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Non-Equilibrium Thermodynamic Approach to Ca^{2+}-Fluxes in Cancer

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Received: 14 August 2020; Accepted: 23 September 2020; Published: 26 September 2020

Abstract: Living systems waste heat in their environment. This is the measurable effect of the irreversibility of the biophysical and biochemical processes fundamental to their life. Non-equilibrium thermodynamics allows us to analyse the ion fluxes through the cell membrane, and to relate them to the membrane electric potential, in order to link this to the biochemical and biophysical behaviour of the living cells. This is particularly interesting in relation to cancer, because it could represent a new viewpoint, in order to develop new possible anticancer therapies, based on the thermoelectric behaviour of cancer itself. Here, we use a new approach, recently introduced in thermodynamics, in order to develop the analysis of the ion fluxes, and to point out consequences related to the membrane electric potential, from a thermodynamic viewpoint. We show how any increase in the cell temperature could generate a decrease in the membrane electric potential, with a direct relation between cancer and inflammation. Moreover, a thermal threshold, for the cell membrane electric potential gradient, has been obtained, and related to the mitotic activity. Finally,we obtained the external surface growth of the cancer results related (i) to the Ca^{2+}-fluxes, (ii) to the temperature difference between the the system and its environment, and (iii) to the chemical potential of the ion species.

Keywords: biophysics; cancer; non-equilibrium thermodynamics; heat and ions fluxes; transport theory; thermodynamics of biosystems

1. Introduction

At present, cancer is still an problem in biophysics, medicine and pharmacology. Indeed, statistical evaluations show a continuous growth in those dead due to cancer [1,2].

In recent years, the analysis of the ion transport phenomenon in cancer has also been substantially developed [3–11] in relation to the consequences for the cells' membrane potential. These experimental and theoretical results have pointed to the regulatory role of ion channels and transporters, in relation to the cell cycle phases, with relevance for neoplastic progression, resistance to apoptosis, and metastasis [12].

Indeed, since 1944, in hyperplastic mouse epidermis, the reduction in Ca^{2+} levels has been shown to be an important aspect of precancerous conditions [13,14]: this feature has represented a first direct correlation between Ca^{2+} and cancer. Today, the study of Ca^{2+} dynamics represents a fundamental aspect of the research on carcinogenesis and tumour evolution.

The development in the comprehension of intracellular Ca^{2+} signalling pathways has allowed biologists and the physicians to identify some important molecular players, with a consequent study of the activity of different cancer-related proteins, with altered functions [14]. Indeed, calcium is

a fundamental second messenger, involved in a variety of cellular processes, such as proliferation and apoptosis.

Considering the experimental evidence [14–31], tumour progression has also been related to the accumulation of some alterations in the Ca^{2+} signal, which inhibits its cytotoxic activity [32].

Calcium signal modulation can change cells' sensitivity to signals [15,32]. High levels of mitochondrial Ca^{2+} concentration for a long time, have been shown to induce the mitochondrial permeability transition pore, a pathological and physiological phenomenon, discovered over 40 years ago, and still not completely understood [15,33]. The mitochondrial permeability transition pore causes the formation of a non-specific channel within the inner mitochondrial membrane, useful for Ca^{2+} release and metabolite exchange, between the mitochondrial matrix and cytosol. However, a prolonged mitochondrial permeability transition pore causes changes in the inner mitochondrial membrane potential, cessation of ATP synthesis, bioenergetic crisis, and apoptotic or necrotic cell death [14,16,33].

All this experimental evidence moves the research interest towards the analysis of the Ca^{2+}-fluxes. At present, most of the mechanisms related to intracellular Ca^{2+} responses have been understood by developing in vitro experiments, but comprehension of the physiological role of these processes, in relation to tumour environment, remains an problem [32].

