Chapter

Melanin, from an Evolutionary Remnant to the Myeloid Lineage Cell's Main Energy Source. The Unsuspected Intrinsic Property of Melanin to Dissociate the Molecule from Water. Possible Implications in the Context of Acute Leukemias

Arturo Solís Herrera, Ruth I. Solís Arias and Luis F. Torres Solís

Abstract

Melanin is one of the most stable substances known. The study of the ink bags of fossilized squid that died 160 million years ago has found it in good condition. Its extraordinary stability is what had prevented, to date; assign a relevant role in biology. Sir Everard Holmes' proposal in London; in the eighteenth century, about the role of melanin as a simple sunscreen, it has permeated to this day, especially among dermatologists. Despite the unique physical–chemical qualities of melanin, its biological role as a simple sunscreen that protects us from the dangerous UV rays remained immutable. Our circumstantial discovery during an observational study that lasted 12 years (1990–2002) and which included the ophthalmologic studies of 6000 patients, about the relationship between the vessels of the optic nerve and the three main causes of blindness (Macular degeneration, diabetes, and glaucoma) allowed us to discern the unexpected and surprising true role of melanin in Biology as an energy transducer. The unsuspected intrinsic property of melanin to transform light into chemical energy through water dissociation, like chlorophyll in plants; opens a new era in Biology and therefore in Medicine. And Acute Leukemias are no exception.

Keywords: energy, mitochondria, melanin, water, hydrogen, oxygen, ATP

1. Introduction

Wasserman described the accidental discovery of lymphocytes containing melanin granules in humans since 1963 [1]. Through the reaction to a non-immune Antigen (Egg white), Wasserman found in all the 46 non-White subjects' investigation of the hourly preparations revealed that melanin became discernible in

neutrophils as well as in the subsequent mononuclear phase. The same pattern of events was observed in all these experiments, differing only in the time of appearance and number of pigmented leukocytes present. Theoretically was thought that Neutrophils, as microphages, phagocytose small particles of melanin, and by shrinkage, prior to the stage of lymphocytic influx, condense these particles to clearly discernible granules.

Pigmented leukocytes could be demonstrated in blood from Amphibians and Reptiles, as reported by several previous investigators [2]. The pigment could be identified as melanin in the ease of amphibians by the highly specific ferrous iron technic of Lillie. More pigmented leukocytes were found in those amphibians suffering from the active skin- and subcutaneous infection than in normal animals.

It is not easy to demonstrate melanin inside cells, especially when cytoplasm is scarce, it had been described the following techniques in this regard:

Mop-Gruenwald-Giemsa -—Formed cutaneous melanin present a green-black color when stained by this procedure. This color was noted in several studies describing melanin-containing cells in the bone-marrow either as melanin-laden macrophages [3].

Distaining Procedure. —May-Gruenwald- Giemsa stained preparations may be distained by immersion in absolute methyl alcohol. In skin- window preparations it was confirmed that the dc-stained melanin granule had the same brown appearance as before any staining procedure was carried out. In freshly stained preparations, the granule loses its green-black color before the nucleus of the cell is decolorized, and this facilitates localization of pigment-containing cells and identification of cell-type.

Dope staining. —Though carried out on blood smears by the method of Laidlaw and Blackberg [4], the nonspecific nature of the reaction is of no practical use in determining the nature of the pigment.

Ferrous Iron Uptake (Lillie). - Gives a dark green color with melanin, while lipofuscins do not stain. The specificity of this reaction for melanin is high [5].

2. Melanin in the bone marrow

Since melanin is the energy source par excellence of eukaryotic cells, it is present in all cell lineages, being its main location in perinuclear space in the form of melanosomes. And its presence in the bone marrow solves the conundrum about the energy source of the yellow bone marrow, which has no blood vessels despite being the most metabolically active part.

Melanin may be easily overlooked in routine histological sections, as it resembles hemosiderin and its presence should be confirmed by Masson-Fontana silver impregnation [6]. Melanocytes may be present even in the apparent absence of melanin [7] and can be recognized by electron microscopy [8] and immunohistochemistry for S-100 protein [9], melanoma-associated antigen [10], or HMB-45 antigen [11]. In fact, all cells require melanin to generate the energy they need to function and preserve form. Some cells contain melanin in greater quantity depending on their location and function (**Figure 1**).

The most used staining in histology is hematoxylin and eosin (H & E), but it does not allow differentiation between melanin and hemosiderin.

Hemosiderin comes from aging erythrocytes, so there must be the history of erythrocyte diapedesis that mainly occur in acute stages of inflammation. Hemosiderin appears to be both intra and extracellular [12]. The abnormal presence of hemosiderin is explained by extravasation and lysis of red blood cells, followed by decomposition of hemoglobin into hemosiderin [13].

Melanin is always intracellular because is easily metabolized by the organism to cysteinyl-dopa, which can be found as a urinary metabolite. However, inside the cell, is a quite important component due to bioenergetic role.

The melanin observed with different wavelengths tends to preserve its dark brown hue. It is a practical way to differentiate it without touching tissue samples (**Figure 2**).

Perl's stain is currently used for the detection of hemosiderin granules because it detects the presence of ferric ions ($Fe3^{+}$) in the tissue due to the production of ferric ferrocyanide which results from the reaction of ferric ions with ferrocyanide [14].12Haemosiderin was visible as granules (siderosomes) within macro-phages (siderophages). These granules appeared brownish or black in H & E sections and blue in Perl's stain (**Figure 3**).

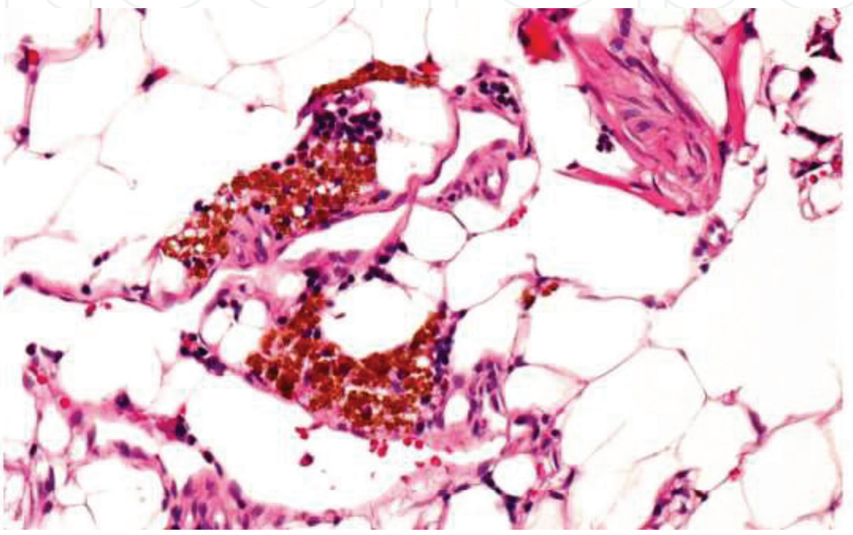


Figure 1.
Melanin in the bone marrow. Melanin appeared as brown, brownish-black, or black granules co-located within cells.

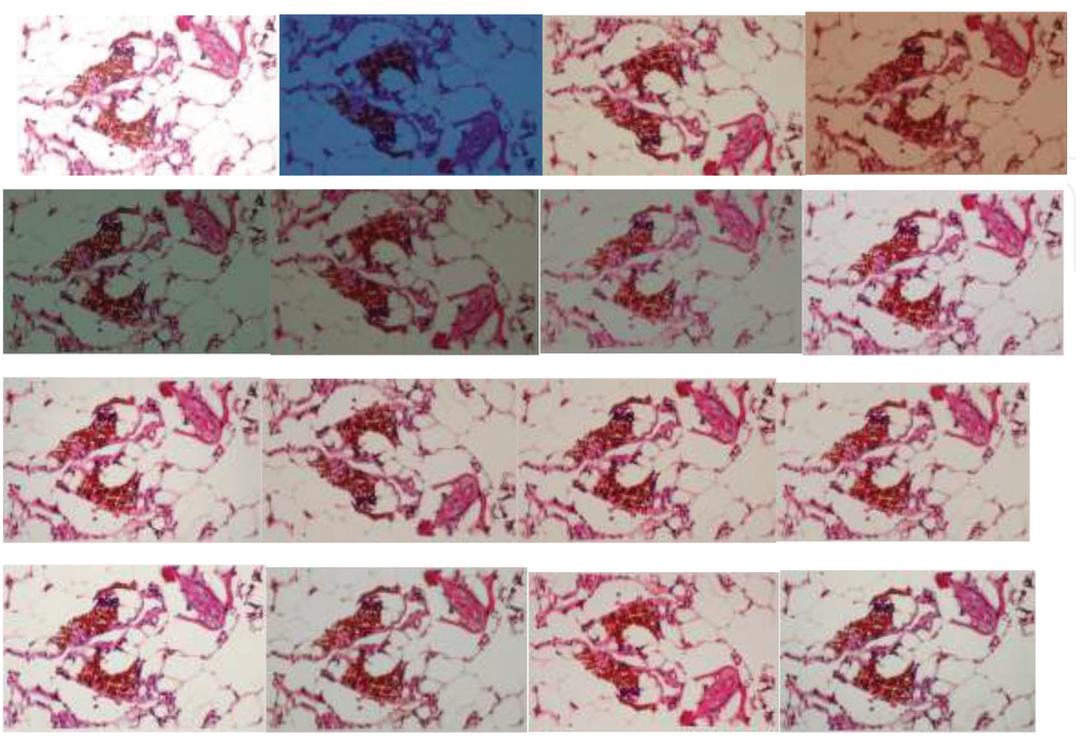


Figure 2.
Melanin observed with different wavelengths.

