Research Article

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Carbonic anhydrase, water dissociation and physiology of Kidney.

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ABSTRACT

Carbonic anhydrase (CA) is a zinc metalloenzyme widely distributed throughout the tissues of the body. In the kidney, this enzyme is thought to play a pivotal role in urinary acidification and bicarbonate reabsorption. Two distinct isozymes of carbonic anhydrase have now been identified in the mammalian kidney. A soluble cytoplasmic form, similar if not identical to human erythrocyte carbonic anhydrase C, accounts for the bulk of the renal carbonic anhydrase activity. In addition, a membrane-bound form constituting only about 2--5% of the renal activity has been found in the brush border and basolateral fractions of kidney homogenates. The activity of carbonic anhydrase enzyme is incessantly, which means a high energy expenditure, but ATP is not the source of energy of this enzyme (CA).

The kidney generates 180 liters of filtrate a day. The process is also known as hydrostatic filtration due to the wrong belief that 180 liters of filtrate a day as result of the hydrostatic pressure exerted, by the left ventricle; on the capillary walls of the kidney. However, the cardiac pressure required to impel the blood stream at adequate level is way beyond 120 mm Hg output force that left ventricle has.

Keywords: Melanin, Kidney, hydrogen, energy, water dissociation.

BACKGROUND

Carbonic anhydrase (CA) is a crucial enzyme that operates in animal cells, plant cells, and in the environment to stabilize carbon dioxide concentrations [1]. Without this enzyme, the conversion from carbon dioxide to bicarbonate (**Figure 1**), and vice versa, would be extremely slow, and it would be nearly impossible to carry out life processes, such as photosynthesis in plants and people exhaling carbon dioxide during respiration. Although it performs a lot of beneficial functions, it can also damage the human body as well, even causing some forms of cancer.

Carbonic anhydrase is one of the key enzymes in life. Its principal function is to facilitate the solvation of carbon dioxide (or its reversible reaction to bicarbonate). It plays, therefore, a central role in facilitating the diffusion of carbon dioxide in photosynthesis, but it is also essential in areas such as respiration (with the transport of carbon dioxide in the blood), ion balance, an important role in kidney function, acid-base balance, biomineralization, and various aspects of bacterial metabolism (e.g. cyanates). It is not only a remarkably fast enzyme, but so central is it to life that one's automatic assumption is that it would be one of the first enzymes to evolve and so employed in diverse circumstances as and when required. Carbonic anhydrase is extremely convergent and may have evolved as many as six times.

CO_2 (aq) ↔ CO_2 (gas)
CO_2 (aq) + H_2O ↔ H_2CO_3 (aq)

However, CA requires, as any other enzyme, it requires a very accurate and highly specific type of energy, and to top it off reversible, to be able to carry out its important and basic function properly (Figure 1) (solvation and desolvation), and not to mention that energy is also required for said enzyme to maintain its shape the most time possible. It is the most active enzyme known [2], and therefore requires a lot of energy, which does not come from ATP.

