Essay: Oncology and Hematology
J Biophys Hematol Oncol **1**, 5:1-93 (2010)

A Unitarian Biochemical and Bioenergetic Theory of Adaptive Oncogenesis: from hypoxia and energy starvation (aerobic *and* ambipolar) to the roles of HIF-1, IGF-I, and Vitamins C and D

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Abstract

The present communication unites in a novel model of the etiology of cancer very diverse contributions to oncology made since discovery of the Warburg effect. The model proposes a unitarian understanding of acquired malignancy as a neo-lamarckian adaptive disorder whereby a cell escapes organism and tissue regulatory controls to adapt to a condition of energy starvation (embodied in a variety of oncogenic pressures that are hypoxic or hypoxia-like in their effects), by abandoning its normal aerobic metabolism and by altering its growth-factor responses to support hyperplastic and neoplastic proliferation, and block normal differentiation. The multifactorial role of oxygen in normal metabolism is underlined by the biological and biophysical effects of its chronic lack in the initiation and promotion of oncogenesis: via the hypoxia-inducible factors (and especially HIF-1), hypoxia activates glycolysis and shuts down oxidative phosphorylation; and it adversely affects not only the oxygen transport roles of hemoglobin and myoglobin, but also their thermal dissipation of absorbed radiant energy, an essential contribution to temperature regulation in homotherms. Nonhypoxic factors may have similar hypoxia-like effects. Lack of vitamin C and iron can block progression of the Krebs cycle, and shut down aerobic respiration. Copper, according to our model of the respiratory chain, may actually work *in vivo* as a respiratory poison. Altered growth factor regulation (eg decreased plasma concentrations of erythropoietin) or enzymic defects (eg lack of citrate synthase) may also induce hypoxia-like effects.

Moreover, according to the analysis we present here, poor oxygenation prevents absorption of the radiant energy needed to inject into the respiratory chain. We propose that absorption of solar-sourced radiant energy in the terrestrial environment - with an ambipolar energy spectrum of 28 to 79 keV - is a key modulating function of biological systems involved in the normal activities of hemoglobin, myoglobin, the cytochrome c oxidase complex, skin production of vitamin D_3 and the differential radiant energy sensors of pinealocytes. It provides the energy thermally dissipated by hemoglobin and myoglobin, as well as the activation energy needed to initiate and terminate the respira-

 ISSN 1920-3799

tory chain, and thus the kinetic energy of the electrons and protons shuttled across mitochondrial membranes. Insufficient absorption of ambipolar energy is tied in to the causation of acquired cancer and, as suggested by the present etiological model, also connected to the deregulation of the Pasteur effect that permits manifestation of the Warburg effect. However, the latter is far from being a universal trait of cancer cells. Recent results by Jacques Sonveaux's group have shown that neoplastic phenotypes are distributed inside a tumor according to an oxygen gradient between lactic fermenters and lactic respirers, with their association being symbiotic. Our model suggests that this is best understood as functions of a unitarian auto-oncogenic vector of cumulative transformations for acquired cancers, and that this vector repeatedly invokes a recursive cell-regulatory circuit, the IGF (insulin-like growth factor) axis, in its effort to de-stabilize the autonomic control of the cell cycle.

At initiation of cancer, aerobic respiration is shut down substantially or entirely, and glycolysis is activated along with lactate dehydrogenase A (LDHA)-driven fermentation, to sustain hyperplastic proliferation and block differentiation. These metabolic shunts are brought about by the HIF axis (in particular by HIF-1) and modulated by the IGF axis (in particular by IGF-I, its binding proteins and its receptor, IGF-IR). They likely involve specific hypersensitive growth factor responses modulated by the IGF axis, including IGF-I hypersensitivity itself as found in *Polycythemia vera*. Such responses involve both epigenetic and post-adaptive alterations. With progression of the auto-oncogenic vector from hyperplasia to neoplasia, something akin to a differentiation of malignantly transformed states takes place which appears to require, in either case, changes in the IGF axis that render its operation independent from physiological control by its ligands, in particular from control by IGF-I, so that the orthosympathetic signals of the IGF axis become permanently turned on and the cell now exerts an organism-independent control over its own cycling. At this juncture, the oncogenic vector undergoes a split. The Pasteur effect coupling glycolysis to aerobic metabolism is severed in both instances of "neoplastic differentiation", but while the more aggressive neoplastic cells to which the Warburg effect applies rely solely on further acceleration of lactate production, other neoplastic cells adapt to the acidification of tumors - and blood - by turning on LDHB to employ the lactate as substrate, via conversion to pyruvate, for their mitochondrial Krebs cycle and respiratory chain. In solid tumors, the lactic respirers activate vascular endothelial growth factor production (controlled by the IGF axis) to stimulate tumor angiogenesis and thus acquire direct access to oxygen from the blood. Ultimately, the lactate fermenters become the highly-tumorigenic metastatic elements of terminal cancer.

We also suggest that serious clinical investigation with properly staged cancer patients should henceforth target the use of novel, or heretofore improperly-tested, non-cytotoxic treatments based on the presently proposed aetherometric model of auto-oncogenesis.

 ISSN 1920-3799

COMMUNICATION

> "My wife, who had accompanied me, said afterwards that she had never before seen a group of medical researchers with less interest in new ideas. They told me that the National Cancer Institute [NCI] would not do anything with vitamin C until studies had been made with animals. Those specialists did suggest, however, that I apply to the National Cancer Institute for a grant to provide support for our institute in California to carry out such a study. I at once applied to the institute [NCI] for a grant to support studies of vitamin C in relation to cancer in mice and guinea-pigs. It was approved as scientifically sound by the institute's [NCI's] consultants, but it was turned down. My next seven applications met the same fate."
>
> L. Pauling, 1986

1. Mechanistic neo-darwinian vs functional neo-lamarckian theories of cancer

As we presented in a previous survey of the last 6 decades of oncology [1] there is no established single model for the genesis of cancer, and it is possible that no unifying understanding of cancer can be formed. The end result of either the master gene or the mitotic aneuploidy theories is still a model of oncogenesis that only invokes (theoretical) inhibition of tumor supressor and repair genes, together with oncogene activation independently of normal controls by growth factors. We examined what role such genetic and regulatory alterations may play in cancer initiation, and concluded that, given degrees of progression in an oncogenic vector, there will be grades of transformation such that while it may be possible to experimentally transform single cells under *in vitro* conditions by seemingly altering very few genetic loci [2-4] (and do so in a variety of ways), the *in vivo* development of fully invasive neoplastic cells affects a multitude of epigenetic and adaptive changes hitting what are essentially recursive circuits of cellular and systemic regulation [1]. Modern oncology today realizes that oncogenesis requires coordinated changes in entire arrays of genes affecting nearly all of the cell's basic functions [5-6], not just responses to growth factors, DNA repair and DNA expression. Most importantly, cancer has become universally understood as a disorder of proliferating cells, so that a state of proliferation appears to be a condition for the initiation of cancer - or for a cell to take the first step into a vector of cumulative transformations that, at the limit, converts it into a neoplastic cell.

To understand the initiation of cancer on the basis of the multitudes of genetic changes so far detected - and the even greater multitudes yet to come - will always be insufficient because one cannot understand what led to the observed restructuring of the genome, nor the steps such process took,

 ISSN 1920-3799

from simply 'reading' the final adaptive alterations that were successfully selected by conferring a growth and metabolic advantage to a malignant clone. How the cell experimented, and with what exactly did it experiment until it committed to transform, still eludes us. It seems that, to understand cancer initiation, one might more advantageously investigate what selection pressures are there that induce diverse types of metazoal cells to engage in oncogenic transformation.

Yet, in presently accepted models of cancer induction and causation, the mechanistic perspective remains virtually intact. Causes are replaced by computation of risk factor probabilities, but it is still the action of actual or reputed mutagens that combine with other factors (viruses, mycoplasma, other mutagens, etc) to increase the number of random mutations in the genome of one or more cells which, if they confer a growth advantage, are positively selected. The role of immunity is considered, but mostly neglected. After all, no universal antibody exists against cancer, and no cancer patient enters into spontaneous remission by production of antibodies against their own neoplastic cells. Note that antibodies against tumor antigens have long been experimentally developed. Today, there are ongoing trials of engineered antibodies that have been shown to block specific segments of the signaling circuits of transformed cells, and which induce apoptosis - rather than differentiation or reversion to a normal cellular phenotype. For example, by blocking a substantial portion of the tyrosine kinase activity of the Abl/Bcr fusion protein, the antibody imanitib induces remission of most patients with early CML [7]. But it is doubtful whether such precision treatments may have a real impact on the treatment of cancer or whether, as has happened with chemotherapy [8-9], they will generate instead novel and yet more aggressive responses by the tumor cells that will likely bypass the segments inhibited by these antibodies. For example, the progression of CML contains mutations that render the cells resistant to the antibody [10]. Mechanistic oncology has, however, also acknowledged this in its particular neo-darwinian way, as Lawrence Loeb put it: in any tumor "there will be cells with *random mutations* that protect them from any treatment you can conceive" [11].

Neo-darwinian, mechanistic oncology holds that, even though no unitary view exists of what are cancer-promoting pressures or oncogenic factors, it is assumed that "selection of the cancer cell which succeeds" is passively performed by the environment on genomes that have been randomly mutated by exposure to mutagens and deficient repair. Cancer is essentially seen as being due to haphazard DNA alterations in key genes (tumor-suppressor genes, oncogenes and repair genes) which are brought about mechanically and passively. This approach invariably doubts the existence of an oncogenic vector proper, fragmenting its notion into a plurality of different mutagenic vectors that have punctually questioned the traditional views of the progression of cancer (eg hyperplasia->neoplasia; cytosis->blastosis; etc [1]). However, if operating within the internal environment of an organism there is a unitarian functional stress - or group of related stresses - that promotes neoplasia, this approach

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is bound to miss it.

Lawrence Loeb enunciated, back in 1974, the basic realization of the failure of all modern neo-darwinian theories of cancer - when he concluded that random, pre-adaptive mutation only hits, on average, a single gene in any given cell during a lifetime, and *thus cannot account for the number of mutations observed in cancer cells* [12]. Cancer by cumulative addition of a few, random somatic mutations was a pipe dream. As William Hahn put it, some biological process must accelerate the mutation rate, "otherwise cells wouldn't accumulate a sufficient number of mutations to form a tumor" (also quoted in [11]). Loeb et al speak of a "mutator phenotype" of the cancer cell that could explain the paradox.

The alternative concept that neo-lamarckian post-adaptive mutation is the real genetic etiology of cancer [13-15], or an essential part of it, rests upon precisely the same realization. In the previous communication [1], we wrote:

> "Assuming oncogenesis to be a neo-darwinian process of random pre-adaptive mutations with the accepted rate of 10^{-7} mutations per gene per cell division, "it is hard to see how any of these cells can acquire enough mutations to become cancerous, unless some process is raising the mutation rate far above its usual value" [16]. The product of two mutations in different genes would exhaust the entirety of the stem cell divisions in the lifetime of a human being. Yet, 1 in 3 human beings presently contract cancer, with an annual rate of 10 million per year which increases with advancing age, and with every human organism thwarting survival of many transformed cells during a lifetime. Thus Cairns suggested that "we should be looking at some other driving force that can be linked to (or triggered by) cell proliferation" in trying to explain the rate of cancer production."

Cairns' conclusion was critical [17]. We should be looking at what hits the proliferating cell, and keeps it proliferating. We should be investigating the force driving the abnormal proliferation, and eliciting the adaptive changes that the cell creatively composes. Given the diversity of biological regulatory signals and their circuits, and the recursiveness that permits the tissue cell to modulate its response and protect itself from uncontrolled mutagenesis, there will be many intersections in cellular circuits that must be targeted to yield transformed phenotypes, by epigenetic control and, mutationally, by adaptive changes. When one further realizes the variety of genetic and epigenetic alterations that are involved even in the same neoplastic phenotype, one is forced to conclude that acquired (nonfamilial) cancer is a biological process of cellular experimentation directed by the 'trans-

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formant cell' itself. This is the core of the concept of auto-oncogenesis that we have proposed [1] - in distinction from viral-induced or mutagen-induced oncogenesis, One might be tempted to say that before a cell is transformed, it is a transformant - a cell in the process of transforming itself. This is also something that neo-darwinian oncology lost sight of - how the cell experiments with the equipment it has at cancer initiation, and directs, to particular extents, its own altered regulation of gene expression and its own mutagenesis. For the essential trait of the cancer cell, as we have said before, is liberation from the tissue regulatory signals of the organism, as acquired in the course of becoming amoeba-like [1].

Investigating what force drives the proliferation of cells engaged into an oncogenic process is stepping into a 'forbidden zone', the sight of which has long been lost by clinical and experimental oncology. We are here referring to the patho-physiological and altered metabolic constraints operating within an 'organism', that might likely promote the onset and development of cancer. It is instructive to remember that, by raising the spectrum of neo-lamarckism with the suggestion of a post-adaptive role for retroviruses in the development of neoplasia, Howard Temin was already begging the question of what the patho-physiological constraints are that, in the cell environment, promote the malignant transformation of cells [18-20]. Indeed, whether experimental, virally-induced or 'wildtype' (auto-oncogenic), neoplasia may well be a perfectly logical, adaptive response to a changing tissue environment, to a changed environment that may not be able to support the degree of histological order and organic function that correspond to a high internal energy of cells, organs and 'organisms' (biological systems). Suppression of regulating genes - involved in modulating or controlling mRNA processing, DNA transcription, DNA repair and replication, or, in particular, the negative regulation of DNA expression - and increased or constitutive expression of oncogenes for growth factors or their receptors, etc, may all be changes secondary to attempts made by the stressed cell to continue to extract high energy from an energy-poor milieu.

Thus, making the case for a single oncogenic vector relating all the main manifestations of acquired cancer largely depends on whether a model of the etiology of cancer succeeds in identifying "the force" - ie the pressure and the coupled, fundamental cellular 'response' - that drives the proliferation of neoplastic cells, and which must be manifested at cancer initiation. It was often stated and taught in the recent past that there is no evolutionary pressure for the adaptations of cancer cells, but this view always took a static perspective of biological functions and processes. Moreover, it has become clear to us that if biologists and oncologists deny the existence of objective pressures in altered, cancer-promoting tissue environments, along with denial of the existence of post-adaptive processes in living systems, then they effectively preclude themselves from any understanding of the functional etiology of cancer. Likely, this failure will be dual - failure to understand the biochemical

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and bioenergetic basis of all cancers (if there is one, as the present communication suggests is largely the case), and failure to understand the articulation of the epigenetic and adaptive processes that transform a cell's genotype.

2. The cell-cycle and a molecular model of cellular autonomic regulation

The cell is the minimum definition of a living system. In a multicellular organism the integration of all organ and tissue activities (metabolic, proliferative and differentiating) is carried out by the functioning of the autonomic (vegetative) nervous system, of its cells and neuroendocrine mediators. In the single cell, this integration is strictly molecular, even if mediated by organelles. The cell still has an autonomic system that regulates action and reaction, but it is embodied by coupled and antagonic molecular signals. Its regulation is not so much multifactorial, as differential. The cell constantly pulsates or alternates, like a vegetative system, between different "neurohormonal" states that are counter-regulated by networks of positive and negative molecular factors operating both at the cell surface and inside the cell: growth is a metabolic expansion with anabolic (ie parasympathetic) and catabolic (orthosympathetic) phases; cell division and proliferation require a metabolic minimization, an orthosympathetic contraction, that ends up splitting a cell; quiescence also shuts metabolism down to a minimum, contracting the whole cell to a dormant state, but recruitment activates it, re-engaging the cell into the growth cycle; and differentiation is the result of multiple cycles of growth and proliferation that eventually either limit cell division and proliferation, or entirely abrogate them, condemning the differentiated cell to eventual apoptosis.

The entire cell cycle is a vegetative system (see **Fig. 1**). At the entry into G_1, the first interphase, the cell either chooses to expand, by growth (during the interphase), or to contract, by entering quiescence (G_0). Recruitment from the quiescent state is another parasympathetic action that returns the cell to G_1. At some point, the G_1 expansion is interrupted by a commitment to proliferate, and the cell growth now includes DNA replication, the S phase. After a brief return to a second interphase, G_2, when the cell prepares to divide, the contraction of mitosis survenes and the cell duplicates itself. At every cellular level, from the regulation of DNA expression to the protein-enzymatic signalling circuits, the molecular 'nervous system' of the cell has its effectors - at once mediators and modulators. And the entirety of the progression of the cell through the cell cycle - from the transition between phases to phase arrests or 'suspensions' - is controlled by these effectors. Whenever these intracellular regulatory circuits intersect or overlap, negative and positive regulators are found. Thus, those ambiguities of microRNA genes and tumor-suppressor genes that might not be artifactual are explained as so many more points of vegetative regulatory control by antagonic molecules or signals of the same families.

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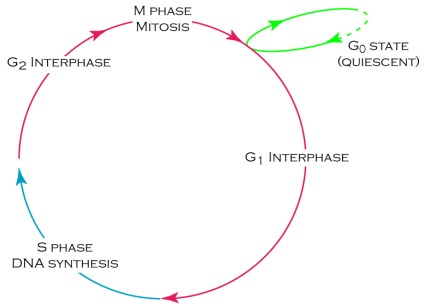

Fig. 1 - The cell cycle and its phases.

Operation of the regulatory network of a cell by the balance of positive and negative stimulatory signals is characteristic of a sustained, stable, homeodynamic G_1 metabolism, one that normally couples pyruvate-producing glycolysis to oxidative phosphorylation (aerobic metabolism). However, the replication event constitutes a special variant of this regulatory or 'vegetative' functioning. In it, one arm of the vegetative system determines the "autoschizis" (the self-splitting) of a cell, not as an apoptotic event (which is the usual sense of autoschizis as coined by Jacques Gilloteaux and his group [21]), but as *a mitotic event*, as the central event of cell proliferation. The cell divides or *splits* as a result of a prolonged contraction, as the replication event (at the level of the DNA and at all other cellular levels, including the replication of organelles and the production of extra plasma membrane) is controlled by a chemically-mediated and invisible orthosympathetic set of molecular effectors. Once the process has seeded two new cells, the homeodynamic processes of metabolism and balanced stimulation restart. It is clear from this that there is an intimate cellular link of orthosympathetic contraction (cellular division) with a model of cell death (apoptotic division or conventional autoschizis), and that it is this link that anchors reproduction (DNA replication, cellular duplication and proliferation). To divide in two, the cell in a way must die. A parallel link to cell death also exists in differentiation processes. Here, the cell loses its proliferative ability by committing to a process of terminal proliferation that either leads to senescence or to differentiated states which endure by slow forms of 'apoptosis' (eg progressive loss of cytoplasts by erythrocytes crossing the spleen, or progressive loss of all cellular structures in the outer skin layers or hair strands, etc).

Now, the homeodynamic metabolism maintained by regulatory factors is based on minimum conditions, which include, precisely, sufficient oxygenation.

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3. The Warburg effect and the Warburg hypothesis

Capitalizing on the work on dehydrogenases carried out by T. Thunberg, and F. Batelli and L.S. Stern in the period of 1910-1920, many researchers during the 1930s found that, in the presence of oxygen, minced-tissue suspensions oxidized a varied of organic acids to carbon dioxide and molecular oxygen. Then, in 1935, Albert Szent-Györgyi ordered these reactions in a manner that effectively identified the second half of the tricarboxylic acid cycle - from succinate to oxaloacetate. He suggested they were all reactions that followed the modification of a physiological substrate which he presumed was glycogen. The relay was picked up by Hans Krebs in 1936, who identified the full cycle (see **Fig. 2**) - from citric acid to oxaloacetate - and the input substrate required for oxaloacetate regeneration as pyruvate, if oxygen was available as electron donor [22]. In 1937, Krebs called it "the citric acid cycle". It is the central circuit of respiratory metabolism. The input is actually the acetyl group of acetyl-CoA, derived from the oxidation of pyruvate, and the converging end-product of all the catabolic reactions that degrade proteins, lipids and polysaccharides (called stage II of catabolism). Even though the "Krebs cycle", as is also known, is considered to form - along with the associated electron transport chain and oxidative phosphorylation (see **Fig. 2**) - a stage III of catabolism, it is better understood as an *amphibolic* circuit. In it, all *catabolic pathways converge* to oxidize to carbon dioxide and water all the products of stage II catabolism, and from it all *anabolic pathways take off in divergent directions* by formation of the smallest molecules or building-blocks - substrates and energy-carriers such as ATP - required for biosynthesis. The Krebs cycle is a pivotal element between catabolism and anabolism, a circuit shared by both metabolic activities - hence amphibolic. Through electron-carrying coenzymes, catabolism generated an oxidized fuel from an input of reduced fuel and oxidized coenzymes, while, conversely, anabolism generated reduced biosynthetic products from reduced coenzymes and oxidized substrates. Finally, in 1948, Eugene Kennedy and Albert Lehninger localized the entire Krebs cycle to mitochondria, by separating them from rat-liver homogenates via differential centrifugation.

In his reasoned approach, Krebs had addressed the poisoning of the citric acid cycle by malonate, which impeded the regeneration of oxaloacetate. The malonate block competitively inhibited (due to structural similarity with the substrate succinate) the enzyme succinate dehydrogenase, leading to accumulation of succinate in malonate-poisoned muscle. This insight converged with the 'Warburg hypothesis', and before the advent of molecular genetics, suggested that there could be a unitarian biochemical explanation for the etiology of cancer. The 'Warburg hypothesis' suggested that 'oxidative enzyme-poisoning' promoted a metabolic switch responsible for neoplastic transformation. Cancer cells from all diverse tumors seemed to share a universal trait - their metabolism was distinctive, which became known as the 'Warburg effect' [23]: their rate of oxygen consumption was lower

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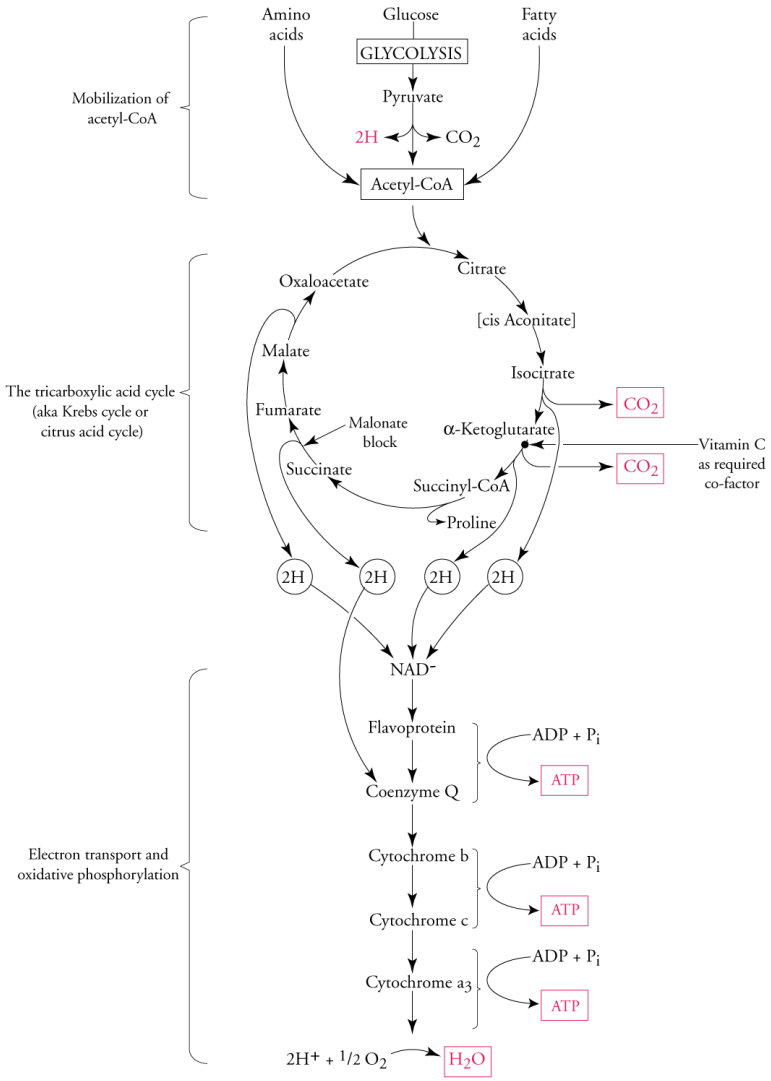
Fig. 2 - Diagram of aerobic metabolism comprising the Krebs cycle (amphibolic circuit), electron transport and oxidative phosphorylation.



than normal cells, yet they consumed 5 to 10 times more glucose, converting most of it into lactic acid and, thereby, depressing respiration (or mitochondrial extraction of energy from the electron transport chain via oxidative phosphorylation).

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The metabolic pathway chosen by cancer cells was termed lactic fermentation or 'aerobic glycolysis' (also 'aerobic fermentation', as opposed to alcoholic fermentation characteristic of anaerobic glycolysis), and it was driven by the enzyme lactate dehydrogenase A (LDHA) (for an introduction see [24]). Aerobic glycolysis goes on in the cytoplasm of eukaryotic cells, and RBCs - which lack mitochondria (ie the organellar sites of oxidative phosphorylation and the Krebs cycle) - entirely rely on aerobic glycolysis for their metabolic energy. The shunt of glycolysis towards lactate production essentially breaks off control of the rate of glycolysis afforded by matching it to the utilization of pyruvate in the Krebs cycle. This led biochemists to the notion that the normal allosteric enzymes regulating the rate of glycolysis and limiting lactate production via the "Pasteur effect" - to match the rate of glycolysis to the rate of utilization of pyruvate by pyruvate dehydrogenase in the Krebs cycle - were defective, blocked or altered in cancer cells. Once glycolysis was severed from both the Krebs cycle and oxidative phosphorylation (respiration), and shunted towards lactic fermentation, the rate of glycolysis could be 'wildly' increased for purposes of sustaining the 'wild' proliferation of the cancer cell. This explained why cancer cells needed to utilize large quantities of blood glucose. Once pyruvate was shunted into lactic fermentation, aerobic respiration was shut down.

