

Mini-review

Mini-review: Respiratory and pH imbalance at the early precancerous stage

Abstract

Cancer prevention is a global priority, yet the proximate causes of most cancers are still little understood even though this is what we want to learn how to avoid. In this review, we bring further evidence in support of the hypothesis on the so-called respiratory and pH imbalance (RpHI) as a cause of long standing hypoxia within the whole organism, characteristic to the precancerous stage. In brief, under hypoxic conditions, the overbusy cells, getting less oxygen than needed, turn into anaerobic adenosine triphosphate (ATP) production, excessive multiplication and, finally, tumor development. Cancer prevention might be associated with an increase in oxygen delivery to overstressed cells, carbon dioxide removing, as well as pH and glucose concentration balancing. The role of RpHI in chemical carcinogenesis and most cancer diseases is also discussed. Literature data also suggest that cancers develop at the physiological level, while the molecular changes in cancer cells are the consequence and not the cause of malign processes.

Keywords: Precancerous stage; Cancer prevention; Hypoxia; Respiratory and pH imbalance; Cachexia; Warburg's theory.

Introduction

Ancient Asian medicine was mostly a preventative medicine, while current western medicine is mainly a curative one. However, the query still remains: should the medical act balance the body or repair the already occurred damage? Imagine that we want to prevent cancer by administering the anti-cancer drug, cyclophosphamide. In a few years we will

have become ill from cancer! Presently, the gap between the two medical approaches seems to be even more evident.

Cancer prevention and etiology are medical priorities, but history study suggests that the journey towards achieving this goal is difficult and full of detours and roadblocks.¹ The proximate causes of most cancers are still less understood even though this is what we want to learn how to avoid.² For instance, it appears difficult to understand why does it have to be such a long time interval between the exposure of a certain cell to a mutagen and the expression of the resulting mutations. It is hard to imagine how the numerous genetic changes found in cancer cells could have been produced in any cell as the result of a single exposure to a DNA-damaging agent, or why months or years have to elapse before the effect of these changes are observed. Malignant tumors exhibit increased dependence on glycolysis, resulting in abundant export of lactic acid, a hypothesized key step in carcinogenesis.^{3,4} It is also well-known that the development of cancer has been associated with microbial infection, injury, and inflammation and tissue repair.⁵⁻⁷ Chronic inflammation conditions, caused by exposure to environmental factors can increase the risk of cancer.⁸⁻¹⁰ Most cancer cases are mainly attributable to long-term exposure to environmental factors, via a multi-year, multi-step, and multi-path process of tumorigenesis.^{11,12} They may involve cumulative genetic and epigenetic alterations in the chronic carcinogenesis of body cells from a non-cancerous stage to precancerous and cancerous stages.¹³ Chemopreventive effects of dietary phytochemicals used at the precancerous stage can hardly be explained in light of current theories.¹⁴ Several dietary phytochemicals such as β -carotene, curcumin, resveratrol, gallic acid, chlorogenic acid, caffeic acid, carnosol, capsaicin, 6-shogaol, 6-gingerol, and their related derivatives have been largely investigated against cancer invasion and metastasis.^{15,16} Since carcinogenesis is a multistep process, these bioactive compounds might act on a variety of stages of the precancerous process to prevent inflammation, enzyme activities, or lactic production.^{17,18} Evidence for cancer-related anti-inflammatory activity of natural and synthetic phenols was provided by Aspirin, which inhibits colorectal carcinogenesis.¹⁹

Warburg stated that there are fundamental differences in the central metabolic pathways operating in tumorigenesis.²⁰⁻²² In recent years, the metabolism of malignant

tissue has once again become one of the most intense areas of research in cancer biology.²³ Considering all these issues, new ideas are needed to clarify what happens during carcinogenesis.

Starting with the observation that the cellular structure is partially broken down during hypoxia, a so-called respiratory and pH imbalance (RpHI) was supposed to be involved in cancer etiology.²⁴⁻²⁶ In principle, the respiratory and pH imbalance, which can be regarded as a physiological reaction against any stress agents, may cause an oxygen crisis within the body, and, consequently, the busiest cells divide faster and faster producing benign or malignant tumors. The tumors thus acquired, as well as their fermentation products, may disturb the normal functions of most cells and tissues in the body.²⁷

Respiratory and pH imbalance was discovered by putting together the main experimental data in cancer with the most important findings in physiology, biochemistry, cybernetics, etc., and treating them as a whole; it represents the mechanism that could create a long standing intracellular hypoxia state, which, over time, generates the conditions for malignant transformation.^{28,29} Nevertheless, it is hard to believe that a tumor is generated starting from a physiological imbalance, prior to the first real malign cell, while the modern biology is looking for precise molecular mechanisms involved in carcinogenesis. However, the impairment of cellular breathing, altered intracellular and extracellular pH, as well as hypoxic conditions might mostly be related to a precancerous pathology. Besides, cancer-associated impairment of respiration and changes in pH are less explored than the genetic mutations.

Therefore, this work aims at presenting evidence for the role of RpHI during the precancerous stage, as well as some concluding remarks that could be useful to design new preventive strategies.

A hypothesis on cancer-related physiological changes

Since the oncogene revolution pushed tumor metabolism to the margins of cancer research,^{20,30} interest in the fundamental differences of central metabolic pathways operating in malignant tissue has only been renewed in the recent years. The potential

causes of deranged metabolism in cancer should be discussed with emphasis on changes in energy metabolism of glucose, fat and protein.³⁰

In brief, various stress agents, such as physical and chemical stressors, viruses, other infectious agents, hormones in excess, but also long lasting anxiety, emotions, conflict states, etc., are able to affect the body as a whole.³¹⁻³⁴ In order to preserve the identity and relative independence of living organisms confronted with the external environment, some co-ordinate physiological processes are involved in keeping their internal states of equilibrium.³⁵⁻³⁷ The living bodies react against aggressions using metabolic energy obtained by the oxidation of organic substances, including organic acids in the mitochondria.^{38,39} Under normal conditions, when the supply of oxygen is sufficient, cells can carry out aerobic respiration.^{40,41} Hence, when busy, cells produce the energy they need from the food stores, including the organic acids they possess. During the Krebs cycle, carbon dioxide, of a weaker acid type, is released, normally out.^{42,43} Since stronger organic acids such as succinic, malic, 2-ketoglutaric and oxalylacetic acids are replaced by carbon dioxide, the cell milieu tends to be more alkaline, just blood and urine.⁴⁴ The tendency of blood alkalization entails retaining of carbon dioxide, so that the pH of blood should alter as little as possible. The retaining of carbon dioxide in blood does not allow the oxygen shift at a normal rate in lungs.^{45,46} As a consequence, oxygen pressure in blood decreases albeit it stays normal all around. However, oxygen may reach quite a low level without affecting cell breathing in any drastic way. Unfortunately, the busiest cells in the body need more oxygen. Oxygen partial pressure getting lower, these cells receive less oxygen than they need, and fermentative processes develop alongside with breathing.

