Understanding the Redox (rH_2) Measurement of the Biological Terrain: Part I

Robert C. Greenberg, D.C., F.A.S.A.

The measurement and assessment of pH in the biological field is well defined and documented.[1,2] It is known and accepted that enzyme kinetics as determined by the Michaelis-Menton equation for the determination of maximum enzyme velocity is pH-dependent.[3] Maximum enzyme velocity and kinetics are also substrate concentration and temperature-dependent as well. Science has clearly demonstrated that assessing and comprehending the factor of pH is a crucial aspect of understanding the biochemistry and physiology of the human body.[4,5]

In contrast, the discernment and dynamic implications of oxidation-reduction (redox) potential is only now coming to the forefront of modern medcine. Even though this field of science as been evaluated and scrutinized since 928, it has not received the attention or edication that pH has afforded.[6] There re many leading authorities who believe nat redox potential is even more signifiant than pH in understanding biologial actions and interactions.[7,8] In fact, ne leading authority has stated that "in oth living and non-living nature, oxidaion and reduction reactions are more nportant than acid and base reactions."[9]

It is, therefore, essential that the full ignificance of redox potential and its ncompassing influence on disease be vell understood. Redox potential even iow is already being viewed by marine biologists as the key factor in sustaining he quality of aquatic life. Its measurement enables scientists to secure the success of sexual reproduction and longevity in aquatic lifeforms.[10,11] The complete understanding of redox potential, the rH_2 factor, and how these values affect human chemistry and physiology must also be researched and established.

According to an assemblage of well respected researchers and authors, oxidative stress is the key factor in many symptoms and disease states e.g., CFIDS, FMS (fibromyalgia syndrome), IBS (irritable bowel syndrome), alterations in nucleic acid sequences which may lead to cancers, environmental sensitivities, accelerated biological aging, food allergies, Leaky Gut Syndrome, energy deficiency, fatigue, sleep disturbances, immune dysregulation, cardiac problems, ocular problems, liver problems, kidney problems, and pancreatic-based problems just to name a few.[12, 13,14] It seems that oxidative stress and the far reaching effects that this biochemical occurrence embraces, can now be directly or indirectly attributed to almost all of life's ailments and physiological dysfunctions.

Dr. Helmut Sies was paramount in categorizing all of these conditions under one united and comprehensive definition. He concluded that any shift in the redox potential toward increased oxidation of cellular macromolecules demonstrated oxidative stress. He further believed that this shift in redox potential could be measured and could provide vital information concerning the progression and degree of cellular damage.[15]

Mitochondrial damage, concentration of reactive oxygen species, anaerobic metabolism, derailment of the oxidative phosphorylation-electron transport chain and production of free radicals are all the results of oxidative stress and redox potential.[16] Therefore, in order to fully comprehend the extent of aberrant cellular function manifested in the conditions stated above, a viable working knowledge of redox potential must be established.

Redox potential is perhaps best described as a representation of overall electron activity. It must be clarified that in the truest state of physics, free electrons do not actually exist in aqueous solutions. They exist in a homogeneous blend and are often times being transferred from one atom or ion to another.[17] In a broader sense, redox potential could be considered as a measurement of the ease with which a substance either absorbs or releases electrons.[18] This donation or acceptance of electrons is correctly termed oxidation and reduction. Simply stated, oxidation is the loss of electrons and/or hydrogen atoms and/or the gain of oxygen. Reduction is the gain of electrons and/or hydrogen atoms and/or the loss of oxygen.[19]

The determination of redox potential is a potentiometric measurement. Within micro-limits, no actual current flows through the sample solution during this measurement process.[20,21] This limitation guarantees that no changes in the solution's chemical composition occur due to electrolysis. This also guarantees that undesirable electrode surface polarization is minimized. During the formation of the redox potential measurement, electrons either flow from the sensing electrode device to the redox system or vice-versa. This separation of electrical charge causes a potential to accumulate on the metal surface of the electrode. This potential represents the propensity of an aqueous solution to gain or lose electrons and is measured in millivolts.[22]