At the time, it appeared that all tumor cells - whether grown in solid tumors in animals or in tissue culture - and most transformed cell lines (eg transformed cells from fibroblast culture foci have increased rates of transport and uptake of glucose, and this is one of the transformation markers) had in common the excretion of much greater quantities of lactic acid and free protons (acid ion) than do normal cells, and consume more glucose than they need to grow. These two "universal facts" indicated that cancer cells fundamentally rely upon a glycolytic metabolism to obtain their energy. James Watson called this increased glycolysis, the "one biochemical difference of real significance" so far found for all cancer cells [25]. Szent-Györgyi proposed a model where the cancer cell switched back from what he called a beta metabolism (aerobic respiration) to a more primitive, oxygen-independent form of metabolism (fermentation as an alpha state of metabolism, anaerobic in prokaryotes and lactic in eukaryotes) [26].

Wilhelm Reich summarized succinctly the 'Warburg hypothesis' by stating: "cancer metabolism is to be viewed as the metabolism of normally growing cells in a condition of anoxia" [27]. However, the basis (poisoning of oxidative enzymes by dynamic clocking) given by Warburg for his hypothesis was not validated. Furthermore, given the heterogeneity of cells even in a single tumor, there was doubt as to whether all malignantly transformed cells really had shunted to lactic fermentation. Louis Siminovich and Arthur Axelrad wrote back in 1960 - in a communication reviewing the then existing problems in the understanding of cancer biochemistry [28]:

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> "The modern era of cancer biochemistry began more than 30 years ago when Warburg demonstrated that the malignant tumors he examined were all similar to each other in having relatively high rates of aerobic glycolysis and relatively low respiratory rates. From these facts Warburg developed the broad concept that an alteration in oxidative metabolism is the essential feature of all cancer cells and that a defect in respiratory metabolism is the basic cause of the malignancy. Although many biochemists did not accept this hypothesis, they did continue to use his technique of comparing a large variety of tumors with normal tissue in the hope of finding one or more common and unique biochemical defects responsible for the malignancy."

Yet, as oncology research progressed, there were no unique biochemical defects to be found. Moreover, it was vexing to realize that cancer cells engaged in lactic fermentation even though they did not lack any of the genes and enzymes required for oxidative phosphorylation or the Krebs cycle. Nor were these genes mutagenized. The increased glycolysis tallied with the much faster than normal rates of replication characteristic of cancer cells, but the variety of genetic alterations observed in cancer cells also did not directly appear to affect metabolism. Worse, still, the universal feature of all cancer cells turned out not to be so universal after all. In fact, oxidative phosphorylation was still going on in many cancer cells. The metabolic switch was not necessary for malignant transformation. This raised even more difficult questions - such as, how do some cancer cells keep oxidative phosphorylation going when tumors are hypoxic (not to mention that tumors occur in bodies affected with systemic hypoxia)?

Despite these facts and spiny questions, and even though the "pessimistic view" once expressed by Siminovich and Axelrad, back in 1960, was well founded and holds true to this day - to quote them, "it may be that even if we accumulated many more facts of the kind that we have now, it might still not be possible to work out a rational explanation of the cancer process in biochemical terms" [28] - we hold instead that oncology has been, ever since Warburg, on the path to understanding the rationale of the main decision that commits a cell to a process that leads to neoplastic transformation (this is so irrespective of the invalidity of oxidative enzyme-poisoning as the mechanism responsible for the Warburg effect, and of the nonuniversality of lactic fermentation as the metabolism of cancer cells). But if it has had the tools, why did the biochemical approach to the etiology of cancer not succeed?

It is true that some discredit rubbed off on the Warburg hypothesis when it became tainted with scientific fraud. In the late-days of the oncogene theory of cancer, it appeared certain - from reports written by Ephraim Racker, then the Albert Einstein Professor of Biochemistry at Cornell

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University - that a membrane-associated ATPase involved in sodium transport was deficient in all tumor cells, consuming excessive ATP, and leading to the Warburg effect. An oxygen-independent explanation had been "found", that effectively explained away the increased glycolysis of cancer cells. Racker's PhD student, Mark Spector, was in charge of conducting the experiments, and he identified a cascade of four membrane-coupled kinases that supposedly activated the ATP-dependent sodium-potassium pump, and thus confirmed Racker's preferred model of cancer causation [29]. When researchers in other laboratories were unable to replicate the findings, an academic investigation was initiated which eventually discovered that Spector had incorporated radioactive iodine into his protein preparations to produce a signal that could be mistaken for the radioactive phosphate that would have been supposedly absorbed by the activated kinases. Racker was exonerated of any wrong-doing, though he was subsequently eased out of Cornell, but all the peer-reviewed journals or magazines, such as *Science*, that had published Racker's papers after 1981, had to retract them.

This contributed to the oblivion of the Warburg hypothesis, but it was not the main factor in its demise. The fact is that no single universal process of cellular transformation exists, and thus a rational explanation in biochemical and metabolic terms of all the steps in each process, or varieties of processes, may well elude us. Thus the Warburg hypothesis does not seem to hold. Only when and if it is possible to integrate all oncogenic processes into a single vector can consideration of a unitarian metabolic hypothesis take place. It is apparent that cells engaged in rapid proliferation must consume more glucose than interphase cells do, and rely exclusively upon glycolysis for extraction of chemical energy; and it is apparent that all cancer cells consume more glucose than normal cells that are dividing. But what are the connections of these changes to varied degrees or intensities of transformation? Moreover, recent discoveries have shown that aerobic glycolysis (the Warburg effect) is not a universal trait of all cancer cells (more on this below). How, then, do neoplastic cells preserve some modicum of oxidative phosphorylation? Confronted with these facts and questions, it would seem that no unitarian biochemical or bioenergetic theory of the etiology of cancer could be possible. It was even unclear (up until recently, that is) how cells regulate uptake of glucose, its transport and utilization. Yet, as we shall see, Warburg was onto something profound. Hypoxia constitutes the main neoplasia-promoting stress in the cellular environment. In an hypoxic environment, the cell has no alternative but to engage in LDHA-driven lactic fermentation, as oxygen is one of the activators required for oxidative phosphorylation. But something else had to be understood - how some cancer cells sustain oxidative phosphorylation and do so, not by appending it to glycolysis, as normal aerobic metabolism does to extract pyruvate, but by expressing hypoxia-independent proteins (eg MCT1) and a different enzyme, LDHB (lactate dehydrogenase B), which permitted them to uptake lactate and use it in the presence of oxygen (at lower partial pressures) to carry on mitochondrial oxidative

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phosphorylation [30]. LDBH is a key enzyme in gluconeogenesis, which reversibly converts pyruvate to lactate and is normally only expressed in liver cells.

4. A unitarian biophysical and biochemical (aetherometric) model of oncogenesis: integration of hypoxic and non-hypoxic cancer-promoting stresses

4.1. The complete view of the biochemical and biophysical effects of hypoxia

Hypoxia may well turn out to be the main factor or condition behind a variety of different diseases including ischemic heart disease, stroke, acquired cancer, chronic lung disease, and congestive heart failure. Apoptosis of tissue (primary) cells can be induced in response to hypoxia [31-32]. However, most tissue cells have mechanisms that respond to hypoxia and evade cell death [33] (as a word of caution, most studies of the apoptotic and necrotic effects of hypoxia have been performed with immortalized or transformed cell lines). If the hypoxia is severe (as in ischemia), primary cells become cyanotic and necrosis sets in. However, in most hypoxic conditions, tissue cells epigenetically adapt by rapidly altering their gene expression program, and this is mainly, but not entirely, carried out by expression of hypoxia-inducible factors (HIFs) that upregulate genes controlling glycolysis and repress expression of genes involved in cellular processes that consume ATP [33]. Through the HIFs, hypoxia stimulates expansion of all hematopoietic progenitors, *increasing their sensitivity to regulatory factors* and factor production by accessory cells [34]. As we shall see below, these responses are primed and modulated by the IGF axis, in particular by the IGF-IR circuitry.

Hypoxia constitutes therefore an adaptive pressure to which the normal cell responds via HIF-dependent and HIF-independent pathways. We now suggest that this is also the main, but not sole, constraint that, upon its aggravation or becoming chronic, selects the adaptive responses involved in neoplasia or, to be exact - involved in both the initiation and the progression of oncogenesis. It may be referred to as an environmental stress, but it is most frequently a dynamic stress of the internal environment of an organism. At some threshold of hypoxia - probably brought about by a prolonged state and its anoxic intensification - survival of tissue cells no longer depends upon the altered gene expression program, but prompts a new adaptive and mutational response capable of supporting a state of rapid proliferation.

Back in 2004 [35], we put forth an hypothesis (the aetherometric model) regarding oncogenesis, and its bioenergetic and biochemical nature, as well as its connection to the analytical model of the repression of biological impulses (rather than "instincts", *Triebe*). In essence, an 'over-organized' biological system (read: "*organismicized*", ie a system subject to chronic orthosympathetic stimulation or sympatheticotonia) becomes progressively unable to continue to accumulate and order its internal energy. This is fundamentally expressed - in a manner largely following that of W. Reich's orgonom-

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ic model of cancer [36]- by deficient oxygenation of RBCs, and their incapacity to provide chemical energy to the tissues. In this respect, however, our aetherometric model [35, 37] contributed the demarcation of two new essential functions for molecular oxygen, one biophysical and the other biochemical:

1) *biophysically*, oxygen is resonant with the main mode of the ambipolar radiation emitted by the Sun [35, 37-38], and can therefore be used as the tuned antenna element of molecular circuits designed to capture the energy of this radiation.

2) connected to this property, but *biochemically* ('*aetherobiochemically*'), molecular oxygen shunts the captured ambipolar energy *either* (1) to complete the electron transport of the respiratory chain [39-40] (in which case the captured energy becomes the kinetic energy of the transfer electrons, which is what is meant by "chemical energy"), *or* (2) to be absorbed by a molecule of hemoglobin or myoglobin (to which oxygen is noncovalently bound) that subsequently radiates the captured energy thermally (true dissipation) [37, 41]. The former is the function of oxygen delivered to the tissues, the latter is the function of oxygen noncovalently bound to hemoglobin in circulating RBCs, or to myoglobin in muscle tissue.

We suggest that disruption of these biophysical and biochemical energy functions of oxygen plays a key role in determining a cell to commit to an oncogenic vector. Lack of oxygen affects the biophysical (thermal dissipation of acquired ambipolar or electromagnetic energy) and biochemical (purveyance of oxygen) functions of hemoglobin, as well as the biophysical (conversion of captured ambipolar energy to the kinetic energy of 'reactive' electrons) and biochemical (oxygen as fuel for biochemical conversion into water) functions of aerobic metabolism. We should note all that this implies. Deficiently oxygenated hemoglobin is unable to provide oxygen to tissues, but is also unable to efficiently capture solar and environmental ambipolar radiation in the beneficial portion of the Tesla wave spectrum [38], and thus unable to directly provide tissues with dissipated thermal energy converted from absorption of ambipolar energy. Moreover, worse still, deficient oxygenation of tissue means that oxygen is not available to sustain respiration and this directly impacts the aerobic metabolism of tissues, specifically by failing to support cytochrome functions. The problem is not just a lack of chemical substrate to complete oxidative phosphorylation (and generate water from oxygen), but also that - according to our aetherometric model of aerobic metabolism [39] - the reaction of pyruvate to form acetyl CoA (see **Fig. 2**) fails to take place. In other words, initiation of the Krebs cycle is not supported.

Thus, according to the aetherometric view, hypoxia entails far more than merely deprivation of oxygen qua source of chemical energy. Hypoxia effectively means that systemically and locally, the main biophysical process of *direct* energy capture from solar radiation in the tissues and RBCs of non-

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photosynthetic aerobes is also blocked, resulting in an inability to complete the electron transport chain and putting an additional stress on the thermal regulation of homotherms (which in turn translates into a still higher organismic demand for energy). Hypoxia intervenes at different levels of cellular function. It is likely the main adaptive pressure to which epigenetic and adaptive strategies involved in non-familial oncogenic transformation respond.

Lack of oxygen thus affects two different enzymatic reactions and two different kinds of allosteric proteins, both of which operate by absorbing ambipolar radiation - or electromagnetic derivatives - when oxygen is available: on one hand, hemoglobin and myoglobin, and on the other, the cytochromes found only in aerobic cells. As we will suggest below, hemoglobin and myoglobin operate by resonant capture of radiant energy (like an antenna) and release of thermal photons (via the pigment function) when molecular oxygen is noncovalently bound to them, at the reduced iron of the heme group. Since they do not undergo valence changes as oxygen is bound or unbound to them, the iron remains in the Fe(II) state and the oxygen can be easily detached and transferred to tissue (when hemoglobin or myoglobin are oxidized to the Fe (III) state, their color changes from red to brown). Conversely, cytochromes undergo reversible Fe(II)-Fe(III) valence changes in their redox catalytic cycles, with their protoporphyrins changing between heme and hemin states, and thus they work as electron carriers rather than oxygen carriers. But, as we will propose below, the kinetic energy of the electrons shuttled by the cytochromes is ultimately transferred from oxygen which, in turn, absorbs it also from environmental radiant energy.

4.2. Ambipolar antenna function of hemoglobin is mediated by oxygen

In general, both myoglobin and hemoglobin absorb blue (blackbody) light at 450 nm and green light at or near 550 nm, when the active site is unoccupied (deoxymyoglobin or deoxyhemoglobin forms). Upon oxygenation their electromagnetic absorption for blue light increases by 150% [42] (see **Fig. 3**), a smaller increase being observed for green light displaced to 535nm, and a third peak, of yellow light, appearing near 580 nm. Yet, arterial blood which is rich in oxygen has a deep red colour (emits red photons) - whereas venous blood looks blue or cyanotic. Why? We have suggested that this is so because the iron porphyrin (heme) and the oxygen noncovalently bound to it respond to very different energy stimulations that are *not* electromagnetic.

From our work, we now know that the atmospheric formation of oxygen is driven by solar ambipolar radiation of 28.8 keV [43] and that the byproduct of this reaction is sensible heat emitted - at the long-wavelength limit of visible light - in the form of photons in the IR range (842nm, near the limit of red visibility). Iron, in turn, turns red-brown when oxidized by hydration, ie it rusts. It is therefore rather likely that, by oxidizing iron, oxygen still retains its property of response to ambipolar radiation in the 28keV range, even if somewhat reduced in wavelength. *A fortiori*, an even more

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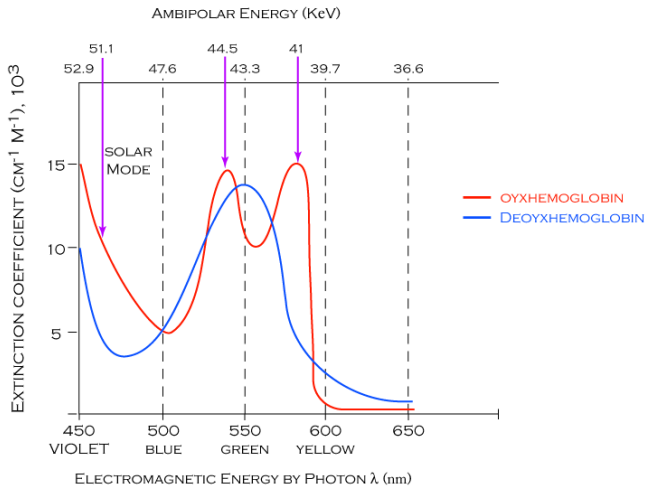

AMBIPOLAR ABSORPTION PEAKS

OXY	DEOXY
52.9 KeV	52.9 KeV
44.5 KeV	43.3 KeV
41.0 KeV	
28.8 KeV	

Fig. 3 - Corresponding visible light energy absorption spectra by wavelength (lower X axis) and dark ambipolar energy absorption spectra in keV (upper X-axis) for hemoglobin.

favorable situation for resonant emission occurs with an iron-oxygen complex that is noncovalently bound by the interference of the so-called "picket fence" structure (discovered by J. Collman [44]) of the iron porphyrin, which prevents the oxidation of iron from taking place. Trapped noncovalently by an hemoglobin molecule with a "fenced in" iron, the oxygen molecule appears therefore to remain particularly affine for ambipolar radiation near 28.8keV, or for ambipolar waves carrying a potential of 28.8kV, such that the very enzyme encapsulating the trapped oxygen luminates red to our eye.

We have therefore suggested that oxygen is a molecule capable of serving as substrate for ambipolar radiation [37, 41], specifically resonant at 28.8 keV, and that the whole art of the vertebrate aerobic cell lies in employing an allosteric protein that resonantly traps oxygen and appropriates its ambipolar resonance to "steal" (re-absorb) the released IR photons or sensible heat, as is optically marked by the very redness of the oxygen-iron complexes. Moreover, since oxyhemoglobin absorbs more efficiently in the blue, green and yellow ranges of optothermal radiation (see **Fig. 3**), hemoglobin is capable of transducing both electromagnetic excitations of wavelength smaller than 600nm, and ambipolar energy in the range of 28-29keV, into longer wavelength red or infra-red photons, ie electromagnetic heat. It is, therefore, a photon-to-photon long-wavelength converter, as much as an ambipolar-to-photon converter. Arterial blood rich in oxyhemoglobin has the highest capacity to

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absorb energy and visible light, and transform either into the heat it continually releases to the organs. Such heat release is essential for the thermal regulation of homeotherms. Thus, lack of oxygen - via the biophysical function of deoxyhemoglobin - introduces a thermal stress.

However, in the aetherometric model, hemoglobin does not just absorb 29keV ambipolar radiation in its oxyhemoglobin form. Though we have not presented this before, the ambipolar absorption spectrum of hemoglobin in both of its dynamic forms spans higher energies than this (see **Fig. 3**), and reaches its peak at 52.9keV, near the solar main mode of ambipolar energy (51.1keV) [38]. Oxyhemoglobin is evidently the main energy absorber of both photon energy and ambipolar energy, with two other peak ambipolar energy absorptions comparable to that of 52.9keV occurring at 44.5keV and 41keV (see **Fig. 3**). Unlike the absorption at 28.9keV, these ambipolar absorptions of oxyhemoglobin probably involve the porphyrin ring, specifically the covalent bonds of iron to nitrogen. Since the main photon emission of oxyhemoglobin lies in the red to infra-red wavelength, oxyhemoglobin must internally reduce the electron kinetic energy obtained from ambipolar absorption at 41, 44.5 and 52.9 keV, down to ~29keV, before red and IR photon emission occurs. As for deoxyhemoglobin, it only has one other ambipolar absorption peak besides the 52.9keV peak, at 43.3keV, the absorption maximum (see **Fig. 3**). It is these energy absorption bands that are responsible for its cyanotic blue color, and its lack of thermal photon emission. This suggests that it carries out no internal downconversion of kinetic energy, emitting a mix of violet and green photons slightly longer in wavelength than those absorbed at 450 and 550 nm, or slightly longer in wavelength than those that would be emitted directly from absorption of ambipolar energy at 43.3 and 52.9keV.

4.3. Ambipolar antenna function of Cyt *c* oxidase is mediated by oxygen, and explanation for the missing energy driving the respiratory chain

Deficient oxygenation of tissues results in the shutting down of aerobic metabolism. Oxygen intervenes at different points in the respiratory chain. According to our aetherometric model, oxygen initiates the chain by injecting electrons with high kinetic energy, and it completes the chain by permitting cytochrome (Cyt) a_3 - and its associated complex - to also act as an ambipolar antenna, while serving as substrate for the production of water. We will next present our argument for these multilevel functions of oxygen in aerobic metabolism.

The overall biochemical equation for the respiratory-chain phosphorylations is usually written as:

$$NADH + H^+ + e^- + 3ADP + 3P_i + 0.5\ O_2 = NAD^+ + 4\ H_2O + 3\ ATP$$

NAD^+ (nicotinamide-adenine dinucleotide) and NADH, like FAD^+ and $FADH_2$, are flavins

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(redox pairs of pyridine nucleotides) that function as local shuttles of charge in the metabolic transfer of electrons, and which are dehydrogenated (or oxidized) by specific enzymes known as dehydrogenases. The overall respiratory chain reaction is decomposed into endergonic and exergonic components. The endergonic component is written for the accumulation of latent heat in ATP once the exergonic component is released, as:

$$3ADP + 3P_i \text{ --> } 3ATP + 3H_2O$$
$$\Delta G^{o'} = 3*7.3 \text{ kcal mol}^{-1} = +21.9 \text{ kcal mol}^{-1} = 91.69 \text{ kJ mol}^{-1}$$

The exergonic component is essentially what drives the reaction - from a caloric viewpoint - since it releases the NAD-H covalent bond (from reduction of the nicotinamide part) to reduce the oxidants O and H^+ to water:

$$NADH + H^+ + e^- + 0.5\ O_2 = NAD^+ + H_2O$$
$$\Delta G^{o'} = -52.7 \text{ kcal mol}^{-1} = 220.6 \text{ kJ mol}^{-1}$$

The driving force of the respiratory chain and its oxidative phosphorylation is the electron-transfer potential of NADH or $FADH_2$ corresponding to the exergonic reaction. The oxidation of NADH by O_2 involves two partial reactions - which, in the notation of the biochemical redox convention (BCC), give:

1. $2H^+ + 2e^- + 0.5\ O_2 \text{ -->} H_2O$
   $E'_0 = +0.816V$

2. $NAD^+ + H^+ + 2e^- \text{ --> } NADH$
   $E'_0 = -0.32V$

Subtracting the second reaction from the first gives:

$$\Delta E'_0 = (+0.816V) - (-.32V) = +1.136V$$

The reader should note that the same reaction could be written in the aetherometrically preferred

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notation of the electrochemical convention (ECC), as:

1. $2H^+ + 2e^- + 0.5\ O_2 \text{-->} H_2O$
$E'_0 = -1.237V$

2. $NADH \text{-->} NAD^+ + H^+ + 2e^-$
$E'_0 = -0.101V$

Subtracting the second reaction from the first gives:

$\Delta E'_0 = (-1.237V) - (-0.101V) = -1.136V$

This, however, no longer qualifies the reaction as an exergonic reaction by its positive $\Delta E'_0$ - as the BCC convention actually forces one to do.

With $n=2e^-$, the Gibbs free energy of oxidation is then obtained by using F, the caloric equivalent of the faraday (23.062 kcal V^{-1} mol^{-1}), to give:

$\Delta F_E = \Delta G^{\circ\prime} = -n\ F\ (\Delta E'_0) = -2\ (23.062\ \text{kcal}\ V^{-1}\ \text{mol}^{-1})\ (-1.136V) =$
$= 52.4\ \text{kcal}\ \text{mol}^{-1} = 219.39\ \text{kJ}\ \text{mol}^{-1}$

The respiratory electron-transport chain is conventionally divided into three spans - referred to as steps I, II and III - that are thought to constitute energy-coupling sites. Each step (see **Fig. 4**) generates a molecule of ATP through the agency of 3 distinct enzyme complexes (NADH-Q reductase or Complex I, QH_2-cytochrome c reductase or Complex III, and cytochrome c reductase or Complex IV), while shuttling a pair of electrons down the chain (in red in **Fig. 4**). The three molecules of ATP generated per electron transport cycle accumulate in the mitochondrial matrix and are transported to the cytosol by specific carrier enzymes, called translocases or porters, that simultaneously import ADP^{3-}.

The entire chain takes place inside mitochondria (see **Fig.s 5** & **6**), in the intermembranous space between the inner and the outer mitochondrial membranes, with all of the cytochromes (heme-containing enzymes) belonging to complexes III and IV being located at two distinct sites on the inner membrane. The ATPase complex that generates ATP from ADP and phosphate, is also localized on the inner membrane.

The chain can be written as a sequential enzyme series of steps:

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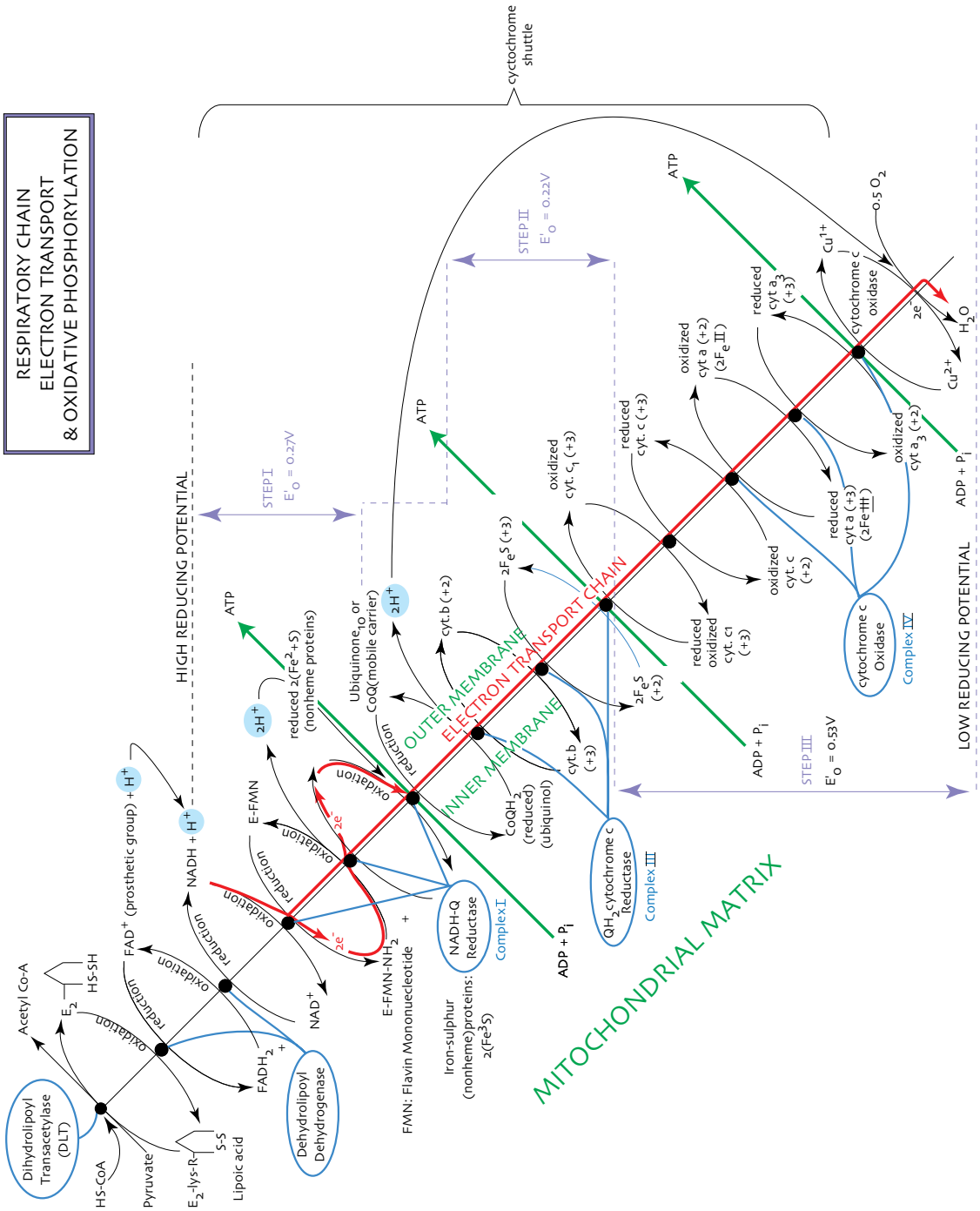

Fig. 4 - Detailed x-section diagram of the respiratory chain: electron transport and oxidative phosphorylation.