Hypoxia is the natural environment in which life appears and proliferates and DNA auto-replication and transcription takes place *in vivo* in all eukaryotes.⁴⁷⁻⁵⁰ Nuclear division unfolds anaerobically using the energy produced by glycolysis.⁵¹⁻⁵³ Consequently, the cells forced to manifest themselves in rather anaerobic conditions will divide more intensely.⁵⁴ Aerobic breathing, which provides cells with a great amount of energy, creates the necessary conditions for the existence of fine structures of the cells, the specific functions running unimpaired.⁵⁵ Lack of oxygen, even partial, causes rupture of these structures, hence the gradual disappearance of specific functions of cells, as well as contact inhibition,

120 while it entails cell-division to a greater extent than necessary for the tissue in question.⁵⁶
121 Then comes the first stage of a so-called respiratory and pH imbalance, which affects the
122 cell respiration and division. Nevertheless, the organism possesses buffer systems, lung
123 ventilation and the kidney mechanisms to control the concentration of hydrogen ions within
124 the cellular milieu (Figure 1).⁵⁷ Lung ventilation is only able to control the blood pH, and
125 not the oxygen pressure.⁵⁸ The kidneys are capable to control both the blood pH and some
126 other blood parameters; however, this process is slow and lasts for several hours or days. If
127 the stress agents act continuously, the blood will become slightly more alkaline than usual,
128 and the blood oxygen concentration will be lower than normal. The overstrained cells,
129 getting less oxygen than needed, will lead to anaerobic fermentation and also to a lower
130 content of $\text{NADH}+\text{H}^+$ and $\text{NADPH}+\text{H}^+$, and a higher one of NAD^+ and NADP^+ . Therefore,
131 a decrease of the oxidation-reduction potential will occur as well. The quantity of
132 sulphhydryl groups in the blood and tissues also decreases. Besides, marked decrease in
133 succinic dehydrogenase and slight increase in cytochrom oxidase levels could be found,
134 suggesting the alteration of Krebs cycle. For this reason, the cell with an excessive
135 fermentation will not reach an upper energetic state, if the neighbor cells and blood cannot
136 interfere with its metabolism.

137 The second stage of the respiratory and pH imbalance is reached when lactic acid is
138 produced due to hypoxic conditions. This time, the CO_2 concentration may decrease;
139 however, part of the produced carbon dioxide is not removed, because the cell content may
140 remain slightly alkaline in spite of the lactate production. Again, the kidneys should control
141 the hydrogen concentration in blood, releasing acidic species, such as NaH_2PO_4 , into urine.
142 The process is complicated by the existence of lactic acid in blood which decreases blood
143 pH, while intracellular pH of overstrained cells is increased.

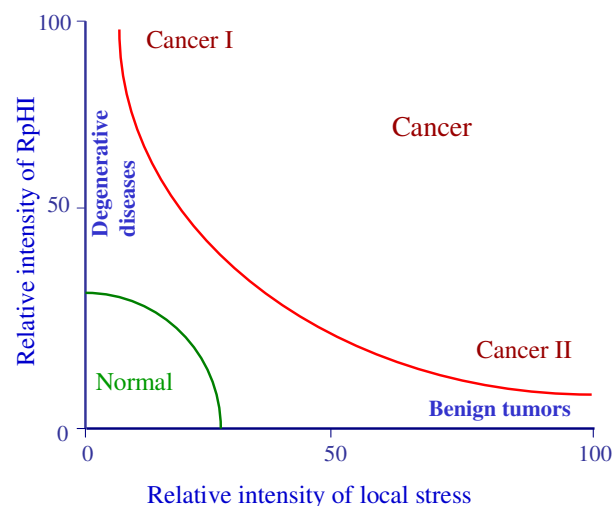


Figure 1. Schematic presentation of the role of the Respiratory and pH Imbalance (RpHI) in the precancerous stage.

However, if the stress agents act continuously, the neighbor cells involved in curing or rebalancing the overbusy cells will work hard, also being overstrained and deprived of oxygen. There follows a third stage of the respiratory and pH imbalance in which a real state of illness (infections, viruses) occurs. A very special balance between the two types of cell (attacked cells and neighbor ones) is established. As a result, the pH value of the blood is little altered. A stronger action of the stress agents could kill the body at this stage. Contrary, a long-standing, still mild action of the stress agents may cause a slow shift of this imbalance, resulting in a stepwise decrease of blood pH value. Most cells become glycolytic while fewer remain normal, but overstressed. This is the pathway to reach the fourth stage of the respiratory and pH imbalance, which is that of tumor formation.

This so-called respiratory and pH imbalance causes an oxygen crisis and the busiest cells divide faster and faster. The tumors thus acquired and their fermentation products can kill the body, if they are not surgically or by other means removed. The respiratory and pH imbalance cannot be easily measured, since the affected individuals seem to be normal and look like they are in good health. Otherwise, if stress agents are more powerful, patients might die during the first stages of body interaction with the environment stressors, without reaching the cancerous stage (IV). To reach cancer, the RpHI should be less strong, yet long-standing in order to get a chronic lack of oxygen in the blood and cells. Cancer could be considered an impairment of the whole body, while malignant cells are quite normal

cells that, because overstrained, get less oxygen than needed and turn into anaerobic fermentation, being divided to a greater extent than what the body requires.

The local/cellular stress means the reaction of the individual cell against the external environmental agents. There is a reciprocal relationship between the respiratory and pH imbalance, which affects the entire body and the local solicitation. Thus, an imbalanced body, found in apparently good health, will die when confronted to a less important local solicitation (Cancer I). On the other hand, as in the case of experimental chemically produced tumors, a long-standing local stress induced by the carcinogen may cause a general imbalance, which could also promote cancer (Figure 1, Cancer II). There are many intermediary states between these extreme situations, giving cancer its characteristic complexity. The respiratory and pH imbalance caused by the difficulty of the lung-kidney system to remove the excess of CO₂ and alkaline ions produced during the stress reaction means/determines a general weakness of the entire body. At the same time, such degree of weakness may be associated either with cancer or other diseases.

The living body can be considered a relatively independent entity, which possesses the physiological mechanisms to preserve its specific structure in order to adapt to the environmental conditions. The respiratory and pH imbalance is the expression of exceeding the adaptive capability of the body. Generally, the body fights against the alien disturbing agents through a well-known phenomenon, involving the humoral immunity and the capacity of immune response mediated by cells. At the same time, the organism possesses metabolizing and transformation systems, which transform alien and complex substances into simpler ones to be released.

The body must also release its own excessive cells because they are no longer necessary for the organism structure and functions. This process is more clearly understood if one considers wound healing. Thus, the injury puts the inner cells and the external environmental agents together. To react against the changes of environmental conditions, these cells use energy, alkalize the surrounding cell environment, get less oxygen and pass to an excessive fermentative (glycolysis) state. Indeed, because of the fundamental role of oxygen in metabolism, a drop in tissue oxygen level to the point where oxygen demand exceeds supply leads rapidly to metabolic crisis.⁴⁰

They divide excessively even after the healing of the wound, but not for too long. Then several cells, which have multiplied in excess for that tissue and are being no longer necessary, are released; thus, the body's own structure is restored. This process takes place in balanced bodies only. Otherwise, the useless cells divide continuously, becoming cancerous. Their neighbors cannot destroy them or help them integrate into the body.