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Figure 1

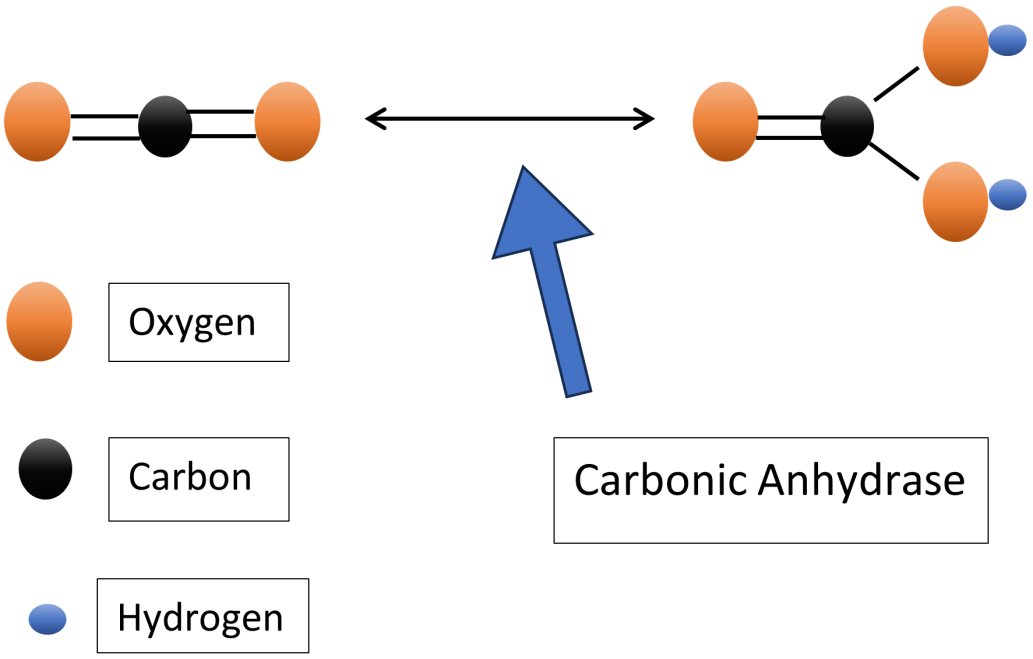

Figure 1. Diagram of the reversible action of the enzyme carbonic anhydrase on CO2 in relation to water. The energy source of this enzyme is not ATP.

INTRODUCTION

Carbonic anhydrase is an omnipresent zinccontaining metalloenzyme which is essential for a lot of physiological activities because of its property to convert CO2 to HCO3- reversibly. It is one of the fastest enzymes known for hydrating 106 molecules of CO2 per second. The rate of reaction of this enzyme is typically limited by the rate of diffusion of its substrates.

Carbonic anhydrase is often arranged in clusters along membranes or localized in extracellular spaces, which may contribute to the ability of carbonic anhydrase to facilitate the intracellular diffusion of carbon dioxide and protons (H^+). By increasing the movement of protons, carbonic anhydrase can dissipate intracellular pH gradients, which means significant energy exchange; thereby helping the cell to maintain the characteristic (and astonishing) uniform cellular pH. [3]

The carbonic anhydrase reaction is involved in many physiological and pathological processes, including respiration and transport of CO2 and bicarbonate between metabolizing tissues and lungs, pH and CO2 homeostasis, electrolyte secretion in various tissues and organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis, and ureagenesis), bone resorption and calcification [4].

The carbonic anhydrase (CA) gene family includes around 10 enzymatically active members, which are major players in many physiological processes, including renal and male reproductive tract acidification, bone resorption, respiration, gluconeogenesis, signal transduction, and formation of gastric acid. The newly identified CA IX (previously called MN) and CA XII are related to cell proliferation and oncogenesis. Carbonic anhydrase isozymes have different kinetic properties, and they are present in various tissues and in various cell compartments. CA I, II, III and VII are cytoplasmic, CA V is mitochondrial, and CA VI is present in salivary secretions. CA IV, IX, XII and XIV are membrane proteins: CA IV is a glycosyl-phosphatidylinositol-anchored protein, and CA IX, XII and XIV are transmembrane proteins. The present work will focus on the roles of CA II and CA IV in transepithelial proton secretion and bicarbonate reabsorption processes [5]. Carbonic anhydrase isozymes have different kinetic properties, and they are present in various tissues and in various cell compartments. CA I, II, III and VII are cytoplasmic, CA V is mitochondrial, and CA VI is present in salivary secretions. CA IV, IX, XII and XIV are membrane proteins: CA IV is a glycosyl-phosphatidylinositol-anchored protein, and CA IX, XII and XIV are transmembrane proteins.

Carbonic anhydrase III (CA III) is a cytosolic enzyme which is known to be highly expressed in the skeletal muscle, [6], tissues that synthesize and/or store fat: liver, white adipose tissue, and brown adipose tissue [7].

We will focus on the roles of CA II and CA IV in transepithelial proton secretion and bicarbonate reabsorption processes. The

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localization of these isoforms in selected epithelia that are involved in net acid/base transport, such as kidney proximal tubules and collecting ducts, and tubules from the male reproductive tract.

CA IV is a glycosylphosphatidyl-inositolanchored membrane isozyme expressed on the luminal surfaces of pulmonary (and certain other) capillaries and of proximal renal tubules[8]. It is essential for acid overload removal necessary for the high metabolic requirements of mammals.