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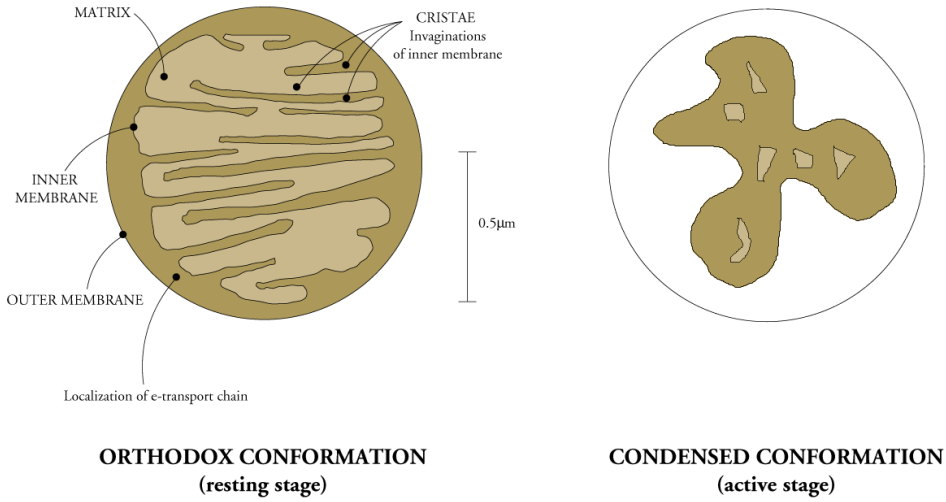

Fig. 5 - Schematic of a mitochondrion in either the orthodox conformation of the resting stage (left) or the condensed conformation of the active stage (right). After A. Lehninger, "Biochemistry", p. 519, Fig 19-10.

$$NADH+H^{+} \rightarrow E\text{-}FMN\text{-}NH_2 \rightarrow Fe\ S \rightarrow QH_2 \rightarrow Cyt\ b \rightarrow Fe\ S \rightarrow Cyt\ c_1 \rightarrow$$
$$\rightarrow Cyt\ c \rightarrow Cyt\ a \rightarrow Cyt\ a_3 \rightarrow O_2$$

Initiation of the chain absorbs two protons per NADH molecule being oxidized; the chain shuttles these protons and finally releases them with the oxidation of ubiquinol ($CoQH_2$), to donate them, together with the pair of electrons from the chain, to the formation of a single water molecule.

There are three main models that explain the electron-transfer process (the "mechanism") of oxidative phosphorylation [45]. The first model - the chemical-coupling hypothesis - postulated the existence of a high-energy compound generated by the electron-transport chain and employed as a reactant to form ATP. But such an intermediate has never been found. The second model - the conformational-coupling theory of P. D. Boyer et al [46] - argues that energy released by the electron chain is absorbed by conformational changes of the coupling ATPase enzyme, ie by the formation of a large number of noncovalent (weak) bonds - the latent heat of conformation being a transient state before its transference into the latent heat of the covalent phosphate bond added to ADP in order to form ATP. The last model - the chemio-osmotic model [47] - postulates that there is a proton pump at work

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through the respiratory chain, pushing protons from within the mitochondrial matrix to the cellular cytosol, as the coupling mechanism for the production of ATP by each of the enzyme complexes. This requires, amongst other modifications, that the re-arrangement of the $FADH_2$, NADH and E-FMN coupled redox pairs be altered. Mitchell's model further requires the translocation of 6 protons per pair of electrons being shuttled (see **Fig. 6**), whereas only two protons are in fact generated through the oxidation of ubiquinol (see **Fig.** 4).

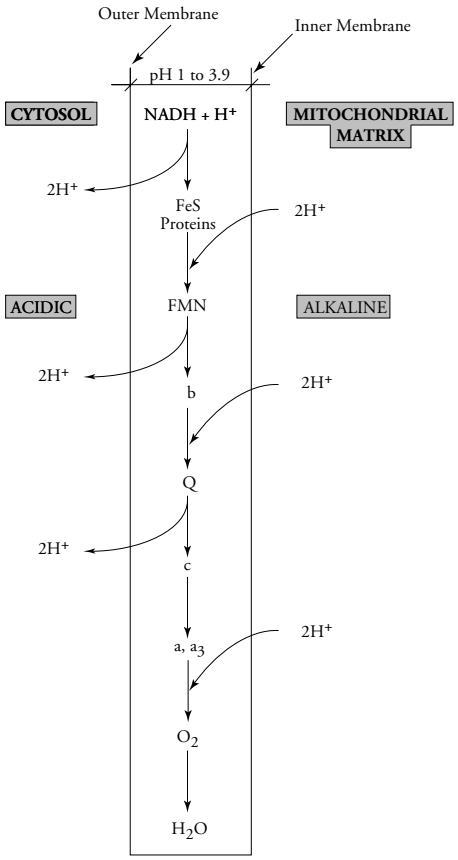

Fig. 6 - The mitochondrial proton shuttle between inner and outer membranes.

The problems with all three models point to the lacunes in our bioenergetic and biochemical understanding of the respiratory chain. Specifically, little is firmly known about the precise location and function of the iron-sulphur proteins (the nonheme proteins) involved in the shuttle process. Cytochrome b has two different forms with distinct redox potentials, and it is unclear whether copper is really present at the end of the chain. Lastly, and as shown by our aetherometric treatment, the energetics of the process such as they are conventionally treated do not exactly tally.

But the conformational-coupling hypothesis has several fundamental virtues - it permits an understanding of a flux of latent heat between noncovalent and covalent structures, it does not require Cu at the end of the chain, and it has a precedent in the actomyosin system responsible for the contraction of skeletal muscle.

More important to our present inquiry is to examine the energetics of the respiratory chain. We have summarized these - differently from their usual representation - in **Fig.** 7, where the standard redox potentials at pH 7.0, by both BCC and ECC conventions, are shown as scales in red, and the aetherometric eV scale is shown in parallel in green. For each of the previously identified steps (see **Fig.** 4) accepted redox potentials $\Delta E'_0$ in volts are shown in square brackets in **Fig.** 7, next to the aetherometric values (obtained from the voltage equivalence of the real log of the electron concentra-

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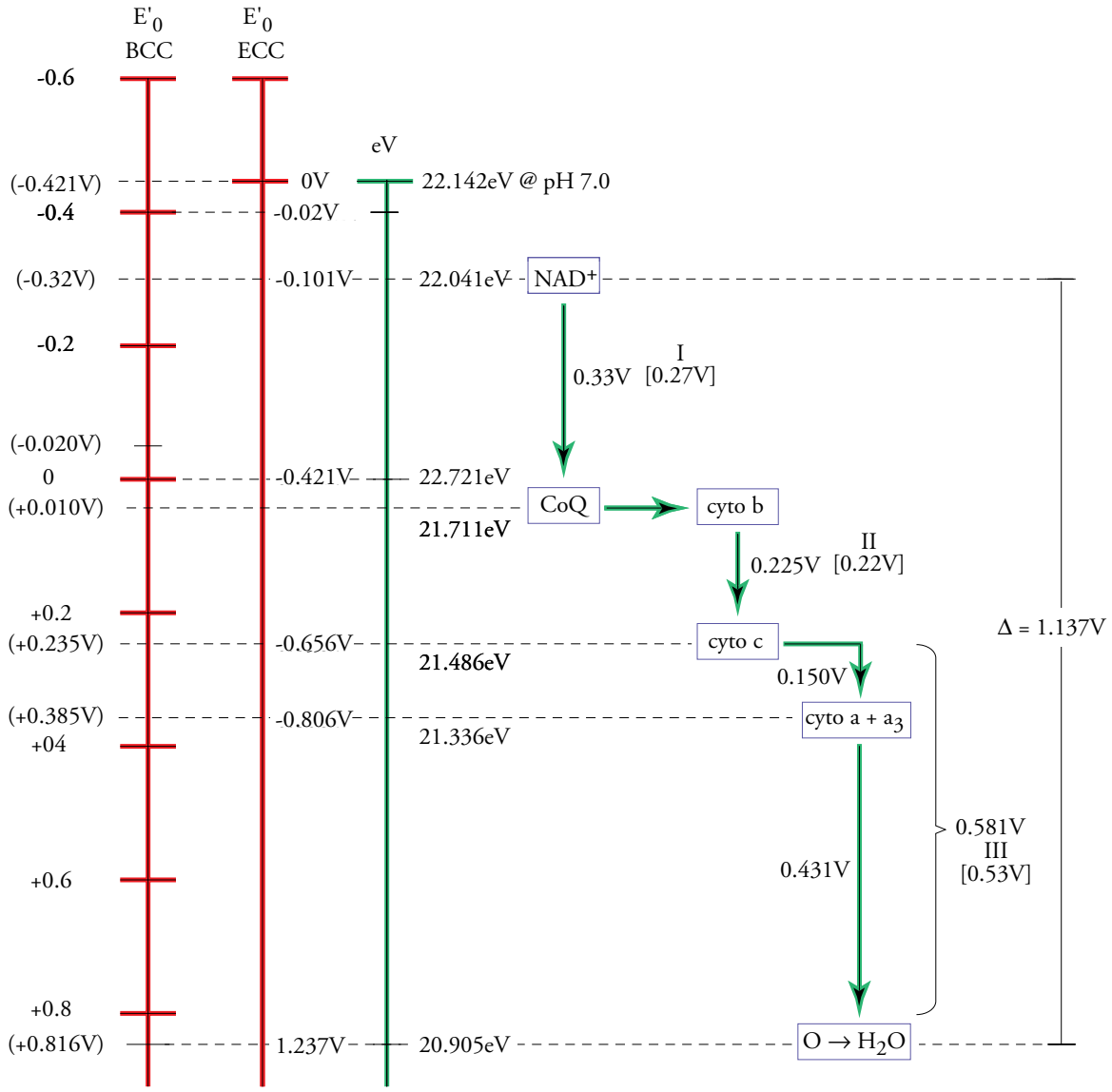

Fig. 7 - Aetherometric correlation of the Biochemical (BCC) and Electrochemical (ECC) conventions for electric potential, with the aetherometric 'true-voltage' scale based on the log of the concentration of electrons, as applied (in green) to the respiratory chain. After P.N. Correa & A.N. Correa [35].

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tion scale (see chapter 6 of reference [35]), which are slightly greater. The same redox potentials $\Delta E'_0$ can also be compared to the caloric values of each step:

Step I: 12.2 kcal mol^{-1} = 51.08 kJ mol^{-1}
Step II: 9.9 kcal mol^{-1} = 41.45 kJ mol^{-1}
Step III: 23.8 kcal mol^{-1} = 99.65 kJ mol^{-1}

To do so, we either use the caloric equivalent of the faraday introduced above, or use, instead, the aetherometric method, which we will detail taking step I as an example. The total caloric energy released by the electron transport is -

$$E_T = 50.6 \text{ kJ mol}^{-1} * (6.022*10^{24} \text{ m}^3 \text{ sec}^{-2} \text{ J}^{-1}) = 3.047*10^{29} \text{ m}^3 \text{ sec}^{-2} \text{ mol}^{-1}$$

Since this is released per transport of two electrons, the energy per transported electron is:

$$E_T/N_A \, n = 2.53*10^5 \text{ m}^3 \text{ sec}^{-2}$$

or, in electron volts:

$$2.53*10^5 \text{ m}^3 \text{ sec}^{-2}/(9.648*10^5 \text{ m}^3 \text{ sec}^{-2} \text{ eV}^{-1}) = 0.26225 \text{ eV}$$

corresponding to a $\Delta E'_0$ of

$$E_T/N_A \, n \, \mathbf{p}_e = 2.53*10^5 \text{ m}^3 \text{ sec}^{-2}/13.97017 \text{ m}^2 \text{ sec}^{-1} = 1.8*10^4 \text{ m sec}^{-1} = \int = 0.26\text{V}$$

(where $\mathbf{p}_e$ = 13.97017 m^2 sec^{-1} is the exact aetherometric value corresponding to the traditional charge e [48]). All three values of $\Delta E'_0$ for each of the steps I to III shown in **Fig. 7**, are compared in **Table 1**.

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TABLE 1
Accepted and aetherometric $\Delta E'_0$ values for the respiratory chain

	REDOX	**CALORIC**	
Steps	**$\Delta E'_0$ conventional (volts)**	**$\Delta E'_0$ (volts)**	**$\Delta E'_0$ aetherometric (volts)**
I	0.27	0.262	0.33
II	0.225	0.206	0.225
II	0.53	0.512	0.581
TOTAL	**1.025**	**0.980**	**1.137**

There is, by the caloric, 14% of the energy unaccounted for, and by the conventional redox ca 10%.

Moreover, the average value of the O-H bond of water is 2.37eV*2 = 4.73 eV. And to form water, two of these covalent bonds must be constituted, which means one needs 2*4.73 eV = 9.46 eV of energy that the two electrons or the two protons - or in fact both pair types - must contribute to the formation of a single water molecule from a single oxygen atom. Yet, when one tallies the energy of the respiratory chain, the proton contribution can be neglected because the high mass ratio between the proton and the electron makes the latter the preferential kinetic vehicle for energy. How, then, could the energy of

1.137eV*2 = 2.274 eV

account for the necessary 9.46 eV that are universally accepted as being equivalent to the value of each covalent O-H bond of water?

It only accounts, in fact, for 1/4 of the energy needed to form water, with ca 7.186 eV of the required energy still missing!!

Even if six protons were output by a proton pump - as Mitchell's hypothesis requires - and even if the mitochondrion sustained an alkaline gradient towards the more acidic outside by pump-

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ing six protons to the cytosol plus a 0.235V transmembrane gradient, the mitochondrion would still need to somehow supply six more electrons to transport down the chain, with the same kinetic energy, and then transfer this energy, somehow, to the two electrons going into the formation of water. In other words, either those two electrons have far more kinetic energy than they are assumed to have, or something else is contributing more than 3/4 of the energy required to drive that electron chain.

This glaring problem which aetherometry has isolated is not entirely unknown to biochemists: the great Lehninger in fact wrote, on this matter, very important words that are rarely quoted or taught to students of biochemistry:

> "Another uncertainty involves the number of electrons transferred in each step of the respiratory chain. It is generally believed that electron transport occurs in two-electron steps between NAD and ubiquinone and in one-electron steps from cytochrome b to oxygen. On the other hand, the reduction of one molecule of oxygen to two water molecules requires a total of four electrons. How electron flow in the respiratory chain is coordinated to yield four electrons to ensure complete reduction of an O_2 molecule is not yet known. One suggestion is that the cytochromes may function in pairs. This is an especially important question since partial reduction of the oxygen molecule yields extremely toxic products." [49].

Lehninger was here thinking of the superoxide and peroxide radicals - and this indeed brings home the impossibility of simply adding more electrons as a means to transport the energy required for the formation of water - once the production of those ATP molecules is deducted, of course.

As it turns out, both propositions are true: the shuttling electrons have more energy than is assumed, and something other than they - or the redox-paired cycle sequence - is contributing energy to the electron chain and the formation of water!

With regard to the first proposition - and without having to assume formation of either superoxides or peroxides - the aetherometric model indicates that for the chain to initiate with the oxidation of NADH, water must be formed and stable, as a low kinetic state in the overall reaction, at an energy level of 20.905 eV. Hence, the real kinetic energy of the electrons, as they shuttle from the high potential for reduction down to the low potential of water, is as shown in **Table 2** below (and correlated to the aetherometric eV scale, shown in green in **Fig.** 7), which also presents their kinetic energies and corresponding aetherometric electron velocities [48]:

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TABLE 2

Aetherometric electron energy, velocity and $\Delta E'_0$ values for the respiratory chain

Steps	Start energy for each step (electronvolts)	$\Delta E'_0$ aetherometric (volts)	$v= (W_k W_v)^{0.5}$ (10^6 m sec^{-1})
I	20.905 + 1.137 = 22.041	0.33	1.969
II	20.905 + 0.807 = 21.711	0.225	1.954
II	20.905 + 0.581 = 21.486	0.581	1.918

This puts matters in perspective, but does not solve the missing 3/4 of the energy required to form water. It limits itself to answering what the real energy levels are in the shuttle, and thus permits an heretofore unknown determination - that of the slightly decreasing high velocity of the chain electrons as they move across the steps down the chain.

To understand the missing energy, we must go back to the beginning of the electron chain - to the role of oxygen, that "mere" electron acceptor which, in the book of biochemists, only appears at the end of the chain. For, it is the partial pressure of O_2 which governs the pyruvate dehydrogenase that begins the conversion of pyruvate into acetyl-Co-enzyme A, and thus initiates both the electron transport chain and Krebs' cycle. Precisely, the aetherometric model suggests that the missing energy is energy contributed by oxygen itself to both the initiation and the termination of the aerobic metabolic cycle, and that this energy is captured by oxygen from atmospheric ambipolar radiation. This proposal set us thinking about the role of the cytochrome enzymes in the electron chain - in particular the 'copper-containing' Cyt a_3 which directly binds oxygen, at the end of the chain (see **Fig. 4**). It is thought that copper plays a role in the noncovalent binding of oxygen and the reduction of oxygen to water - concomitant with the oxidation of Cu^+ to Cu^{2+}. What characterizes the cytochrome complexes is precisely their absorption maxima that are narrowly distributed between 412 nm and 443 nm, ie, in the range of blue light. Cytochrome a_3 absorbs at 443 nm (for 2.8 eV photons), and this is a clue to the ambipolar energy absorbed by the noncovalent bond established between the cytochrome and oxygen. Indeed, the a-a_3 cytochrome complex contains heme A (instead of proto-heme, as do other cytochromes), which is structurally close to the porphyrin of chlorophyll - a fact suggestive precisely of a residual photosynthetic-like activity having remained critical for the respiratory chain of heterotrophic aerobes.

What now proves simply fascinating is that the ambipolar absorption range (53 to 58keV) of cytochrome c when it serves as substrate to cytochrome c oxidase lies very closely to the peak modal

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solar ambipolar radiation (51.1 keV), or to its modal range that spans 45 to 58 keV. Cytochrome c oxidase is the enzyme complex of cytochromes a and a_3 (see **Fig.** 4), which, in the oxidized or hemin form (Fe(III) or F_e^{3+}) of the complex component Cyt a, couples to reduced cytochrome c so as to accept its electrons, which will be eventually donated by Cyt a_3 (in its reduced form, via its heme group) to oxygen, together with two protons, to form water. Since it is only Cyt a_3 that binds oxygen, and since Cyt c functions as a substrate for the enzyme complex, we suggest that the heme of (oxidized) Cyt c, in turn, only gives off its electrons to Cyt a, when oxygen is bound to the associated Cyt a_3, as is typical of an allosteric regulation that involves conformational changes of associated proteins. Accordingly, the aetherometric model further holds that it is likely the combined energy absorption by Cyt a_3 and Cyt c that drives the last three steps of the electron transfer circuit, *when* molecular oxygen binds to Cyt a_3. While Cyt a_3 would absorb either photon energy at 443 nm or the corresponding ambipolar energy at 53.8keV to directly generate water, Cyt c would absorb either photon energy at the gamma peak of 415-425 nm (see **Fig. 8**) or ambipolar energy at the corresponding 56 to 57.9 keV.

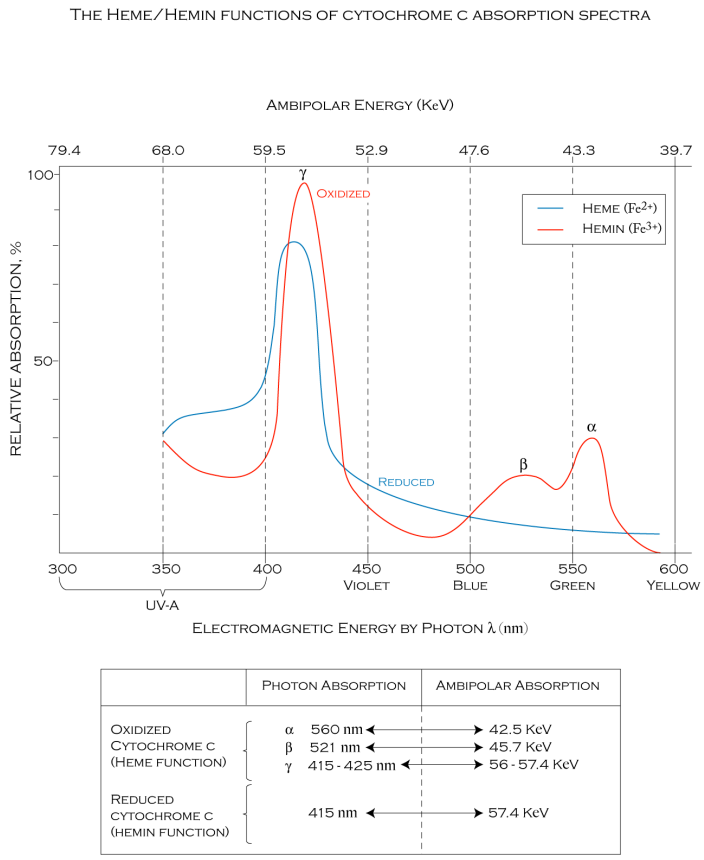

Fig. 8 - Cytochrome c visible light energy absorption spectra by wavelength (lower X axis) and dark ambipolar energy absorption spectra in keV (upper X-axis), when it serves as substrate for cytochrome *c* oxidase while cytochrome a_3 binds oxygen.

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As was found for hemoglobin, CO also binds at the active O_2-binding site to inhibit the reoxidation of reduced Cyt a_3, and - most interesting in what concerns the photosynthetic-like action of this pigment of aerobic heterotrophs - this inhibition is reversed by irradiation with visible light in the 300 to 500 nm range. In our aetherometric model of the double role of pigments, this would mean that aerobic animals owe their metabolism - including their oxygen transport - to enzymes that have retained a plant-like quality in their operation, because this quality permits at once the reception of visible light and reception of ambipolar radiation. Our suggestion, then, in this context, is that it is the oxygen-cytochrome complex which functions as an ambipolar antenna to feed the minimum 45.5 keV (that corresponds to a kinetic energy of 2.37 eV per electron, see below) needed for the actual process of shuttling electron pairs along the respiratory chain.

The aetherometric contention, then, is simple: molecular oxygen is not simply the acceptor, but - together with the catalytic action of Cyt a_3 and its enzymic complex plus substrate - the real initiator and energy injector of the respiratory chain. Right at the onset, (1) it feeds part of the energy of the process to NADH, or to the real starting point somewhat upstream from the reducing potential of NADH; and (2) it retains the remainder (the 'missing ~7.186 eV) of the energy required to produce water while, presumably, (3) dissipating the rest as blackbody photons. We write 'presumably', because we suspect that there are alternative pathways that suppress and control the release of this apparent exothermic heat from oxygen - and which involve precisely molecular cooperativity in the absorption and re-emission of decaying modes of ambipolar radiation (this was discussed in detail in [35]). It is further likely that this cooperativity vis-a-vis ambipolar radiation may be mediated precisely by the conformational changes in the Mg^{2+} F_1 ATPase membrane complex which Boyer's conformational-coupling hypothesis for oxidative phosphorylation suggested - in which case the cooperativity will involve both molecular oxygen and critical redox enzymes such as ATPase and the cytochromes. Hence, if we consider the whole process as being actually initiated by the ambipolar capture and transfer properties of molecular oxygen, the whole respiratory chain will, per real cycle, have to churn out a final production of two molecules of water, and thus account for the formation of 4 OH bonds, from the shuttle of 4 electrons and 4 protons, as shown in **Fig. 9** and in accordance with the overall master equation:

	Blackbody production: hυ	-	[(Respiratory Chain)	+	(Directly Absorbed Energy)]
{45.52 keV - [4(4.74 eV)]} =>	α^{-2} (2.37 eV)	-	{[4(1.185 eV)]	+	[6(2.37 eV)]}

This suggests at once that the chain may well start a little upstream of NADH (since 1.185>1.137eV),

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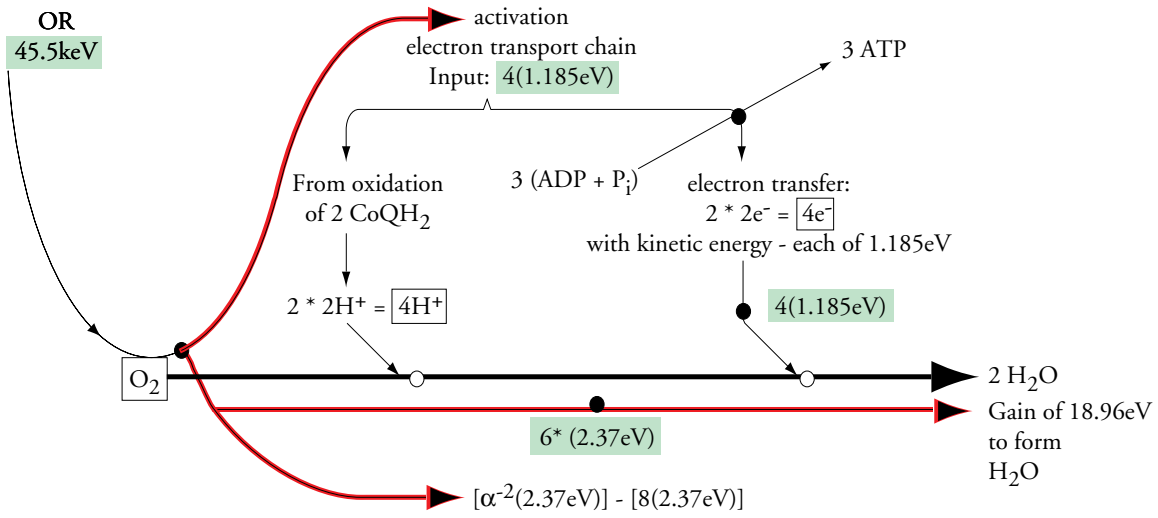

Fig. 9 - Average formation of 2 H_20 molecules in the aetherometric model of the respiratory chain driven with a modal ambipolar energy input of 45.5 keV. The respiratory chain modal input is some 5 keV less than the modal peak of solar ambipolar radiation. After P.N. Correa & A.N. Correa [35].

and that the missing energy leads directly to what actually drives aerobic respiration - the capture of energy from modal solar ambipolar radiation by molecular oxygen, and its transfer to the production of ATP and the formation of water.