In order for maintaining its specific structure, the body tends to remove the existing malignant cells, too, even if they are its own cells. This process seems to be dependent on the size of the respiratory and pH imbalance. The surgical removing off the tumor cells causes a decrease in this imbalance. As a result, both the immunity and the capacity of restoration of the body's own structure, will increase because the tumors are strong stress agents, as well.

Cancer cells can thus be considered as normal; however, they behave abnormally under hypoxic conditions. Consequently, carcinogenesis only develops in exhausted organisms, hence where a respiratory and pH imbalance occurs. A carcinogenic promoter acts in a similar way as those agents that provoke a wound, but lasts longer; a local respiratory and pH imbalance causing cellular hypoxia develops.

Chemical carcinogenicity and the abnormal metabolism of carcinogens

The carcinogenic substances may cause a respiratory and pH imbalance that causes in its turn a slight intracellular hypoxia, which provides the normal specific conditions for expressing the ancestral gene existing in every cell. It follows that a carcinogen touching a living tissue must be released at any price, because the affected living cell will become exhausted in its effort to destroy and release that compound and its metabolites. Also, this effort is subsequently assumed by the neighbor cells and a respiratory and pH imbalance at the level of the whole organism takes place. The RpHI will cause in its turn the cellular hypoxia that represents the specific medium of malignant transformation.

Initiation involves a brief and irreversible interaction between a carcinogen and the target tissue, and implies a local cellular stress/solicitation. Gradually, the stress caused by carcinogen removal may promote an overstraining of the neighbor cells, and then of the whole body, causing a general imbalance. The two stages of cancer occurrence provoked

by carcinogenic substances can easily be explained by the existence of the respiratory and pH imbalance.

Suppose that a small amount of carcinogen enters a cell. Cell will degrade the foreign compound through a series of eight consecutive chemical reactions within the microsomal mixed function oxidase system.⁵⁹ The activity of these enzymes requires nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen; in a typical reaction, one molecule of oxygen is consumed (reduced) per substrate molecule, with one oxygen atom appearing in the product and the other in the form of water. However, noxious reactive oxygen species (ROS) and H_2O_2 may appear. Some other degradation reactions may also be possible, depending on the type of cell. To form NADH from NAD^+ , glucose, fatty acids, amino acids, citric acid, malic acid, etc., are consumed. During this process, cell content will become more alkaline, and, if oxygen supply is not enough for removing the carcinogen, fermentation will develop alongside with respiration. Glucose from blood will help the fermentative processes, because it forms acetyl-CoA, both in the presence and absence of oxygen. Acetyl-CoA cannot be used in the absence of oxygen; therefore, glucose will give only pyruvate, which, in turn, is reduced by NADH to lactate.

Whenever a cell is damaged, its neighbors undergo inflammation by which this cell can be destroyed. In this process, the need of oxygen of the neighbor cells is increased. They undergo an inflammatory process, during which they become slightly alkaline, and retain carbon dioxide. A hypoxia state may occur in the surrounding tissue. Paradoxically, blood will provide a cell with less oxygen if it acts against a carcinogen than if it is in the neighborhood. If the carcinogen does not react directly with the cell chemical components, so as to be degraded and removed, the cell will continue to act against it. The degradation process, in which the carcinogen is transformed into metabolites may last long enough. Whereas a long standing hypoxia state is created, the fine cellular structure is damaged by ROS, and the lactic acid is released from the contaminated cell, being then reconverted into glucose. The neighbor cells support the carcinogen-contaminated cell, undergoing an inflammatory reaction. Both the degradation and the inflammatory reaction may damage the involved tissue. Omega (omega)-3 polyunsaturated fatty acids have been recognized as having anticancer activity,⁶⁰ probably as a consequence of their anti-inflammatory effect.

Logically, both the carcinogen and the poisoned cell should be removed. Then the tissue will recover. If not, more oxygen is needed to help cells fight against carcinogen.

Chromosomal instability during precancerous stage

Epidemiology and clinical evidence clearly indicate that specific genetic, environmental and behavioral factors are associated with an increased risk for cancer development.¹ However, our findings are concordant to the observation that intracellular alkalizing and extracellular acidification play a key role in the growth and metastasis of tumor cells.⁶¹ The stepwise progression from an early dysplastic lesion to full-blown metastasis malignancy seems to be associated with increases in genomic instability.⁶² Still, at each step of carcinogenesis, there are natural mechanisms protecting the body against development of cancer.⁶³ Besides, carcinogens can also be removed from the body. If DNA damage occurs, it is repaired in most of the cases.

Changes in DNA methylation consisting of either DNA hypomethylation or hypermethylation are commonly seen in cancer cells. The significance of these alterations in the genesis of cancer is, however, unclear.⁶⁴ Nevertheless, hypermethylation of tumor-suppressor genes in cancer could merely be a secondary event to help maintain gene inactivity which has been induced by some other mechanism.

Nevertheless, renal carcinogenesis is associated with neither chronic inflammation nor persistent viral infection, and any histological change is hardly evident.⁶⁵ However, regional DNA hypermethylation on C-type CpG islands has already accumulated in such non-cancerous renal tissues, suggesting that, from the viewpoint of altered DNA methylation, the presence of precancerous conditions can be recognized even in the kidney.

Malign process is preceded by the genetic deficiencies, yet, only 0.3 per cent of the recessive-lethal mutations give neoplasm in *Drosophila melanogaster*.^{66,67} Moreover, tumor cells of this species become easily reversible because of the fact that these mutations do not seem to be the cause of cancer. In addition, even if cancer occurrence is directly related to the recessive-lethal mutations, the resulted abnormal proteins are normally hydrolyzed to amino acids.

The accumulation of unfolded proteins in the endoplasmic reticulum (ER) represents a cellular stress induced by multiple stimuli and pathological conditions. These include hypoxia, oxidative injury, high-fat diet, hypoglycemia, protein inclusion bodies and viral infection.⁶⁸

According to Eugen Macovschi,⁶⁹ the structure in the cancer cell is found under an altered, abnormal state; the investigation of this state is essential for the knowledge of carcinogenesis. The RpHI hypothesis is concordant with Macovschi's statement, because it considers the role of hypoxia in the malign transformation of cell biostructure. Genomic instability may also be associated with hypoxia, which might be the causal phenomenon.

Increased glucose metabolism of cancers

In solid regions of tumors the most consistent finding is: a higher glucose utilization rate, a higher lactate production, and a higher pH than in the surrounding tissues.⁷⁰ Tumor ATP was found to be slightly higher and glucose slightly lower than in the neighbor tissues. Most measurements of regional glucose and oxygen consumption by positron emission tomography have confirmed that oxygen extraction is lower than in normal tissue, and that the oxygen/glucose uptake ratio corresponds to a glycolytic rate of more than 65%.⁷¹

Since ATP production by glycolysis is less than 6% of that of oxidative phosphorylation, restriction of glucose delivery to the tumor by the blood was supposed to be more important than acidosis for inducing energy failure and subsequent tissue necrosis.⁷⁰

It was suggested that glycolysis is elevated because it produces acid, which provides an evolutionary advantage to cancer cells vis-à-vis normal parenchyma which they invade.⁷² The RpHI hypothesis states that the slight hypoxia in the precancerous stage will induce an increased consumption of glucose in order to produce the same amount of ATP as that obtained by normal respiration.