The carbonic anhydrase active sites contain a zinc ion, and all share a similar catalytic activity. In the carbonic anhydrase enzyme, a zinc prosthetic group is coordinated in three positions by histidine sidechains. The active site of the enzyme "E" contains a specific pocket for CO2 which brings it closer to the hydroxide group attached to the zinc. Therefore, this causes the electron-rich hydroxide ion to attack the CO2 thereby creating a bicarbonate molecule.

$$CO_{2\ (gas)} + H_2O_{\ (liquid)} \leftrightarrow H_2CO_{3\ (liquid)} \leftrightarrow HCO_3^{-}{}_{(solid)} + H^+$$

KIDNEY

In the kidney, this enzyme is thought to play a pivotal role in urinary acidification and bicarbonate reabsorption[9]). Acidification of urine could be brought about either by the secretion of hydrogen ions into the tubular fluid or by the selective absorption of a buffer base. Both filtration and secretion are essential to hydrogen ion excretion and both proximal and distal convoluted tubules are involved. The bulk of the bicarbonate filtered at the glomerulus is reabsorbed in the proximal tubule, from which it passes back into the peritubular capillaries. This mechanism is designed to keep the normal plasma bicarbonate concentration constant at about 25 millimoles per liter. When the plasma concentration falls below this level, no bicarbonate is excreted, and all filtered bicarbonate is reabsorbed into the blood. When the plasma bicarbonate rises above 27 millimoles per liter, bicarbonate appears in the urine in increasing amounts.

The brush borders of the cells of the proximal tubules are rich in the enzyme carbonic anhydrase, which means that these cells have significant amounts of available energy needed to drive and maintain the constant activity of that enzyme, as well as to maintain their shape, which also requires energy, and this energy does not come from ATP.

The CA enzyme facilitates the formation of carbonic acid (H_2CO_3) from CO_2 and H_2O, which then ionizes to hydrogen ions (H^+) and bicarbonate ions (HCO_3^-). The starting point for bicarbonate reabsorption is probably the active secretion of hydrogen ions into the tubular fluid. These ions are formed under the influence of carbonic anhydrase from CO_2 liberated from oxidation of cell nutrients and H_2O already in the cells. The filtered base, bicarbonate, accepts the hydrogen ions to form carbonic acid, which is unstable and dissociates to form CO_2 and H_2O. The partial pressure of CO_2 in the filtrate rises, and, as CO_2 is highly diffusible, it passes readily from the tubular fluid into the tubular cells and the blood, and the water is either dealt with in the same way or is excreted. In the meantime, the proximal tubular cells are actively reabsorbing filtered sodium, which is balanced by the HCO_3^- formed within the cells from the CO_2 generated by the hydrogen ions in the luminal fluid. Thus, the bicarbonate reabsorbed is not that which was originally the filtrate, but the net effect is the same as if this was the case.

Thus, Carbonic anhydrase isozymes perform different functions at their specific locations, and their absence or malfunction can lead to diseased states, ranging from the loss of acid production in the stomach to kidney failure. This enzyme, since was first identified in 1934, in red blood cows [10], and in plant leaves as component of chloroplast in 1939 [11] it has been extensively studied. However, in no one article, its source of energy is analyzed, defined, or at least mentioned.

Water dissociation and reformation, the unexpected source of energy of carbonic anhydrase

Our observation that our body does not take oxygen from the air around it, but from the water that our cells contain inside, through the dissociation of water, like plants; It is a disruptive knowledge that forces us to rethink biology and biochemistry, almost in its entirety or at least in a good part [12]. This observation was not the result of chance, but came from a descriptive, observational study about the morphological characteristics of the tiny vessels that enter and leave the human eye through the optic nerve. This study began in 1990 and ended in 2002 and included the ophthalmological records of 6000 patients.

The unsuspected role of intracellular water as a source of oxygen in eukaryotic cells led us to reflect that gas exchange in the lung only occurs in relation to CO_2, since this toxic gas requires constant expulsion since it is continuously formed inside the cells, and it is so fundamental for human physiology that it is expelled, that from the moment we are born we breathe constantly without the need to learn. The air we introduce during inspiration only works as a transporter of the CO_2 that is formed on the surface of the pulmonary alveoli, where the enzyme carbonic anhydrase intervenes decisively; but the lung does not take in the oxygen we need from that air, because our requirements in this regard are five times higher than the concentration of oxygen contained in the atmosphere, which ranges between 18 and 21%. Lung and oxygen have nothing to do with each other. Breathing is only to expel CO_2, and that's where our interest in delving deeper into the enzyme carbonic anhydrase was born. And each cell takes in the oxygen it requires from the water it contains inside.