The fact that each electron in the chain can only collect a maximum of 1.137 eV kinetic energy above the baseline of 20.905 eV, is likely a clue to understanding what happens to the ambipolar energy absorbed by molecular oxygen and, equally, a possible indication of the cooperativity modes involved in absorption and re-emission of ambipolar energy (for a full discussion of these see [35]). Indeed, if the energy absorbed were directly determined from the captured mean ambipolar radiation, we should expect each transported electron to have retained, at the start of the chain, a kinetic energy of at least 2.37 eV, instead of half that much. Accordingly, it is likely that the absorbed ambipolar energy is split into 2*22.759 keV fluxes by molecular oxygen itself, upon the latter's very splitting into two atomic oxygens to form the OH bonds of water.

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The energy values presented in **Table 3** below are slightly adjusted so that one may keep to the correct aetherometric values of fundamental constants, but one can see how, with respect to $\Delta E'_0$ and the caloric-equivalent value of the enzymatic steps of the electron chain, the energy values remain essentially as they exist in modern biochemical theory.

TABLE 3

Accepted (conventional) and aetherometric $\Delta E'_0$ and chemical energy values for the respiratory chain per production of 3 molecules of ATP

	REDOX		CHEMICAL ENERGY	
Steps	**Conv. $\Delta E'_0$ (volts)**	**AToS $\Delta E'_0$ (volts)**	**Conv**	**AToS**
			kJ mol^{-1}	**kJ mol^{-1}**
I	0.27	0.331	51.08	63.68
II	0.225	0.225	41.45	43.42
II	0.53	0.581	99.65	111.92
TOTAL	**1.025**	**1.137**	**192.18**	**219.02**

The aetherometric model is perfectly compatible with the biochemical model, which claims that 52.7 kcal mol^{-1} = 220.6 kJ mol^{-1} of chemical energy is released by the electron transport chain per atom of oxygen that is reduced. But this chemical energy only accounts for 1/4 of the energy required to reduce oxygen to water, and ignores, therefore, the real value of the energy absorbed and required to produce those 3 molecules of ATP every time that a pair of electrons circulates down the chain. So the final proposed aetherometric model can be tabulated as follows using the 1.137 eV as reference:

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TABLE 4

Aetherometric $\Delta E'_0$ and chemical energy values for the respiratory chain per production of 3 molecules of ATP, for a total $\Delta E'_0$ = 1.137V

Steps	$\Delta E'_0$ per electron (volts)	Chemical contribution Input kJ mol^{-1}	per $2e^-$ eV	Ambipolar contribution Input kJ mol^{-1}	per $2e^-$ eV
I	0.331	63.68	0.66	191.04	1.98
II	0.225	43.42	0.45	130.26	1.35
II	0.581	111.92	1.164	335.76	3.49
TOTAL	**1.137**	**219.02**	**2.274**	**657.06**	**6.82**

Thus, for the formation of water, 2.274eV+6.82 eV = 9.094 eV will have been allocated at a minimum.

We can also tabulate our model using the 1.185 eV value that corresponds to one quarter the energy of the average O-H bond, and taking into account that some other step - probably present upstream of the oxidation of NADH, and denoted in **Table 5** as X - accounts for the missing 0.048eV, to make up the difference between 1.185eV and 1.137 eV:

TABLE 5

Aetherometric $\Delta E'_0$ and chemical energy values for the respiratory chain per production of 3 molecules of ATP, for a total $\Delta E'_0$ = 1.185V

Steps	$\Delta E'_0$ per electron (volts)	Chemical Input kJ mol^{-1}	per $2e^-$ eV	Ambipolar contribution Input kJ mol^{-1}	per $2e^-$ eV
X	0.048	9.26	0.096	27.79	0.29
I	0.33	63.68	0.66	191.04	1.98
II	0.225	43.42	0.45	130.26	1.35
II	0.581	111.92	1.164	335.97	3.49
TOTAL	**1.185**	**228.28**	**2.37**	**685.06**	**7.11**

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Hence, for the formation of a single molecule of water, 2.37eV+7.11 eV = 9.48 eV will have been allocated on average for the formation of 2 O-H bonds, corresponding to a staggering release of 685+228.3= 913.3 kJ mol^{-1}, or more than 4 times the accepted value of the energy released by the respiratory chain - precisely because only 1/4 of the required energy is chemically released, and the remaining 3/4 is essentially contributed from the ambipolar energy absorbed by molecular oxygen.

This aetherometric model of the respiratory chain, we should note, is also compatible with other energy conversions. For instance, excess electron pairs flowing down the chain could also result in the production of hydroxide ions along with water. This could, therefore, provide an alternative to the chemio-osmotic model of a proton pump which exports protons from the mitochondrial matrix - and requires, in Mitchell's hypothesis, a redox potential of 0.235V, which corresponds, in fact, to a pH difference of (0.235V/0.06V) = 3.917 pH units - with the mitochondrial matrix being the alkaline term. Mitchell suggested the difference was just 1 pH unit, since 0.15V would be contributed by a transmembrane potential that would be generated by the electron transport chain (possibly at the Cyt c to Cyt a interface). But we can hardly see how the chain can account for this, when it only accounts for 1/4 of the energy required to form both covalent O-H bonds of water. Yet, in the presence of solutions with high partial pressure of oxygen, excess production of shuttled charges could result from the decreasing concentration of protons in the mitochondrial matrix (required for its export of water to the cytosol). This would result in an increase of OH^- inside the matrix, and could constitute another pathway for the utilization of the greater part of the split ambipolar radiation captured by molecular oxygen.

Respiration is not merely a biochemical process, but also one that couples the chemical bioenergetics to the bioenergetics of massfree electricity, where oxygen is, on one hand, and ambipolar energy absorber and an electron acceptor, and, on the other hand, a re-radiator (thermal dissipation, ambipolar resonant transmission), distributor (emission cooperativity phenomena) and carrier (latent heat) of massfree energy. Hence, it is not just the entire electron chain of the oxidative phosphorylation cycle that is driven by electrons with high kinetic energy that are made to resonate to specific energy levels of absorbed ambipolar radiation, but that it is the entire aerobic metabolism that is driven by the absorption of ambipolar energy - all chemical combustion ultimately depending upon the presence of oxygen and its capture of ambient ambipolar radiation. In other words, respiration is a method to graft the absorbed ambipolar (and electromagnetic) energy, in that it feeds this energy to the completion of aerobic metabolism

4.4. An integrated approach to cancer-promoting stresses, hypoxic and nonhypoxic

Another key feature of the aetherometric model is that it proposes that hypoxia is not the only stress that promotes the epigenetic and adaptive changes associated with the oncogenic vector. Auto-

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oncogenic stresses may not be hypoxic. Yet, we propose that there is unity of function, and that the possibility exists for an integrated approach to the understanding of the oncogenic vector because all these auto-oncogenic stresses (hypoxic and nonhypoxic) converge into the same pathways of response that are triggered by hypoxia (more on this below).

How many blocks in the overall circuit of aerobic metabolism can trigger lactic fermentation?

Traditionally, there is a potential blockage of the Pasteur effect. This effect is mostly controlled by ATP, NADH and citrate in the presence of molecular oxygen. These molecules decrease the rate of glycolysis by acting as inhibitory modulators of phosphofructokinase. Conversely, as part of the same regulatory effect, the rate of glycolysis is accelerated by both AMP and ADP. It is possible for impaired citrate synthase to derail the Pasteur effect, and this seems to be the case in splenic lymphocytes of diabetic rats [50].

Any molecule that can replace oxygen's role in oxidative phosphorylation, or that can block the latter or the citric acid cycle, has the potential to derail the Pasteur effect, as well as block the utilization of pyruvate to form acetyl CoA. One of the suggestions of the aetherometric model is that this is precisely what copper poisoning does, by hitting the cytochrome c oxidase complex of oxidative phosphorylation. It simulates the effects of hypoxia, but is hypoxia-independent.

Another aetherometric suggestion - this time in line with the approach of orthomolecular medicine to cancer - is that appropriate lack of dietary vitamin C and iron effectively block progression of the Krebs cycle half-way through it. This is a nutritional deficiency that, once again, can mimic hypoxia, but is hypoxia-independent.

Evidently, there is a variety of factors, some strictly toxicological, which can shut down or decrease the activity of mitochondrial respiratory complexes. Nitric oxide, for instance, shuts down complexes I, II and IV, but not citrate synthase or ATP synthase [51].

But there are other ways in which regulatory processes that involve central signaling networks can shut down aerobic metabolism - such as the insulin/(Insulin-like growth factor-I) IGF-I axis. Quite independently from hypoxia, this axis can activate and modulate hypoxia-dependent genes, activate glycolysis, shut down respiratory metabolism and engage cells into proliferative states that, dependent upon other conditions, range from hyperplasia to the terminal proliferation associated with processes of differentiation. Thus the IGF-I axis can mimic the effects of hypoxia in a hypoxia-independent manner, as we shall see below.

Even though hypoxia is the main neoplasia-promoting adaptive pressure in acquired cancer, it is not the only adaptive pressure that is oncogenic. Yet, all of the above non-hypoxic factors act by shutting down oxygen-driven respiration. Thus, any and all these factors add further pressure to the hypoxic constraint, and together promote a cellular switch, gradual and eventually definitive or cata-

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strophic, toward oxygen-independent lactic fermentation. By keeping itself in a proliferative state, the oxygen- and energy-starved cell commits to an oncogenic process to ensure that its own metabolism remains strictly glycolytic, and since no pyruvate can be shunted to the Krebs cycle, which is down-regulated and eventually shut down, pyruvate is increasingly converted into lactic acid instead (this, as we shall see below, is not yet the full picture).

4.5. Copper as a blocker of aerobic metabolism in cancer cells

Most provocatively, our aetherometric model of the contribution made by ambipolar radiation to the completion of the electron transport chain in the oxidative phosphorylation performed inside mitochondria can be used to predict that poisoning of the cytochrome c oxidase complex is sufficient to induce hemolytic anemia, since it will directly attack the aerobic metabolism of erythroblasts. Indeed, lack of copper-binding proteins causes copper-poisoning of cytochrome a_3 which is part of the complex [40]. In fact, cancer cells appear to concentrate copper, presenting copper levels ~50% higher than normal cells, and this has been cited as evidence that copper is likely a factor responsible for the malignant process [52].

However, general consensus has been that the malignant action of copper is due to its induction of oxyradicals that damage DNA. But this can hardly be the main malignancy-inducing effect of copper, since it has been experimentally shown in two distinct animal models of breast cancer that induced copper deficiency actually suppresses both tumor growth and angiogenesis (associated with increased metastatic potential of tumor cells) - and thus that copper has acquired some critical metabolic role in cancer cells [53]. Could it be precisely that cancer cells need copper to block the aerobic metabolism, by poisoning Cyt a_3? A copper block to oxidative phosphorylation could effectively force glycolysis to overflow and shunt to lactic fermentation, even in the presence of oxygen [54].

As a consequence, our aetherometric model of the etiology of cancer is bound to emphasize that hypoxia, while a fundamental factor, is not the only factor involved in the promotion of neoplastic conversion of cells. There are oxygen-dependent, or *hypoxia-dependent* processes, but in oncogenesis these combine or synergize with other processes that are *hypoxia-independent*. Poisoning with copper - or analogous metals - is another hypoxia-independent process.

4.6. Role of vitamin C in aerobic metabolism and the treatment of cancer, and role of its lack in auto-oncogenesis

L-ascorbic acid (see **Fig. 10**) - the lactone of a sugar acid - was first synthesized by Szent-Györgyi in 1928, but only identified as vitamin C in 1932 by Charles King and William Waugh. Today there is a tendency to think the concept of vitamins as distinct from that of growth factors, but as Stanier put it in his homage to Lwoff, vitamins - in Lwoff's view - only constituted "a subclass of the larger class of organic growth factors", with the difference that the organism itself could not syn-

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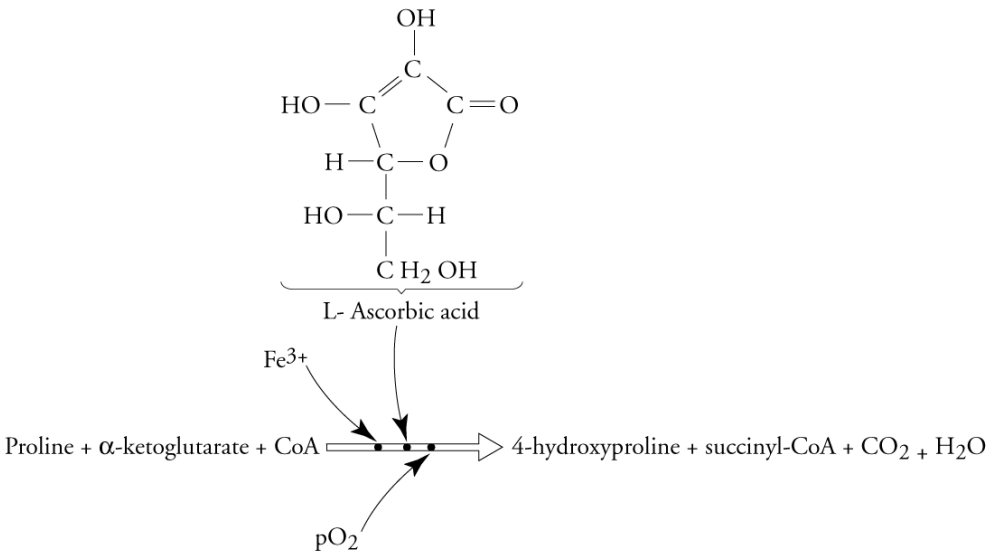
Fig. 10 - The role (and chemical structure) of Vitamin C in the amphibolic Krebs cycle of aerobic metabolism.



thesize them from scratch [55]. The last part of the definition (regarding its phenomenological component), of course, was eventually revised, since some organisms synthesize vitamins and others do not. For example, man and other primates are some of the few animals that do not produce vitamin C (all plants produce it). Moreover, it is easily destroyed by cooking, so it must be obtained from fresh food (citrus fruits and tomatoes). Vitamin C, along with vitamin E (d-alpha-tocopheryl, and "d-alpha alone"), are the only *growth factors* that have anti-oxidant activity. Generally, the activity of vitamin C is attributed to its anti-oxidant properties. As we shall see, these are likely the least important of its critical *in vivo* functions.

Even though the dynamics of a respiratory poisoning invoked by Warburg in his hypothesis was not verified, something akin to malonate poisoning should have been considered as potentially having a parallel role. We are referring to the production of succinate (catabolically) and proline (anabolically) from Succinyl-CoA (see **Fig. 2**), half-way through the Krebs cycle, for which ferric iron (Fe3+) and vitamin C (ascorbic acid) are necessary. Lack of these factors, or degrees in their unavailability, can effectively block completion of the Krebs cycle, and thus turn off aerobic metabolism.

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Conversely, their availability should facilitate progression through the Krebs cycle, and this should preclude the auto-oncogenic tendencies of cells. In fact, vitamin C has been observed to inhibit the malignant transformation and oncogenic progression of cells *in vitro* [56], and this effect is likely tied in to the activation of respiratory metabolism. Rarely is this crucial role of vitamin C in respiratory metabolism mentioned or explored, not even by Linus Pauling and his collaborators. Recent reviews gloss over it entirely [57].

Vitamin C is most often considered just for its role in the development of healthy connective tissue, cartilage and bones, its prolonged lack resulting in scurvy. Milder deficiency of vitamin C leads to collagen loss, alterations in the structure of connective tissue, and decreased immune resistance to some infections. Note that, without L-proline, no collagen or elastin fibers can effectively be produced. But, evidently, vitamin C has a much deeper role in the control of aerobic metabolism, its lack being another factor that can block respiration and promote oncogenesis. Moreover, it acts at a multiplicity of levels - at the level of the organism's immunity; as a required cofactor for oxidative metabolism; and as an apoptotic factor for cancer cells [58-59] that can directly kill transformed cells i*n vitro* [60]. Finally, in the ester form, ascorbate functions as an element composing the matrix of collagen and bone. In its quality of electron donor, ascorbic acid is a cofactor for 8 different enzymes involved in the synthesis of collagen, carnitine, proline and norepinephrine, and in tyrosine phosphorylation and amidation of peptide hormones. As an antioxidant, it protects low-density lipoproteins from oxidation and reduces tissue and stomach oxidants (producing the unstable dehydroascorbic acid).

It is generally supposed that the nutritional need for ascorbic acid is small, but seldom, if ever, is it ventured in clinical research papers that different beneficial effects of ascorbic acid depend on different concentrations. *In vitro* it has become apparent that ascorbic acid only kills cancer cells directly at high concentration. That is likely the reason why breakthrough treatment of pancreatic carcinoma, a particularly aggressive cancer, with IV injection of 50 gm of vitamin C is possible [61]. Similarly, high plasma concentrations of ascorbic acid are necessary for the prevention and treatment of viral infections (for a review see [62]). Cameron and Pauling calculated that required daily intake may be as high as 12-20 gm [63], a far cry from the ridiculous RDA value of 60 mg. In recent years, Steve Hickey and Hilary Roberts have led the fight to change the RDA value and force the clinical and research establishment to investigate the action of mega doses of vitamin C. Hickey summarizes the ongoing suppression of findings:

> "Since Linus Pauling's death, there seemed to be a great deal of misinformation coming into the area. The NIH had performed some questionable experiments and were making the apparently ridiculous statement that blood plasma and tissues

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> became saturated with low doses of vitamin C. There was no mainstream research on high doses and the establishment was making wild extrapolations from their low dose data. We could not see how a clinical trial with 200 mg of vitamin C, for example, could be used to suggest that higher doses were not effective." [64]

Yet, clinical use of mega-doses is reported by a variety of physicians in individual case histories that are relegated to the realm of the anecdotal (see [62]). Even less studied is the indirect modulation of the effects of ascorbic acid at given concentrations by the partial pressure of oxygen. Utilization of vitamin C to sustain the Krebs cycle is modulated by oxygen concentration in tissue (and thus by the dynamics of breathing and the quality of air), and it must compete with other biosynthetic uses of vitamin C. Utilization of vitamin C is also modulated by other factors - such as the presence of NADH and FADH "bioflavonoids" that are critical for the citric acid cycle, and the synergism with vitamin E. Thus, one may legitimately speak of a "vitamin C complex". In this context, it is important to note that gut absorption of vitamin C can also be modulated by time-release preparations that permit a more efficient and stable delivery of mega-dose ascorbic acid, and thus sustain a higher plasma concentration of the vitamin. Nearly all clinical or nutritional literature on vitamin C fails to emphasize the importance of using time-release preparations as the preferred method for oral intake.

In light of the role of ascorbic acid in the citric acid cycle, we should keep in mind how the oncogenic vector of leukemias frequently initiates on the backdrop of some anemic state, with not only impaired tissue oxygenation but often low iron levels. This led Cameron and Pauling to suggest that "the symptoms of acute leukemia are identical to the symptoms of scurvy" [65]. The lack of iron and ascorbic acid translates into weak aerobic metabolism, and thus a backlog of pyruvate substrate that is eventually discharged as lactic acid. Since ascorbic acid promotes the absorption of iron, there is an intimate connection between them, deficiency of vitamin C leading to impaired erythropoiesis and hypochromic anemia, often macrocytic, sometimes normocytic and even microcytic. In cardiovascular disease, ascorbic acid deficiency has been shown to play the primary causative role [66], but no such studies of leukemic or cancer patients have been systematically conducted to ascertain dietary intake and plasma concentrations. Still to this day, all one is left with is the study of Cameron's group at Vale of Leven Hospital which showed that the ascorbate content of leukocytes of healthy subjects who took more than (the miserly quantity of) 150mg of vitamin C per day were three times greater than found in leukocytes of cancer patients [67].

Even more impressive were the results of the study also carried out by Cameron's group at the Vale of Leven Hospital regarding the effect of daily intravenous intake of 10 gm of vitamin C in prolonging median survival time for a wide variety of terminal cancer patients judged untreatable (see

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Fig. 11) [68-70]. Other research groups disputed these findings. The most common criticism is that the trial was not randomized. Randomized trials at the Mayo Clinic have claimed they found no such vitamin C effect. Yet, in one case [71], not only much lower quantities of ascorbic acid were used, but most of the cancer patients tested had received prior cytotoxic chemotherapy. Thus, the observed short survival (80% death in 12 months) was meaningless with reference to the vitamin C intake. In the other case [72], even though the selected patients (100 patients with advanced colorectal cancer) had not received chemotherapy, vitamin C was only administered for 2.5 months, and by oral route (without being a time-release preparation). Little wonder the study concluded that with 95% confidence intervals, the possibility that vitamin C could improve survival by 25% or more was excluded... For a young student of oncology who has little perspective on these matters, it is hard not to believe that these faulty replications may not be the result of a malignant intent (conspiracy, say, to deny a non-patentable treatment for cancer, etc), rather than stupidity and lackadaisical complacency, because the poverty of the so-called scientific effort is so evident. But what to say of supposedly dispassionate reviews that state with evident bad-faith that "Pauling speculated that vitamin C improved 'host resistance' to cancer" [73]? Yet, all Pauling did was put together the facts adduced by those investigators who carried out the basic science without flinching or committing easy errors in setting parameters. In the same isolated pool of subjects, those with high intake of vitamin C present a significantly greater production of IgG and IgM antibodies [74]. This relationship holds for cancer patients treated with vitamin C [75]. Phytohemagglutinin-stimulated lymphocyte blastogenesis can be enhanced twofold by ascorbic acid [76]. Administration of 6 gm per day of vitamin C was sufficient to induce 87% of the population infected with the common cold to shorten the symptomatic period from 14 days to 2 days [77]. Production of prostaglandin PGE1, which regulates T-lymphocyte function, requires ascorbic acid [78]. The killing and phagocytic functions of leukocytes are corrected by administration of ascorbate [79]. Leukocytes - and lymphocytes - of cancer patients have very low concentrations of ascorbic acid, and untreatable cancer patients that receive mega-vitamin C therapy long outlive patients in the ordinary medical course (untreated or treated with chemotherapy) [80]. If you put all these facts together - as Pauling did - you conclude that, if vitamin C stimulates host immunity, then, if there is a chance the host may mount an immune response to its own auto-oncogenic disorder, it is bound to require vitamin C to do so [81]. That's called, not speculation, but a well reasoned prediction.

What is even less forgivable in Vickers' review [73], is that, since the time Pauling, Cameron and their co-workers conducted their studies, and the time Vickers reviewed the evidence, at least one other study was conducted with high-dose vitamin C which she neglects to mention. Donald Lamm and his group performed a double-blind clinical trial with randomized cancer patients treated by the

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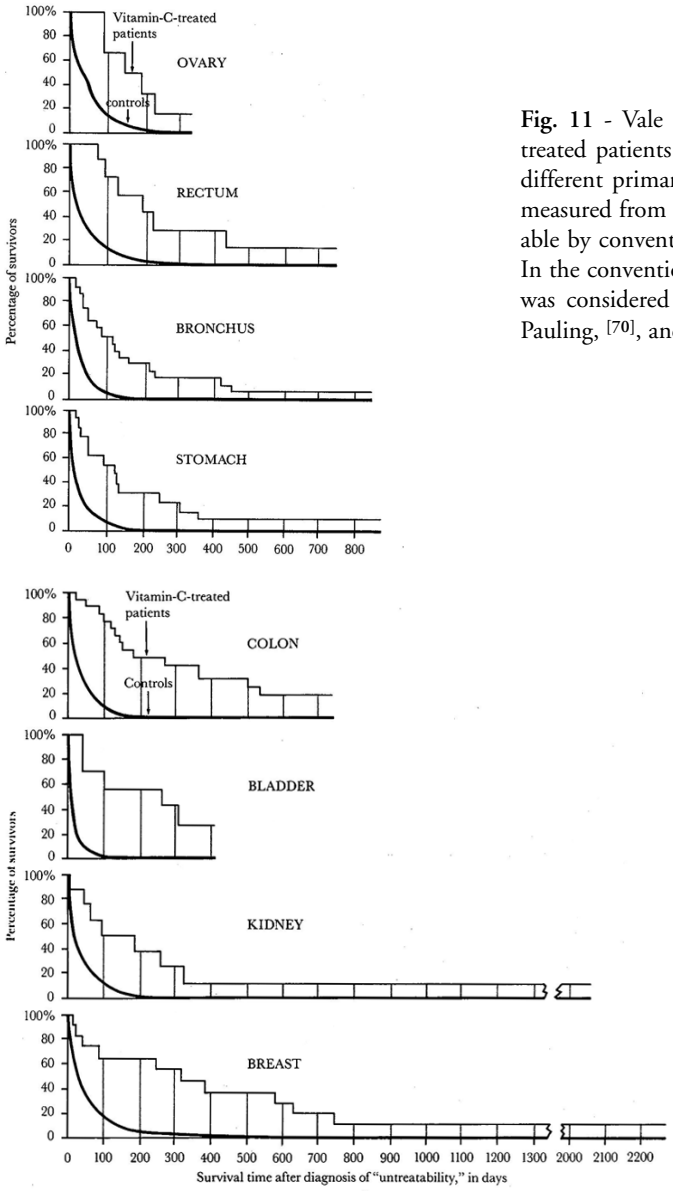

Fig. 11 - Vale of Leven survival times of vitamin C-treated patients compared to untreated controls, for 8 different primary sites of somatic cancer. Survival was measured from the day the patient was judged untreatable by conventional methods (ie terminal cases only). In the conventional cancer statistics of the time, "cure" was considered survival of 5 years or more. After L. Pauling, [70], and E. Cameron and L. Pauling [69].

traditional BCG therapy (Bacillus Calmette-Guérin), together with either the recommended daily allowance (RDA) of vitamins (RDA for vitamin C is 60 mg) or with 2 gm of vitamin C (along with 40,000 units of vitamin A, 100mg of vitamin B6, 400 units of vitamin E and 90 mg of zinc), following surgical removal of transitional epithelial carcinoma of the bladder [83]. Vitamin intake was

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continued for a mean treatment duration of 45 months. Lamm et al found that 5-year recurrence was cut down by more than half in the megavitamin-treated group (41% vs 91%), when compared to the group treated with BCG and the RDA. Another study surveyed nearly a million bladder cancer patients and claimed that vitamin C did not affect mortality; however, it defined the parameter for regular vitamin C supplementation, as intake of >15 times per month (thus not even a daily use) at sub-RDA levels [84]. This effectively invalidated the value of the study, all the more as Lamm et al had already shown that the RDA regime did not affect the 5-year recurrence rate of bladder carcinoma.