There are two different types of redox reactions:

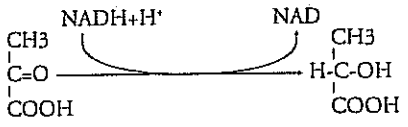
Example #1

$$2Fe^{+3} + 2I^{-} \longrightarrow 2Fe^{+2} + I_2$$

This is an example of a reaction in which electron movement occurs due to alterations in ionic valences. In this reaction, iron which begins in the ferric state (Fe+3) gains an electron and is reduced to the ferrous state (Fe+2). Simultaneously, iodine in the -1 state loses an electron and is oxidized to atomic iodine. This reaction represents the "typical" form of a redox reaction. This

type of redox reaction occurs most frequently outside the physical body. [23]

Example #2



This is an example of a redox reaction that occurs without the visible exchange of electrons. Instead, the redox reaction transpires as a result of the exchange of hydrogen atoms. This occurs at the expense of NADH + H+ being converted into NAD and liberating 2H's. These 2H's are then picked up by pyruvate which subsequently causes a conversion to lactate. In this reaction there are no visible exchanges of electrons or valence states as is demonstrated in example #1. Nevertheless, a complete redox reaction has occurred. In this example NADH + H+ has been oxidized to become NAD. Subsequently, pyruvate has been reduced to become lactate. While the first example occurs more frequently outside the human body, the second example predominates inside the human body. Most redox reactions that are seen inside the human body occur not as an exchange of electrons that cause valence alterations, but instead as a production of energy rich reducing agents, in the form of NADH + H+ or $FADH_2$. [24]

All redox reactions, whether they are similar to example #1 or #2, conform to Nernst's law which states:

$$E = E^0 + 2.3\frac{RT}{nF} \ln \frac{(ox)^n}{(red)}$$

In this equation:

- E = redox potential in millivolts
- E^0 = the standard electrode potential when all activity is equal to unity
- R = the gas constant or 8.314 J/°mol
- T = absolute temperature
- n = number of electrons involved in the reaction
- F = Faraday's constant or 96000 coulombs

When an exchange of hydrogen atoms does not occur, the redox reaction is considered pH independent.[26,27] (See example #1) When hydrogen atoms play a key role in the redox reaction, it is pH dependent. (See example #2) The relative concentration of hydrogen ions will, in fact, play a significant role in the overall E or redox potential.[28] An analysis of these stated scientific facts clearly documents that redox reactions that occur inside the body which are dependent upon the transference or exchange of hydrogen atoms are completely dependent upon changes and adjustments in relative pH conditions. Redox reactions which are independent of the transference or exchange of hydrogen atoms are completely independent of changes and adjustments in relative pH conditions. This vital fact can be demonstrated when Nernst's equation is solved with direct reference to the relative exchange of hydrogen atoms. To solve Nernst's equation under these parameters it is essential to understand the following reaction expressed by the equation:

$$H_2 \longrightarrow 2H^+ + 2e^-$$

In this equation, atomic hydrogen dissociates into hydrogen ions and simultaneously liberates electrons. This reaction is termed the "universal reference reaction" because it represents the basis of all universal life.[29] Therefore, when solving Nernst's equation utilizing the "universal reference reaction" it becomes:

$$E = E^0 + 2.3\frac{RT}{nF} \ln \frac{(H^+)}{(H_2)}$$

When expressed in this form, the log of the hydrogen ion concentration (H+) is equal to the pH. However, because the molecular hydrogen concentration (H_2) is not a measurement that can be evaluated in terms of concentration like pH, it must instead be measured in terms of partial pressure. This mode of measurement is essential since H_2 at room temperature or greater, e.g., body temperature, is in a gaseous state. A gas is easily defined in terms of partial pressure and is represented by the symbol r. The partial pressure of molecular hydrogen is therefore represented by the symbol rH_2 and is measured in terms of atmospheric pressure known as "bar."[30,31]

REFERENCES

1) Aihara H. *Acid and Alkaline.* George Ohsawa Macrobiotic Foundation, 1511 Robinson Street, Oroville, California 95965, Fifth Edition. 1986.