However, hypoxia occurs in physiological situations such as during embryonic development, as well as in pathological conditions such as ischemia, wound healing, and cancer.³²

The link between hypoxia and the regulation of angiogenesis is an area of intense research. The molecular details of this connection are still being elaborated.⁷³ A primary effector of the adaptive response to hypoxia in mammals is the hypoxia-inducible family factor of transcription regulators. These proteins activate the expression of a broad range of genes that mediate many of the responses to the decreased oxygen concentration, including enhanced glucose uptake, increased red blood cell production, and the formation of new blood vessels via angiogenesis.⁷³

The limiting role of glucose - and not of oxygen - for maintenance of energy state is also reflected by the relationship between ATP and glucose content under conditions of critical blood perfusion. Diabetics treated with an insulin-lowering drug, known as metformin, have less chances of cancer than those who receive insulin as treatment.⁷⁴ However, the undertaken studies are incapable of establishing a causal relationship. A relationship between this drug and glucose concentration may be suspected.

The tumors were shown to be slightly more alkaline than normal brain tissue, although lactate was consistently increased.⁷⁵ Thus, in animals with pronounced peritumoral brain edema, the latter is distinctly more alkaline than the healthy tissue. Therefore, increased lactate content from tumors is usually not associated with an acid shift of pH.⁷⁰ Moreover, high CO₂ levels decrease O₂ consumption and ATP production, and impair cell proliferation independently of acidosis and hypoxia.⁷⁶ Quantification of tissue pH in solid regions of glioma reveals an average value of 7.26. This value is higher than in the cortex of the opposite hemisphere and lower than that in adjacent edematous cortex.

In tumors, ATP content generally decreases when glucose begins to fall, whereas in the normal brain, ATP declines before glucose is reduced.⁷⁷ Although these considerations and the observed lactate increase are in support of a preferential glycolytic pathway for ATP production, tumor pH was slightly more alkaline than in normal brain tissue.⁷⁸⁻⁸⁰ Nevertheless, at an extracellular pH of 7.25, the oxygen consumption rate of cells increases by nearly a factor of 2 as the glucose concentration decreases from 5.5 mM to 0.4 mM.⁸¹ This effect of glucose concentration on the oxygen consumption rate, however, was slight at an extracellular pH of 6.95 and disappeared completely at an extracellular pH of 6.60. Hence, low glucose concentrations, associated with a normal extracellular pH will increase

oxygen utilization, slowdown glycolysis, increase respiration and aerobic ATP production. An ill animal will consume less food and will avoid effort. This behavior could be a lesson for human beings to reduce blood glucose concentration according to physiological requirements.

The new approach against cancer, as represented by inhibition of proton pumps, changes in pH, and a mechanism used by tumor cells to avoid intracellular accumulation of toxic substances was already reviewed.⁸²

Respiration and glycolysis

It is well known that respiration and fermentation are two independent biochemical processes, which may occur simultaneously in the living cell. Given an oxygen concentration of 10 per cent or more in the surrounding atmosphere, respiration occurs in the living cell. Given a concentration below 3% of oxygen, fermentation (glycolysis) will occur. Both processes occur in the range from 3% to 10% oxygen (Figure 2). The growth and lifespan of human diploid cell strains at oxygen concentrations of less than 20% is enhanced, and an extension of about 25% of the lifespan of both cell types was carried out by long term cultivation below 10% oxygen.⁸³

Whenever a cell within the metabolic system (muscle, liver, kidney, lung etc.) uses oxygen at a faster rate than it can be supplied by the circulatory system, the cell begins functioning anaerobically, reducing the pyruvate to lactate instead of further oxidizing it, as it would occur if oxygen supplies were adequate. Lactate thus accumulates in that cell, diffuses out through the bloodstream, and eventually finds its way to the liver, where it is reoxidized to pyruvate and converted back to glucose by the gluconeogenic pathway operative there.

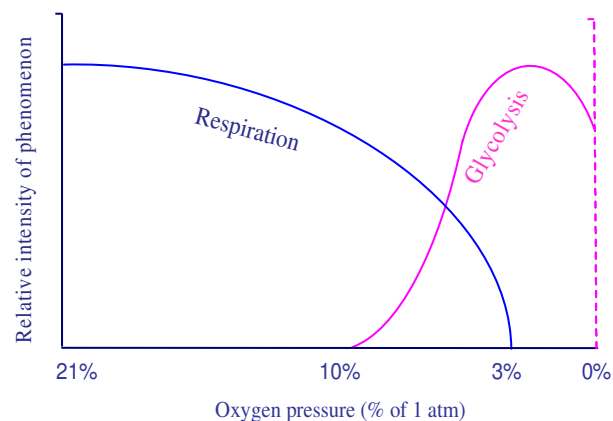


Figure 2. Schematic presentation of the relative intensities of cellular respiration and glycolysis as a function of oxygen partial pressure.

Mitochondria play an important role in the functional responses of eukaryotic cells to changes in O_2 concentration, being involved in the adaptation to hypoxia. Previous investigations showed that when eukaryotic cells are exposed to hypoxia, glycolysis is stimulated and it can compensate the low ATP production within the oxidative phosphorylation process. Moreover, Otto Warburg showed that normal cells would become irreversibly cancerous if the environment they rested in had their oxygen levels lowered by 35% for two days. Besides, long-term cell cultures may become malignant without any other influences.

The glucose consumption rate of cancer cells increases much when the oxygen concentration is reduced;⁸¹ however, severe conditions are required to stop cell growth (8.2 μM oxygen and an extracellular pH of 6.6).

Stressed cells and neighbor cells

As the metabolic control mechanism which regulates the transition from aerobic to anaerobic metabolism seems to be of key importance in the development of pathological changes, several studies were initiated to correlate the effects of anoxia on normal and aged mitochondria to the various biochemical and functional parameters of these organelles.

In isolated mitochondria inhibition of respiration and energy production have been demonstrated to be important factors in cell injury especially when nitrogen-induced anaerobiosis was investigated.⁸⁴ It was observed that aging, especially under nitrogen, leads to a decrease of the affinity constants of all mitochondria. The effect of anaerobiosis is

increased by the combination with decreased pH; it was demonstrated that decreased pH exerts an effect on the redox state of cytochrome *a* in anaerobic, ATP-treated intact mitochondria.⁸⁵

Cytochrome *c* oxidase (EC 1.9.3.1.) acts as a final electron acceptor in the mitochondrial respiratory chain and catalyzes the molecular oxygen reduction to water. The fact that the radical intermediates of oxygen reduction have a carcinogenic potential calls to a special consideration of the cytochrome *c* oxidase pathway.⁸⁶

The cytochrome *c* oxidase activity is the highest in all forms of host liver mitochondrion; aging of mitochondria was associated with a decrease in enzyme activity, which was more intense in host liver mitochondria than in the tumor ones. As depicted in Figure 3, ageing and anaerobiosis provoke a partial breakdown of the mitochondrial biostructure; the cytochrome *c* oxidase activity mirrors this process.⁸³ The biostructure of ascitic mitochondria is also broken-down; anaerobiosis and carcinogenic factors induce a similar breaking down of mitochondria. Synthesis of mitochondrial-encoded subunits of cytochrome *c* oxidase in *Saccharomyces cerevisiae* is also suppressed in response to oxygen deprivation both *in vivo* and *in vitro*.⁸⁷ Cytochrome *c* oxidase activity among other respiratory enzymes monitored an imbalance, which characterizes both a precancerous stage and cancer. While cancer cell is hypoxic, the surrounding cells are overstrained.