Epidemiological studies have also consistently indicated a correlation of higher intake of vitamin C with decreased incidence of carcinomas of the stomach [85], esophagus [86] and cervix [87].

Given that intravenous administration of ascorbic acid is substantially more powerful than oral intake [57, 88], it behooves one to consider what might be the results of studies carried out with administration of high-dose time-release vitamin C to cancer patients and controls - since, as a viable alternative to intravenous injection, it also maintains constant and high plasma levels of the vitamin. No systematic investigation of these questions has been pursued since the work of Pauling, Cameron and Rath. Investigation of the effects of vitamin C does not benefit the pharmaceutical industry, and detracts from the effort to develop chemotherapies. When this lack of interest is combined with complacent and sloppy 'scientific' work, it kills the desire to learn for a long time to come.

As we said already, understanding how vitamin C works in the stimulation of immune responses does not even begin to address the role of vitamin C in promoting and controlling aerobic metabolism, or *a fortiori* in killing cancer cells. If the concentrations of vitamin C which are required by the former are bound to vary with oxygen availability and physical demand, it is now established that specific antitumor activity of ascorbic acid against cell lines from breast, bladder and oral carcinomas, endometrial tumors and mouse hepatomas, depends on very high concentrations, even when synergism with other vitamins (E and K) enters into play and considerable reductions in concentration can be used [59, 89-90]. Parallel *in vivo* results for the antitumor activity of vitamin C were obtained with human prostate cancer cells implanted in nude mice [91] and with the inhibition of aggressive tumor xenografts in mice by pharmacological doses of vitamin C [92] . Since the cancer cell killing property of ascorbic acid is abrogated by catalase, it is mediated by hydrogen peroxide (H_2O_2). Increased peroxide production augments the pool of intracellular calcium, activating at least one DNAse involved in degrading tumor cell DNA [59]. In this context it is fascinating to keep in mind that, in hypoxic conditions (5% oxygen tension), low concentrations of H_2O_2 mediate the action of granulocyte and monocyte growth factors secreted by adherent cells [34].

As reviewed previously [1], transformed cell lines have substantial ongoing cell death. In the human bladder carcinoma line employed by Gilloteaux and his group, 8.6% of the cells were dying,

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with the largest group being apoptotic, followed by autoschizic (2.9%) and necrotic (1.6%) cells. Exposure of the transformed cell to vitamin C increased the autoschizic proportion, from 3% to 16%, and together with administration of vitamin K_3, to 29%, without altering the apoptotic or necrotic fractions [59]. Cancer cells found in G_1 during vitamin treatment arrested in G_1, and this constituted 89% of the cells. Cells that were in the S phase continued through it and arrested at G_2/M, and no cells arrested at the S phase. Thus, most of the cells undergoing autoschizis had arrested in the first interphase. Conversely, ongoing cell death in the untreated cells presented 70% arrested at G_0/G_1, 19% in the S phase and also 11% at G_2/M. This suggests that most of the interphase arrests in the untreated cells lead to apoptosis, but that, upon treatment, the 19% population that would otherwise arrest at the S phase, instead of continuing to the G_2/M interface (as the authors suggest, in our view erroneously, is the case), arrest *earlier*, in G_1, to undergo autoschizis. Apparently, the action of vitamin C is the most intense during G_1, when it promotes both the Krebs cycle and biosynthesis (anabolism). This constitutes a signal to stop proliferating (observed 86% decreased thymidine incorporation with the two vitamin combination [59]) and re-engage normal metabolism - something which the cancer cell ostensively can no longer do, thus resulting in arrest at either interphase prior to cell death.

4.7. Critical roles of Vitamin D in the etiology and treatment of cancer

Another vitamin, besides C and K, which has antiproliferative activity and is able to kill cancer cell lines *in vitro* is vitamin D, specifically the natural analogue 1α,25-dihydroxyvitamin D_3 (1,25-D_3). Vitamin D has no role that we know of in aerobic metabolism per se, but, like hemoglobin, myoglobin and the cytochromes, is involved in the absorption of ambipolar radiation. In fact, the process of vitamin D production is, in skin, the main absorber of high energy solar ambipolar radiation. Yet, the ambipolar absorption does not employ a pigment-antenna, but a covalent reordering of the vitamin D precursor molecule, which is a cholesterol derivative.

Vitamin D_3 (cholecalciferol) is naturally synthesized by skin cells from 7-dehydroxycholesterol when exposed to solar radiation (see **Fig. 12**). Lack of vitamin D in children that are exposed to little sunshine (as in regions with long winters) is the common cause of rickets, as it affects the development and maintenance of bone and gums. But increased incidence of various cancers with higher latitude also seems to epidemiologically correlate with decreased production of vitamin D [93], suggesting that there is an etiological connection between vitamin D and cancer. Moreover, Japanese people, up until recently, largely avoided this connection to a variety of cancers [94] (colorectal, prostate, etc) by eating a diet rich in raw fish oils containing vitamins A and D. Vitamin D deficiency in the general population has aggravated in the recent past due to the irrational fear of solar exposure for mostly imaginary risks of melanoma, and to widespread usage of cytotoxic sun-screens lacking

 ISSN 1920-3799

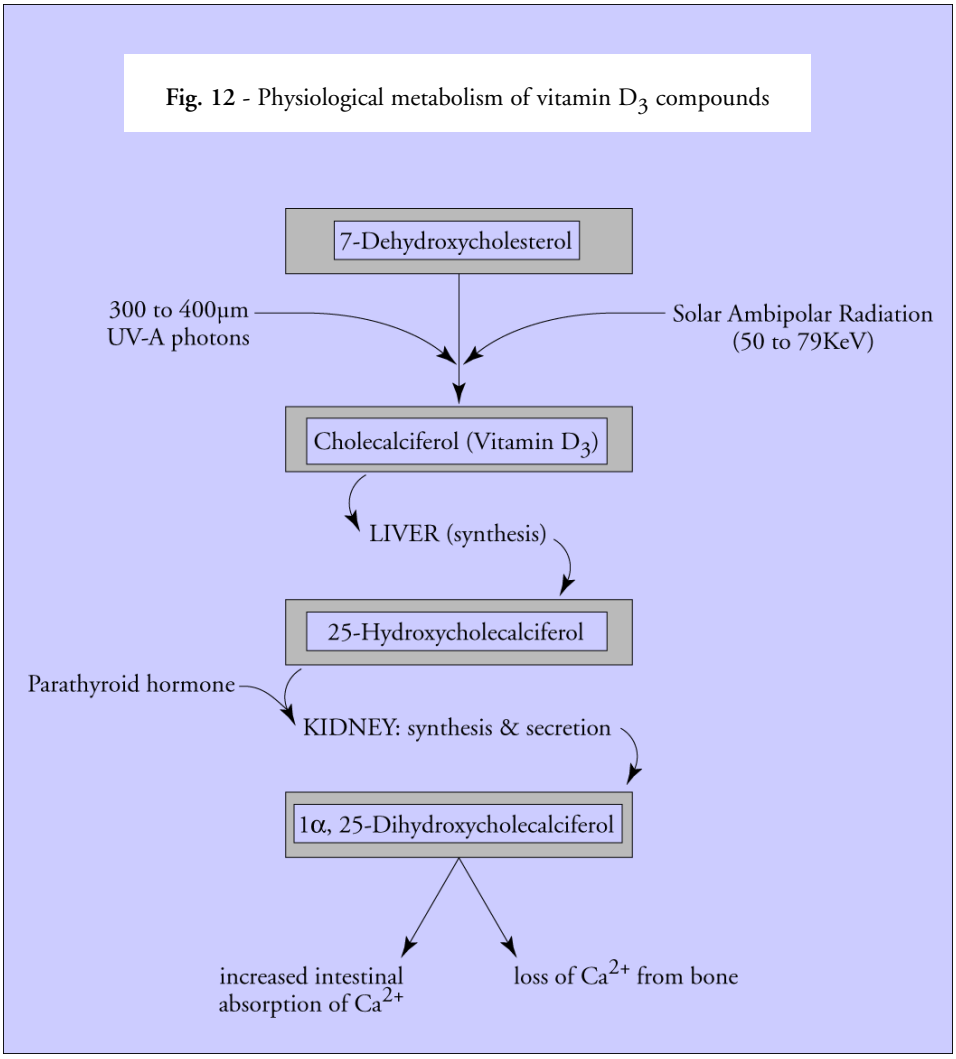

Fig. 12 - Physiological metabolism of vitamin D_3 compounds

proper regulatory checks. Vitamin D deficiency is also tied in to the causation of psoriasis, an epithelial hyperplasia.

In normal skin cells, absorption of UV-A photons converts the cholesterol substrate to cholecalciferol (vitamin D_3). This is part of a "natural parasympathetic" metabolism of epithelial cells, where an orthosympathetic mediator (cholesterol) that serves as precursor to most steroid hormones, is converted to factors (cholecalciferol and, subsequently, its liver and kidney derivatives, see **Fig. 12**) that inhibit proliferation, and induce a parasympathetic response. Even more fundamentally, and

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from the viewpoint of the aetherometric etiology of cancer, the chemical production of cholecalciferol is brought about with skin exposure to ambipolar radiation in the range of 50 to 79 keV, ie in the supramodal range of solar ambipolar radiation in the OR subspectrum [38]. Thus, 7-dehydroxycholesterol is one of the body's main absorbers of supramodal ambipolar energy. In the process of production of vitamin D_3, absorption of radiant energy, whether electromagnetic or ambipolar, plays the critical role of the parasympathetic effector and generates ("participates in the generation of") its own chemical mediator or parasympathetic signal (cholecalciferol). Treatment of vitamin D deficiency has employed absorption of electromagnetic energy generated by plasma UV lamps (*viz*. in tanning beds). However, this unfortunately involves skin exposure to oncogenic and melanoma-inducing photon wavelengths shorter than 300 nm, in particular those that cause production of ozone and nitrogen free radicals. Our discovery that it is possible to generate tuned ambipolar emissions with special induction coils (Tesla coils) [38, 49, 95], and that when these coils are *resonantly-loaded* they produce no ozone [49, 95], raises the unexplored possibility of therapeutic exposure to (lampless) tuned coil radiation inside a resonant cavity as an alternative method to induce skin production of cholecalciferol. Again, this is a specifically aetherometric suggestion to be further investigated.

Skin exports cholecalciferol to the bloodstream, and via the latter to the liver, where it is metabolized to the vitamin D_3 analogue 25-hydroxycholecalciferol or calcidiol (see **Fig. 12**). This form is more active at targeting tissues than cholecalciferol is, but its further conversion - by the proximal tubules of the kidneys in response to the action of parathyroid hormone (PTH) - to 1α,25-dihydroxyvitamin D_3 (1,25-D_3 in short). produces a still more potent biological factor, one that actually constitutes a steroid hormone by the classical definition of endocrine secretion and delivery. These forms of vitamin D are highly calcemic - and they induce calcium loss from bone. Most studies today claim that 1,25-D_3 is the biologically active form of vitamin D. We take exception to this, for the simple reason that each stage in the conversion of vitamin D employs a biologically active form of the molecule with rather distinct functions. Moreover, it implies that 1,25-D_3 is the "real vitamin", when, in fact, its administration is dangerous - both for its hypercalcemic and immunosuppressive effects, but also for its negative feedback effects on the kidneys, liver and parathyroid, which entirely inhibit processing of cholecalciferol, and likely turn off skin absorption of ambipolar radiation. Moreover, 1,25-D_3 production is a tightly controlled process that responds to the physiological demands for calcium and phosphate, essential buffers and transmitters of (ortho)sympathetic activity. The PTH induction of 1,25-D_3 itself is subject to a direct feedback control loop, where 1,25-D_3 turns off the *PTH* gene in parathyroid cells [96-98]. Moreover, it is far from clear whether 1,25-D_3 might not have a mitogenic and oncogenic potential, as it increases the expression for Epidermal Growth Factor Receptor (EGF-R) in breast carcinoma cells [99]. High doses (generally more than 1,000 IU) of vita-

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min D compounds also have toxic effects, causing nausea, vomiting, diarrhea, calcium deposition and anemia.

Vitamin D in its $1,25(OH)_2$-D_3 form is not just an hormone in the most technical sense of the term, but it is also a steroid, a steroid precursor, and a regulatory growth factor. The critical importance of vitamin D is emphasized by data showing that vitamin D_3 in all of its *physiological* isoforms constitutes a *normal negative regulator of proliferation* and a positive regulator of interphase growth. It is distinct therefore from the action of classical hormones which have a mitogenic effect even when they induce a commitment to terminal proliferation and differentiation. Vitamin D compounds are generally distinct from tumor-suppressors because they appear to lack different forms associated with antagonistic effects. Studies have shown that breast, prostate and colorectal cancers present increased risk and/or increased mortality in patients with low serum 25(OH)-D_3 [100-102]. Other studies have shown that 1,25-D_3 can provoke tumor regression, inhibit metastases, suppress tumor angiogenesis and prolong survival time in animal models (reviewed in [103]). Vitamin D_3 treatment of cancer cell lines extracted from a variety of tumors arrests the cell cycle progression at the G_1 interphase, decreasing the number of cells that enter the S phase, but leaving cells at G_2 and M unaffected (for a review see [104]).

Aside from vitamin D_3 compounds present in fish liver oils, most synthetic vitamin D available on the market of "nutritional health products" used to be vitamin D_2 (ergocalciferol) produced by far-UV irradiation of ergosterol (not from 7-dehydrocholesterol). There is today a growing awareness that such preparations are cytotoxic. Currently, manufacturers of health products offer cholecalciferol preparations, but even well-established and respected manufacturers are unwilling to divulge their purity and method of production (trade secret), and to identify the presence of other vitamin D analogues and their concentration in the cholecalciferol preparations they sell to the public. This should be unacceptable to consumers and regulatory agencies. We contacted four different vitamin D_3 (cholecalciferol) manufacturers - Swiss Herbal Remedies, Natural Factors, Puritan and Jamieson - and not one of them was willing or able to divulge the purity of the preparation or its inclusion of other vitamin D compounds. Even though we asked for a certificate of analysis - a trivial matter for any chemical product - none was ever provided at the date of this writing. Moreover, from what little we could ascertain, present preparations of cholecalciferol are derived from sheep lanolin and employ UV irradiation in its synthesis. It is most certain that this will result in the production of a variety of vitamin D analogues, and it also raises questions regarding the potential cytotoxicity of these cholecalciferol preparations.

The most important difference between the antitumor activity of vitamin C and vitamin D, is that, unlike vitamin C, vitamin D has immunosuppressive effects - and so do all of the vitamin D_3

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analogues that have been synthesized in the hope of finding a more effective and marketable vitamin D-based cure for various types of cancer [105]. The immunosuppressive effects are mediated by the vitamin D receptor (VDR), but these results have consistently been carried out in culture systems that employ serum, which itself is contaminated with vitamin D and a host of other compounds that interact with it. 1,25-D_3 induces an increase in the intracellular free calcium and protease-induced (calpain) cell death that is not caspase-mediated but morphologically still looks like apoptosis [106]. 1,25-D_3 is able to induce growth arrest, differentiation and cell death in a variety of cancer cell lines [107-109], and these anti-oncogenic effects appear to be mediated by VDR. 1,25-D_3-induced differentiation of leukemic cells is also synergistically enhanced by addition of ATRA (all-*trans*-retinoic acid) and other retinoids [109]. In colon cancer patients, expression of *VDR* correlates with an observed high-degree of inducible tumor cell differentiation and a good prognosis [110-112]. Yet, 1,25-D_3- or analogue-induced arrested cancer cells can be rescued by the antiapoptotic proteins Bcl-2 and Bcl-X_L - though not by the p53 protein [113]. This suggests that the killing action of 1,25-D_3 or its analogues is under a control parallel to that of hypoxia-induced apoptosis. The killing action may well be linked to the activation of the gene for IGF-II (Insulin-Like Growth Factor II), a factor that increasingly appears to negatively control proliferation and the effects of IGF-I (see below).

However, as we have stressed, one should be careful in making inferences from direct in vitro effects upon cancer cell lines, not only because of the nature of these lines (for an overview see [1]), but also because these studies are regularly conducted in serum-contaminated cultures. Recent results obtained with VDR-null mice indicate increased sensitivity to autoimmune diseases and susceptibility to oncogenic mutations [114]. Yet, FDA-encouraged widespread addition of vitamin D (mostly still as D_2) and its analogues to milk and food products has raised the concern that this practice may well be contributing to current epidemics of obesity and immune dysfunction [115]. No studies that we are aware of have, in fact, compared the effects of cholecalciferol intake in normals and cancer patients, to the intake of other vitamin D analogues, in particular 1,25-D_3.

4.8. Pineal tumors and insufficient ambipolar energy exposure

The pineal gland is a neuroendocrine organ that, in mammals, develops from both mesoderm and neural crest cells. The pineal "complex" is found in all vertebrates. In "evolutionarily lower" vertebrates, where the complex can be found subepidermally on the median dorsal aspect of the head, the main activity of the pineal gland (epiphysis) has been suggested to be photoreception (a "third eye"), but the fact remains that even in these species the eye-like structure remains diminutive and buried under the skin. In "higher" vertebrates the gland "evolutionarily" migrated inward to the center of the brain and is thought to have a "vestigial" photoreceptor "function". In mammals, the pineal gland is one of the key components of the neuroendocrine system. Within it, neural (norepinephrine

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synthesized by the orthosympathetic fibers innervating the pineal body) and hormonal (indoleamines such as melatonin or its precursor, serotonin; and peptides or proteins) components interact to regulate the operation of the gland. The two major functions of the gland are the control and modulation of the circadian and circannual biological rhythms, and thermoregulation of the internal environment.

The great mystery of the functional role of the pineal body is that, while it has been shown to control the seasonal regression of reproductive organs in photoperiodic species, *the circadian function of the gland is in fact independent of light and photo-control* [116-118]. The biological clock of serotonin is "generated independent of environmental light-dark schedules" [117]. This has led to the notion that the pineal was once a photoreceptor but has now ceased to respond to direct illumination, even if (chick) pinealocytes grown in tissue culture respond to constant light by suppressing the nighttime increase in N-acetyl serotonin synthesis [119]. Precisely however, retina cells do not exhibit this behavior, thus differing from pinealocytes in the light-response [120].

We previously suggested that the pineal body could function as key autonomic modulator and integrator of the overall neuroendocrine functions of an organism, and that it functioned not really as a photoreceptor organ, but as an ambipolar sensor organ. We proposed that it worked as a differential detector of ambipolar radiation emitted from sources outside and inside the body [121], the solar ambipolar flux being the obvious, most intense diurnal source of input energy variation in the atmosphere monitored by the pineal gland. Further, we proposed that the parasympathetic effector in this gland was the input ambipolar radiation itself (as happens with skin production of vitamin D). Our model dovetailed with the old results of Gould, who experimentally shifted the circadian rhythm in bees with a synthetic 23-hour period of diurnal magnetic field variation created by employing, precisely, a coil system [122]. We have shown that such coil systems are emitters of ambipolar radiation [38, 49, 95], and can thus constitute an outside ambipolar source that can interfere with the differential sensing abilities of the pineal body and its synchronization to the solar ambipolar flux. We have also indicated that disruption of pinealocyte detection of the ambipolar flux - by the keV ambipolar emission associated with the destabilization of the local gravitational field that initiates seismic events - is the explanation for pre-seismic anomalous animal behaviour that anticipates even the so-called primary seismic wave [123].

In the aetherometric model, cellular photoreceptors may have specialized in two distinct directions:

1) those that receive both photic stimuli in the outer environment (optical vision, or ordinary vision) *and* ambipolar radiation from without - radiation capable of triggering on the surface of special cells a local production of photons; this would be the role of retinal cells and largely why they

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differentiated into two different types. And

2) those which only receive ambipolar radiation, the pinealocytes, and specialized therefore in forming "dark receptors"; these could recede to the innermost cavity of the body *to receive attenuated* ambipolar radiation in the *upper frequency or supramodal energy range of the solar ambipolar spectrum*. Electronic coupling of pinealocytes is in fact suspected to control the flow of the synthetic output of pinealocytes [124]. Moreover, pineal modulation of the thermal control exerted by the hypothalamus may further suggest that the same pinealocytes may also receive or sense the ambipolar radiation coming from the internal environment (via the blood system), and this may well be the evolutionary sense of their close coupling to blood capillaries in the perivascular spaces.

This proposed model admits entirely to the possibility of photon production inside the two subcellular processes of pinealocytes: in both cases the receptors would absorb ambipolar radiation and release photons *in situ*, but there is no need to invoke any reception of external light (photic) signals. Moreover, our model also suggests that the intraparenchymal and perivascular processes of pinealocytes may well have differentiated to detect, respectively, the attenuated external ambipolar radiation, and the internal ambipolar radiation. Or, it may be that the perivascular process is capable of detecting directly the ambipolar-radiation-induced photon production from, for example, hemoglobin (the lizard *Sphenodon* has an intracranial pineal gland equipped with a lens and pigment-containing pinealocytes). Either way, this would explain why the function of the pineal body is effectively independent of external light cycles, and why its cells are capable of being "entrained" by temperature manipulation of an *in vitro* simulated internal environment [125]. Any increase of the internal temperature detected by pinealocytes (either as internally produced ambipolar radiation or as photon production, as from hemoglobin), would be compared with the reception of attenuated ambipolar radiation from the external environment, and if the latter matched or more than matched the former, it would trigger a functional hyperthermic entrainment of the body "thermostat" setting that fit the amount of irradiation. Such an upward thermostat drift would follow for example direct skin exposure to solar radiation.

In essence, then, the "vagotonic balance" or "counterweight" to the sympathetic innervation of the pineal gland would be provided by the ambipolar external stimuli. Finally, the pineal independence from light variation and the light/dark cycle, and its capacity to retain a circadian clock and resynchronize it, would be simply pineal responses to the diurnal variation of the intensity of "dark" solar ambipolar radiation reaching the Earth's surface. The endogenous clock would be but a comparative ambipolar clock.

This model also dovetails with the more general orgonomic and aetherometric contention that the most fundamental oncogenic pressure of acquired cancers is hypoxia caused by sympatheti-

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cotonic disturbances. Lack of blood oxygenation is bound to derange the differential ambipolar comparator function of the pinealocytes. And it will result in no activation of the parasympathetic system, putting a sympatheticotonic pressure on the whole organism. Pineocytomas in children present unusually high levels of melatonin at night [126]. Being an orthosympathetic effector, along with its precursor, serotonin, this is coadunate with overexpression of sympathetic mediators.

In keeping with the cytosis/blastosis distinction (see below and [1]), pineal tumors also distribute between 45% pineocytomas of the pineal parenchyma that present differentiation (these are more frequent in adults), 10% intermediate differentiation pinealomas and 45% pineoblastomas (these are more frequent in children and young people), showing a spread of oncological disorders that affect progenitors at different levels of potentiality, with a strong dependence upon patient age [127]. In light of our model of the etiology of cancer, the more frequent manifestation of pineoblastomas in young people suggests that insufficient absorption of, and/or exposure to, ambipolar radiation may be highly auto-oncogenic for the pineal parenchyma and hinder its development or proper differentiation. It also suggests that absorption of solar ambipolar radiation is required for the proper function of the pineal body, as well as for balanced autonomic regulation.

4.9. The aetherometric etiology of cancer (1): initial expression of the oncogenic vector

In keeping to the initial insight of Warburg, the aetherometric model of oncogenesis suggests that inception of chronic hypoxia created the main adaptive pressure in the tissue environment that promoted the malignant transformation of cells and the switch in metabolism, from respiratory to lacto-fermentative. But our model also took into account the tremendous discoveries of molecular oncology, and thus suggested that the genetic alterations characteristic of all malignantly transformed cells were directed changes - changes that articulated complex modifications of cellular metabolism and proliferation geared from the beginning to block differentiation and promote rapid growth and proliferation. Given the recursiveness of regulatory biological circuits, and the tissue-specificity of the genetic alterations, one necessarily expects that there will be a variety of 'transformed cellular circuits' putting different proliferative loads on the energy metabolism of transformed cells.

The reader should note that, in presenting our model, we are not arguing that chemical and physical insults do not damage the DNA, nor that, at the end of the day, cancer-induction by these stimuli is not ultimately a matter of their dosage; on the contrary, we accept perfectly well what has been learned about the induction of carcinogenesis and leukemogenesis, whether caused by intense exposure to chemical or physical mutagens, induced by infection with retroviruses or other microorganisms, alone or in combination with other factors, or the result of hereditary transmission of truly 'oncogenic' defects (some being Mendelian, like human retinoblastoma). But hereditary cancer and

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well-defined occupational or toxicological neoplasias - including iatrogenic ones - are a very minor proportion of all human cancers. Most instances of neoplastic disease confront one with a functionally acquired disease, an adaptive disorder devoid of hereditary history and with a great diversity of alterations hitting very different organs, tissues and tissue lineages. From a medical viewpoint, the matter is even more complicated, since one should realize that nearly every cancer has an associated 'true neoplastic syndrome'. But our more immediate concern is understanding whether there is, or not, a unity of function to the initiation of auto-oncogenic processes.