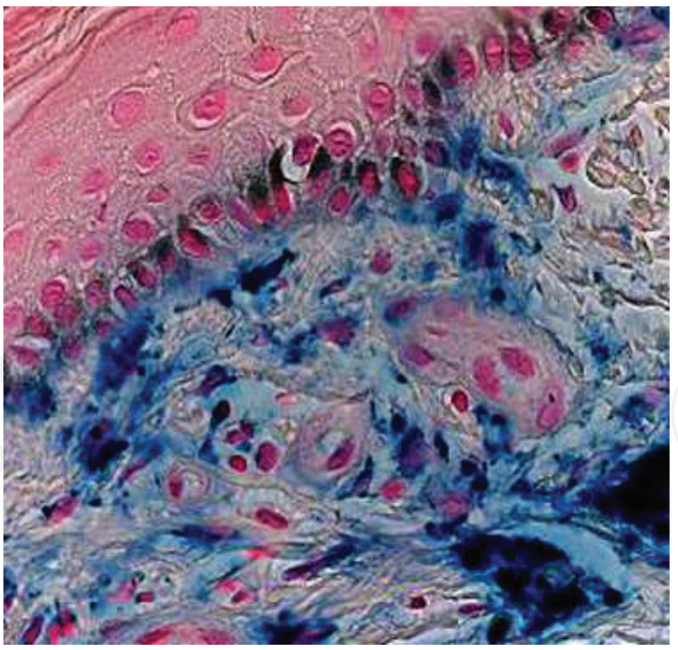


Figure 3.
Perl's stain. Hemosiderin is seen as deep blue. Excess hemosiderin accumulates in the liver and spleen causing impaired organ function culminating in death. Iron poisons melanin itself.

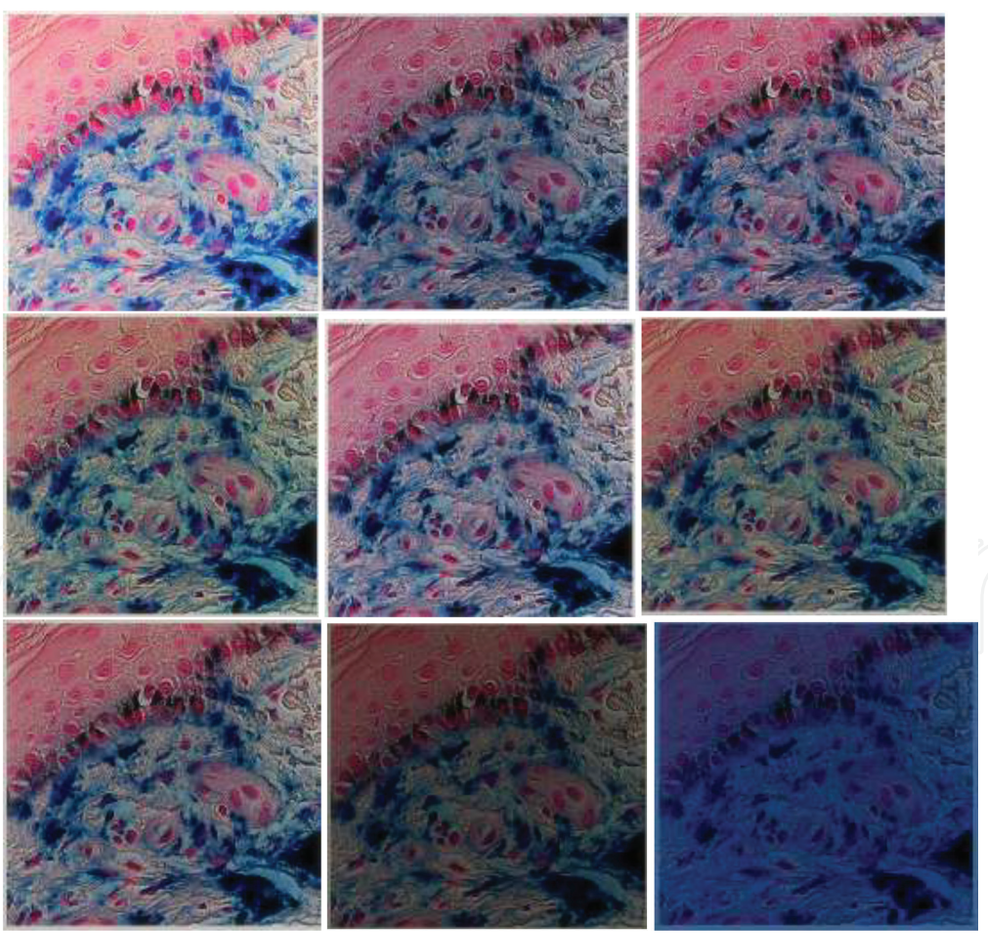


Figure 4.
The same tissue in ***Figure 3*** *observed with different wavelengths, allows us to identify the dark brown color characteristic of melanin happens with any wavelength.*

Hemoglobin is a conjugated protein found in erythrocytes, that stains vividly with acid (anionic) dyes such as eosin. Hemoglobin breaks down into two parts: globin (protein that is returned to the amino acid pool) and heme (iron-containing

pigment part). The heme portion splits again into iron (hemosiderin) and bile pigments (biliverdin). Hemosiderin (stored ferric iron) is a breakdown product of hemoglobin; if the iron is not needed immediately to produce new hemoglobin, hemosiderin is stored in bone marrow and splenic red pulp. If the production and destruction of red blood cells is not balanced, there may be increased deposition of hemosiderin in tissues. Hemosiderin is differentiated from other yellow to brown pigments with the Prussian blue reaction which detects ferric ($Fe3^{+}$) iron.

Observation of tissue sections with different wavelengths, allow us to differentiate reasonable the presence of melanin from hemosiderin without the need to add different chemical compounds to the tissues (**Figure 4**).

The distinction between hemosiderin and melanin pigmentation is difficult in routine histological examination. Excessive amounts of splenic hemosiderin are seen when erythropoiesis is reduced (less demand for iron) or from the rapid destruction of erythrocytes in hemolytic anemias (increased stores of iron), such as those caused by immune-mediated hemolytic anemias or hemotropic parasites. Excess splenic hemosiderin may also occur in conditions such as chronic heart failure or injections of iron dextran or as focal accumulations at the sites of old hematomas, infarcts, or trauma-induced hemorrhages. The precise reason for deposition of iron is not always clear. Aniline and related agents may also increase splenic iron content.

3. Energy plays a fundamental role in all processes of the body

Energy is defined as everything that produces a change. Metabolism means continuous change. Therefore, our body requires energy constantly, all the time, day, and night. Glucose is the universal precursor to any organic matter in plants and animals, but it cannot provide the energy that its own metabolism needs.

Our body takes the necessary energy from light, dissociating the molecule from water, such as chlorophyll in plants. A reliable test is that two of the most active metabolic areas - the layer of cones and rods in the retina and the yellow bone marrow - do not have blood vessels.

Being the beginning of everything, events that alter the generation and distribution of energy from melanin, cause important effects on health depending on the nature of the physical, chemical, and/or biological agent.

The generation and distribution of energy from melanin is an astonishingly accurate process and has not changed since the beginning of time. The energy requirements of the bone marrow are incessant, day and night. Only in this way does the balance between mass and energy happen properly so that the bone marrow produces 2 to 3 million blood cells per second.

Therefore, there are physical factors (electrical trauma, extreme temperatures, ionizing radiation, etc.) and chemical factors such as contaminated water, contaminated air, pesticides, herbicides, fertilizers, metals, plastics, solvents, industrial waste, solvents, alcohol, addictive drugs, anesthetic agents, etc., which alter the process, resulting in the balance between mass and energy being disturbed and the body, or in this case, the bone marrow is disorganized and cannot perform its functions efficiently, as it has millions of years ago.

For clarity purposes, we will refer especially to water, for two reasons: 1) water is the perfect substrate for melanin and 2) any type of contamination eventually reaches the water.

When water becomes contaminated, the first thing it loses is viscosity and then other characteristics, and as the physicochemical properties of water are altered by contaminants, efficiency and accuracy in that melanin dissociates and re-associates

the water molecule is disturbed resulting in intracellular biochemical processes beginning to disorganize.