In this paper, we wish to develop a new viewpoint in the analysis of ion fluxes, recently published in [34], based on thermodynamics, with particular regards to the non-equilibrium thermodynamics. Our aim is to suggest an approach which takes into account the ion fluxes, in relation to the membrane electric potential gradient, in order to analytically describe the link between ion fluxes and membrane potential, in relation to cancer behaviour. We will focus our analysis on Ca^{2+} fluxes, due to the fundamental role of this ion in the regulation of a great number of cell functions.

2. Materials and Methods

The living cell membrane is characterized by a different permeability in relation to the distinct ions (Na^+, K^+, Cl^-, Ca^{2+}, etc.) which cause an electric potential difference, $\Delta\phi$, between the cytoplasm and the extracellular environment, measured in reference to the environment [35,36].

Since 1956, it has been clear that cancer cells are electrically different from normal ones [37]. Cone Jr. pointed out that hyperpolarization:

- Characterises the start of the cell M phase [38];
- Can reversibly block the synthesis of DNA and the mitosis [39];
- Was found to be a characteristic of the normal cells: the lowered membrane potential was identified as a cause of an increase in proliferation of the cancer cells [40].

Consequently, in 1971, Cone Jr. conjectured a relation between the cell cycle progression and the membrane electric potential changes [40]: this hypothesis has always been experimentally confirmed [41–45].

Moreover, the fundamental role of the membrane electric potential has recently been highlighted in relation to the control of the critical cell functions (proliferation, migration, and differentiation) [46–48]. In this context, the role of the ion fluxes has also been highlighted; indeed, an increase in the Na^+ intracellular concentration in tumour causes a depolarisation, during malignant transformation of normal cells [49,50]. On the other hand, the K^+ intracellular concentration remains approximately constant [51].

The membrane electric potential can be theoretically described by the Goldman–Hodgkin–Katz equation [52–54]

$$\Delta\phi = \frac{RT}{F} \ln\left(\frac{P_{Na^+}[Na^+]_{outside} + P_{K^+}[K^+]_{outside} + P_{Cl^-}[Cl^-]_{outside}}{P_{Na^+}[Na^+]_{inside} + P_{K^+}[K^+]_{inside} + P_{Cl^-}[Cl^-]_{inside}}\right) \quad (1)$$

where [A] is the concentration of the ion A, $R = 8.314$ J mol^{-1}K^{-1} is the universal constant of ideal gasses, T is the absolute temperature, $F = 96,485$ C mol^{-1} is the Faraday constant, and P is the relative permeability [55–57], such that $P_{Na^+} = 0.04$, $P_{K^+} = 1$ and $P_{Cl^-} = 0.45$ [55–57].

In order to develop a non-equilibrium thermodynamic analysis of the cell membrane, we must consider the interrelationship between the fluxes through the cell membrane (heat and ion fluxes) and the potentials at the borders of the membrane itself (temperature and electric potential). To do so, we follow the Onsager approach, by introducing the phenomenological equations [34,58–61]

$$\begin{cases} \mathbf{J}_e = -L_{11}\frac{\nabla\phi}{T} - L_{12}\frac{\nabla T}{T^2} \\ \mathbf{J}_Q = -L_{21}\frac{\nabla\phi}{T} - L_{22}\frac{\nabla T}{T^2} \end{cases} \tag{2}$$

where $\mathbf{J}_e$ is the current density [A m^{-2}], $\mathbf{J}_Q$ is the heat flux [W m^{-2}], T is the living cell temperature, and L_{ij} are the phenomenological coefficients, such that $L_{12} = L_{21}$ in the absence of magnetic fields, and $L_{11} \geq 0$ and $L_{22} \geq 0$, and $L_{11}L_{22} - L_{12}^2 > 0$ [34,58–63]. The phenomenological coefficients in the Equations (2) are constant over the range where the linear laws hold, and they must be determined experimentally [60,64]: L_{11} is named the heat conductivity, L_{22} is commonly called the electrical conductivity, while L_{12} and L_{21} are named the cross coefficients. Moreover, the cross coefficients are independent of both L_{11} and L_{22} [62,63].