Our main suggestion is that, confronted with a variety of blocks to aerobic metabolism, the cell first steps into an oncogenic vector (first commits to transform) after being exposed to chronic hypoxia, or to factors that are hypoxia-independent but mimic the effects of hypoxia. In other words, chronic hypoxia and hypoxic-independent factors translate into an insufficiency of biophysical and biochemical energy for the cell to implement the normal tissue requirements for growth and differentiation.When sufficient oxygen is lacking or an hypoxia-independent factor blocks oxygen utilization, the cell becomes unable to directly absorb energy from its radiant environment (including the thermally-poor internal environment of the organism), as well as unable to chemically extract energy with high efficiency. The unity of function of all acquired cancers would therefore lie in the sustained production of hypoxia-like effects by epigenetic and adaptive means, in response to the chronic presence of hypoxia and/or hypoxia-independent constraints. The first such hypoxia-like effect is to activate glycolysis, and the second is, in all probability, to turn on proliferation, immediately committing all cellular biosynthesis to deliver what is needed to enter and progress through the S phase, and then reach mitosis. In this view, then, the initiation of cancer must take place with cells that are either proliferating already, or able to make the decision to proliferate. The antiproliferative effect of vitamin C, its inhibition of S-phase DNA replication, and capacity to reverse transformed and oncogenic phenotypes [56], are, in this model, most probably tied in to its stimulation of aerobic metabolism. Likewise, the documented role of SOD (superoxide dismutase) in inducing differentiation of Friend erythroleukemia cells (see, for example, reference [128]) points to the trivial, but not unimportant, fact that *oxygen functions as the main negative regulator of proliferation*. Indeed, there is an *in vitro* correlation, on one hand, between low oxygen levels and increased proliferation status [129-130] and, on the other hand, between differentiation, high oxygen partial pressures and the presence of enzymes - like SOD - that restore molecular oxygen, *viz*. from superoxide free radicals (differentiation of Friend cells has also been shown to be accompanied by increased expression of the *SOD* gene, forming a positive feedback circuit [131]).

Thus, we can think of factors that promote aerobic metabolism as antiproliferative (parasympathetic) signals, and of their lack, or of factors that shut down aerobic metabolism, as promoters,

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inducers or triggers of proliferation, or orthosympathetic signals. The criterion the cell employs to select and coordinate a multiplicity of changes in its path towards malignant transformation comes down to the requirement to implement *rapid energy extraction* methods *under hypoxic conditions* (which, in our model, include decreased oxygen-mediated absorption of ambipolar energy) *or non-hypoxic conditions that simulate the effects of hypoxia*. Loyal to Reich's vision, the cell that engages into an auto-oncogenic process, is a cell prey to an orthosympathetic disorder (chronic sympathetico-tonia) [36]. Accordingly, our model explains why most of the selected targets of retroviral insertion, transduction and excision turned out to be growth-factor or growth-factor receptor genes involved in metabolic control of growth - suggesting, in the process, that *ontogenic* viral emission may well have been (evolutionarily speaking), and continue to be, a cellular process designed to increase the activity of metabolically-critical genes (and thus, merely a subspecies of acquired cancer). It is equally noteworthy that hypoxia significantly increases the susceptibility of target cells to infection with Friend virus [132]. A similar logic of hitting metabolically-critical genes seems to underlie most genetic mutations and aberrations [1], as these permit the neoplastic cell to churn out much greater protein outputs; in particular, greater outputs of phosphorylating enzymes typically linked to growth factor receptors that have been truncated or modified, so as to gain metabolic and autonomic independence from control by the physiological receptor ligands - the 'non-vitaminic' and 'hormonal' growth factors.

All factors that adversely and systemically impacted aerobic metabolism at critical junctures - whether in the Krebs cycle or oxidative phosphorylation - induced proliferative states and increased glycolysis, and the oncogenic process at its initiation simply seized this response to increasingly shunt the glycolysis to lactate production, rather than to the Krebs cycle, so as to sustain rapid proliferation. Chronic hypoxia translates therefore into chronic proliferation and a block to differentiation, the markers of *hyperplasia*. In this view, the oncogenic vector is first expressed by a pre-neoplastic lesion of an hyperplastic nature, in turn initiated by adaptive changes that secured some degree of lactate fermentation (LDHA-driven) in response to hypoxia, or hypoxia-like constraints. Similarly, progression of the oncogenic vector and acquisition of a fully transformed, malignant or neoplastic phenotype is an adaptive response to further aggravation of the hypoxia or hypoxia-like constraint to the status of effective anoxia, which explains why fully tumorigenic, neoplastic cells have even greater rates of glucose consumption and lactic acid production, turning into completely anaerobic, amoeba-like or independent organisms.

Progression of the oncogenic vector appears therefore to be associated with total abandonment of an energy metabolism that relied on oxygen and pigments to extract chemical energy and absorb radiant energy, whether ambipolar or electromagnetic. Effectively the cancer cells engages in a

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form of physiological regression, opting to extract energy, albeit in large quantities, from less energy efficient processes than those employed in aerobic metabolism.

4.10. The aetherometric etiology of cancer (2): progression of the oncogenic vector from cytosis to blastosis

In our approach, hyperplastic cells still possess some degree of aerobic respiration or retain the capacity to do so (eg retain their mitochondria), even as their rate of glycolysis increases. Only malignantly transformed cells can afford to sever aerobic metabolism from glycolysis entirely, so as to shunt the latter towards lactic fermentation and tremendously accelerate it. Malignant transformation would be incident upon this shunting and the increased glycolysis. Thus the aetherometric model of the etiology of cancer suggests that transformed cells that still retain *pyruvate-based oxidative phosphorylation* may not yet necessarily be neoplastic or malignant. Some are pre-neoplastic hyperplastic cells, while others are tumorigenic and thus neoplastic. Initial "recruitment" by an oncogenic vector consists, in our model, of setting a cell into a hyper-proliferative state. It may recruit the cell into the cell cycle, but more likely it "recruits" cells that are already cycling, or even committed to proliferating. This oncogenic "recruitment" is ongoing with the progression of cancer or the auto-oncogenic vector - in blood disorders, it appears to progressively operate on increasingly more primitive stem cells.

The notion that a pre-neoplastic hyperplastic state results from cancer initiation is essentially a view that suggests that cytosis or cytotic proliferation precedes, in the oncogenic vector, the manifestation of a blastosis or 'blast-crisis'. Take the diverse chronic myeloproliferative disorders (CMPDs, which include PV, *Polycythemia rubra vera*; JCML, Juvenile Chronic Myelogenous Leukemia; JMML, Juvenile Myelomonocytic Leukemia; ET, Essential Thrombocythemia and IMF, Idiopathic Myelofibrosis): they all seem to have a unifying mutation in the *JAK2* gene [133-137]. Following the Correa & Axelrad model of cytokine hypersensitivity of CMPDs [138-140], this type of hyperplastic disorder is driven not by independence from growth factor control, but by hypersensitivity to normal growth factors or recursive alternative growth factors (for an overview, see table 1 of reference [1]). In PV, the phenomenological independence from regulation by EPO (erythropoietin) results from hypersensitivity to IGF-I (Insulin-Like Growth Factor I) which is characteristic of PV erythroid progenitor cells [138, 141]. In JCML and JMML, the disorders are mediated by hypersensitivity to GM-CSF (the normal regulator of granulopoiesis) on the part of granulocyte and macrophage precursors [142-143]. In ET, the hyperplasia is mediated by hypersensitivity to MGDF/TPO (thrombopoietin) on the part of megakaryocytic precursors [144-145]. And in IMF, by SCF (Stem Cell Factor or KIT ligand) hypersensitivity on the part of the same cells [140, 146].

As we presented it in the previous communication [1] and will return to below, the unification

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of the CMPDs indicates that they form consistent hyperplastic disorders, each producing a cytosis that affects only relatively late blood cell progenitors and still permits differentiation, or some dysplastic variation thereof, to occur. Further, the fact that they all subsequently evolve into different types of acute myeloid leukemia indicates that the oncogenic vector progresses to blastosis, or blastoses (several types at once), affecting more primitive, stem or stem-like cells and presenting a lack of differentiated cells, or only metaplastic cells. One might conceive of a process which involves the concept of commitment to neoplastic transformation in a manner analogous to the well-studied developmental commitment of cells to differentiation pathways. As happens with a differentiation inducer, the varying distribution of oxygen over time (the changes in its partial pressure) may define a "primary kinematic wave" [147], or be associated with one, and according to its time-varying concentration threshold and rate of flux, (1) be permissive of interphase growth and aerobic metabolism (matching glycolysis to respiration), (2) determine an epigenetic commitment to differentiate and terminally proliferate, or (3) epigenetically upregulate glycolysis, with some LDHA-driven lactate production and a commitment to replicate and proliferate (see **Fig. 13**). If the primary kinematic wave is suppressed - or if, as induced by hypoxia-independent factors, the effects of the primary wave are suppressed - and over time the oxygen concentration becomes constantly hypoxic, then, within the framework of option 3, the cell begins to search for a more radical solution (see **Fig. 13**), for a metabolic reorganization, and with neoplastic alteration it eventually comes to enforce the upregulation of glycolysis and the commitment to proliferate with adaptive changes that sever aerobic metabolism. We can follow this process in the oncogenesis of blood disorders. Generally, at initiation, the first cells to feel the pressure of the cancer-promoting constraints are the late blood progenitor cells on the forefront line of normal commitment to differentiate in response to stimuli that require delivery of more differentiated cells. Thus the hyperplastic diseases (CMPDs, indolent CML, CLL) are cytoses that inadequately respond to the need for differentiated cells by producing excesses of dysfunctionally-differentiated cells. As the oncogenic vector progresses, more primitive blood stem cells are now mobilized to respond to the oncogenic constraints, and their malignant transformation results in blastoses that are increasingly more metaplastic and refractory to differentiation. When more primitive, blast-like cells are malignantly transformed, more aggressive neoplasias result.

In the context of the present model of auto-oncogenesis, the evolution of CMPDs to various forms of AML constitutes precisely the progression of the oncogenic disorder from cytosis to blastosis. But CMPDs may evolve towards CML instead, as found recently in a *JAK2V617F*-positive PV patient who developed a *bcr-abl* translocation that transiently inhibited the underlying PV phenotype [148]. This work, in fact, confirms our model in also another way, since the CML masked the occult PV, and treatment of the former exposed the latter, in an instance of regression of the

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oncogenic vector from blastosis back to cytosis (see **Fig. 13**).

Similar progressions would be at work in the development of solid tumors or 'somatic' malignancies. One could cite, for example, the typical evolution of bladder transitional cell papillomas to carcinomas, and how the evolution seems to localize at first near the epithelial basement membrane, hitting the more primitive or less differentiated epithelial stem cells.

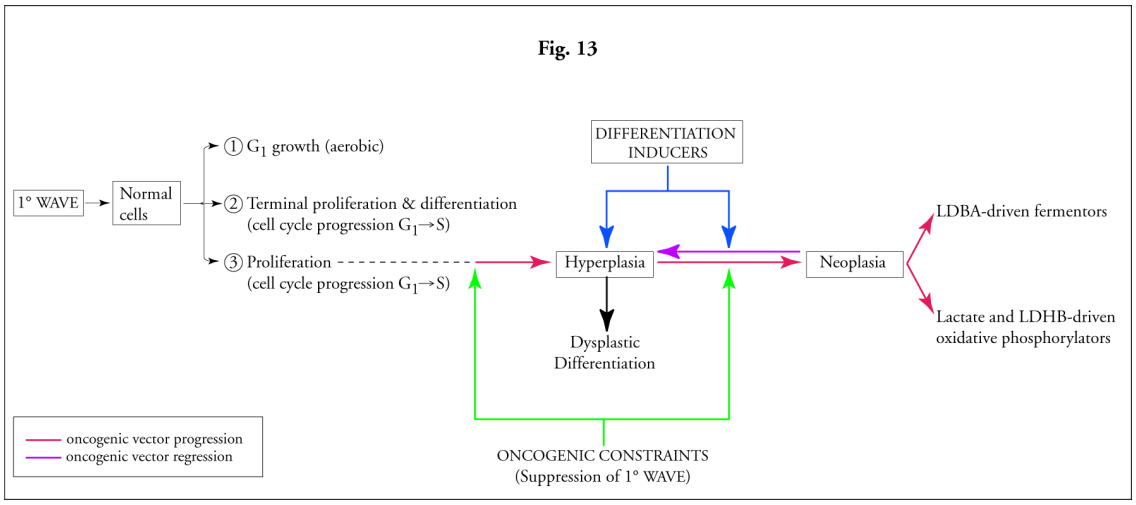

Fig. 13 - Basic vectors of the aetherometric model of cancer initiation and progression.

However, the notion that neoplastic cells may not be irreversibly transformed and may, in fact, be able to differentiate normally, appears to contradict this model of two main stages in the progression of blood cell oncogenesis. Leo Sachs and coworkers have argued that, since most neoplastic alterations are mediated by DNA de-methylation, it is possible to epigenetically reprogram neoplastic cells to undergo differentiation [149]. This, however, begs the question of whether cells that appeared to be malignantly transformed but are so only epigenetically, are really malignantly transformed cells. In APL, transformation hits the *RARα* gene, blocking it from undergoing conformational change and recruiting histone deacetylase activity in response to its ligand, ATRA, a vitamin A analogue, with the result that DNA remains inaccessible to transcriptional factors and myeloid differentiation is blocked. Inhibition of the histone-chromatin complex overcomes this differentiation block. In most instances, treatment with pharmacological doses of ATRA induces differentiation of the APL cells, with complete remission if elimination of the abnormal clone is successful (ie if all the cells of the abnormal clone differentiate) [150-151]. In this case, remission of the blastosis of an acute

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leukemia is brought about by pharmacological treatment with the physiological factor to which the oncogene product (the ATRA *RAR*α receptor) should be responding at normal or physiological concentrations, seemingly reversing the entire oncogenic vector. Differentiation-inducing treatment of APL with ATRA remains the ideal paradigm in the treatment of cancer: finding treatments that do not kill the patient while killing the cancer cell, or better, that do not even kill the cancer cell but reverse its transformation, "cure it" by inducing its differentiation. Yet, the facts suggest that the cells differentiated from malignant clones are not normal but dysfunctional (for a review see [1]). What, according to our model, is happening in the ATRA treatment of APL is that the oncogenic vector again regresses from the blastic to the cytotic states (see **Fig. 13**), with the latter succumbing to dysplastic differentiation. The very fact that the disease frequently returns some time later, with more aggressive forms still, further indicates that the treatment of the malignant clone did not result in the cure of the leukemia, or in removal of the oncogenic selection pressures from the patient's environment. It simply regressed the blastic or neoplastic clone to its cytotic phase, permitting it to reacquire the inducibility of differentiation.

Thus, while the normal cell has a 3-position commitment switch between aerobic growth, differentiation or proliferation, the hyperplastic cell commits to proliferate and grow by accelerated glycolysis - which is the initial commitment to transform. Yet, it keeps open some of the DNA and enzymic circuits involved in differentiation, and can therefore be induced to commit to differentiate instead. It also keeps open the as of yet undefined possibility of a commitment to undergo further transformation, ie to malignantly transform, but its switch is really a 2-position switch - either continue to proliferate, or commit to differentiate. The more dysplastic the differentiation turns out to be, the faster the cell dies, either by apoptosis, autoschizis or necrosis. Fully neoplastic cells have foreclosed the differentiation circuitry - but have not necessarily lost it - and only by regression to the hyperplastic stage of the 2-position switch could they re-engage the differentiation circuitry again (see **Fig. 13**). The more aggressive they become, the less likely it is that such regression will occur (or, therefore, that such cells will respond to differentiation-inducing treatments). The cells eventually lose altogether the property of responding to the induction of differentiation with a commitment to do so.

Another question this raises is whether chronic hyperplastic stages are always nonmalignant, or sometimes malignant. Immortalized cells have hyperplastic characteristics, but are not neoplastic or tumorigenic (for review, see [1]), even if all neoplastic cells are immortalized. Likewise, it is doubtful whether embryonal carcinoma cells are truly malignant, even though they form carcinomas in transplant hosts [1]. If acquisition of adaptive changes that involve DNA mutations and genomic alterations - and are therefore not simply epigenetic in nature - is a criterion needed to define malignant

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transformation, then many of the hyperplastic disorders - like all the CMPDs - are already neoplastic. They have, at any rate, already engaged the transformation vector of oncogenesis.

All of the above provides a novel approach to the understanding of cancer. The cancer cell, in its many varieties, is an eukaryotic adaptation to energy-poor environments. In the absence of a need for mutagenic exposure or a viral infection, a cell engages in a neoplastic process in response to systemic and enduring pressures of energy starvation, whether hypoxic, dietary or toxicological. Once engaged, it becomes an hyperplastic cell - a genomically-unstable neo-Lamarckian element that directs post-adaptive mutations to increasingly rely upon glycolysis as a source of energy. At first, the hyperplastic cell is likely hypersensitive to regulatory controls. However, with progression, it transforms further into a neoplastic cell that is independent of regulatory controls and shuts down aerobic respiration.

5. Control of the Warburg effect is mediated by HIF-1

5.1. The hypoxia gradient of tumors, and the symbiosis of metabolically heterogenous cancer cells

Very recent research has finally illuminated the complex nature of the Warburg effect and - in our view - confirmed the essential contention common to the orgonomic [36]. and aetherometric models of oncogenesis, of a pivotal role in the induction of cancer being played by chronic depression of the local and systemic partial pressure of oxygen.

Tumors present inversely reciprocal internal gradients of pO_2 and pH, the oxygen concentration decreasing and acidity increasing distally with distance to the closest blood supply [152]. These gradients correlate with both the topological and the metabolic distributions of cancer cells: those that are most hypoxic have high levels of the HIF-1 factor in the nucleus - and thus increased glycolysis, LDHA-driven lactate production and acid secretion - and are located furthest from the nearest blood vessel; while the aerobic metabolizers are found nearest the blood supply (see **Fig. 14**).

As we have already mentioned above, one of the main problems with the Warburg effect is that it was not truly universal; that, while glycolysis is increased in all tumor cells, there was metabolic heterogeneity (Siminovich's and Axelrad's argument), with some cells being lactate fermenters while others preserved some form of aerobic respiration. By 2004 it was definitively established that, in solid tumors, hypoxia constitutes the main environmental oncogenic pressure, so that after repeated cycles of its intensification, selection for resistance to hypoxia-induced apoptosis occurs among the tumor cells [153]. Then, in 2007, Peter Vaupel and colleagues showed that the partial pressure of oxygen inside tumors is lower than in surrounding normal tissue, and that development of more aggressive cancer cells capable of faster rates of division and greater metastatic potential correlate with the

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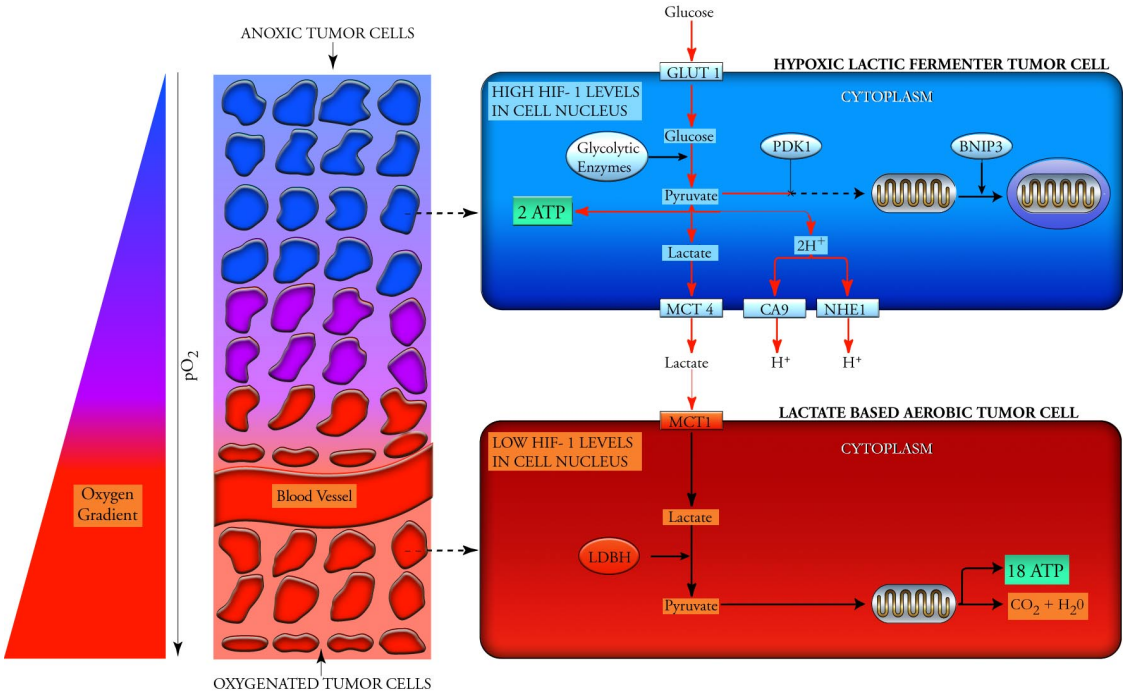

Fig. 14 - Metabolic tumor cell heterogeneity between lactate fermentors and lactate respirers, as a function of oxygen partial pressure. Discovered by J. Sonveaux et al [**30**]). After G. Semenza [**155**].

degree of intratumoral hypoxia along the oxygen gradient (see **Fig. 14**) [154]. Finally, in 2008, Sonveaux et al showed that inside each tumor there is a "metabolic symbiosis" between anaerobic and aerobic cancer cells [30]. Whereas, with low energy efficiency (2 moles of ATP generated with one mole of glucose) and under the action of hypoxia-*inducible* factor 1 (HIF-1)-controlled LDHA, fermenter tumor cells convert glucose into lactic acid and acid ion which they excrete, the tumor cells that lie near the blood vessels and still rely upon aerobic metabolism for energy extraction uptake lactate via the monocarboxylate transporter 1 (MCT1), and, through expression of LDHB, utilize it as substrate for oxidative phosphorylation (generating 36 moles of ATP per 2 moles of lactate consumed, see **Fig. 14**). Tumor heterogeneity finally could be explained by adaptive and symbiotic responses to localization with respect to the oxygen gradient, and the symbiotic relationship was mediated by high levels of expressed HIF-1 in the nucleus of the lactic fermenters, and by the activation of LDHB and low nuclear levels of HIF-1 in the lactate "respirers".

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Previously, it was thought that, if all cancer cells engaged in lactic fermentation, then all cancer cells were effective parasites. Since the formation of a molecule of glucose from lactate (through the so-called "uphill" conversion of pyruvate to glucose that takes place only in the liver) requires input of six molecules of high-energy phosphate, whereas the lactic fermenter cancer cells only obtain two molecules of ATP per molecule of glucose consumed (see **Fig. 14**), cancer cells were thought to place tremendous energy and lactic acid loads on the liver of the host, and thus drain the energy of the "organism" to satisfy their high glucose requirement. But Sonveaux et al showed that this was not exactly the case, or even the main story, as the heterogeneity of cancer cells inside a tumor exhibited a symbiotic, metabolism-based "differentiation" or separation. This, in our view, is one more fact that attests to the adaptive nature of the neoplastic process. The lactic fermenters did not so much rely upon the host liver to convert lactate back to glucose, as they symbiotically fed their lactate to the lactic "respirers", which, instead of sustaining their oxidative phosphorylation from glucose-derived pyruvate (as normal aerobic cells do), sustained it instead from lactate-derived pyruvate produced 'in reverse' by LDHB. All happens as if the metabolic heterogeneity and synergism of neoplastic cells inside a tumor were the result of two kinds of possible "differentiation" of malignantly transformed cells.

In light of what we wrote above about dynamic poisoning of the respiratory chain by copper and the higher levels of copper observed in cancer cells [52], one cannot help but wonder whether the distribution of copper in solid tumors may not present a gradient inverse to that of the gradient of oxygen, and thus parallel to the gradient of acid ion - with the lactic fermenters being the cells that concentrate copper and employ it to block aerobic respiration. Note that the current biochemical contention that copper is required to complete the respiratory chain, leads precisely to the opposite contention - that, in tumor cells, copper should be concentrated in the tumor respirers, rather than the tumor fermenters. A determination of which contention is correct would clarify the role played by copper in the etiology of cancer.

5.2. The physiological role of Hypoxia-inducible Factor-1 (HIF-1)

HIF-1 regulates the transcription of genes whose products play critical roles in energy metabolism, erythropoiesis, angiogenesis, cell survival and apoptosis (cell death). It is the first line of response to hypoxia, and any proliferative stimulus will invoke its expression. HIF-1 acts as transcriptional activator that mediates and modulates multiple homeostatic processes, including those involving iron metabolism, and expression of the gene encoding vascular endothelial growth factor (VEGF) [155]. The expression and transcriptional activity of HIF-1 alpha subunit gene is regulated by cellular oxygen concentration.

HIF-1 induces the expression of proteins that increase uptake and transport of glucose (such

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as GLUT1); that convert glucose into pyruvate (glycolytic enzymes); that generate lactic acid and free protons [156-157] (eg LDHA), and export these two molecules out of the cell [158-160] (eg carboxic anhydrase IX, monocarboxylate transporter 4, MCT4, and the sodium-hydrogen exchanger 1). HIF-1 also shunts away pyruvate from mitochondria (through PDK1) [161], and downregulates mitochondrial utilization of oxygen [162], including a reduction of mitochondrial mass as a result of autophagy [163].

5.3. The modulating function of HIF-1 in controlling cancer cell metabolism

The hypoxia gradient within tumors corresponds to a gradient of metabolic reprogramming largely "orchestrated" by HIF-1 - with the fermenters being activated by HIF-1 expression, and the respirers having HIF-1 turned off by their exposure to higher oxygen concentrations. There is thus an intra-tumoral gradient of nuclear HIF-1 inverse to that of the oxygen gradient. The HIF-1 gradient responds to our "primary kinematic wave", and thus the localization of tumor cell heterogeneity itself is an expression of the primary wave. The aerobic cancer cells (the lactate "respirers") take advantage of the inverse HIF-1 gradient because hypoxia represses expression of MCT1, and thus only "respirer" cancer cells can employ it as an intake transporter of lactate. This permits the lactate "respirers" to symbiotically coexist with the more aggressive fermenter cells inside the same tumor.

Remarkably, hypoxia also induces a proportion of cancer cells to undergo cell death. This is likely less frequent amongst tumor cells *in vivo* than with derived cell lines *in vitro*. In the latter, as we have seen, the proportion of spontaneous cell death is high [1]. In a variety of cell lines, the hypoxic cell death, whether necrotic or apoptotic, can be blocked by overexpression of the antiapoptotic proteins Bcl-2 and Bcl-X_L [164].