In any system, when the problem is power, the failure is widespread, and the bone marrow is no exception. Several histological alterations must occur almost simultaneously for acute leukemia to manifest.

Therefore, contaminated water can produce all kinds of diseases, mainly because it affects the generation and distribution of energy from melanin.

4. Melanin in leukocytes

Pigmented leukocytes are considered rare so far, but now that we are aware of the importance of bioenergetic role of melanin in cell biology, we think it is rather the difficulty in identifying the microscopic melanin granules (**Figure 5**).

Until today, it is abnormal to have a greater number of pigmented leukocytes, for instance more than two percent [15] and frequently there is also histiocytes in the peripheral blood with massive amounts of pigment granules.

There was an impression about that the abdominal vein and renal portal vein transported more melanin, the abdominal vein and renal portal vein often had cells densely packed with melanin granules. The pigment in large cells, and present in large amounts, was shown to be mostly melanin. In some of these cells only a positive Schmorl reaction was obtained, and such pigment may be lipofuscins rather than melanin.

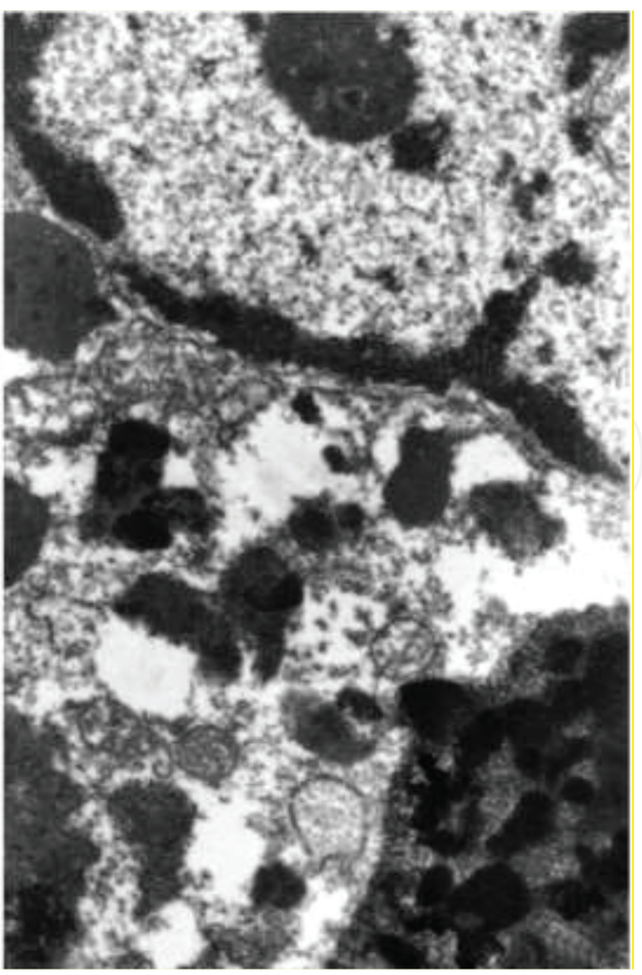


Figure 5.
The cytoplasm of macrophage loaded with melanin. X 10000.

In some cells a few black granules were seen but could not be identified as melanin. Most of the pigmented leukocytes were mononuclear cell types. The neutrophils of amphibians contain none or only a few specific granules, and it is impossible to decide on the actual nature of some very dark, slightly larger granules in some neutrophils.

Some cells with a basophilic cytoplasm but a nucleus resembling a granulocytic nucleus should probably be classified as a monocytic cell type rather than granulocytic. But it would be difficult to conceive how such granules of melanin reached the blood.

The organs most heavily laden with melanin are the liver, the spleen, and the lung approximately in this order, but the kidney and myocardium also contain some melanin. These melanin deposits are not P.A.S.-positive, a reaction obtained from phagocytosed melanin in macrophages, but the granules vary in size, which does suggest phagocytized melanin. All cells in your body have the necessary genetic information to synthesize melanin. Wandering cells require an energy source, so it is not uncommon for synthetize and carrying it with them, for instance histiocytes.

Cells that form in the bone marrow have unique characteristics since their generation, starting with the central part of the bone marrow (**Figure 6**), the area of greatest metabolic activity; and paradoxically has no blood vessels at all. According to the current dogma, glucose has a double paper (theory), a) universal precursor of 99% of organic matter and b) the energy source par excellence of the eukaryotic cell.

Glucose carbon chains are the building blocks of any organic matter, but glucose cannot provide the energy that its own metabolism requires.

Bone fluorescence is a known phenomenon, and occurs when short-wavelength photons, such as ultraviolet; are absorbed by some molecule containing a fluorophore and is re-emitted at longer wavelengths (**Figure** 7). Hemoglobin from the blood vessels that cover the periosteum absorb this emission. Since hemoglobin and chlorophyll are remarkably similar molecules, it is perfectly possible that hemoglobin also dissociates the molecule from water, transforming luminous energy into chemical energy, such as chlorophyll in plants. The energy that is released by

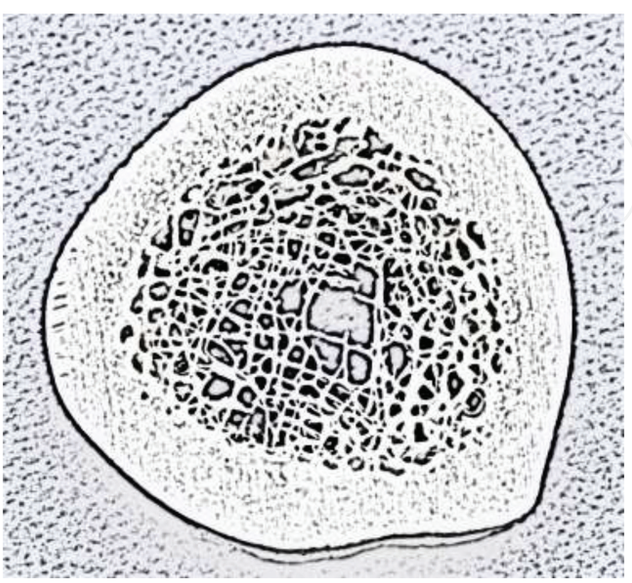


Figure 6.
The bone marrow has a part called a red bone marrow because it contains blood vessels and bone spicules (spongy bone) that function as a vessel supporter. The other part called yellow bone marrow that does not contain vessels or spicules and is the most metabolically active part. It is surrounded by a compact outer shell located in the medullary cavity of the diaphysis. The periosteum (membrane covering bone) is richly vascularized.

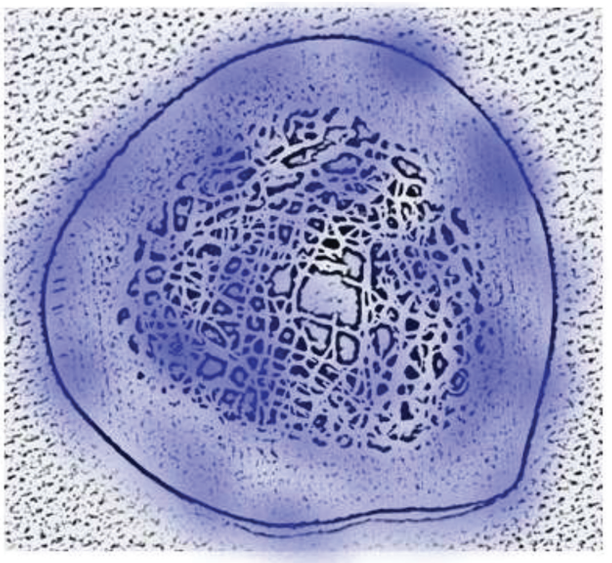


Figure 7.
Bone fluorescence (violet) when illuminated with wavelengths close to UV-A. the absorbed energy is re-emitted at different wavelengths. Erythrocyte hemoglobin completely absorbs UV wavelengths, and hemoglobin dissipates absorbed energy by dissociating the water molecule.

breaking down the water molecule is transported by hydrogen, the energy carrier par excellence in the entire universe. Oxygen is actually a necessary evil, a waste of reaction, this is: a byproduct.

Bone marrow is a relatively hypoxic microenvironment. Oxygen tensions fluctuate through the medullary cavity and along the endosteal and periosteal surfaces [16]. Despite being a highly vascularized tissue, the bone is a particularly hypoxic environment. Oxygen tension in most normal tissues falls between 2% and 9% (14–65 mm Hg) [17], however, in the bone it is widely accepted that oxygen levels range from <1–6% (~7 mm Hg – 43 mm Hg) [18] like in the umbilical artery.. Oxygen tension in the bone is likely determined by the level of cellularity and oxygen consumption rate in particular regions of the bone [19], however, the body's cells cannot take and do not require oxygen from the blood. The main reason is that oxygen is not used as we have so far believed, this is to combine it with glucose and obtain energy, because our body takes energy from light directly; and the second reason is all the body's cells have the ability to dissociate the molecule from the water by means of the pigments they possess, that is: the cells of the organism are able to produce their own oxygen by dissociating the molecule from the water, such as chlorophyll in plants.