Angiogenesis is a necessary component of all healing wounds. Tissue hypoxia is a well-known stimulus to angiogenesis.⁸⁸ There are, however, inconsistent data and widely differing hypotheses concerning how much (what pressure or concentration) is required to support cellular metabolism and how the oxygen levels in cells within tissues are regulated.⁸⁹ There is a wide disparity in the published data and in the hypotheses used to interpret the cell and tissue responses to changes in oxygen pressure.^{90,91}

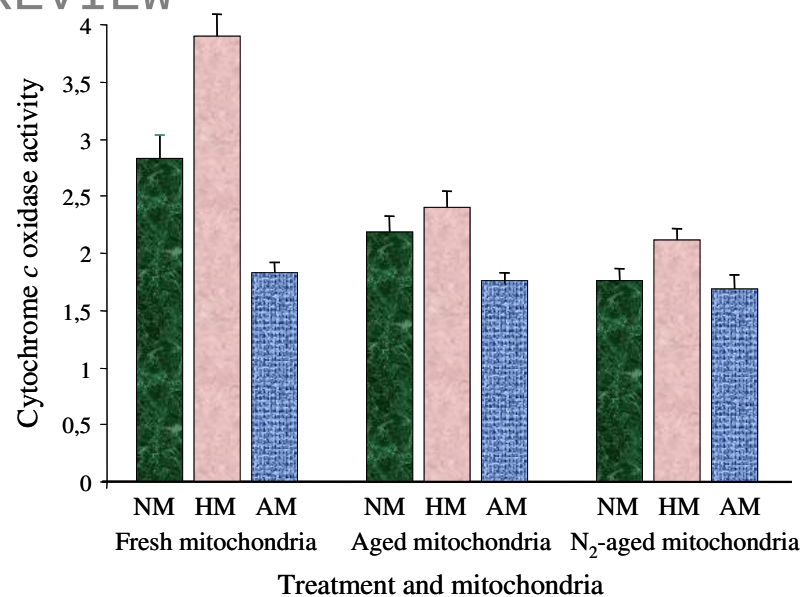


Figure 3. Cytochrome *c* oxidase activity (expressed as micromoles oxidized cytochrome *c*/mg protein/min) of normal mitochondria (NM), host liver mitochondria (HM) and ascitic mitochondria (AM) under different conditions (Fresh and aged mitochondria for 15 min at 22 °C in air or nitrogen-based anaerobiosis. See the experiments by C. Mihai for details.⁸⁴

Life style and cancer

The American Cancer Society has recently published the Nutrition and Physical Activity Guidelines, which are developed by a national panel of experts in cancer research, prevention, epidemiology, public health, and policy. They reflect the current scientific evidence related to dietary and activity patterns and cancer risk.⁹² Moderate to vigorous levels of physical activity lower the risk of developing several cancers. Overweight and obesity are clearly associated with an increased risk of developing many cancers; maintaining a healthy weight, staying physically active, and consuming a healthy diet can substantially reduce the risk of developing, or dying from cancer. These same behaviors are also associated with a decreased risk of developing cardiovascular disease and diabetes. All these recommendations are concordant with the RpHI hypothesis. Moreover, anxiety, rather than depression, seems to be a problem at long-term cancer survivors and their spouses, compared with healthy controls.⁹³

It was suggested that cancer cells have dysfunctional mitochondria, which prevent them to use the citric acid or other compounds in the Krebs cycle.^{42,94} We also consider that

the mitochondrial function can be altered in the precancerous stage. However, if cell content becomes more alkaline due to the continuous work under stress conditions, it will retain carbon dioxide and will get less oxygen regardless of its concentration. The RpHI approach is in full compliance with the findings on cancer occurrence reported by other authors.⁹⁵ A more comprehensive theory on cancer etiology to highlight not only the molecular aspects but also the biostructural ones, and possibly psychic-related processes as Macovschi stated, is expected from a more integrative life science.⁹⁶

Hypoxia prevents normal ATP production by oxidative phosphorylation.⁹⁷ Hypoxic cells swell, whereas the swollen mitochondria produce less energy. Chronic inflammation begins following continuous exposure to noxious exogenous influences.⁹⁷ Most cancers are mainly attributable to long-term exposure to environmental factors, via a multi-year, multi-step, and multi-path process of tumorigenesis.¹¹ Despite ATP synthesis rate is reduced when oxygen availability decreases, the exposure of cells to low oxygen level do not significantly affect the total ATP content, which is quite the same level of normoxia.⁹⁸ These data suggest that cells respond to lowered oxygen tension by increasing the activity of glycolytic enzymes and glucose transporters via the hypoxia-inducible factor-1 (HIF).⁹⁸ HIF upregulates pyruvate dehydrogenase kinase 1, that inhibits the pyruvate dehydrogenase decreasing TCA cycle and consequently oxidative phosphorylation in mitochondria. HIF, which mediates the transcriptional response to hypoxia, is a strong promoter of tumor growth and invasion.⁹⁹⁻

101

Concluding remarks

Literature findings reviewed here support the hypothesis that a so called respiratory and pH imbalance previously described to explain the long interval between exposure of a certain cell to a mutagen and the expression of the resulting mutations, as well as carcinogenesis without any apparent genetic alteration. A large body of information suggests that the lethal mutations play an important role, yet not a decisive one. The hypothesis discussed here

takes into consideration the long standing hypoxia within the whole organism, characteristic to the precancerous stage. Moreover, under hypoxic conditions, the overbusy cells, getting less oxygen than needed will lead to anaerobic adenosine triphosphate (ATP) production, excessive multiplication and, finally, tumor development. Such assertion is consistent with most literature data. Consequently, new strategies of cancer prevention should be drafted, based on a physiological approach of precancerous stage. Cancer prevention may be associated with oxygen delivery to the cells, carbon dioxide removal as well as pH and glucose concentration control. Natural mechanisms protecting against cancer should be taken into consideration. Future medicine can also be seen as caring for physiological balances and not only as an emergency surgery.

Further studies are required to highlight the essential mechanisms characteristic to the precancerous stage hidden in the extremely complex oncology. At least, our theoretical approach suggests that oxygen tension, hypoxia, and carbon dioxide loading are key parameters that may influence cancer development in tissues.