6. Hypoxia-independent circuits in HIF-1 control and in malignancy

6.1. The insulin-like circuit (IGF axis) mediates HIF-1 expression

Most interesting, in light of the IGF-I hypersensitivity of PV circulating erythroid progenitor cells. and of the IGF-I-independence of *src*-transformed cells and autocrine leukemic cell lines [1], is that insulin and insulin-like growth factors (IGFs) induce transcription of metabolic and hematopoietic genes via expression of the gene for HIF-1α [165-166]. In other words, the same HIF-1α gene that responds to oxygen concentration and is turned on by hypoxia can also be activated by the insulin/IGF-I axis quite independently of the oxygen concentration. Induction of the gene for HIF-1α by organomercurial compounds is abrogated if the gene encoding IGF-IR is eliminated by targeted mutation [167], so, normally, IGF-I activation of its receptor is required for hypoxia-independent induction of HIF-1α. In fact, it seems that all three factors - insulin, IGF-I and IGF-II - induce expression of the *HIF*-1α gene, which, in turn, as in a self-amplifying loop, is required for expression of the genes for IGF-II and for two of the IGF-binding proteins (3 and 5) [168]. It is the entire IGF

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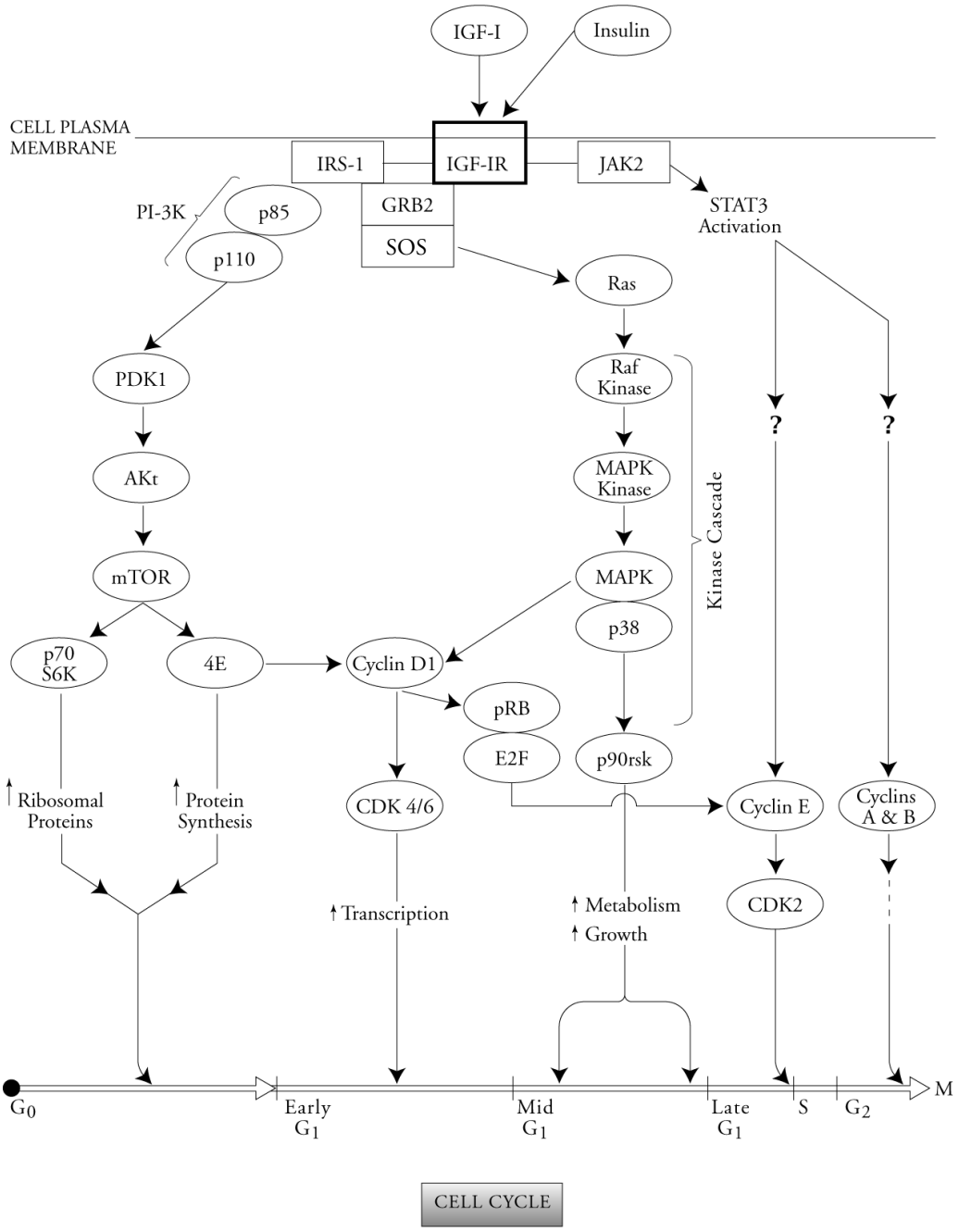

Fig. 15 - IGF-I and cell cycle control exerted via IGF-IR-downstream signal effector pathways involved in recruiting cells into the cell cycle, activating glycolysis, mediating and modulating HIF-1 activity, controlling metabolism, activating DNA transcription, DNA replication, mitosis and cell proliferation.

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axis - with its interplay of signals emitted inside the cell by the cytoplasmic domains of both receptors, IGF-IR and IGF-IIR - that is implicated in the hypoxia-independent regulation of HIF-1. The IGF-I receptor (IGF-IR) has intrinsic tyrosine kinase activity [169], and is normally involved in controlling uptake of glucose, activation of glycolysis and metabolism - tasks that it shares with the insulin receptor - as well as progression through the cell cycle, growth, differentiation, and inhibition of apoptosis [170] (see **Fig. 15**).

Cells from human breast and colon cancers have in common HIF-1 stimulated activation of

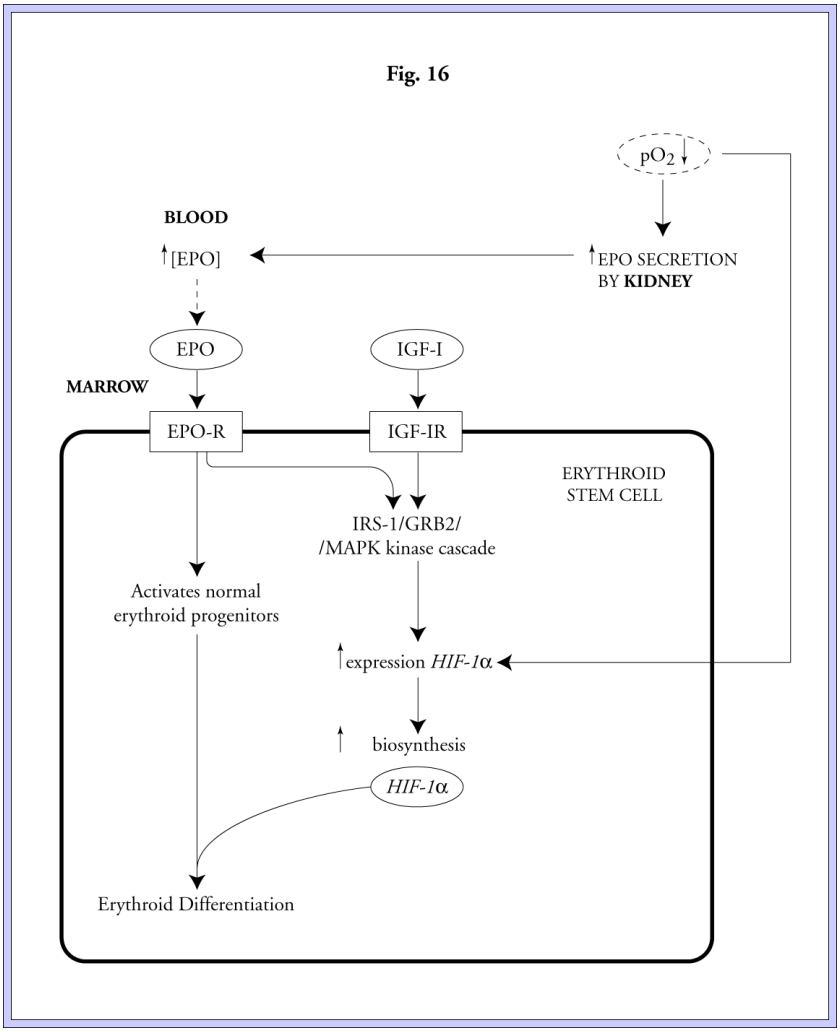

Fig. 16 - Joint control of normal erythropoiesis by EPO and IGF-I.

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different receptor tyrosine kinases and HIF-1-mediated increased expression of the *VEGF* gene [166]. In these cancer cells, the hypoxia-independent IGF axis compounds the effects of hypoxia. Whereas hypoxia inhibits ubiquination and degradation of HIF-1α, IGF-I increases the HIF-1α protein synthesis and *VEGF* mRNA expression. Moreover, the IGF-I induction of *HIF-1α* gene expression was shown to be MAPK kinase-mediated [166], and thus to operate via the insulin receptor substrate 1 (IRS-1) [171] (see **Fig. 15**). Evidently, through this pathway, the IGFs can either up- or down-modulate the HIF-1-mediated effects of hypoxia. Indeed, whereas, for example, the latter has apoptotic effects, activation of IGF-IR has an anti-apoptotic effect [170, 172-173]. The MAPK kinase pathway controls G_1 metabolism and growth [174] (see **Fig. 15**).

6.2. A dynamic model of erythropoiesis and the role of hypoxia

6.2.1. IGF-I modulates HIF-1 control of EPO-responsive erythropoiesis

When the preceding findings are coupled to the fact that HIF-1α deficient embryos have altered iron homeostasis which simultaneously correlates with decreased expression of EPO receptors and decreased transcription of the EPO gene [175], one is led to conclude that in these embryos the IGF-I pathway is not recursively engaged by erythropoiesis once the EPO pathway fails, because the IGF-I downstream HIF-1 circuit is turned off. Thus, the embryonic erythroid response to EPO depends on insulin and insulin-like factor induction of HIF-1 (see **Fig. 16**), with the insulin and insulin-like growth factors priming the erythroid progenitor cells whenever hypoxia is sensed. In other words, the IGF axis is not simply independent of hypoxia, but also the modulator of the response to hypoxia, including the erythropoietic response to EPO that normally induces differentiation and maturation of RBCs.

Insulin-like growth factors, and IGF-I in particular, are likely the agent (or the main one) that was found, back in 1981, to greatly enhance the effect of EPO in IGF-I-contaminated serum-containing cultures of BFU-Es grown under hypoxic conditions [176]. In fact, the existence of an EPO-IGF-I synergism in normal erythroid growth and differentiation has been demonstrated in a practically defined, serum-free medium (see **Fig. 17**) [139]. Moreover, normal adult erythropoiesis can be driven either by EPO in the absence of IGF-I (see **Fig.s 17 & 18A**), or by IGF-I in the absence of EPO (see **Fig.s 17, 18B & 18C**) [139].

It is known that relative EPO sensitivity of BFU-E increases up to 8x with hypoxia [177], but this has not been confirmed in serum-free tissue culture, and thus has not been distinguished from either the EPO-enhancing effect or the HIF-1-induction effect of IGF-I, a known serum-contaminant. Whether a parallel enhancement of cytokine response - or a "conditional modulation" - by insulin and insulinlike growth factors is also at work in the kidney peritubular cells that normally secrete EPO is unknown.

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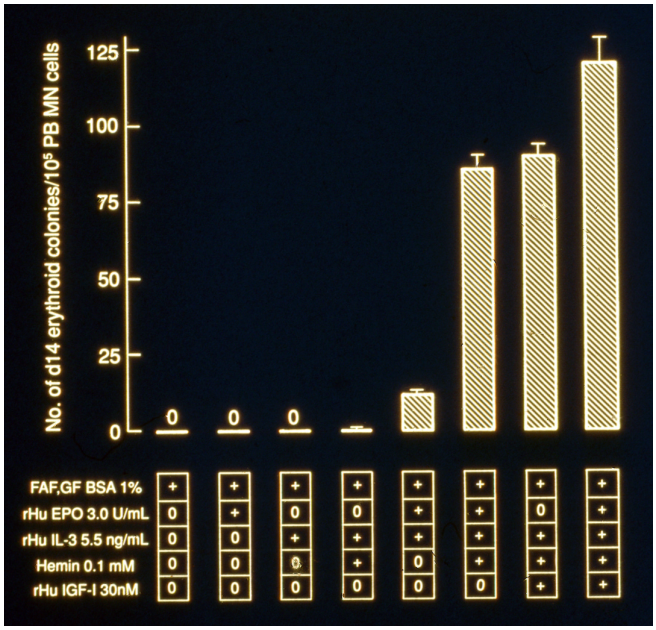

Fig. 17 - Synergism between EPO-induced and IGF-I-induced d14 erythroid burst-component colony formation in a true serum-free medium (S_0Z_0) devoid of background growth activity and employing defined growth factor additions. After P.N. Correa & A.A. Axelrad [139]).

6.2.2. IGF-I hypersensitivity drives EPO-independent erythropoiesis independently from hypoxia

These findings suggest that there is a recursive regulation of erythropoiesis, as Correa and Axelrad have suggested [138, 140-141], employing alternative but synergistic EPO-driven and IGF-I-driven pathways that overlap in normal erythropoiesis.

Increased EPO production in response to hypoxia is a central controlling process of normal erythropoiesis, the erythroid progenitor cells responding to EPO with proliferation, increased glycolysis and aerobic respiration, and a 'clocked' differentiation and maturation after a series of rounds of cell division. This response, however, is mediated by the transcriptional activator HIF-1, which, in turn, depends on activation of the insulin and insulinlike growth factor circuit.

When EPO production fails, or its concentration is low (as in PV, probably due to the negative feedback from the erythrocytosis), the IGF-I circuit takes up the entire load of the hypoxic pressure to produce oxygen carriers, ie differentiated erythrocytes (see p. 197 of reference [138]). This puts pressure to resort to an IGF-I-hypersensitive pathway, which is the result of the JAK2 adaptive mutation in PV [137]. These findings were stark confirmation of the Correa & Axelrad discovery of IGF-I hypersensitivity in PV (the resulting model of PV erythropoiesis is shown in **Fig. 19**). The IGF-I hypersensitivity is mediated by tyrosine-phosphorylation of the β-subunit of the IGF-I receptor [178].

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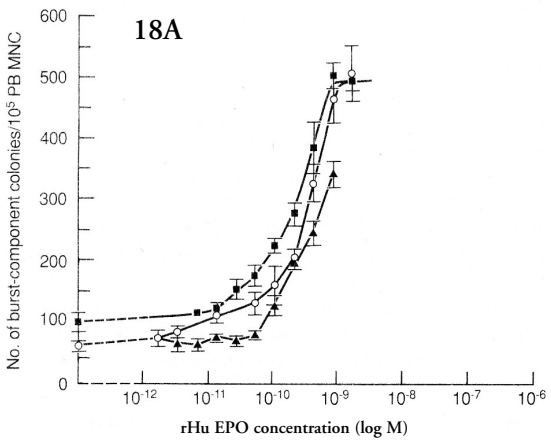

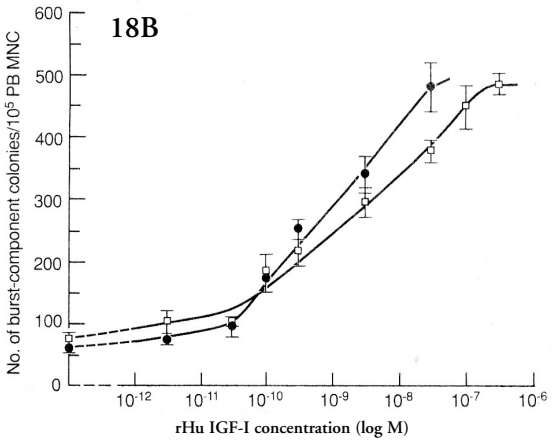

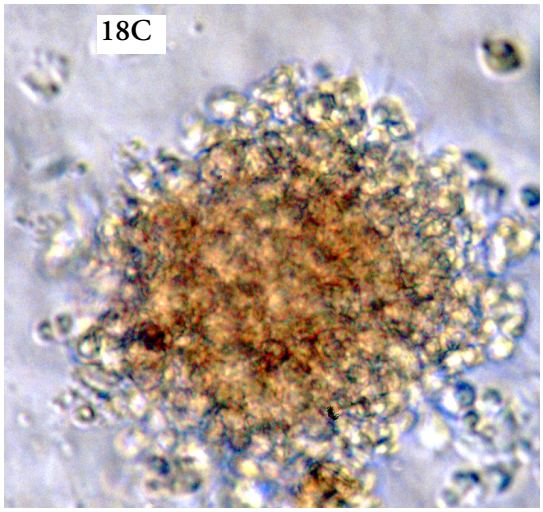

Fig. 18 - Normal erythroid burst-component colony (BCC) formation by circulating blood mononuclear cells in a serum-free medium with defined growth factor activities, induced either by EPO in the absence of IGF-I (**Fig. 18A**) or by IGF-I in the absence of EPO (**Fig. 18B**). After Correa & Axelrad [139]. These findings proved that normal erythroid progenitors respond to IGF-I as a recursive driver of erythropoiesis under EPO-independent conditions. **Fig. 18C** -High power view of a live, naturally hemoglobinized, IGF-I-induced, EPO-independent, single-colony burst cloned from a circulating blood mononuclear progenitor cell of a normal donor. Mag.: ~300x.

Correa, Axelrad and co-workers also found that overexpression of *SOCS-2* and *SOCS-3* genes is able to reverse both the IGF-I hypersensitivity and the PV erythrocytosis [179].

Moreover, via the hypersensitive response to IGF-I, PV cells are able to induce HIF-1 (and probably also HIF-2) *independently of hypoxia*. Erythropoiesis can thus be turned on constantly, the result being a primary erythrocytosis.

The reader should note that this model *explains just as well why PV patients are refractory to hyperoxic treatment*, given that the IGF-I circuit - *and its induction of HIF-1* - does *not* depend on oxygen concentration.

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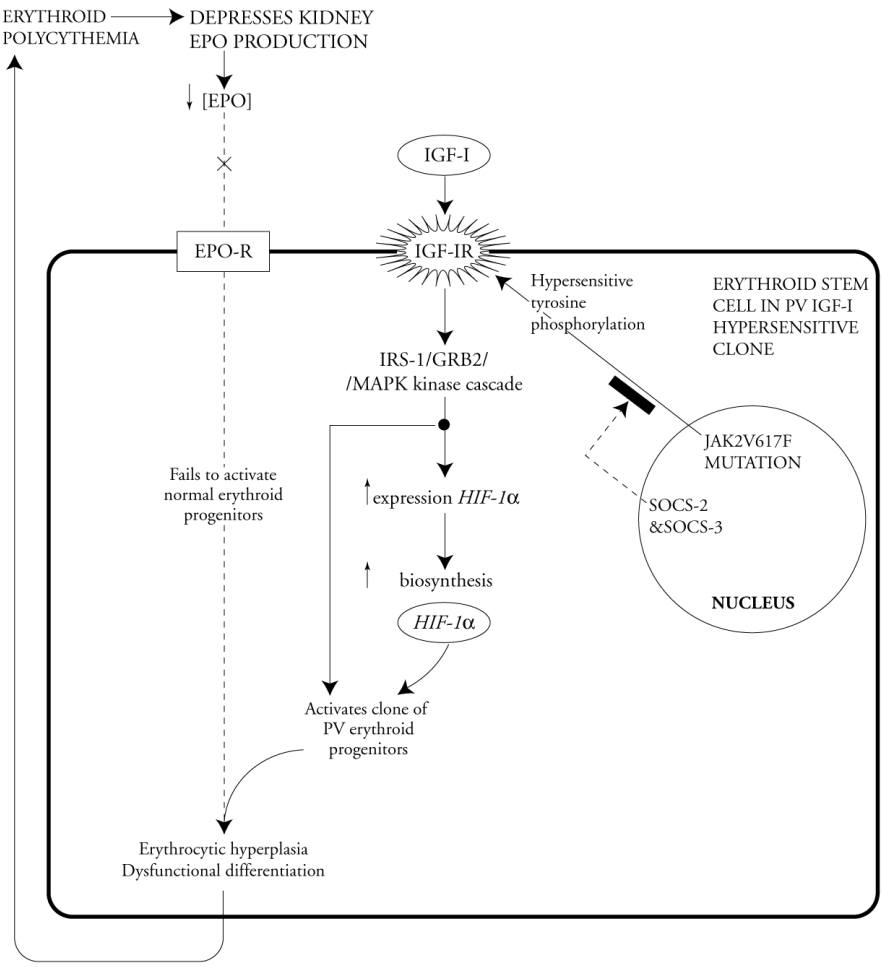
Fig. 19 - The Correa & Axelrad model of hypersensitivity to IGF-I in *Polycythemia vera*.



6.3. Role of insulin-like factors in the initiation and progression of malignancy

Regulation of IGF-I signaling and response(s) is a complex subject. Extracellularly, the IGF-I signal is regulated by both inhibitory and stimulatory IGF binding proteins (IGFBPs). Some of these IGFBPs (the inhibitory IGFBP-3 and the stimulatory IGFBP-5) are expressed in response to the binding of IGF-I to its receptor, IGF-IR. The subject is incredibly involved, but it appears that IGFBP-3 mRNA expression, but not IGFBP-5 mRNA, is mediated by the MAPK pathway, and that,

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upstream of this pathway, expression of mRNA for both IGFBP-3 and IGFBP-5 is mediated by another pathway, the phosphatidylinositol-3 (PI-3) kinase pathway [180].

Intracellularly, as we have seen, the IGF-IR circuit operates via the IRS-1/GRB2/MAPK kinase cascade pathway to induce expression of *HIF*-1α and control metabolism, growth and differentiation (see **Fig.s 15, 16 & 19**). Most importantly, IGF-I modulation of the kinase cascade via IRS-1 converges, upstream of the cascade, with the signal from a variety of other growth factors (eg GH [181], IL-4 [182] and EPO) at the GRB2 protein (the IGF-I signal converges with the signal of insulin at the IRS-1 step, see **Fig. 15**). This crosstalk between signals of cytokine receptors means that various cytokine signaling circuits invoked at different stages of hematopoiesis (and not just those of erythropoiesis) are modulated, positively or negatively, via the IGF-IR. Lymphopoiesis is directly stimulated by IGF-I via the IGF-IR, so the entirety of the lymphoid immune response is also modulated by the IGF axis [183].

From the IRS-1 element there open up at least two distinct pathways for the action of IGF-IR. As shown in **Fig. 15**, aside from the MAPK kinase pathway that, through the GRB-2/SOS protein complex, controls G_1 metabolism and growth [174], there is the phosphatidylinositol-3 (PI-3) kinase pathway that recruits cells into the cell cycle, from G_0 to G_1. IGF-IR enhances protein synthesis and exerts anti-apoptotic effects through the PI-3 kinase pathway [184]. Through both of these pathways, IGF-IR exerts its control of the G_1/S interphase, via cyclins D1 and E (see **Fig. 15**) [185]. It is thought that the regulatory effect of IGF-IR at the G_2/M interface is mediated by cyclins A and B and Cdc2 synthesis [186]. Thus, mediation by cyclins, seems essential for induction of replication (S phase) and proliferation (M phase) by the activated IGF-IR.

But, at least in some cell types, there seems to be still a third pathway for IGF-IR signaling inside the cell [185]. In some cell types, IGF-IR can phosphorylate directly the Janus kinases, JAK-1 and JAK-2, that are involved in mediating cytokine signals [187] and in activating the STAT (signal transducers and activators of transcription) proteins. In transformed cell lines, STAT3 is commonly activated by the IGF-IR [188]. Activated STAT3 is sufficient to induce cellular transformation *in vitro* [189], and it has been shown that STAT3 is constitutively activated in granulocytes of some PV patients [190]. Thus STAT 3 activation appears to be an essential mediator of the transforming activity of IGF-IR.

Thus, it appears that IGF-IR plays a central role in malignancy. Aside from the documented IGF-I hypersensitivity of PV cells, the IGF circuit plays a role in a large catalogue of malignancies, mostly carcinomas (breast, colorectal, pancreatic, bladder), melanomas, neuroblastomas, glioblastomas, meningiomas and medulloblastomas, as well as - via control of HIF-I and the VEGFs - in both tumor angiogenesis and lymphangiogenesis. Increased IGF-I, IGF-II and IGF-IR expression has

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been reported in all these malignancies, but, as documented extensively in a recent review [185], nearly three decades of data has yielded rather conflicting evidence for the role of IGFs in cancer. The existence of multiple molecular mechanisms, both inside and outside the cell, regulating IGF-IR expression and function greatly complicate the interpretation of the data. It has been suggested recently that, rather than expression of IGF-I, IGF-II or IGF-IR, the IGF-I/IGFBP ratios may be better predictors of the outcome of these various malignancies [185], but given the complexity of the IGF-IR signaling inside the cell, and what is still to be found, this may well be precocious. Nevertheless, it seems that overexpression of IGF-IR is significantly linked with the development of acute blastosis [191] and metastatic malignant phenotypes [192-194], and may thus serve as a predictor of a poor outcome in some types of cancer. And it has been shown that signals from both the kinase and the C-terminal domains of IGF-IR are necessary for tumor invasiveness of malignant cells and upregulation of matrix metalloproteinase-2 (MMP-2) [195-196]. But, given the status of the current knowledge regarding the role of IGFs in the promotion and maintenance of cancer cells, it is far too early to think, clinically, in terms that simplify the matter down to the notion that suppression of positive effectors of the IGF system will result in inhibition of tumor growth, as many have suggested it may happen. With a molecular system this complex, and given its interaction with so many other gene and cytokine circuits, it seems that there is no simple suppression of a positive effector that could have unequivocal therapeutic results.

6.4. The IGF axis in the aetherometric etiology of malignancy

It is apparent that HIF-1 plays a critical role in sustaining the proliferative states of normal and malignant cells, whether these are normal cells, leukemic cells or somatic cancer cells. It is equally apparent that, aside from the interplay of IGF binding proteins and the various IRS-1-downstream pathways, the role of HIF-1 is controlled both by the concentration of oxygen and by the state of activation of the insulin and IGF circuit, in particular by the interplay of IGF-IR and IGF-IIR. The latter may, in fact function as a negative regulator of IGF signaling and its overexpression to have an antiproliferative effect on cancer cell lines (reviewed in [185]).