In fact, we must consider oxygen as an indirect indicator of the molecular hydrogen levels of tissues, because by dissociating water, our body obtains hydrogen and oxygen at the same time, but the really valuable is hydrogen since it is the energy hauler per excellence in the entire universe (**Figure 8**).

Therefore, low oxygen levels are not caused by high cellular consumption, but by the low light conditions of the microenvironment of the bone marrow, and because hemoglobin and chlorophyll are not as efficient as melanin to dissociate the molecule from water. What goes according to the significant differences in hematological parameters [20] and in the prevalence and incidence of blood diseases between the different skin phototypes, as well as their geographical location according to the amount of sunlight [21]. The absolute oxygen concentration around blood stem cells was indeed low, despite an exceptionally large density of the blood vessels. The marrow is extremely densely populated by various cell types (not just hematopoietic stem cells or HSCs), so that the oxygen concentration drops steeply just microns away from feeding capillaries, but this is not due to an higher oxygen consumption,

but oxygen, like any other gas; is not combined with water and is far from easily crossing the different barriers between blood vessels and tissues. It is reported that oxygen does not cross the blood/brain barrier [22].

On the other hand, just as the umbilical artery has a saturation similar to that of the bone marrow, about 40%, and elevated oxygen levels in the region in question, regardless of where they came from, would have toxic effects on surrounding tissues. The fundamental processes of the body are astonishingly accurate.

The analysis of peripheral blood values and bone marrow cell populations in newly born and young infants is particularly suited for the detection of racial differences. Such subjects have experienced limited impact from extrauterine factors that might be responsible for environmentally induced differences in hemopoietic cell distributions. American black infants have a consistently lower hemoglobin level than their American white counterparts. This lower hemoglobin level in black infants is compatible with the relative erythroid hyperplasia of their bone marrow. This 0.5–1.0 gm difference in hemoglobin levels has been found at all ages, even when corrected for sex, dietary intake, socioeconomic status, and place of residence. There are reports of a higher 2–3 DPG level in healthy black males and females [23]. Racial differences in peripheral blood leukocyte counts have also been well documented [24]. As in the older individuals, this population of black infants had consistently lower total leukocyte and total neutrophil values. The racial differences in peripheral blood leukocyte counts were not reflected in the prevalence of bone marrow myeloid or small lymphocyte cell compartments. So far, it is

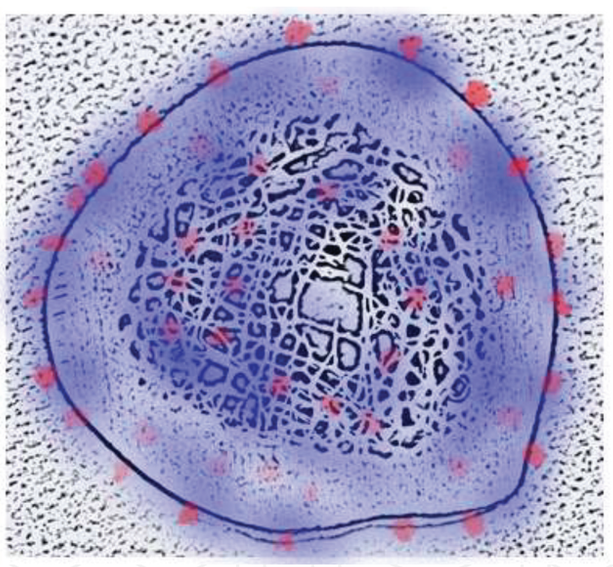


Figure 8.
The red dots repress the hemoglobin present in the periosteum, in the cortex, in the endosteum, and in the compact and spongy bone. Hemoglobin well absorbs wavelengths close to 300 μm, and the energy absorbed by hemoglobin is dissipated by dissociating the water molecule, transforming photonic energy into chemical energy, which is transported by diatomic hydrogen (H_2). Cells and tissues use this energy in many ways.

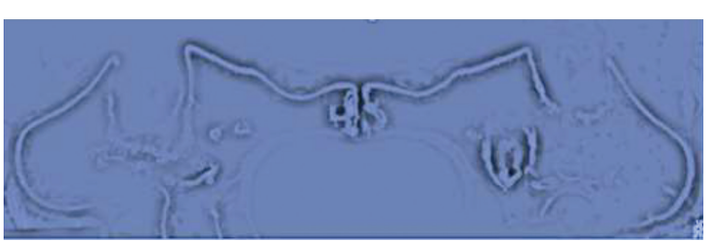


Figure 9.
Fluorescence of bone is a well-known phenomenon. Furthermore, is used to identify and differentiate healthy from necrotic bone tissue during resection surgeries [25].

not possible to explain this discrepancy, except if we consider that the higher the amount of melanin, the greater the proportion of water dissociation and therefore greater energy availability in tissues (**Figure 9**).

5. Melanin, considered for a long time an evolutionary remnant, participate substantively in energetic cell's metabolism

The synthesis of melanin is a multistep and highly regulated pathway, and so far, considered as a simple sunscreen that protect against UV-induced damage [26]. Although some research had shown that the presence of melanin pigment affected the elastic properties of the cells as well as the transmigration abilities, they concluded that inhibitory effect being mechanical in nature [27].

Theoretically, melanin granules can attenuate the movement of cells due to mechanical (physical) effect of loading. However, the induction of melanogenesis is accompanied by the dramatic changes in cyto-architecture such as round morphology which correlates positively with the melanin content of the cell [28]. Interestingly, SKMMEL – 188 cells, supplemented with L-tyrosine, increase melanin content, and can easily detach from the culture substratum, which contrasts with the amelanotic cells cultured in Ham's F10 medium [29].

This significant change in adhesiveness and form due to the presence of melanin, include a complex and reciprocal interactions that are context dependent and are nonlinear in nature with multitude of regulatory pathways/factors operating in a form that cannot be explained in a reductionistic form. We must keep in mind that melanin presence induces dramatic changes in the metabolic status of the cells and their behavior both on biochemical and on molecular levels **Figure 10**.

6. Leukocytes are wandering cells that require an energy source at its own

The dynamic functions of peripheral blood leukocytes (Eosinophils, Basophils, Neutrophils, monocyte, Erythrocytes, T-cells, B-cells) and platelets require an integrated metabolic machinery to meet energetic demand during normal physiology which is likely (theoretically) to involve both glycolysis and mitochondrial oxidative phosphorylation. The role of both these important ATP generating pathways in supporting supposedly the biological function of platelets and leukocytes has been postulated but have not been integrated into an overall understanding of these cell types in human subjects.

The myeloid lineage supports the greatest variety of differentiated circulating cells which include erythrocytes, platelets, neutrophils, basophils, eosinophils, and monocytes (**Figure** 7). Two stages of some cells are described once they are secreted by the bone marrow: M1 or pro-inflammatory and M2 or anti-inflammatory. The difference that is handled relates undoubtedly to energy levels.

Thus, the metabolic programs of monocyte/macrophage populations are highly plastic and adapt to facilitate the changing function of these cells in the inflammatory process. However, the possibility of detecting these important changes through current laboratory methodology is not clear. Typically, differentiation of the M1/M2 macrophages occurs at the site of inflammation not in the circulation.

From the translational perspective the pre-differentiated monocyte is the dominant form in the circulation. What there is no doubt about is that energy levels are what make the difference. Supposedly, there is something like a switch to a metabolic phenotype with an increase in both glycolytic function and mitochondrial

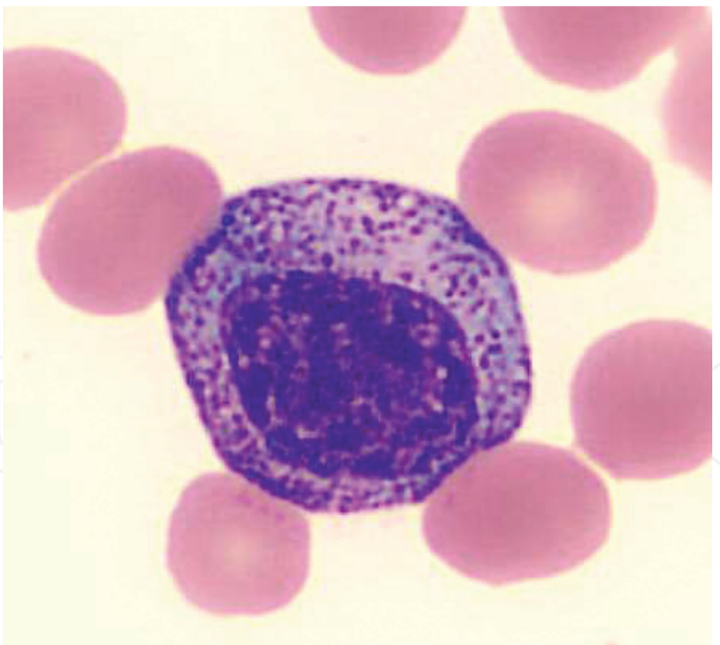


Figure 10.
Typical aspect of a myelocyte. The usual stains do not demonstrate or rule out the presence of melanin, which on the other hand is something that seems to be of no interest to researchers.

oxygen consumption, which is essential for their diverse immunological functions, which includes clonal expansion and the production of cytokines and antibodies. It should be noted that mitochondria are not proper organelles that produce energy, their resemblance to the heat sinks of electronic circuits leads us to think that they are rather temperature regulators, and on the other hand, animal species that require exposure to the sun to start moving, characteristically have a very small number of mitochondria.