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The enzyme carbonic anhydrase is also present in the erythrocytes of mammals. And yet, these do not have mitochondria [13], so ATP can be ruled out as an energy source for AC. CO_2 produced within skeletal muscle has to leave the body finally via ventilation by the lungs. To get there, CO_2 diffuses from the intracellular space into the connective transport medium, blood with the two compartments, plasma, and erythrocytes [14]. Within the body, CO_2 is transported in three different forms: physically dissolved, as HCO_3 -, or as carbamate.

Carbonic anhydrase accelerates around 25 times the normal speed of hydration/dehydration reaction between CO_2, HCO_3^-, and H^+ . In skeletal muscle, various isozymes of carbonic anhydrase are localized within erythrocytes but are also bound to the capillary wall, thus accessible to plasma; bound to the sarcolemma, thus producing catalytic activity within the interstitial space; and associated with the sarcoplasmic reticulum. In some fiber types, carbonic anhydrase is also present in the sarcoplasm. In exercising skeletal muscle, lactic acid contributes huge amounts of H+ and by these affects the relative contribution of the three forms of CO_2.

ATP is not produced inside the erythrocyte because there are no mitochondria, so what drives the activity of carbonic anhydrase inside the erythrocytes?

The answer is that the human body has several molecules capable of transforming light energy into chemical energy, through the dissociation of water, like plants.

In 1844, Verdeil [15] reported that acid treatment of chlorophyll or haem yielded apparently similar red compounds. Hoppe-Seyler [16] confirmed the apparent similarity of acid derivatives of haems and chlorophylls from their light absorption characteristics (**Figure 2**), a point rather overshadowing the discovery at the same time by McMunn [17] of cytochromes, another group of haem proteins. (**Figure 3**)

Figure 2

Figure 2. Most people do not know, but within 30 to 50 feet of water, human blood appears green instead of red. Source: Shivans, QUORA.

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Figure 3

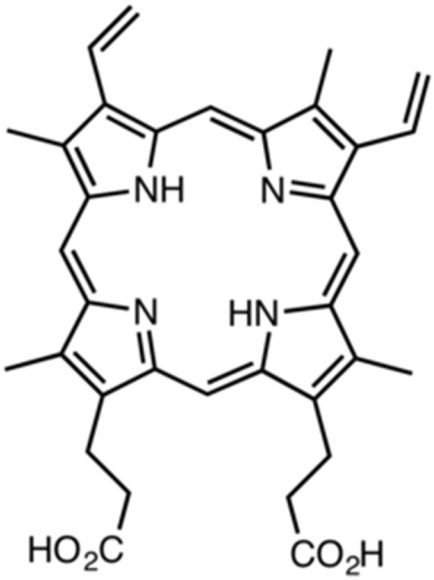

Figure 3. Protoporphyrin PPIX formula.

It was the demonstration by Nencki and coworkers [18] that the degradation of both chlorophylls and haems yielded monopyrroles that led them, in true neo-Darwinian fashion, to postulate a common origin for animals and plants.

Today we can show the similarity between haem and chlorophyll based on their common precursor, protoporphyrin IX. Protoporphyrin IX (PPIX) is ubiquitously present in all living cells in small amounts as a precursor of heme. [19]

For over a century, scientists have been aware of the existence of the numerous types of porphyrin-based compounds to be found in a wide range of eukaryotic and prokaryotic organisms[20]. The earth-bound biological porphyrins are diverse and range in color from grey blue (bacteriochlorophyll), green (chlorophyll), and red (protoheme) to yellow and brown (avian egg porphyrins). Compounds derived from protoporphyrin IX have the intrinsic property of irreversibly dissociating the water molecule [21].

Many tetrapyrroles, particularly those of avian eggshells, have no central complexed metal. There are only five metals commonly found in natural porphyrins: copper in a uroporphyrin III derivative in the flight feather of the tropical Musophagidae family of birds; cobalt as the metal component of vitamin B12 (cobalamins) [22]; iron in the metal complex in haems including hemoglobin, myoglobin, catalase, peroxidases, and cytochromes. The fourth metal, magnesium, is characteristic of all chlorophylls and bacteriochlorophylls. A fifth metal, zinc, may complex enzymically or non-enzymically to many porphyrins and to the porphyrin breakdown product biliverdin (it is not known if complexing occurs before or after ring cleavage). Zinc is ease inserted non-enzymically into porphyrins in vitro would make it surprising if zinc porphyrins did not exist in vivo.