At the level of erythropoiesis and the organism's response to oxygen starvation, the action of IGF-IR is essential for the production of properly differentiated erythrocytes. Chronically depressed EPO levels (without hypoxia, as in response to primary erythrocytosis, see **Fig. 19**) eventually trigger an hypoxia-independent adaptive response driven by IGF-I hypersensitivity, as is observed in PV. Even though this response is not observed in secondary erythrocytosis [1], it may also be induced by chronic hypoxia. In myeloproliferative disorders, the *JAK2V617F* mutation mediates the IGF-IR induction of hyperplastic proliferation. JAK proteins may also phosphorylate IRS-1 with the result that specific activities (probably mediated by the cyclins) of IGF-IR through the other two pathways

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are enhanced. Hypoxia, acting via HIF-1, can only aggravate the role of IGF-I in sustaining hyperplasia, by further stimulation of the MAPK kinase pathway that also enhances the role of the cyclins. If the oxygen starvation becomes even more acute, systemically and/or locally, the adaptive pressures, hypoxic and nonhypoxic, will likely push a targeted cell towards independence from regulation by IGF-I, either via constitutive expression of the *IGF-I* gene in autocrine loops, or by mutations of the *IGF-IR* gene that result in constitutive activation of the IGF-I receptor - along with other alterations in any downstream elements of the IGF-IR circuits.

So, while on one hand, the effects of hypoxia and the IGF axis converge, to the point that they unify in the same pathway in response to hormonal mitogens such as GH or EPO, on the other hand, real or phenomenological ligand-independence from the IGF axis invokes still other pathways that affect cell replication and proliferation. One may therefore superimpose a gradient of IGF-IR activation along the IGF axis upon the oncogenic vector. In hypoxia, the EPO axis is primed by both the IGF-IR and the HIF-1 circuits, through the MAPK and PI-3K pathways. Upon initiation of the oncogenic vector, lactic fermentation and proliferation are accelerated by mutations in JAK and associated genes, which mobilize other pathways for IGF-IR and permit ligand-hypersensitive response. In mild but chronic hypoxia, with depressed EPO levels, the PV hypersensitive response to IGF-I would be a prototypical adaptive change involving still aerobic but now transformed cells. In deep chronic hypoxia, ie anoxia, one would encounter autocrine IGF-I mechanisms - as found in leukemia cell lines - or IGF-I-independent self-phosphorylating receptor activity - as found in a variety of tumorigenic cells. Similarly, one would expect to more frequently find, inside tumors, critical mutations that activate the IGF-IR among the lactic fermenters, than among the lactic respirers. In this scenario, all the different adaptations encountered in the course of unfolding the neoplastic vector, are neo-lamarckian adaptive changes designed to respond outside of tissue constraints to the growth requirements of cells in energy-stressed (including, first and foremost, oxygen-deprived) environments. The very fact that PV is not hypoxia sensitive indicates that the oncogenic vector points towards the development of *hypoxia-independent* responses that are increasingly more malignant, to the point that, *by becoming independent from IGF-I control, the cell also becomes independent of regulation by the hypoxia-independent circuits of the IGF axis*.

However, we are not saying that alteration of the insulin-like factor circuit is a universal trait of cancer cells. It might turn out to be such once we are able to better understand the roles of IGF-IR and IGF-IIR in development, hematopoiesis and malignancy. Similarly, it seems that the roles of HIF-1 and HIF-2 may also prove to be central to the induction and maintenance of all forms of malignant transformation (their role in leukemia needs further investigation). Yet, it is unclear, for instance, what role either the IGF axis or the HIF axis play at the onset, or in ATRA-induced

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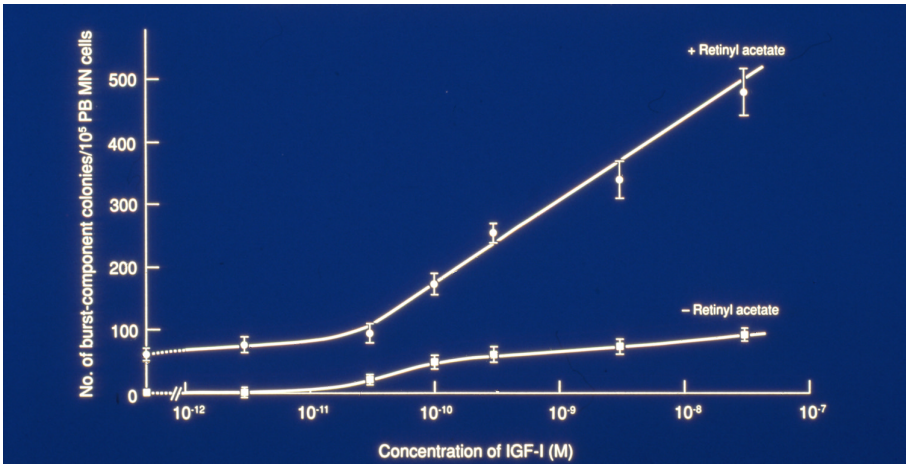

Fig. 20 -Number of d14 BCCs from circulating erythroid progenitors in complete serum-free medium as a function of IGF-I concentration and the addition of retinyl acetate (vitamin A). A similar enhancement of erythroid colony formation is observed with ATRA [197]. At concentrations above 10^{-7} M, the retinoids suppress colony formation [197]. After Correa & Axelrad [139].

remission, or in the relapse, of APL. Such investigations could be advantageously conducted with primary cells in a true serum-free medium. In the growth of both myeloid and erythroid progenitors, vitamin A and its analogues, including ATRA, play an essential role in promoting both terminal proliferation and differentiation. Retinyl acetate and ATRA act as co-inducers of erythroid and myeloid differentiation that dramatically increase the response to EPO and IGF-I in serum-free studies of human BFU-E from normal peripheral blood [197]. Both retinoids have also been shown - in serum-free medium - to enhance EPO-independent, IGF-I-driven erythropoiesis of normal progenitor cells (see **Fig. 20**), but to have a negligible effect on the EPO-independent, IGF-I hypersensitive erythropoiesis of PV progenitor cells [141]. This is strongly indicative of an as of yet uninvestigated interaction between response to vitamin A compounds and the IGF axis, whether in normal cells undergoing differentiation, or in myeloproliferative and neoplastic cells where the IGF axis undergoes different kinds of alterations.

As we have seen, there is mounting evidence that the IGF axis may be involved in all stages of the oncogenic vector - in cancer promotion, initiation and progression. It is likely that the danger of hypoxia has favoured a cellular strategy that modulated all of the cell's activities at once (1) in a manner *convergent with that of the effects of hypoxia* (thus hypoxia-independent stresses can induce a hypoxic-like response through intracellular pathways shared with the hypoxic response), and (2) in

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manner that is *hypoxia-independent but parallel to the effects of hypoxia* (thus some of the IGF-IR mitogenic abilities do not converge with the pathway conveying the mitogenic stimulus of hypoxia). As we have briefly discussed above, the insulinlike circuit is subject to a complex and delicate balance of recursive controls: outside the cell, the IGF-I signal is modulated by both inhibitory and stimulatory binding proteins, the gene expression and protein synthesis of some of these binding proteins being controlled by IGF-I via a positive feedback loop; inside the cell, IGF-IR signaling depends not just upon positive interaction with its ligand, but also upon negative interaction with type II IGF receptor (IGF-IIR) - which therefore functions as a negative effector of IGF-IR [198]; moreover, inside the cell, there are at least three distinct pathways (PI-3K, MAPK, and JAK) that transmit the IGF-IR signal, with very different and sometimes antagonistic effects. Any alteration(s) of these balancing systems at any of the levels in the signaling circuits has the potential to lead to malignancy. Likely, this potential emerges at every stage of the insulinlike signal pathways downstream from the IGF-IR. Upstream from the kinase cascade, it can diminish or suppress, and likely potentiate, any cytokine signal of GRB-2-converging cytokine pathways, including those triggered by hypoxia; downstream from it, it can induce *HIF*-1α independently of hypoxia, and thus induce any HIF-1-mediated activation of gene expression (eg *VEGF*); in parallel, through the PI-3K pathway, it can amplify the effects of hypoxia by recruiting quiescent cells into the cell cycle and, by activation of cyclins D1 and E, induce cell replication and proliferation; through another parallel kinase cascade, the JAK pathway, it can activate STATs and control the phosphorylation of various signal transduction pathways - making this pathway a preferred target for adaptive changes that suppress differentiation and promote malignant transformation.

At the level of the organism, autonomic control of blood glucose and its cellular uptake is performed by antagonistic hormonal signals. Orthosympathetic effectors - such as glucagon and adrenalin - increase blood glucose and activate lactic acid production, whereas parasympathetic effectors - such as insulin and IGF-I - activate enzymatic co-factors that permit glucose to be taken up from the blood by fat cells (triggering the fatty-acid shuttle) and liver cells (triggering the same shuttle and glycolysis, plus glycogenesis). While insulin is an endocrine production from the pancreas, IGF-I is a paracrine production from liver cells, triggered solely in response to GH, and which can also bind to the insulin receptor. Now, with reference to **Fig. 15**, where the IGF-I axis or IGF-IR circuits 'hit' the cell at different checkpoints of the cell cycle, we suggest that - with reference to the conceptualization of the cell cycle as part of an autonomic control of cellular activity (as present above in section 2) - the PI-3K and MAPK pathways constitute, inside the cell, the parasympathetic 'nervous' system that corresponds to the parasympathetic effect of insulin and IGFs at the level of the organism. These effects recruit the cell into the first interphase and ensure its progress. They activate glycolysis, but so

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as to couple it to aerobic metabolism. However, either as part of the normal regulation of the IGF axis (control of cyclins) or as the result of adaptive changes that engage an auto-oncogenic vector, the IGF-IR can also function as an intracellular orthosympathetic effector, by inducing progress of the cell cycle from the first interphase to the S phase, or from the second interphase into mitosis. It is in the orthosympathetic functions of the IGF axis - functions that control both G_1/S and G_2/M interfaces - that the oncogenic potential of this axis lies. What is fascinating in this respect is that vitamin D compounds block precisely the mitogenic ability of IGF-I embodied by these two orthosympathetic functions. This is accompanied by a downregulation of proliferation and an increase of apoptosis in cancer cell lines [199-200]. It is very likely that the ability of vitamin D to block some of the IGF-IR signals and decrease *IGF-IR* gene expression [201] may be mediated by both the IGFBPs and IGF-II.

Accordingly, in the aetherometric model of cancer, the hypoxic stress is regulated by HIFs in tissue, and HIFs and EPO in erythropoiesis, and modulated in both cases by the IGF axis. But when either the hypoxic stress or the nonhypoxic energy stress (as caused by a nutritional deficiency, a respiratory poison, or other factors impacting energy metabolism) becomes chronic, the IGF axis takes over, activating glycolysis and proliferation, through functions and pathways that then become a preferred target for oncogenic adaptive changes. It is in this process that the orthosympathetic functions of the IGF axis gain pre-eminence over its parasympathetic functions. Thus, the IGF axis appears to be a general recursive system, rather than a master axis, whose alteration is essential for oncogenesis. The recursiveness operates both within the IGF axis and outside of it, that is, across most signal regulatory circuits of the cell. Within the IGF axis, we should keep in mind that insulin also acts via the IGF-IR circuitry, and that the insulin receptor, besides being structurally nearly identical to IGF-IR, shares some of the IGF-IR downstream pathways. Outside of the IGF axis proper, the IGF signals prime different growth factor circuits, potentiate them and, if some of them fail, pick up the slack, as happens when normal erythropoiesis is driven by IGF-I (via the IGF-IR) in the absence of EPO. But if the energy-stress persists, whether it is hypoxic or otherwise, or a combination, the adaptive response eventually severs control of the IGF axis from physiological control by its ligands, in particular from control by IGF-I, so that the orthosympathetic signals of the IGF axis become permanently turned on and the cell now exerts an organism-independent, autonomous control over its own cycling. Epigenetic and adaptive alterations of the IGF axis appear to be intimately linked with the initiation and progression of the oncogenic vector. Onset and maintenance of hyperplastic states can be brought about by alterations of the IGF axis, and further changes affecting this axis can determine the subsequent evolution of hyperplasia to full neoplasia.

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7. CONCLUSION:

7.1. The nature of the oncogenic stresses and their unity of action

Despite the myriad of genetic alterations of cancer cells, we find there is a functional bioenergetic and biochemical unity to the diversity of malignant adaptations or disorders. The unity of all adaptive or acquired cancers lies in the minimum denominator of its causation, induction or etiology, which is a cellular and organismic energy stress. This stress has many manifestations, all of which tend to converge in the cancer patient. It is, first of all a stress on aerobic metabolism brought about by hypoxia having become chronic. But it may also be a stress on aerobic metabolism brought about by nonhypoxic constraints, which may be nutritional (lack of vitamin C, vitamin D, vitamin A, iron) or toxicological (as we suggest is the case with copper).

However, hypoxic stress does not simply mean lack of oxygen qua chemical energy. For, according to the aetherometric model of the etiology of cancer that we have proposed above, lack of oxygen means first and foremost lack of absorption of solar and atmospheric ambipolar energy, which translates into lack of thermal energy radiated by hemoglobin and myoglobin, and lack of kinetic energy needed by the electrons in the respiratory chain. Likewise, nutritional stresses are not necessarily nutritional per se. As if to underline the importance of ambipolar energy absorption by the biomolecular antennas involved in delivering oxygen in blood or tissue or in employing it for respiration, deficiency of cholecalciferol (vitamin D_3) is strictly the result of lack of skin exposure to solar radiation. In the normal function of a solar irradiated skin, the captured ambipolar radiation is not converted into thermal energy or kinetic energy, but is converted directly into covalent bond (chemical) energy. Thus, the energy stress of an organism and its cells is first of all the result of *starvation of massfree ambipolar energy*, irrespective of whether this energy is captured by oxygen-mediated enzymic action or directly by chemical substrates, and of whether it is injected into the respiratory chain (role of the cytochromes), or converted into thermal photons (role of hemoglobin and myoglobin), or captured by a chemical bond (role of cholecalciferol).

Yet, the oncogenic energy stress can also be caused by genuine nutritional deficiencies - as is the case with lack of adequate vitamin C - that affect aerobic metabolism and other critical functions of the organism, such as iron absorption and immunity. Lack of vitamin C blocks progression of the Krebs cycle, and this functions as a nonhypoxic constraint that has an hypoxia-like effect.

But oncogenic stresses can be caused by still other factors which our model equally takes into account. They may not be energy factors, but affect just as well energy metabolism. Hypoxia can be brought about by hemorrhage or senescence of RBCs, for example. A decreased EPO production, as found in PV patients, is another such factor. With low EPO levels, the normal hypoxia-controlled mechanism fails to induce erythropoiesis. This is bound to aggravate any hypoxic episode, or induce

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a chronic hypoxia, the very condition that elicits and selects the IGF-I hypersensitive PV clone. The altered clone in turn results in an erythrocytosis - which translates into a greatly increased red cell mass - that further turns off EPO production by kidney cells via negative feedback, and thus further aggravates the condition. Thus the oncogenic constraint here is not hypoxia per se, but a failure to respond normally to hypoxia. It is this failure that eventually forces the recursive IGF axis to adaptively devise the hypersensitive polycythemic response that entirely bypasses the EPO circuit. The chronic hypoxia remains, but given the erythrocytosis, its intensity is mild.

The cell seems to have two main axes of response to these energy stresses - the HIF and IGF axes. The latter, however, normally seems to function as a general recursive axis that is called forth by a great variety of growth factor receptor activations. In hypoxia, it also primes and modulates the HIF-1 circuitry. But, in the presence of persistent hypoxia-like effects, whether caused by hypoxia or not, the IGF axis takes over the cell circuitry, at first replacing other hormone signals, and later becoming the target of mutations that make the entire axis independent of physiological control. It is as if the cell had come to recognize that it was no longer going to be able to obtain energy from ambipolar sources or oxygen-mediated processes - that it was no longer going to be able to respire and thus was going to suffocate (which is the essence of Reich's argument [36]) - and thus it decided that control of its growth and proliferation could no longer abide by the physiological regulators, and thus had to transfer these functions to an emergency system (the IGF axis) which activated glycolysis and lactate production (via HIF-1 induction of LDHA).

It is equally of note that the diversity of transformed cells in a tumor obeys an hypoxic gradient distributed between lactic fermenters and lactic respirers, with both types of cancer cells having altered their cellular metabolism. There is evidence that suggests that HIFs also induce the expression of the LDHB gene on the short arm of chromosome 12. Evidently, both lactic respirers and lactic fermenters avail themselves of the HIF circuits that are modulated by the IGF axis to sustain their transformed phenotype. But since the LDBH gene appears to be encoded by mitochondrial DNA and lactate respiration takes place in the mitochondria [202], the respirers found a way to keep mitochondria in a metabolic functional state for as long as the lactate fermenters kept producing lactate. In all likelihood, the IGF axis is involved in regulating this symbiosis between tumorigenic cells committed to very different metabolisms, with the respirers moderating the Warburg effect of the fermenters.

7.2. Adaptive cancer appears to be unitarily caused by persistent energy starvation

Thus, if energy starvation is the most basic aspect of oncogenic selection pressures, the disturbance of aerobic metabolism is its earliest manifestation. From there onwards, the responses or solutions found by the affected cells (whether it is an acceleration of glycolysis, or also an acceleration of lactate production as in the Warburg effect, or instead the activation of a muscle-like form of lac-

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tate respiration), are all solutions to the disturbed aerobic metabolism driven by lack of oxygen and poor absorption of ambipolar energy. This, then, is the unity that vectorially underlies the cause of all auto-oncogenic cancers: prolonged and systemic energy starvation shuts down aerobic metabolism. The universality of cancer - the unity of its manifestation as a multiplicity - does not lie in a particular transformed cellular state, but in the impairment of aerobic metabolism. This unity is also expressed at the terminus of the oncogenic vector, by the universal characteristic shared by *all terminal, metastatic neoplastic cells*: the most malignant and aggressive cells are strict lactic fermenters (Warburg effect). Even here, there might be a chance for benign therapeutic interventions, because immortal and aggressive as these terminal neoplastic cells might be, they can be killed with oxygen, high dose vitamin C or vitamin D treatments, or induced to regress and differentiate - as with vitamin A compounds.

The various lines of evidence discussed in the present communication are slowly operating a veritable unspoken revolution in the understanding of the cancer cell and how a malignancy can be acquired. In the process, the fundamental insights and discoveries of many oncological approaches were enjoined - Warburg's effect and hypothesis, the orgonomic model of cancer, Szent-Györgyi's theory of a metabolic switch between alpha and beta states of metabolism, the molecular genetics of experimental viral oncogenesis, the discoveries of the oncogene theory of cancer and the biology of growth factors, and our aetherometric model where oxygen figures prominently as provider of chemical energy (in aerobic metabolism and the functioning of hemoglobin), and as an absorber of solar ambipolar radiation and emitter of IR photons [38, 43]. But we have gone beyond all these findings and theories of oncogenesis, to integrate their findings according to a vector of metabolic alterations that responds to the vector of energy starvation. Each approach made contributions that have not yet been properly integrated and acknowledged, all the more so because none of the approaches actually held the solution to the unitarian understanding of cancer in all of its acquired forms. It is as if the discoveries had to illuminate one another long past each one's epoch, throwing light on each other before any sense could begin to emerge, ever so tentatively. The discovery that the Warburg effect does not put a load on the host because of intratumoral metabolic symbiosis illuminates how the cancer cell is above all an adaptive element experimenting with metabolic responses. At last, hypoxia appears to constitute the fundamental neoplasia-promoting pressure which the very structure of a tumor addresses, even if this realization simply opens the doors to consider the greater variety of energy-deprivation disorders and in the process obliges scientific research to inject some heretofore unknown basic biophysics. The etiology of cancer is ultimately bioenergetic, and this constraint is at play not only etiologically, but even inside of tumors, in the topological separation of lactic fermenters and 'lactic respirers', and in the organization of their metabolic symbiosis. And it is now also admitted at

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last that oncogenesis and its progression involve a metabolic adaptive switch required for cellular adaptation to hypoxia [161]. Semenza [155] summarizes the situation (ie the "signal intelligence" of the cancer cell) by explaining that the fermenter-type cancer cells downregulate the oxidative metabolism in order to retain redox homeostasis [161], and consume large quantities of glucose in order to maintain energy homeostasis that can keep up with their fast rates of growth and proliferation. These tumor cells have entered into another universe of evolution and sense-detection, innovating adaptive responses that are ultimately protozoal-like. And in this universe, activation of the IGF axis appears to be a general oncogenic strategy.

Back in 2004, we wrote that:

"From this [aetherometric] perspective, A. Szent-Györgyi's notion of two fundamental metabolic states - alpha and beta, corresponding to [lactate-producing] glycolysis and to aerobic respiration - may no longer appear so far-fetched, for the two metabolisms might well, in fact, describe the two polar responses of non-photosynthetic eukaryotic life that apply to the fundamental range of *livable* conditions it can endure. Differentiation or transformation then appears as a Lamarckian switch that has been built into every eukaryotic cell. The variety of transformed DNA alterations gives the breadth of the neoplastic 'solutions' whose groupings, amazingly enough, permit emergence of ordered patterns that define specific clinical neoplastic conditions (...) Cancer may well, in this sense, turn out to be simply a Lamarckian response of metazoic eukaryotes to chronic anoxia [and energy starvation]" [35].

With the proper amendments, these words still stand, for there is a bioenergetic unity to the metabolic and genotypic diversity of transformed cells, hyperplastic and neoplastic. It is this bioenergetic unity that the variety of oncogenic epigenetic and adaptive changes address. Thus it would appear - in an irony that makes us smile - that, *in general terms*, admittedly, Wilhelm Reich was correct in concluding that cancer is a disease induced by oxygen and energy starvation, and is caused by an hyper-orthosympathetic condition in an organism and in cells. He was also correct in thinking that malignant transformation resulted from the Warburg effect promoted by energy starvation, even if his heterogenic theory of malignant transformation [27] proved to be in error [36] and his theory of energy deeply flawed. It would take molecular biology, molecular genetics and aetherometric biophysics before one could begin to decipher the cellular, molecular and biophysical paths of response to energy starvation, their difference and their unity, and how they work in detail, in complete integration with the biological circuits of absorption and conversion of massfree energy. This, at last, seems to identify the profound unity of the auto-oncogenic vector.

It appears that the only unitary approach to understanding acquired cancers is a biophysical energy-based analysis of auto-oncogenesis as a metabolic disorder that responds to energy-starvation

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and hypoxia above all. Furthermore, only an adequate understanding of acquired cancer - of what launches the oncogenic vector and what promotes its progression - may lead towards a unitary approach to treatments no longer based, as conventional cancer therapy does, on killing cancer cells with chemical poisons and ionizing radiation that indiscriminately kill healthy cells involved in normal tissue production and regeneration. Future treatments to be further investigated must include at least long-term administration of oxygen and mega-dose vitamin C, ambipolar induction of cholecalciferol or ingestion of non-toxic cholecalciferol preparations, controlled exposure to solar radiation and/or tuned sources of ambipolar radiation, proper diet and essential amino-acid supplements (eg L-lysine, L-arginine), and diuretic (non-ozonized) water intake. They should also include only patient populations that have been properly staged (histologically, by biopsy, *and* in accordance with the proposed staging of the oncogenic vector), have *not* been treated by cytotoxic methods, and whose complete nutritional and toxicological consumptions can be ascertained and controlled.

Unfortunately, there are no grants available for such endeavours, and few physicians who are interested in pursuing these approaches. The greater mass of the public is indifferent to the problems of the science and the clinics of oncology. And when affected by cancer and turned into the greater mass of cancer patients, all that most seek is a fast cure, and in desperate hope at not getting such a cure from established medicine, they willfully deliver their lives to unscrupulous predators peddling asinine notions and useless, when not dangerous, alternative "treatments". This is an intolerable situation. Only a better understanding of the etiology of cancer has the potential to reverse it. And that is the ethical responsibility of the science of oncology and of oncologists.

Abbreviations:

$1,25\text{-}D_3$ = $1,25(OH)_2\text{-}D_3$ = 1alpha,25 dihydroxyvitamin D_3
$25(OH)D_3$ = 25-hydroxyvitamin D_3
AP-1 = activator protein-1
AML = acute myeloid leukemia
AMP = adenosine monophosphate
ADP = adenosine diphosphate
ATRA = all-*trans*-retinoic acid
ATP = adenosine triphosphate
APL = acute promyelocytic leukemia
BCC = erythroid burst-component colony
BFU-E = erythroid burst-forming unit
Cdk = cyclin-dependent kinase
CKI = cyclin-dependent kinase inhibitor
CLL = chronic lymphocytic leukemia
CML = chronic myelogenous leukemia

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CMPDs = chronic myeloproliferative disorders
EGF = epidermal growth factor
EGFR = epidermal growth factor receptor
EPO = erythropoietin
FAD = flavin adenine dinucleotide
FMN = flavin mononucleotide
GH - growth hormone
GLUT = glucose transporter
GM-CSF = granulocyte-monocyte colony stimulating factor
HIF-1alpha = hypoxia-inducible factor 1alpha
IGFBP = insulin-like growth factor binding protein
IGF-I = insulin-like growth factor-I
IGF-II = insulin-like growth factor-II
IGF-IR = insulin-like growth factor-I receptor
IL-3 = interleukin 3
IL-4 = interleukin 4
IR = infra-red
IRS-1 = insulin receptor substrate 1
JAK = Janus kinase
LDHA = lactate dehydrogenase A
LDHB = lactate dehydrogenase B
MAP kinase = mitogen-Activated Protein Kinase
MCT1 = monocarboxylate transporter 1
MCT4 = monocarboxylate transporter 4
MGDF/TPO = thrombopoietin
MMP = matrix metalloproteinase
NAD = nicotinamide-adenine dinucleotide
PDK-1 = 3-phosphoinositide-dependent protein kinase-1
PI-3K = phosphatidylinositol 3-kinase
PTH = parathyroid hormone
PV = *Polycythemia vera*
RAR = retinoic acid (ATRA) receptor
RBC = red blood cell, erythrocyte
SCF = Stem Cell Factor or KIT ligand
SOD = superoxide dismutase
STAT= signal transducers and activators of transcription
UV = ultraviolet
UV-A = near UV (wavelength >300nm)
VDR = vitamin D receptor
VDRE = vitamin D response element
VEGF = vascular endothelial growth factor
Vitamin D = vitamin D compounds in general

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