Thereby, the relationship of bioenergetics with the disease processes associated with inflammation still has numerous mysteries. For instance, Neutrophils have very few mitochondria which do not play a role in energy metabolism, by other side, the space available in the mitochondrial matrix calls into question the existence and location of enzymes required by their supposed energetic function.

Hereby, it is argued that the energy required for neutrophil chemotaxis and activity is derived from glycolysis with very few mitochondria, which is paradoxical. It is interesting that patients with septic shock demonstrated a strong association between decreased mitochondrial function, specifically loss of ATP synthase activity in peripheral blood mononuclear cells and increased mortality. But we must keep in mind that mitochondria are not an energy-autonomous organelle. Mitochondria requires energy to perform its function, requiring energy even to preserve form.

The ATP synthase enzyme does not use ATP to perform its function, but that does not mean that it does not require energy, because of course it requires it, because any chemical reaction needs it. Whether in the form of activation energy, or termination, or reaction support, whatever you want to call it. Enzymes that do not use ATP to perform their function use another form of power that is not yet determined by the orthodox science; however, it is accepted. By the way, energy is defined as everything that causes a change.

It has also been shown that platelets from patients with type 2 diabetes have lower mitochondrial membrane potential and higher ATP content compared to controls, which does not make sense. Furthermore, a normal mitochondrial membrane potential requires energy, and regulation of ATP content requires energy also.

A study of mononuclear cells in type 2 diabetes showed that the mitochondrial mass was decreased and that the mitochondria were hyperpolarized; but in any system, when the energy decreases, the mass tends to disappear, so we can think that the energy source of the mitochondria is compromised and therefore the mass is tending to disappear, like in any system; and hyperpolarization would be explained simply because the normal electronic behavior of the mitochondria requires adequate levels of energy, and if it is disturbed, then changes happen that are not compatible with the normal functional balance of the organelle. This is: the balance of charges requires energy.

It has been reported that leukocytes from patients with leukemia have higher numbers of circular dimer mitochondrial DNA compared to healthy controls, which is surprising, because the number of circular dimers of mitochondrial DNA is a process strictly regulated by millions of years of evolution, but such controls require the right energy, we could say accurate in time and form. Just as melanin generates and distributes it from the dissociation of the water molecule, such as chlorophyll in plants. It is an astonishingly accurate process, which is the same since the beginning of time, and after millions of years of evolution has not changed, it is not going to change, it cannot change.

But it is disturbed by contaminated water, with polluted air, with pesticides, herbicides, fertilizers, metals, plastics, solvents, industrial waste, alcohol, abuse drugs, anesthetic agents, etc.

It is therefore not surprising that leukemias are significantly more common in populations exposed to such pollutants. Because when the generation and distribution of energy from melanin is disturbed by the aforementioned factors, the whole organism begins to disorganize until, eventually, some sicknesses appear, which are nothing more than a manifestation of imbalance between mass and energy from melanin, not glucose.

7. Melanin laden macrophages in CSF

Vogt- Koyanagi- Harada Syndrome (VKH) is suspected to be systemic immunological reactions in various organs containing melanocytes [30], suggesting that the cell mediated immune process involving melanocytes plays an important role in the pathogenesis of VKH [31]. Supporting this idea, Nakamura et al., reported the existence of melanin laden macrophages (MLMs) in the cerebrospinal fluid of VKH patients [32]. Few months later, Nakamura et al. reported a VKH patient whose CSF examination reported pleocytosis (cell counts 273 X 10^6/L) and a large number of MLMS (**Figure 5**) [33] and after three months of initial administration of corticosteroid, visual acuity recovered and cell counts in the CSF had decreased (cell count 13 X 106/L), to within normal range, but MLMS were still present and after fourth months ophthalmologic manifestations recurred (**Figure 11**).

Pleocytosis in VKH is considered a sign of the focal immune response against melanocytes in meninges. Although lymphocytes are predominantly observed in the CSF and uveal tract of the eye in patients with VKH, a small number of macrophages are detected.

It has been reported that patient with melanin granules in the cytoplasm of macrophages in CSF of patients with VKH that appears in early stage and disappear after steroid treatment have, supposedly, a better prognosis. However, small melanin granules are not routinely looking for. Thereby, we could think that macrophages that are "activated" requires more energy to carry out their function, and therefore synthesize melanin more than phagocyte it. An errant cell, such as the

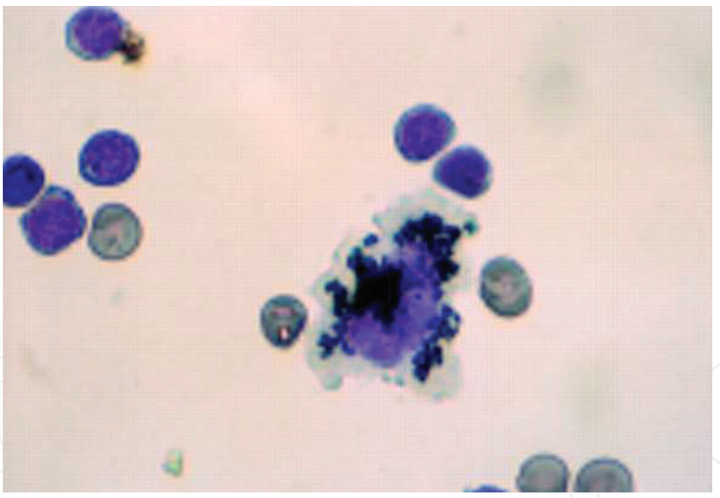


Figure 11.
Morphological characteristics of a melanin laden macrophage (May-Grünwald staining, original magnification × 400) in CSF from the VKH patient obtained 2 weeks after the initial corticosteroid therapy was started. Melanin pigments are identified as basophilic granules in the cytoplasm of a macrophage. (Takayuki Takeshita et al. Br J Ophthalmol 1997; 81: 1113 reprinted with permission, license number: 4852140023803).

macrophage, requires carrying its energy source with it, because the proximity to the supposed source of energy (glucose carried by the blood vessels) is not usual.

8. Biological characteristics of acute leukemias and its relationship to the energy of melanin

They were 13, 780 new cases of acute myeloid leukemia (AML) and 6050 new cases of acute lymphocytic leukemia (ALL) in the United States in 2012 [34]. Supposedly, acute leukemia is the result of a series of mutational events occurring in an early hematopoietic precursor that theoretically prevents the progeny of that precursor from maturing normally. Although advanced age, white ancestry, and family history of hematologic malignancies are risk factors, the etiology of acute leukemias is unknown. This explanation only takes structural issues into account but does not mention at any time the energy needed or where it comes from.

Patient with at least one affected relative is considered "familial" [35], which means that more than hereditary, they are groups of people exposed to similar toxics. Familial cases have an earlier age of onset than sporadic cases [36]. This leads us to think that exposure to environmental pollutants is more marked in family cases, and therefore develop alterations to minor age and therefore at a shorter time of exposure than sporadic that require longer exposure time. The generation and distribution of energy that comes from melanin is an astonishingly accurate process that has not changed since the beginning of time, because the components of the process: light, melanin and water, in order of abundance in the universe, are extremely stable, because they do not show even the minimum data suggesting evolution.

There is no consistent pattern of illness that can be explained by a simple mode of genetic transmission [37]. Strong familial aggregation favored the relatives sharing environmental risk factors. For example, relatives of patients with colon cancer are at increased risk for developing colon cancer. An astonishingly accurate process (water dissociation by melanin) is easily disturbed by contaminated water, contaminated air, pesticides, herbicides, fertilizers, metals, plastics, solvents, industrial

waste, etc. And when the liquid–gas cycle is altered, then the tissues of the organism begin to disorganize and eventually appear what we call sick, which do not actually exist as such, it all comes down to an imbalance of the fundamental process of life that is the generation and distribution of energy from melanin.

And in colon cancer as in acute or chronic leukemias labeled as familial disease, what we see are groups of people exposed to similar toxics, because they lived in the same house, they took the same water, they had the same habits, the same air, the same soil, the same environment, etc.