When ion fluxes occur $\mathbf{J}_e \neq \mathbf{0}$, it follows that [58,59]

$$\frac{dc_i}{dt} = -\nabla \cdot \mathbf{J}_i \tag{3}$$

where c_i is the concentration of the i-th ion (Na^+, K^+, Ca^{2+}, Cl^-, etc.), t is the time, and $\mathbf{J}_i$ is the current density of the i-th ion. In this condition, considering the Equation (2), it follows that [34,58,59]

$$\frac{d\phi}{dT} = -\frac{L_{21}}{L_{11}}\frac{1}{T} \tag{4}$$

which highlights that a Peltier-like effect occurs [58], and a related heat flux is also generated [58,59]

$$\frac{du}{dt} = -\nabla \cdot \mathbf{J}_u \tag{5}$$

where u is the specific internal energy. Living cells exchange heat power towards their environment by convection, therefore, following the First Law of Thermodynamics, we can write [65]

$$\frac{du}{dt}\,dV = \rho\, c\,\frac{dT}{dt}\,dV = \delta\dot{Q} = -\alpha\,(T - T_0)\,dA \quad \Rightarrow \nabla \cdot \mathbf{J}_u = \alpha\,\frac{dA}{dV}\,(T - T_0) = \beta\,(T - T_0) \tag{6}$$

where $\rho \approx 10^3$ kg m^{-3} is the cell density, $c \approx 4186$ J kg^{-1} K^{-1} is the specific heat of the cell, $\alpha \approx 0.023 Re^{0.8} Pr^{0.35} \lambda / \langle R \rangle$ is the coefficient of convection, with $\lambda \approx 0.6$ W m^{-1}K^{-1} conductivity, $Re \approx 0.2$ the Reynolds number and $Pr \approx 0.7$ the Prandtl number [66], A area of the cell membrane, V is the cell volume, and $\beta = \alpha\, dA/dV$ is constant. Therefore, considering Equation (2), we can obtain [34]

$$\frac{d\phi}{d\ell} = -\frac{\alpha}{\left(L_{22}\frac{L_{11}}{L_{12}} - L_{12}\right)}\,T\,(T - T_0) = -\frac{\alpha}{k}\,T\,(T - T_0) \tag{7}$$

which links the membrane electric potential to the temperature of the cell, with ℓ being the length of the cell membrane. Moreover, considering the Schrödinger approach to living systems [67], we can point out that life always requires $T - T_0 > 0$, and, consequently, $d\phi/dr < 0$. This last inequality explains hyperpolarization in cells [41–44].

The model obtained allows us to describe life as [34,67]

- A continuous metabolic generation, characterised by ion and metabolite fluxes, for which a Peltier-like effect occurs, and $d\phi/dT = -L_{21}/L_{11}T$
- A continuous heat exchange, towards the environment, for which a Seebeck-like effect occurs, and $d\phi/d\ell = -\alpha\, T\,(T - T_0)/k$

Consequently, a specific entropy rate is generated [68]

$$T\,\frac{ds}{dt} = \nabla \cdot \left(\mathbf{J}_u - \sum_{i=1}^{N} \mu_i\, \mathbf{J}_i\right) - \sum_{i=1}^{N} \mathbf{J}_i \cdot \nabla \mu_i \tag{8}$$

where s is the specific entropy, T is the temperature, $\mathbf{J}_S = \mathbf{J}_u - \sum_{i=1}^{N} \mu_i\, \mathbf{J}_i$ is the contribution of the inflows and outflows, and $T\sigma = -\sum_{i=1}^{N} \mathbf{J}_i \cdot \nabla \mu_i$ is the dissipation function [58], and μ is the chemical potential, defined as

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,p,n_{k\neq i}} \tag{9}$$

where G is the Gibbs energy, n is the number of moles, and p is the pressure. The entropy outflow σ is fundamental to generate order from disorder, as Schrödinger himself pointed out [67].