Protoporphyrin IX has come to be placed in a central role in the study of haem and chlorophyll metabolism. Protoporphyrin isomer IX, of the fifteen possible isomers, is believed to be a precursor at the branch point of biosynthesis of both haems and chlorophylls. a major pathway gives rise to a series of haem compounds, of which the cytochromes, in one form or another, are present in nearly all organisms. Hemoglobin, a characteristic pigment of vertebrates, is present in a wide range of organisms.

Leptocephalus eel larva has no haemoglobin until it reaches the elver stage [23]. A form of haemoglobin is found in plants under the name of leghaemoglobin. It is, however, confined to those plants which can fix nitrogen in co-operation with the bacterium Rhizobium. Such plants include the legume family. Leghaemoglobin appears to help to maintain a constant, but low, level of oxygen within nitrogen fixing cells. Haem synthesis appears to take place in the bacteria, globin in the plant, the completed molecule being located on the interface of the two organisms. [24]

Plants do not, (neither mammal) have a complex gas transport system; they obtain their oxygen from solution in cell wall water films. An excellent review of the occurrence of haieigilobins and myoglobin in invertebrates are provided by Kennedy [25]. Myoglobin is present throughout the vertebrates but appears only sporadically in the invertebrates.

Oxygen-carrying Pigment

We must banish the idea that these pigments transport oxygen and begin to interpret molecular biology since they are pigments that irreversibly dissociate the water molecule, and that is why they are always associated with oxygen, but not because they transport it, but because they are producing it throughout the constant irreversibly dissociating of the water molecule.

It is quite clear, then, that alternative systems to hemoglobin for oxygen production, more than its transportation, have existed for hundreds of millions of years, probably long before the appearance of the first land animals.

All fungi and plants contain haem pigments, the cytochromes. One third or more of the total tetrapyrrole content of dark-grown barley seedlings consists of protoheme [26]. All chlorophylls are magnesium tetrapyrroles and it is likely that they are formed by successive modifications of magnesium protoporphyrin [27].

In higher plants (the flowering plants or angiosperms), the porphyrin protochlorophyllide (**Figure 4**) accumulates if seedlings are germinated in the dark. In most other plants (some algae being an exception) chlorophyll is formed even in the dark, with no detectable protochlorophyllide accumulation.