9. Acute promyelocytic leukemia (APL) and energy from melanin

Few thousand people worldwide are diagnosed each year of Acute Promyelocytic Leukemia (APL). Once considered the most malignant human leukemia as well as the one associated with the worst prognosis, APL has been transformed in the past few decades into the most frequently curable one.

"Most outstanding feature was its very rapidly downhill course of few weeks' duration, a white blood cell picture dominated by promyelocytes and severe bleeding caused mainly by fibrinolysis" [38]; it was the description of Hillestad, in 1957, about acute promyelocytic leukemia (APL). J Bernard, in 1959; reported a series of 20 patients, with the full definition of the disease and its association with promyelocytic proliferation, hyperacute onset and catastrophic hemorrhagic events [39].

The life-threatening coagulopathy was recognized as the defining clinical feature of APL accounting for most deaths at presentation and during initial cytotoxic treatment, with most fatal events being intracranial and pulmonary hemorrhages. The hemostatic abnormalities were attributed to a disseminated intravascular coagulopathy in which fibrinolysis and procoagulant activity triggered by APL blasts played a major role, and hematologic remission resulted in the resolution of the coagulopathy [40].

The impressive improvement in outcome achieved after the advent of all-trans retinoic acid (ATRA) (**Figure 12a** and **b**) the early death rate in APL has remained elevated even in recent years, mostly because many patients die even before they can start treatment [41], and older age remains a prominent negative prognostic factor [42].

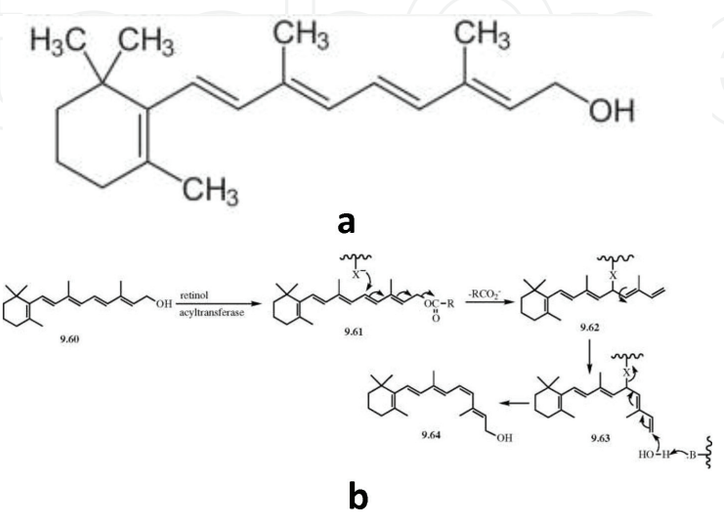
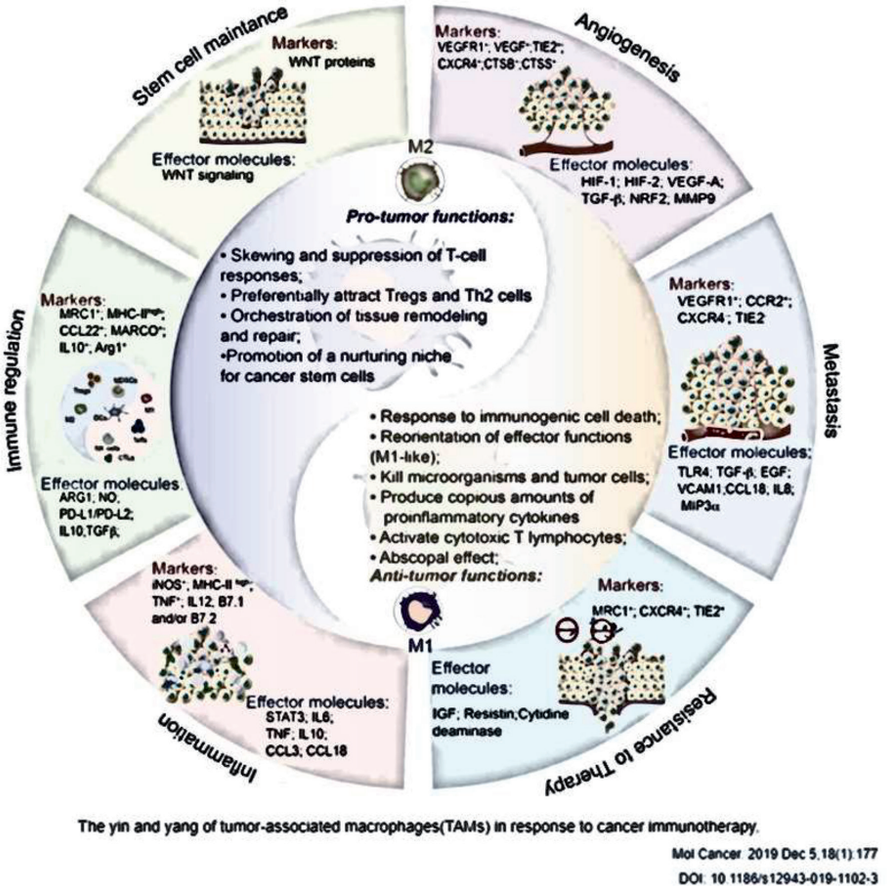


Figure 12.
(a) Structure of all-trans retinol. (b) Proposed mechanism for the reaction catalyzed by retinol isomerase. Note two things: (a) the scheme is theoretical and (b) does not mention energy flow at all. Retrieved in June 122,020 from: https://www.sciencedirect.com/science/article/pii/B978008051336250005X



The yin and yang of tumor-associated macrophages(TAMs) in response to cancer immunotherapy.
Mol Cancer. 2019 Dec 5;18(1):177
DOI: 10.1186/s12943-019-1102-3

Figure 13.
Literature is abundant in terms of structures, processes, and sequences; but it is silent about the energy required by each chemical reaction that incessantly happens inside each cell.

All *trans*-retinol is converted (energy required) to 11-*cis*-retinol by retinol isomerase (enzymes also require energy) in a covalent catalytic process [43]. Control of retinoic acid levels is maintained (energy required) by a suite of proteins that control synthesis and degradation of retinoic acid (**Figure 13**) Again, the control of retinoic acid levels requires energy, in several ways (suite of proteins that control synthesis) and degradation of retinoic acid.

10. Energy production, the true role of melanin in biology

Einstein said it is easier to break the atom and break a prejudice. Therefore, breaking the dogma so rooted that glucose and ATP are the energy source of the eukaryotic cell is not an easy challenge. Given the space limitations we will only mention a few demonstrative examples.

Glucose is the universal precursor of any organic compound of the organism, its carbon chains are the fundamental basis of the anabolic processes of cell synthesis that culminates in a myriad of extraordinary complex compounds. But it is not possible for glucose to provide the energy that your own metabolism requires. Therefore, the double role assigned to glucose since centuries ago should be discarded.

Our body takes glucose, from food; the building blocks, the precursors, with which it builds, rebuilds, and replaces those molecules that continually wear out throughout the day, but the energy needed for such metabolic activities is taken from light.

The passage of carbon chains in the body's metabolism ends with the formation of CO_2 (O=C=O), the most oxidized form of carbon, suggesting that our body uses the carbon chains present in food exhaustively, oxidizing them to their lowest expression. And said process uses intensively the energy that comes from light, but it must first be transformed into chemical energy by dissociating the water molecule, which happens inside melanin.

The dissociation of water is a process that requires a lot of energy, in the laboratory we require heating the water to two thousand degrees Celsius. But melanin and chlorophyll do it daily at room temperature. The equation would be as follows:

$$\text{Chlorophyll}: 2H_2O_{(\text{Liquid})} \rightarrow 2H_{2(\text{gas})} + O_{2(\text{gas})} \quad (1)$$

Both melanin and chlorophyll are certain that this is the case [44], but the mechanisms involved are far from understood. But melanin is hundreds of times more efficient than chlorophyll, as chlorophyll only absorbs the ends of visible light, but melanin absorbs the entire electromagnetic spectrum. Moreover, in chlorophyll the dissociation of water is irreversible, and the proof is that the plants expel the toxic oxygen into the atmosphere; but in Melanin, water dissociation is reversible. This is from liquid to gas, and back to liquid, which is possible because melanin supports oxygen toxicity. Then the equation would look as follows:

$$\text{Melanin}: 2H_2O_{(\text{Liquid})} \rightarrow 2H_{2(\text{gas})} + O_{2(\text{gas})} \rightarrow 2H_2O_{(\text{Liquid})} + 4e^- \quad (2)$$

For every two water molecules that are reformed, 4 high-energy electrons are generated. High-energy electrons are easily exchanged, as they travel at near-light speeds are difficult to control, so what is thought to be absorbed quickly, especially by the fibrous parts of the cell, that is, those close to the perinuclear space that is the main location of the melanosomes. On the other hand, molecular hydrogen (H_2) is the main energy hauler in the entire universe, and since it is not combined with water, once it is generated, molecular hydrogen follows the laws of simple diffusion, and targets the areas of highest concentration (where it occurs continuously) to the areas of lower concentration, simply moving through the cytoplasm and reaches even the cell membrane. It is redundant to say that during its path, said molecular hydrogen is captured by the different cellular organelles and chemical reactions that use its precious energy load and its powerful antioxidant effect, being consumed continuously.