In relation to Ca^{2+} fluxes, we rewrite Equation (8) as follows

$$T\,\frac{ds}{dt} = -\nabla \cdot \left(\mathbf{J}_u - \mu_{\mathrm{Ca}}\, \mathbf{J}_{\mathrm{Ca}}\right) \tag{10}$$

which, considering T constant, and following Prigogine ($ds/dt = 0$) [69], becomes

$$\nabla \cdot \left(\mathbf{J}_u - \mu_{\mathrm{Ca}}\, \mathbf{J}_{\mathrm{Ca}}\right) = 0 \tag{11}$$

Now, considering that $\nabla \cdot \mathbf{J}_u = \beta(T - T_0)$, we can write

$$\beta(T - T_0) + \nabla \cdot (\mu_{\mathrm{Ca}} \mathbf{J}_{\mathrm{Ca}}) = 0 \Rightarrow \nabla \cdot (\mu_{\mathrm{Ca}} \mathbf{J}_{\mathrm{Ca}}) = \beta\,(T - T_0) = \frac{\delta \dot{Q}}{dV} \tag{12}$$

3. Results

In this paper, we have developed a non-equilibrium thermodynamic analysis of the cell membrane electric potential, in order to obtain an analytical model for the comprehension of the role of the ion fluxes in relation to cancer behaviour, with particular interest in Ca^{2+} fluxes.

Some general statements can be introduced; indeed, Equation (7) points out that:

- Any increase in cell temperature generates a decrease in the membrane electric potential; in the case of cancer, it is caused by inflammation;
- The possible existence of this, due to the thermal threshold ($T > T_0$) for the cell membrane electric potential gradient, is related to the mitotic activity [40].

In relation to Ca^{2+}, these results link the external surface growth of the cancer to Ca^{2+} fluxes, to the temperature difference between the internal of the system and its environment, and to the chemical potential of the ion species. Indeed, Ca^{2+} outflow is a flux against the gradient, so, it is negative, and the heat exchange decreases. In this case, the cell must use the energy stored in other ways (proteins formation, etc.). If the Ca^{2+} inflows into the cell, the sign changes, and the cell can outflow heat, decreasing its energy value. Consequently, Ca^{2+} inflow should allow the cell to prevent cancer development, because the cell can decrease the chemicals that are useful for proliferation, in accordance with the experimental evidence [13,15,70–72].

4. Discussion and Conclusions

Hyperpolarization determines the activation of the Ca^{2+}-K^+ channel, which increases the Ca^{2+} intracellular concentration [45]. Consequently, the Ca^{2+}-K^+ channel results in a fundamental controller of the membrane electric potential.

Proteins play a fundamental role in ion transport. Proteins in the cytosolic can be modified in their functions by phosphorylation or dephosphorylation. In this context, the H^+-ATPase plays a fundamental role, because, it generates inflows of positive charges into the cell [73–76]. Consequently, protein phosphorylation results in an important cellular regulatory mechanism, because many enzymes and receptors [77,78] are activated or deactivated by phosphorylation, by involving kinase and phosphatase.