References

1. Bode, A.M., Dong Z. (2009) Cancer prevention research - then and now. *Nat Rev Cancer*, **9**, 508-516.
2. Brash, D., Cairns, J. (2009) The mysterious steps in carcinogenesis. *Br J Cancer*, **101**, 379–80.
3. Le Floch, R., Chiche, J., Marchiq, I., Naiken, T., Ilk, K., Murray, C.M., Critchlow, S.E., Roux, D., Simon, M.-P., Pouyssegur, J. (2011) CD147 subunit of lactate/H(+) symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. *Proc Natl Acad Sci USA*, **108**, 16663-16668.
4. Martin, N.K., Gaffney, E.A., Gatenby, R.A., Gillies, R.J., Robey, I.F., Maini, P.K. (2011) A mathematical model of tumor and blood pHe regulation: The $\text{HCO}_3^-/\text{CO}_2$ buffering system. *Math Biosci*, **230**, 1-11.
5. Rakoff-Nahoum S. (2006) Why cancer and inflammation? *Yale J Biol Med*, **79**, 123–130.

- 538 6. Ioannou, S., Voulgarelis, M. (2010) Toll-like receptors, tissue injury, and
539 tumourigenesis. *Mediat Inflamm*, ID 581837, doi: org/10.1155/2010/581837
- 540 7. Niwa, T., Tsukamoto, T., Toyoda, T., Mori, A., Tanaka, H., Maekita, T., Ichinose, M.,
541 Tatematsu, M., Ushijima, T. (2010) Inflammatory processes triggered by
542 *Helicobacter pylori* infection cause aberrant DNA methylation in gastric
543 epithelial cells. *Cancer Res.*, **70**, 1430-1440.
- 544 8. Mantovani, A, Allavena P, Sica A, Balkwill F. (2008) Cancer-related inflammation.
545 *Nature*, **454**, 436-444.
- 546 9. Solinas, G., Germano, G., Mantovani, A., Allavena, P. (2009) Tumor-associated
547 macrophages (TAM) as major players of the cancer-related inflammation. *J*
548 *Leukoc Biol*, **86**, 1065-1073.
- 549 10. Ricciotti, E., FitzGerald, G. A. (2011) Prostaglandins and inflammation. *Arterioscl*
550 *Throm Vasc*, **31**, 986-1000.
- 551 11. Rathore, K. (2011) Suppression of chronically induced breast carcinogenesis and role of
552 mesenchymal stem-like cells. PhD thesis, University of Tennessee.
- 553 12. Rathore, K., Wang, H. C. (2012) Green tea catechin extract in intervention of chronic
554 breast cell carcinogenesis induced by environmental carcinogens. *Mol Carcinogen*,
555 **51**, 280-289.
- 556 13. Rakoff-Nahoum, S., Medzhitov, R. (2009) Toll-like receptors and cancer. *Nat Rev*
557 *Cancer*, **9**, 57-63.
- 558 14. Mattarei, A., Azzolini, M., Carraro, M., Sassi, N., Zoratti, M., Paradisi, C., Biasutto, L.
559 (2013) Acetal derivatives as prodrugs of resveratrol. *Mol Pharmaceut*, **10**, 2781–
560 2792.
- 561 15. Weng, C.-J., Yen, G.-C. (2012) Chemopreventive effects of dietary phytochemicals
562 against cancer invasion and metastasis: Phenolic acids, monophenol, polyphenol,
563 and their derivatives. *Cancer Treat Rev*, **38**, 76-87.
- 564 16. Wang, P., Liu, H.-J., Mei, X.-Y., Nakajima, M., Yin, L.-J. (2012) Preliminary study
565 into the factors modulating beta-carotene micelle formation in dispersions using an
566 in vitro digestion model. *Food Hydrocolloid*, **26**, 427-433.
- 567 17. Manson, M. M., Linseisen, J., Rohrmann, S., Sotirioudis, T. G., Kyrtopoulos, S. A.,

- Hayes, J. D., Kelleher, M. O., Eggleston, I. M., de Kok, T. M., van Breda, S. G.,
Rafter, J. (2007) Bioactive components in foods. In: Dietary vitamins,
polyphenols, selenium and probiotics: biomarkers of exposure and mechanism of
anticarcinogenic action. Ed. Bjorn Akesson, Per Mercke, Lodz, pp. 115-177.
18. Grill, A. E. (2002) Examining and improving the chemopreventive efficacy of
curcumin. PhD Thesis, University of Minnesota.
19. Benamouzig, R., Uzzan, B., Deyra, J., Martin, A., Girard, B., Little, J., Chaussade, S.
(2012) Prevention by daily soluble aspirin of colorectal adenoma recurrence: 4-year
results of the APACC randomised trial. *Gut*, **61**, 255-261.
20. Cairns, R. A., Harris, I. S., Mak, T. W. (2011) Regulation of cancer cell metabolism.
Nat Rev Cancer, **11**, 85-95.
21. Cairns, R.A., Harris, I.S., Mak, T.W. (2011) Regulation of cancer cell metabolism. *Nat*
Rev Cancer, **11**, 85-95.
22. Santos de Souza A. C., Zenker J. G., Ribeiro de Araújo D., Martins C. A.D. (2011)
Defining the molecular basis of tumor metabolism: a continuing challenge since
Warburg's discovery. *Cell Physiol Biochem*, **28**, 771–792.
23. Bissell, M. J., Hines, W. C. (2011) Why don't we get more cancer? A proposed role of
the microenvironment in restraining cancer progression. *Nat Med*, **17**, 320–329.
24. Drochioiu, G. (1987) Considerations on biochemical mechanism of malignant
transformation. *Proc Symp Comp Oncol*, Timișoara, România, **1**, 116-117.
25. Drochioiu G. (2006) Eugen Macovschi's concept of biostructure and its current
development. In *Life and mind. In search of the physical basis*. (S. Savva ed.)
Trafford Publ., Canada, USA, Ireland & UK, pp. 43-60.
26. Drochioiu, G. (2008) Chronic metabolic acidosis may be the cause of cachexia: Body
fluid pH correction may be an effective therapy. *Med Hypotheses*, **70**, 1167- 1173.
27. Drochioiu, G. (2008) *Biological systems: a structural-phenomenological approach*, in
Cybernetics and Systems, Proceedings of the Nineteenth Meeting on Cybernetics and
Systems Research, Trappl R. (ed.) Austrian Society for Cybernetic Studies, Vienna, **1**,
203-208.