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Figure 4

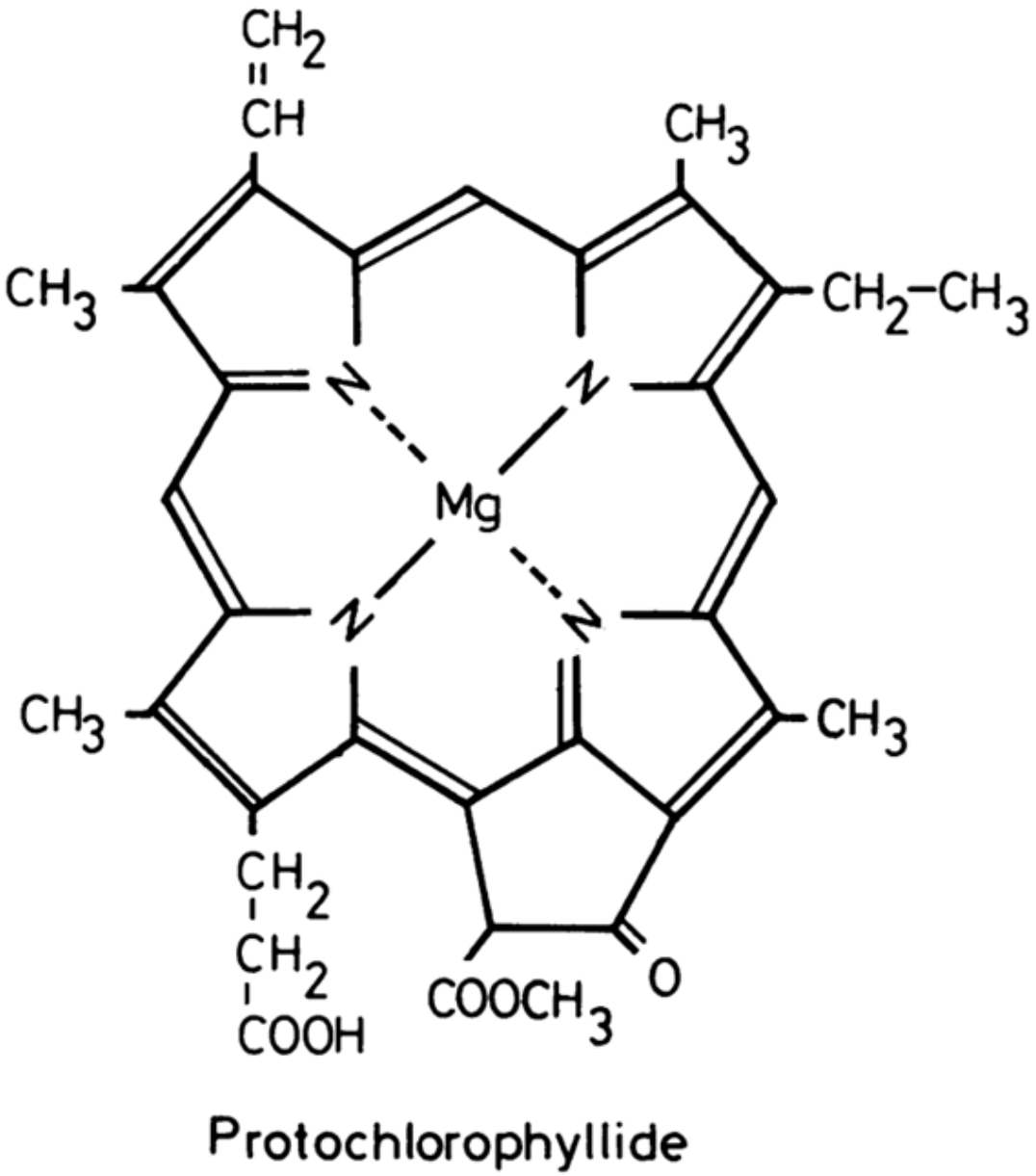

Figure 4. The precursor of chlorophylls and bacteriophylls.

Among the iron porphyrins the substituent at position 4 is constant. The original vinyl group persists in hemoglobin, cytochromes a, b, and d, catalase, and most peroxidases. Certain degradation products of porphyrins are well known[28] as bilins or bile pigments. The structural similarity between biliverdin, bilirubin, and mesobilirubin suggests that these compounds are derived from the breakdown of protoheme (from hemoglobin, myoglobin, cytochrome P-450, and cytochrome b) and this has been confirmed in vitro. The prokaryote blue-green algae and the eukaryote red algae contain bile pigments (probably as accessory pigments in photosynthesis), two of which have been named phycocyanobilin and phycoerythrobilin [29].

It is interesting the absence of any reports of bile pigments derived from chlorophylls. Furthermore, it is not known how chlorophylls are degraded in vivo. Chlorophyll degradation might involve the loss of magnesium (to form phaeophytin) and the removal of the phytol side chain (to form pheophorbide). One of the great mysteries of porphyrin biochemistry is the natural fate of chlorophylls in autumn leaves. Some 1012 tonnes [30] of chlorophyll disappear annually, much of it during autumn.

And this is, quite probably, the dissociation of water. The only known molecule capable of dissociating and reforming the water molecule, both outside and inside a living entity, is melanin:

$$2H_2O_{(liq)} \rightarrow 2H_{2\,(gas)} + O_{2\,(gas)} \rightarrow 2H_2O_{(liq)} + 4e^-$$

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And melanin gives rise to protorphyrin IX (PP IX), a molecule presents in all living beings, just like melanin. All molecules derived from PPP IX irreversibly dissociate the water molecule, but both PP IX and its derivatives, to carry out their water dissociation function, require being inside a living entity, for example a cell, but not melanin.

The amino-acid 5-aminolaevulinic acid (ALA) was first shown to be an intermediate in porphyrin synthesis by Shemin and Russell [31]; it is formed by ALA synthase (the suffix synthase means that it does not use ATP as an energy source) by avian and mammalian erythrocytes, bone-marrow cells, and liver, by yeast, bacteria, and insects [32]. It was also shown in a photosynthetic bacterium Rhodopseudomonas sphaeroides [33], where its activity was found to be greatest at times of maximum bacteriochlorophyll synthesis [34].

It is striking that no enzyme has been described from plants which could account for the rate of synthesis of chlorophyll in actively greening tissues [35], and melanin can fill this gap, since melanin requires a trillionth of a second to dissociate a water molecule.

