example is the region in the interior of tumours, where oxygen is scarce. Moreover, some yeasts, for example *Kluyveromyces marxianus* and *Pichia fermentans*, use respiration under aerobic conditions and fermentation when no oxygen is available [6]. Such a constraint has been used earlier in FBA [39,48].

Kareva and Berezovskaya [29] established a differential equation model describing the population dynamics of tumour and immune cells and considering the Warburg effect in both cell types. Their model corresponds to cause (6) mentioned above, i.e., poisoning of healthy cells by lactate. The outcome of these interactions includes tumour elimination, tumour dormancy and unrestrained tumour

growth. The model also predicts periods of oscillatory tumour growth. Archetti [37] proposed a related model and derived conclusions about manipulating acidity as a potential anti-cancer therapy.

Discussion

Here, we have reviewed several models for explaining the Warburg effect. Various optimal metabolic regimes in energy metabolism can be predicted by the optimality criteria of maximizing ATP production rate or biomass production rate. In several models, the optimization problems are linear with respect to the fluxes because ATP and biomass production rates are linear functions of fluxes and also the side constraints are linear. Note that the underlying rate laws can be non-linear. The role of non-linear kinetics in these resource allocation problems was analysed by Müller et al. [25]. Other models were based on non-linear differential equation systems rather than linear programming (see above).

Recently, it has been questioned whether cancer cells always show an increased ATP production rate [49]. A more detailed theoretical analysis should consider that the coefficients in eqn (1b) can change upon tumorigenesis. A decrease in ATP production rate could then be compensated by a shift to glycolysis. Else it is possible that the system is satisfied if the ATP production completely meets its demand for ATP, so that the Warburg effect allows the cell to invest excess metabolic capacity into other functions, such as NADPH or nucleotide synthesis.

The models reviewed above clearly show the two current tendencies in theoretical Systems Biology. One tendency is to use large-scale models, mainly motivated by the availability of -omics data. The model by Shlomi et al. [2] falls into this category. Such models have the advantage of being more comprehensive and nearer to the real system. They are more data-driven than hypothesis-driven. On the other hand, they are more cumbersome to analyse and often involve unnecessary detail. The opposite tendency is to establish minimalist models, motivated by the principle of Ockham's razor. A useful feature of minimal models is that they can largely be treated analytically. The models proposed in [3,26,45], as well the models based on evolutionary game theory [28], belong to that category. The model published by Molenaar et al. [19] is a medium-scale model lying in between. Such models ideally use the positive features of both extreme types of models.

All the reviewed models are quite powerful. In particular, several models show that depending on protein costs and substrate availability, pure respiration, pure fermentation or respiro-fermentation are predicted. A re-allocation of protein to the high-yield pathway only pays if the synthesis costs for that pathway are low enough. Most of the models reviewed here can, by a few modifications, be adapted to many other cell types and organisms including microbes.

Pure respiration is predicted to occur if the respiratory enzymes are cheap. However, this is not likely to be the case in many cell types. Another possibility is that the upper bound on glucose availability is even lower than shown in Figure 4. If that bound is low enough, the admissible region is a triangle delimited by the abscissa, ordinate and the line corresponding to that bound [2]. This is in agreement with the observation that baker's yeast, many other yeasts, and *E. coli*, among other cells, use pure respiration at very low glucose levels.

It is more complicated to explain the usage of pure respiration in many cell types of multicellular organisms (animals, plants, multicellular fungi). Non-proliferating cells show a low nutrient consumption and, thus, pure or predominant respiration [11]. It can be assumed that they have evolved towards a more co-operative, economical use of glucose, i.e., towards using high-yield pathways. Moreover, optimization criteria other than maximizing ATP production rate are likely to be relevant for them, since they do not proliferate at all or not as fast as microbial or tumour cells. The 'selfish' usage of glucose by glycolysis is avoided in many cells of multicellular organisms by regulatory mechanisms, which are not explicitly considered in our model. Implicitly, some mechanisms may be considered by choosing appropriate parameter values in the side constraints. Other regulatory mechanisms may include the action of the immune system, which serves, among other purposes, for cleaning the body from tumour cells.

Pure fermentation (in higher organisms usually called pure glycolysis) is predicted if the respiratory enzymes are costly and sufficient glucose is available and can be taken up. This regime is observed, for example, in lactobacilli and mammalian erythrocytes. The latter are very small to pass thin capillaries and are packed with haemoglobin. Thus, space constraints have led to expulsion of mitochondria, in agreement with the predictions of our model.

Respiro-fermentation is relevant in tumour cells, activated lymphocytes and many other cells (see 'Introduction'). It is predicted if the respiratory enzymes are costly and glucose uptake is limited. Limited substrate availability is especially relevant for the region inside of tumours. Other examples are provided by animal blood platelets and spermatozoa. Even in the resting state, they rely both on oxidative phosphorylation and (to a significant extent) on glycolysis. Platelets contain small numbers of fully functional mitochondria, which serve not only for energy generation, but also for redox signalling [50]. Since platelets and sperm cells are very small, space constraints certainly play a role [expressed by side constraint (eqn 1b)]. In sperm cells, the contribution of glycolysis appears to be particularly high in the head and principal piece of the flagellum [51].

The approach of maximizing the ATP synthesis flux is based on the assumption that cells producing ATP (and/or biomass) as fast as possible should have a selective advantage [52–54]. This is likely to be particularly relevant for micro-organisms, which can out-compete other species or strains when growing fast [20,42,43] and for rapidly proliferating cells in multicellular organisms.

Acknowledgements

We thank Michael Bauer, Regine Heller, Jean-Pierre Mazat, Stefan Müller, Reinhard Wetzker and several members of the Bioinformatics Department in Jena for stimulating discussions. We are grateful to Jan-Hendrik Hofmeyr for drawing our attention to Aristotle's causae.

Funding

This work was supported by the Deutsche Forschungsgemeinschaft [RTG 1715 (to P.M. and S.S.)]; a University of Jena fellowship to P.M.; the Excellence Cluster "Inflammation at Interfaces" EXC 306 (to C.K.)]; the Federal Ministry of Education and Research, Germany [grant number 0315890 C (to H.S. and S.S.)]; and the National Science Foundation [grant number IIS-1319749 (to D.B.)].

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Received 24 July 2015
doi:10.1042/BST20150153