- 597 28. Drochioiu, G. (1985) Respiratory and pH imbalance in carcinogenicity (Romanian).
598 *Știință și tehnică* (Science and Technology-Bucharest), **9**, 38-39.
- 599 29. Drochioiu, G. (1987) The carcinogenic action of some chemicals. *Proc Symp Comp*
600 *Oncol* Timișoara, România, **1**, 115-116.
- 601 30. Douglas, R. G., Shaw, J. H. F. (1990) Metabolic effects of cancer. *Br J Surg*, **77**, 246–
602 254.
- 603 31. Aldwin, C. M. (2007) *Stress, coping, and development: an integrative perspective*.
604 Guilford Press, pp. 37-54.
- 605 32. Lovallo, W. R. (2005) *Stress and health: biological and psychological interactions*.
606 Sage.
- 607 33. Stranks, J. (2013) *Stress at work*. Jeremy Stranks, Routledge.
- 608 34. Bendelow, G. (2009) *Health, emotion and the body*. Polity Press, UK.
- 609 35. Silverthorn, D.U., Ober, W.C., Garrison, C.W., Silverthorn, A.C., Johnson, B.R. (2009)
610 *Human physiology*. Person, Benjamin Cummings, USA.
- 611 36. Piaget, J. (2013) *Behaviour and evolution*. Routledge.
- 612 37. Csepregi, G. (2006) *The clever body*. Calgary Press, Canada.
- 613 38. Shiraiwa, M., Selzle, K., Pöschl, U. (2012) Hazardous components and health effects of
614 atmospheric aerosol particles: reactive oxygen species, soot, polycyclic aromatic
615 compounds and allergenic proteins. *Free Radical Res*, **46**, 927-939.
- 616 39. Gong, B., Boor, P. J. (2006) The role of amine oxidases in xenobiotic metabolism.
617 *Expert Opin Drug Met*, **2**, 559-571.
- 618 40. Taylor, C. T. (2008) Mitochondria and cellular oxygen sensing in the HIF pathway.
619 *Biochem J*, **409**, 19–26.
- 620 41. Erecińska, M., Silver, I. A. (2001) Tissue oxygen tension and brain sensitivity to
621 hypoxia. *Resp Physiol*, **128**, 263–276.
- 622 42. King, A., Selak, M. A., Gottlieb, E. (2006) Succinate dehydrogenase and fumarate
623 hydratase: linking mitochondrial dysfunction and cancer. *Oncogene*, **25**, 4675–
624 4682.
- 625 43. Wiklund, L. (1996) Carbon dioxide formation and elimination in man. *Upsala J Med*
626 *Sci*, **101**, 35-68.

- 627 44. Williamson, J. R., Jákob, A., Scholz, R. (1971) Energy cost of gluconeogenesis in rat
628 liver. *Metabolism*, **20**, 13–26.
- 629 45. Robin, E. D. (1963) Abnormalities of acid-base regulation in chronic pulmonary
630 disease, with special reference to hypercapnia and extracellular alkalosis. *New Engl J*
631 *Med*, **268**, 917-922.
- 632 46. West, J. B. (2012) *Respiratory physiology: the essentials*. Kluwer, Canada.59. Ershov,
633 E., Shlosberg, A., Hanji, V., Kagan, J. (1994) The role of microsomal mixed function
634 oxidases in the metabolism and mechanism of action of certain organophosphorus
635 insecticides. *Drug Metabol Drug Interact*, **11**, 139-152.
- 636 47. Das, M., Bouchey, D. M., Moore, M. J., Hopkins, D. C., Nemenoff, R. A., Stenmark,
637 K. R. (2001) Hypoxia-induced proliferative response of vascular adventitial
638 fibroblasts is dependent on G protein-mediated activation of mitogen-activated
639 protein kinases. *J Biol Chem*, **276**, 15631-15640.
- 640 48. Hall-Stoodley, L., Costerton, J. W., Stoodley, P. (2004) Bacterial biofilms: from the
641 natural environment to infectious diseases. *Nat Rev Microbiol*, **2**, 95-108.
- 642 49. Boutilier, R. G. (2001) Mechanisms of cell survival in hypoxia and hypothermia. *J Exp*
643 *Biol*, **204**, 3171-3181.
- 644 50. Bergers, G., Benjamin, L. E. (2003) Angiogenesis: tumorigenesis and the angiogenic
645 switch. *Nat Rev Cancer*. **3**, 401-410.
- 646 51. Martinou, J. C., Green, D. R. (2001) Breaking the mitochondrial barrier. *Nat Rev Mol*
647 *Cell Bio*, **2**, 63-67.
- 648 52. Morowitz, H., Smith, E. (2007) Energy flow and the organization of life. *Complexity*,
649 **13**, 51–59.
- 650 53. Embley, T. M., Martin, W. (2006) Eukaryotic evolution, changes and challenges.
651 *Nature*, **440**, 623-630.
- 652 54. Schmitt, C. A. (2007) Cellular senescence and cancer treatment. *BBA - Rev Cancer*,
653 **1775**, 5–20.
- 654 55. Nyakas, C., Buwald, B., Luiten, P. G. M. (1996) Hypoxia and brain development. *Prog*
655 *Neurobiol*, **49**, 25–27.
- 656 56. Damjanov, I. (2009) *Pathology secrets*. 3rd Ed., Mosby, Inc., an affiliate of Elsevier Inc,

- 657 USA.
- 658 57. Hall, J. E. (2010) *Guyton and Hall textbook of medical physiology: enhanced E-book*.
659 Elsevier Health Sciences.
- 660 58. Reglin, B., Secomb, T. W., Pries, A. R. (2009) Structural adaptation of microvessel
661 diameters in response to metabolic stimuli: where are the oxygen sensors? *Am J*
662 *Physiol*, **297**, H2206-H2219.
- 663 59. Ershov, E., Shlosberg, A., Hanji, V., Kagan, J. (1994) The role of microsomal mixed
664 function oxidases in the metabolism and mechanism of action of certain
665 organophosphorus insecticides. *Drug Metabol Drug Interact*, **11**, 139-152.
- 666 60. Cockbain, A.J., Toogood, G.J., Hull, M.A. (2012) Omega-3 polyunsaturated fatty acids
667 for the treatment and prevention of colorectal cancer. *Gut*, **61**, 135-149.
- 668 61. Kim, J., Namkung, W., Yoon, J.S., Jo, M.J., Lee, S.H., Kim, K.H., Kim, J.Y., Lee,
669 M.G. (2009) The role of translation elongation factor eEF1A in intracellular
670 alkalization-induced tumor cell growth. *Lab Invest*, **89**, 867-74.
- 671 62. Schwartzman, J.M., Sotillo, R., Benezra, R. (2010) Mitotic chromosomal instability and
672 cancer: mouse modeling of the human disease. *Nat Rev Cancer*, **10**, 102-115.
- 673 63. Jakóbisiak, M., Lasek, W., Gołab, J. (2003) Natural mechanisms protecting against
674 cancer. *Immunol Lett*, **90**, 103-122.
- 675 64. Laird, P.W., Jaenisch, R. (1994) DNA methylation and cancer. *Hum Mol Genet*, **3**,
676 1487-1495.
- 677 65. Arai, E., Kanai, Y. (2011) Genetic and epigenetic alterations during renal
678 carcinogenesis. *Int J Clin Experim Pathol*, **4**, 58-73.
- 679 66. Finnegan, D. J. (2011) Oogenesis: active heterochromatin. *Curr Biol*, **21**, R630- R632.
- 680 67. Vincent, J.P., Kolahgar, G., Gagliardi, M., Piddini, E. (2011) Steep differences in
681 wingless signaling trigger myc-independent competitive cell interactions. *Dev Cell*,
682 **21**, 366-374.
- 683 68. Kim, I., Xu, W., Reed, J. C. (2008) Cell death and endoplasmic reticulum stress:
684 disease relevance and therapeutic opportunities. *Nat Rev Drug Disc*, **7**, 1013-1030.
- 685 69. Macovschi, E. (1984) The biostructural theory of carcinogenesis. *Rev Roum Biochim*,
686 **21**, 3-11.