The heavy cloud of unknowns around the processes that seem to constitute the common origin of living beings is based on academics trying to adjust biochemical logic to ATP as a source of energy, which is theoretical. However, the dissociation of water constitutes the first spark of life, but it could not be included in the scheme of the origin of life, given that chlorophyll requires being inside a living entity, such as a cell, which pointed out that life came first and then chlorophyll or the dissociation of water. But by identifying the intrinsic property of melanin to dissociate and reform the water molecule [36], both inside and outside of a living entity, the problem is solved. The dissociation of water is the common origin of all living entities, and melanin is what makes it possible. Everything else comes later.

It is fascinating to weave together the work of past and present researchers considering the water dissociation process, but I would go on too long, so we will return to the topic of carbonic anhydrase, water dissociation, and the biology of the kidney.

Kidney biology traditionally explained

The following is a brief general description of kidney function, according to prevailing texts.

Filtration, which takes place at the renal corpuscle, is the process by which cells and large proteins are retained while materials of smaller molecular weights [37] are filtered from the blood to make an ultrafiltrate that eventually becomes urine. The kidney generates 180 liters of filtrate a day. The process is also known as hydrostatic filtration due to the hydrostatic pressure exerted on the capillary walls. But the hydrostatic force required to force glomerular filtration is very far from the 120 mm Hg that the contraction force of the left ventricle provides.

The nitrogenous wastes urea, from protein catabolism, and uric acid; from nucleic acid metabolism, tend to make inefficient the generation and distribution of energy (from melanin, hemoglobin, myoglobin, cytochromes, and other molecules derivate of PP IX capable of dissociate irreversible the water molecule)), thereby the water is scarce attracted to the intra-cellular medium, and easily pass to proximal convoluted tubule and then to descending limb of loop of Henle. The high waste concentration induces a low level of energy, so, the reabsorption of water and other molecules is diminished in a significant way. The constant dissociation of the water molecule that occurs inside the cells and tissues of both the kidney and the entire body, induces a vacuum that attracts molecules and liquids to itself.

As the waste molecules are diluted for the increasing presence of water, the generation and distribution of energy from melanin, experience an elevation in their efficiency, allowing a greater availability of energy (from water dissociation and not from ATP) and therefore an increasingly efficient reabsorption in the path of the Henle handle and its upward portion.

When urine reaches the distal contoured tubule, more efficient reabsorption, due to the lower concentration of waste, allows the reabsorption of a greater amount of water and other elements, so when urine pass into the collection tube, there are only present substances whose nature significantly decreases the generation and distribution of energy from melanin and other pigments throughout water dissociation, so the reabsorption of such toxic compounds is minimal or no, due to low levels of available energy so the processes relatives to mechanisms of reabsorption cannot take place.

The ability of mammals and some birds to concentrate wastes into a volume of urine much smaller than the volume of blood from which the wastes were extracted is dependent on an elaborate countercurrent multiplication mechanism. This requires several independent nephron characteristics to operate: a tight hairpin configuration of the tubules, water, and ion permeability in the descending limb of the loop (low levels of energy), water impermeability in the ascending loop (even less available energy), and active ion transport out of most of the ascending limb. In addition, passive countercurrent exchange by the vessels carrying the blood supply to the nephron is essential for enabling this function. But the amount of energy required by such processes, as well as their precision, has not been explained by mitochondria or ATP as an energy source. However, the dissociation of water, the source of energy par excellence of life, can be explained given the numerous molecules capable of carrying it out, as well as their location, which is not random.

The kidney generates 180 liters of filtrate a day, which means that amount of energy expenditure is huge, so it is not possible to explain it, based in the 120 mm Hg of contraction

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force of the left ventricle. It is more plausible that the incessant dissociation of water, provide the adequate energy levels in time, form, and location, for instance, descend of it allowing the pass-through cell membranes of water and other molecules of small size. Let us remember that the processes of both the kidney and all the organs and tissues that make up us are strictly regulated by millions of years of evolution.

We must keep in mind, that in any system, it is easier to lower energy levels than to raise them, and the kidney is no exception. By the way, energy is defined as anything that brings about change.

Urine formation begins in the glomeruli, where the concentration of waste products resulting from the body's metabolism is elevated in part by the glomeruli anatomy (a double layer of cuboidal cells), which induces in the surrounding cells a decrease in the available energy, thereby the suction forces inside the cell cannot maintain liquid (water) and small molecules inside the blood vessels and such fluid tends to be directed to the light of the proximal contoured tubule (**Figure 5**), which is thicker-walled than the descending portion of the loop of Henle. It is not by chance, that renal chloride and bicarbonate homeostasis are closely linked, because both processes depend on water dissociation.