Cancer and normal cells have different metabolic pathways; indeed, cancer cells must increase their metabolism in order to support their growth [79]. Consequently, we can consider that glycolysis is the cytoplasmic catabolism of glucose ($C_6H_{12}O_6$) and it finishes with the inflow of pyruvate (CH_3COCO_2) into the Krebs cycle and the mitochondrion in the presence of oxygen, but, when oxygen supply is scarce, the pyruvate is converted into lactate ($CH_3CHOHCO_2$), and pumped out of the cell. As a consequence, the production of Hydrogen ions (H^+) causes acidification, with the consequence of the stabilization of the Warburg metabolic cycle. In cancer cells, there is a net conversion of serine to glycine, catalysed by the cytosolic (SHMT1) or mitochondrial (SHMT2) serine hydroxymethyl transferase, and correlated with the cell proliferation rate and the DNA synthesis rate [79,80]. Protein synthesis, proportional to the inflow rate of amino acids, requires energy: in cancer, around the 70% of glucose is converted to lactate during aerobic glycolysis [79,81]. Therefore, our results point to the fundamental role played by ion transfer in any protein cycle. Indeed, ion channels transduce surface events to the cytosolic protein machineries. They couple the sensitivity of cooperative allosteric proteins to chemical and electrical signals: to do so, they use the energy released during the passive ions flows. Here, we have developed the study of the Ca^{2+} flows and the related membrane potential variations, because changes in membrane potential can regulate Ca^{2+} influx, which can impact T cell activation. This process is triggered by an elevation of the cytosolic free calcium concentration, which activates the Ca^{2+}/PKC-dependent pathways that regulate progress from G0 into mitosis, with a related lymphocyte proliferation, as an effective immune response to cancer. The Ca^{2+} inflow is obtained by means of hyperpolarization, which is induced through K^+ channel activation [5].

Here, a theoretical model to analyse the ion fluxes was developed using non-equilibrium thermodynamics. It represents a useful tool for future analysis, in order to develop a new approach to anticancer therapies, based on ion fluxes.

Recently, the key role for Ca^{2+} was shown to be in regulating cancer, in relation to oncogenes protecting against cell death, and perturbing intracellular Ca^{2+} homeostasis. Indeed, oncoprotein B cell lymphoma 2 over-expression has been shown to be able to reduce steady-state Ca^{2+} levels within the endoplasmic reticulum, reducing Ca^{2+} transfer to the mitochondria, during apoptotic stimulation, and inhibiting apoptosis initiation [20,21,56]. Moreover, the protein mitogenic kinase Akt has been linked to Ca^{2+} homeostasis control, pointing out its modulation function on the phosphorylation state of IP3R, by inhibiting its Ca^{2+}-channel activity, and reducing the transfer of Ca^{2+} from the endoplasmic reticulum to the mitochondria [25].

In conclusion, our results agree with the experimental evidence in the literature [32,79,82–84], and could represent their biophysical explanation based on non-equilibrium thermodynamics. Moreover, this approach could support the new frontier in cancer therapies [14,32,85–89].

Last, we can evaluate the Ca^{2+}-fluxes in Equation (12) as follows:

$$J_{Ca} = \frac{\ell \cdot \alpha}{\mu_{Ca} \cdot \langle R \rangle} (T - T_0) = \frac{0.004 \times 0.023 \times 0.2^{0.8} \times 0.7^{0.35} \times 0.6}{-552.79 \times 10^3 \cdot \langle R \rangle^2} (T - T_0) = -\frac{0.97 \times 10^{-11}\ [\text{mol s}^{-1}]}{\langle R \rangle^2\ [\text{m}^{-2}]} \quad (13)$$

where $\ell \approx 0.004$ µm is the depth of the cell membrane [90] and $\langle R \rangle$ is the mean radius of the cell, considered, in the first approximation, as a sphere, $\mu_{Ca} = -552.79$ kJ mol^{-1}, and $T - T_0 \approx 0.4$ °C [91]. The numerical result depends on the mean size of the cell. Considering that the mean radius for human cell is of the order of 10^{-6}–10^{-5} m, it follows that the Ca^{2+}-flux is of the order of 21–450 mmol s^{-1}m^{-2}, which can be expressed as ∼0.010 mol s^{-1}kg^{-1}, in agreement with the experimental results obtained in [92].

Author Contributions: Conceptualization, U.L.; methodology, U.L. and G.G.; software, G.G.; validation, U.L. and G.G.; formal analysis, U.L.; investigation, G.G.; resources, U.L.; data curation, G.G.; writing—original draft preparation, U.L. and G.G.; writing—review and editing, U.L. and G.G.; visualization, G.G.; supervision, U.L.; project administration, U.L.; funding acquisition, U.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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