- 687 70. Hossmann, K. A., Mies, G., Paschen, W., Szabo, L., Dolan, E., Wechsler, W. (1986)
688 Regional metabolism of experimental brain tumors. *Acta Neuropathol*, **69**, 139-147.
- 689 71. Rhodes, C. G., Wise, R. J. S., Gibbs, J. M., Frackowiak, R. S. J., Hatazawa, J., Palmer,
690 A. J., Thomas, D. G. T., Jones, T. (1983) In vivo disturbance of oxidative metabolism
691 of glucose in human cerebral gliomas. *Ann Neurol*, **14**, 614-626.
- 692 72. Gillies, R. J., Robey, I., Gatenby, R. A. (2008) Causes and consequences of increased
693 glucose metabolism of cancers. *J Nucl Med*, **49**, 24S-42S.
- 694 73. Hickey, M. M., Simon, M. C. (2006) Regulation of angiogenesis by hypoxia and
695 hypoxia-inducible factors. *Curr Topics Develop Biol*, **76**, 217-257.
- 696 74. Taubes G. (2012) Cancer research. Cancer prevention with a diabetes pill? *Science* 335,
697 29.
- 698 75. Chiche, J., Le Fur, Y., Vilmen, C., Frassinetti, F., Daniel, L., Halestrap, A., Cozzzone, P.
699 J., Pouyssegur, J., Lutz, N. W. (2012) In vivo pH in metabolic-defective Ras-
700 transformed fibroblast tumors: Key role of the monocarboxylate transporter, MCT4,
701 for inducing an alkaline intracellular pH. *Int J Cancer*, **130**, 1511-1520.
- 702 76. Vohwinkel, C.U., Lecuona, E., Sun, H., Sommer, N., Vadász, I., Chandel, N.S.,
703 Sznajder, J.I. (2011) Elevated CO₂ levels cause mitochondrial dysfunction and impair
704 cell proliferation. *J Biol Chem*, **286**, 37067-37076.
- 705 77. Paschen, W., Hossmann, K. A., van den Kerckhoff, W. (1983) Regional assessment of
706 energy-producing metabolism following prolonged complete ischemia of cat brain. *J*
707 *Cereb Blood Flow Metab*, **3**, 321-329.
- 708 78. Junck, L., Blasberg, R., Rottenberg, D. A. (1981) Brain and tumor pH in experimental
709 leptomeningeal carcinomatosis. *Trans Am Neurol Assoc*, **106**, 298- 301.
- 710 79. Rottenberg, H. (1984) Membrane potential and surface potential in mitochondria:
711 uptake and binding of lipophilic cations. *J Membr Biol*, **81**, 127-38.
- 712 80. Mies, G., Paschen, W., Ebhardt, G., Hossmann, K. A. (1990) Relationship between of
713 blood flow, glucose metabolism, protein synthesis, glucose and ATP content in
714 experimentally-induced glioma (RG12.2) of rat brain. *J Neuro-Oncol*, **9**, 17-28.

- 715 81. Casciari, J. J., Sotirchos, S. V., Sutherland, R. M. (1992) Variations in tumor cell
716 growth rates and metabolism with oxygen concentration, glucose concentration, and
717 extracellular pH. *J Cell Physiol*, **151**, 386–94.
- 718 82. Fais S. (2010) Proton pump inhibitor-induced tumour cell death by inhibition of a
719 detoxification mechanism. *J Intern Med*, **267**, 515-525.
- 720 83. Packer, L., Fuehr, K. (1977) Low oxygen concentration extends the lifespan of cultured
721 human diploid cells. *Nature*, **267**, 423-425.
- 722 84. Mihai, C., Dinescu-Romalo, G. (1977) Anaerobiosis-induced modifications of normal
723 rat liver mitochondria. *Rev roum Biochim*, **14**, 31-34.
- 724 85. Higuti, T., Sugiyama, Y., Sato, M., Muraoka, S. (1975) *Biochem Bioph Res Co*, **62**,
725 602-607.
- 726 86. Mihai, C., Dinescu-Romalo, G. (1979) The influence of some factors upon cytochrome
727 c oxidase activity of ascites and Guérin T8 ascites host rat liver mitochondria. *Rev*
728 *Roum Biochim*, **16**, 49-53.
- 729 87. Kwast, K. E., Hand, S. C. (1996) Oxygen and pH regulation of protein synthesis in
730 mitochondria from *Artemia franciscana* embryos. *Biochem J*, **313**, 207-213.
- 731 88. Knighton, D.R., Silver, I.A., Hunt, T.K. (1981) Regulation of wound-healing
732 angiogenesis-effect of oxygen gradients and inspired oxygen concentration. *Surgery*,
733 **90**, 262-270.
- 734 89. Wilson, D.F. (2008) Quantifying the role of oxygen pressure in tissue function. *Am J*
735 *Physiol*, **294**, H11-H13.
- 736 90. Wilson, D. F. (2008) Quantifying the role of oxygen pressure in tissue function. *Am J*
737 *Physiol*, **294**, H11-H13.
- 738 91. Cameron, N. E., Cotter, M. A. (1994) The relationship of vascular changes to metabolic
739 factors in diabetes mellitus and their role in the development of peripheral nerve
740 complications. *Diabetes Metab Rev*, **10**, 189–224.
- 741 92. Kushi, L.H., Doyle, C., McCullough, M., Rock, C.L., Demark-Wahnefried, W.,
742 Bandera, E.V., Gapstur, S., Patel, A.V., Andrews, K., Gansler, T. (2012) Reducing
743 the risk of cancer with healthy food choices and physical activity. *CA Cancer J*
744 *Clin*, **62**, 30-67.

93. Mitchell, A. J., Ferguson, D. W., Gill, J., Paul, J., Symonds, P. (2013) Depression and anxiety in long-term cancer survivors compared with spouses and healthy controls: a systematic review and meta-analysis. *Lancet Oncol*, **14**, 721–732.
94. John, A. P. (2001) Dysfunctional mitochondria, not oxygen insufficiency, cause cancer cells to produce inordinate amounts of lactic acid: the impact of this on the treatment of cancer. *Med Hypotheses*, **57**, 429–431.
95. Nijsten, M. W. N., van Dam, G. M. (2009) Using the Warburg effect against cancer by reducing glucose and providing lactate. *Med Hypotheses* **73**, 48–51.
96. Murariu, M., Drochioiu, G. (2012) Biostructural theory of the living systems. *BioSystems*, **109**, 126-132.
97. Damjanov, I. *Pathology secrets*. 3rd ed., Mosby, Inc., 2009.
98. Del Sole, M. *Effect of hypoxia and hyperglycemia on cell bioenergetics*. PhD Thesis, University of Bologna, 2012.
99. Kim, Y., Lin, Q., Glazer, P. M., Yun, Z. (2009) Hypoxic tumor microenvironment and cancer cell differentiation. *Curr Mol Med*, 9(4), 425-434.
100. Kunz, M., Ibrahim, S. M. (2003) Molecular responses to hypoxia in tumor cells. *Mol Cancer* **2**, 23, doi:10.1186/1476-4598-2-23
101. Wisea, D. R., Warda, P. S., Shayb, J. E. S., Crossa, J. R., Gruberb, J. J., Sachdevab, U. M., Plattb, J. M., DeMatteoa, R. G., Celeste Simonb, M., Thompson, C. B. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α -ketoglutarate to citrate to support cell growth and viability. *Proc Nat Acad Sci USA*, 108, 19611–19616.