Melanin releases energy symmetrically, in all directions, like increasing bubbles of energy. And such spheres also flood the cell nucleus, constituting its source of energy, since it contains no mitochondria, nor ATP.

So is this, that when the generation and distribution of energy from melanin is affected by environmental pollutants, the functions and posteriorly the structures´ cells begin to affect, including genes. Because the functions of the nucleus also depend entirely on the energy coming from melanin. Therefore, nature's insistence on placing melanin in all cells of all living things now has a meaning: energy production.

11. CO_2 levels highest than normal should impoverish melanin's bio-energetic functions

In all lung life forms, mechanisms are in place to accelerate CO_2 expulsion, such as carbon dioxide anhydrase. Elevated CO_2 levels have such a marked effect on the generation and distribution of energy from melanin that they cause death in less than 60 seconds.

That is why we observe in different life forms a rush of nature to expel the carbon dioxide that it continuously generates because of cellular metabolism, which is also continuous, day and night. And the figures are revealing in the air we breathe in, CO_2 makes up 0.04% of the inspired volume, but in the exhaled air, The CO_2 rises to 4%, that is a hundred times.

Conversely, only 5% of inspired oxygen is absorbed as inspired air contains about 21%, and 16% exhaled air. If oxygen were used as intended by biochemistry texts, liters, and liters of oxygen per minute would be needed, which is not the case. In addition, no mechanism has been identified to accelerate the passage of atmospheric oxygen into the body, in any form of life. So, the poor lung absorption of oxygen is due to simple diffusion.

Finally, two of the body's most metabolically active tissues known to date: the cones and rods of the retina and the central portion of the yellow bone marrow, they are completely devoid of blood vessels, which contradicts the dogma that glucose is the universal energy source, because if so, glucose would have to be transported through the blood vessels, and both tissues indicated above, under normal conditions; they completely lack them.

12. Conclusion

Our incidental discovery about the true energy source of the eukaryotic cell, breaks into a thousand pieces the dogma of double glucose paper as a universal precursor to any organic compound of the human body and which, at the same time, serves as an energy source. The discovery of the unsuspected bioenergy role of melanin opens new possibilities in the study, diagnosis, and treatment of disturbances of the functioning of the body that we call acute leukemias.

Aromatic compounds, ionizing radiations, ozone (O_3), Sulfur Dioxide (SO_2), carbon monoxide (CO), nitrogen Oxide (NO_2), Methyl Mercury, PCBs, lead, cadmium, phthalates, brominated flame retardants, heavy metals, per-fluorinated compounds (PFCs), nonylphenol ethoxylates, fungicides, polyvinyl chloride plastic, Arsenic, Aflatoxins, blue-green algae in recreational waters, cyanogenic glycosides in cassava, foxglove, oleander; persistent organic pollutants (POPs),; HCB: hexa-chlorobenzene; HCH: hexachlorocyclohexane; PCBs: polychlorinated biphenyls; DDT: dichlorodiphenyltrichloroethane; PBDEs: polybrominated diphenyl ethers; they are toxic agents whose mechanism of action begins by damaging the distribution and generation of energy from the dissociation of the water molecule by melanin. The effects on bone marrow depend on the dose and whether the poisoning was acute, unique, multiple, chronic, acute over chronic, etc.

It is surprising to find that tissues as metabolically active as cones and rods and the central portion of the yellow bone marrow, which require 10 times more energy than the cerebral cortex in the case of the retina, or the bone marrow that produces about 2.5 million blood cells per second, do not contain any blood vessels under normal conditions.

According to the dogma currently prevalent, they should have numerous blood vessels since glucose is transported that way. But now that we know that the bone marrow gets the energy of light, like any other tissue of the human body, then the focus changes radically and the explanation about the lack of blood vessels is evident.

Since the transformation of sunlight into chemical energy through the dissociation of the water molecule is a grimly accurate process that has not changed in the very way since the beginning of time, we can now unlearn the biochemistry of the usual textbooks and broaden our landscape about diseases, because in reality, such

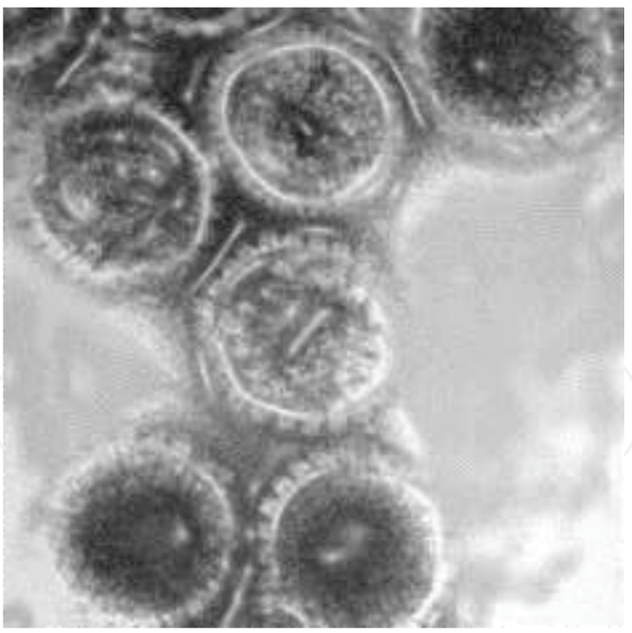


Figure 14.
Melanin is a great electron-acceptor, so in a TEM microphotograph, melanin is seen as a dark area.

diseases do not exist as such as the vast majority are reduced to an imbalance in the generation and distribution of energy from melanin.

The fundamental processes of life, such as energy collection, are astonishingly fast and accurate, so even minor perturbations that we might call transients, for example by the cold, induce a disorganization first of the functioning of the body and then real histochemical alterations that grow and spread rapidly by not being the adequate balance between the mass and energy.

In a few sheets it is not possible to explain all that it involves reordering our thoughts and concepts based on a different form of energy, which we had not even imagined, so it is necessary for the interested reader to deepen and the subject by looking for more information in other articles and books so that concepts about the flow of mass and energy of the body, which we now know is totally different from how it had been considered to date (**Figure 14**), yields fruits that will be reflected in a better quality of life in our patients.

Welcome to a new era in Biology and medicine.

Acknowledgements

This work was made possible thanks to the support of the Center for the Studies of Human Photosynthesis (R) S.C. Aguascalientes 20000, Mexico.

Conflict of interest

There is no conflict of interest to disclose.

Author details

Arturo Solís Herrera[1*], Ruth I. Solís Arias[1] and Luis F. Torres Solís[2]

1 Human Photosynthesis(R) Research Center, Aguascalientes, México

2 Universidad Autónoma de Aguascalientes, Aguascalientes, México

*Address all correspondence to: comagua2000@gmail.com

IntechOpen

References

[1] Wasserman, H. P. Lymphocytes, and the transport of melanin. *J. Invest. Derm*. 41: 473, 1963. Pp: 377-385

[2] Pienaar, U. de V.: Haematology of some South African reptiles. Witwatersrand Univ. Press, Johannesburg, 1962.

[3] Sundberg, R. D.: Metastatic malignant melanoma of the bone marrow. *Anat. Rec*., 124: 368, 1956.

[4] Laidlaw, G. F. and Blackberg, S. N.: Mela- noma studies: Simple technique for dopa reaction. *Amer. J. Path*., 8: 491, 1932.

[5] Pearse, A. G. E.: Histochemistry—Theoretical and applied. 2nd Edition, London, J. & A. Churchill, 1960

[6] Warter A, George-Diolombi G, Chazal M, Ango A (1990) Melanin in a dentigerous cyst and associated adenomatoid odontogenic tumor. *Cancer 66*, 786-788

[7] Soames JV (1982) A pigmented calcifying odontogenic cyst. *Oral Surg Oral Med Oral Pathol* 53, 395-400.

[8] Buchner A, David R, Carpenter W, Leider A (1996) Pigmented lateral periodontal cyst and other pigmented odontogenic lesions. *Oral Dis* 2, 299-302

[9] Takeda Y, Sato H, Satoh M, Nakamura S, Yamamoto H (2000) Pigmented ameloblastic fibrodentinoma: a novel melanin-pigmented intraosseous odontogenic lesion. *Virchows Arch* 437, 454-458.

[10] Kitano M, Tsuda-Yamada S, Semba I, Mimura T, Nozoe E, Setoyama M (1994) Pigmented ameloblastic fibro-odontoma with melanophages. *Oral Surg Oral Med Oral Pathol* 77, 271-275.