Figure 5

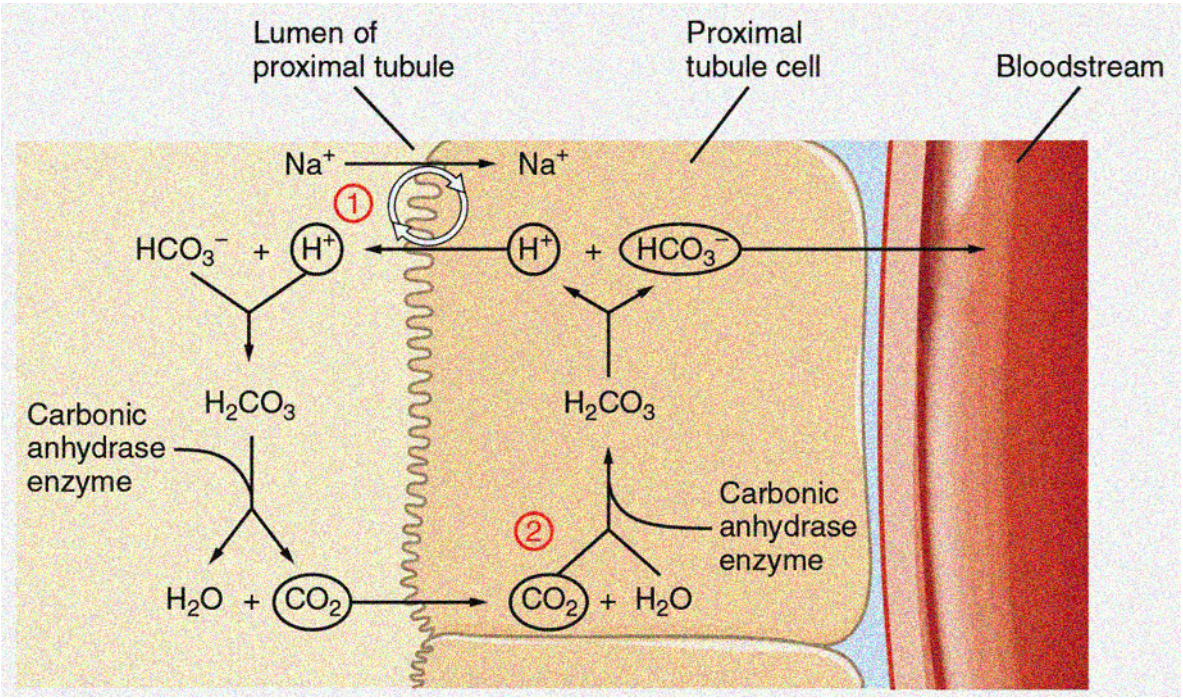

Figure 5. This is a scheme frequently used in textbooks, where one of the main locations and actions of the enzyme carbonic anhydrase is outlined. However, the source of energy that allows carbonic anhydrase to carry out its function and at the same time maintain its shape is not mentioned.

Figure 6

Figure 6. The erythrocytes have a characteristic shape, like a biconcave disk, as if there were suction or vacuum inside it, and vacuum characteristically appears when water is dissociated.

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CONCLUSION

From now on, we can be certain that the energy source of the enzyme carbonic anhydrase, as well as other enzymes that do not use ATP, is undoubtedly the dissociation of water, which is carried out by various molecules normally present in tissues., all of them derived from protoporphyrin IX (PP IX), except melanin.

So, the intense activity of carbonic anhydrase, of about 106 CO_2 molecules per second, is sustained completely by the even more intense dissociation of water, that are in the range de nano (1 X 10^{-9} seconds) and picoseconds (1 X 10^{-12}).

On the other hand, the dissociative activity of hemoglobin on plasma water, in the bloodstream, contributes with molecular oxygen and molecular hydrogen (loaded with energy captured during water splitting) and transports it to its environment, which drives the activation energy required by the complex chemical reactions that occur constantly and incessantly in the bloodstream, since blood is a bioreactor in constant motion, in which very precise chemical processes occur and therefore, they are driven by the chemical energy that the hydrogen molecule traps at the moment in which, hemoglobin, separates the water molecule (from liquid to gas), and transports it in its microenvironment, following the laws of simple diffusion.

It is plausible that the vacuum that is generated when the water molecule dissociates is the explanation for the shape of the erythrocyte, like a biconcave disk, and not so much the absence of the cell nucleus, mitochondria, and other organelles.

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Conflict of interest: None.

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