[11] Üzüm N, Akyol G, Asal K, Köybaşioğlu A (2005) Ameloblastic carcinoma containing melanocyte and melanin pigment in the mandible: a case report and review of the literature. *J Oral Pathol Med* 34, 618-620.

[12] Caggiati, A. Rosi, C. Franceschini, M. Innocenzi, D. The Nature of Skin Pigmentations in Chronic Venous Insufficiency: A Preliminary Report. *Eur J Endovasc Surg* 35, 111-118. DOI: 10.1016/j.ejvs.2007.08.007

[13] Farmer, ER. Hood, AF. Pathology of the skin. 2nd ed. New York: McGraw-Hill, Health Professions Division; 2000. pp. 795

[14] Pearse, AGE. Histochemistry. Theoretical and applied. 3rd ed., vol.2. Edinburgh London: Churchill Livingstone; 1972. pp. 1130

[15] Jordan, H. E.: A source of origin of pigmented leucocytes in Amphibia. *Am. Ass. Anat. Abstr*. 20(a) Anat. Rec., 29: 387, 1925

[16] Johnson, Rachelle W. Sowder, Miranda E. Giaccia, Amato J. Hypoxia and Bone Metastatic Disease. *Curr Osteoporos Rep* 2017 August; 15(4): 231-238. Doi: 10.1007/s11914-017-0378-8

[17] Mohyeldin A, Garzon-Muvdi T, Quinones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell*. 2010; 7(2):150-61. DOI: 10.1016/j.stem.2010.07.007 [PubMed: 20682444]

[18] Harrison JS, Rameshwar P, Chang V, Bandari P. Oxygen saturation in the bone marrow of healthy vol . Rankin EB, Giaccia AJ, Schipani E. A central role for hypoxic signaling in cartilage, bone, and hematopoiesis. *Current osteoporosis reports.* 2011; 9(2):46-52. DOI: 10.1007/s11914-011-0047-2 [PubMed: 21360287] unteers. *Blood*. 2002; 99(1):394. [PubMed: 11783438

[19] Chow DC, Wenning LA, Miller WM, Papoutsakis ET. Modeling pO2 Distributions in the Bone Marrow Hematopoietic Compartment. I. *Krogh's Model. Biophys J.* 2001; 81(2):675-84. [PubMed: 11463616]

[20] Kraemer, Michael J. Hunter, Robert. Rosse, Cornelius. Smith, Nathan J. Race. Related differences in Peripheral blood and in Bone Marrow Cell Populations of American Black and American White Infants. *JAMA*, Vol. 69, No. 5, 1977.

[21] O Landgren, B I Graubard, J A Katzmann, R A Kyle, I Ahmadizadeh, R Clark, S K Kumar, A Dispenzieri, A J Greenberg, T M Therneau, L J Melton, N Caporaso, N Korde, M Roschewski, R Costello, G M McQuillan, S V Rajkumar. **Racial disparities in the prevalence of monoclonal gammopathies: a population-based study of 12 482 persons from the National Health and Nutritional Examination Survey**. *Leukemia*, 2014; DOI: 10.1038/leu.2014.34

[22] Kassissia, Ibrahim G. Goresky, Carl A. Rose, Colin P. Schwab, Andreas J. Simard, André. Huet, Pierre. Michel. Bach, Glen G. Tracer Oxygen Distribution is Barrier-Limited in the Cerebral Microcirculation. *Circ Res* 1995: 77: 1201-1211. Doi: 10.1161/01.RES.77.6.1201.

[23] Garn SM, Smith NJ, Clark DC: The Magnitude and the implications of apparent race differences in hemoglobin values. *Am J Clin Nutr* 28:563-566, 1975

[24] Caramihai E, Karayalcin G, Aballi AJ, et al: Leukocyte count differences in healthy white and black children. *J Pediatr* 86:252-254, 1975

[25] Wehrhan, Falk. Weber, Manuel. Neukam, Friedrich W. Geppert, Carol-Immanuel. Kesting, Marco. Preidl, Raimund H.M. Fluorescence-guided bone resection: A histological analysis in medication-related osteonecrosis of the jaw. *J Cranio- Maxillofacial Surg.* Volume 47, Issue 10, October 2019, pp. 1600-1607. Doi: 10.1016/j.jcms,2019. 07.012.

[26] Slominski, Radomir M. Zmijewski, Michal A. Slominski, Andrzej T. The role of melanin pigment in melanoma. *Exp Dermatol.* 2015 April ; 24(4): 258-259. doi:10.1111/exd.12618.

[27] Sarna M, Zadlo A, Hermanowicz P, et al. *Exp Dermatol.* 2014; 23:813-818. [PubMed: 25180917]

[28] Slominski A, Zmijewski MA, Pawelek J. *Pigment Cell Melanoma Res*. 2012; 25:14-27. [PubMed: 21834848]

[29] Schallreuter KU, Kothari S, Chavan B, et al. *Exp Dermatol*. 2008; 17:395-404. [PubMed: 18177348]

[30] Albert DM, Jacobiec FA. Svitra PP, Perry H. (1994) Vogt–Koyanagi–Harada (uveomeningitic) syndrome. in *Principles and practice of ophthalmology.* eds Albert DM, Jacobiec FA (Saunders, Philadelphia), pp 481-488

[31] Norose K, Yano A, Aosai F, Segawa K. (1990) Immunologic analysis of cerebrospinal fluid lymphocytes in Vogt–Koyanagi–Harada disease. *Invest Ophthalmol Vis Sci* **31**:1210-1216.

[32] Nakamura S, Nakazawa M, Yoshioka M, *et al.* (1996) Melanin-laden macrophages in cerebrospinal fluid in Vogt–Koyanagi–Harada syndrome. *Arch Ophthalmol* **114**:1184-1188

[33] Takeshita, Takayuki. Nakazawa, Mitsuru. Murakami, Kazuko. Tamai, Makoto, Nakamura Shozo, A patient With long standing melanin laden macrophages in cerebrospinal fluid in Vogt- Koyanagi- Harada syndrome. BJ Ophthalmol. Volume 81, issue 12. dx. doi.org/10.1136/bjo.81.12.1113b

[34] Appelbaum, Frederick R. Acute Leukemias in adults. **Abeloff's Clinical Oncology (Sixth Edition)** 2020,

Pages 1783-1797.e1 /doi.org/10.1016/B978-0-323-47674-4.00095-5

[35] Houlston RS, Sellick G, Yuille M, Matutes E, Catovsky D. Causation of chronic lymphocytic leukemia--insights from familial disease. *Leuk Res* 2003; 27:871-876. [PubMed: 12860003]

[36] Ishibe N, Sgambati MT, Fontaine L, et al. Clinical characteristics of familial B-CLL in the National Cancer Institute Familial Registry. *Leuk Lymphoma* 2001; 42:99-108. [PubMed: 11699227]

[37] Goldin, Lynn R. Landgren, Ola. Marti, Gerald E. Caporaso, Neil E. Familial aspects of chronic lymphocytic leukemia, monoclonal B-cell lymphocytosis (MBL) and related lymphomas. *European J Clin Med Oncol*. 2010 February; 2(1): 119-126

[38] Hillestad LK. Acute promyelocytic leukemia. *Acta Medical Scandinava.* 1957; 159: 189-194. http://dx.doi.org/10.1111/j.0954- 6820. 1957. tb00124.x

[39] Bernard J, Mathe G, Boulay J, Ceoard B, Chome J. Acute promyelocytic leukaemia: a study made on 20 cases. *Schweiz Medical Wochenschrifte.* 1959; 89: 604-608. PMid:13799642

[40] Rand JJ, Moloney WD, Sise HS. Coagulation defects in acute promyelocytic leukemia. *Arch Intern Med.* 1969 Jan;123(1):39-47. http://dx.doi.org/10.1001/archinte.1969.00300110041007 PMid:5248747

[41] McClellan JS, Kohrt HE, Coutre SS, Gotlib JR, Majeti R, Alizadeh AA, Medeiros BC. Treatment advances have not improved the early death rate in acute promyelocytic leukemia. *Haematologica*. 2011 Oct 11

[42] Lengfelder, Eva. Hofmann, Wolf-Karsten. Nolte, Florian. Management of elderly patients with acute promyelocytic leukemia: progress and problems. *Ann Hematol* (2013) 92:1181-1188 DOI 10.1007/s00277-013-1788-z

[43] Silverman, Richard B. Organic Chemistry of Enzyme-Catalized reaction. Chapter 9 – Isomerizations. Elsevier, 2002, pages 359-397. doi.org/10.1016/B978-0-08-051336-2.50014-0

[44] Solís- Herrera, A. Arias- Esparza, MC. Ashraf, Ghulam Md. Zamyatnin Jr, Andrey A. Aliev, Gjumrakch. Beyond mitochondria, what would be the energy source of the cell? CNS Agents Med Chem. 2015, Vol. 15, No. 1; pp. 32-41. **DOI** : 10.2174/1871524915666150203093656 PubMed: 128159