2) Wingate P, Gifford C, Treays R. *Essential Science.* EDC Publishing, 10302 E. 55th Place, Tulsa, Oklahoma 74146. 1995.

3) Stryer L. *Biochemistry.* W.H. Freeman Co., New York, New York, 1988.

4) Aickn CC. Intracellular pH regulation by vertebrate muscle. *Annu Rev Physiol,* (48) 349:1986.

5) Seldin DW, Giebisch G. *The Regulation of Acid-Base Balance.* Raven Press, New York, New York, 1989.

6) Hanke M, Tuta J. Studies on oxidation-reduction potential of blood. *J Biol Chem* (78)36:1928.

7) Carter D, Philips A, Silver J. Measurement of oxidation-reduction potentials and pH of tissues. *J Physiol* (129)33:1955.

8) Ingold W. Redox Measurement, Principles and Problems. *Ingold Messtechnik AG,* CH-89002 Urdorf, Switzerland, 1982.

9) Clark WM. *Oxidation-Reduction Potentials of Organic Systems.* Williams & Wilkins Company, Baltimore, Maryland, 1960.

10) Chapelle FH, Lovley DR. Rates of microbial metabolism in deep coastal plan aquifers. *App Env Microbiol.*(56):1865-74.

11) Gorby YA, Lovley DR. Electron transport mechanisms in the dissimilatory Fe (III) - reducing microorganism. *App Env Microbiol.*(57):867-70.

12) Grisham MB. Reactive oxygen metabolism. *Reactive Metabolites of Oxygen and Nitrogen and Medicine.* Armstrong Printing, Austin, Texas, 4-19.

13) Halliwell B, Gutteridge JM. *Free Radicals in Biology and Medicine.* Claverder Press, second edition, Oxford,

England, 1991:31-34.

14) Bandy B, Davison AJ. Mitochondrial mutations may increase oxidative stress: implications for carcinogenesis and aging? *Free Radical Biology and Medicine.* 1990, 8:523-39.

15) Sies H. Strategies of antioxidant defense. *Eur J. Biochem.* 1993; 215(2):213-19.

16) Bland J. *New Perspectives In Nutritional Therapies.* HealthComm, Inc., Gig Harbor, Washington, 1996.

17) Ingold W. *Redox Measurement Principles and Problems.* INGOLD, Urdorf, Switzerland, 1982.

18) Stryer L. *Biochemistry,* 3rd Edition, W. H. Freeman and Company, New York, New York, 1981.

19) Stryer L. ibid.

20) Clark WM. *Oxidation-Reduction Potentials of Organic Systems.* The Williams & Wilkins Company, Baltimore, Maryland, 1960.

21) Harbury HA. *A Potentiometric Investigation of the Horse -Radish Peroxidase System,* Dissertation, Johns Hopkins University, 1953.

22) Ingold CK. *Structure and Mechanism in Organic Chemistry,* Cornell University Press, 1953.

23) Clark WM. ibid.

24) Stryer L. ibid.

25) Nernst W. *Theoretical Chemistry.* 4th ed., Translated by RA. Lehfeldt, 1904.

26) Carter D, Phillips A, and Silver J. ibid.

27) Carter D, Phillips A, and Silver J. *Apparatus and technique for measurement of oxidation-reduction potentials, pH and oxygen tension in vitro.* Proc Roy Soc (Biol.), (146)289:1957.

28) Clark WM. ibid.

29) Ziegler E. *The Redox Potential of the Blood in Vivo and in Vitro: Its Measurement and Significance.* Charles C. Thomas Publisher, Springfield, Illinois, 1960.

30) Cohen B. et al. See Studies on Oxidation-Reduction.

31) Zerfas LG, Dixon M. An improved cell for measurements of oxidation-reduction potential. *J Bio-chem.* 34: 365.

Understanding the Redox (rH_2) Measurement of the Biological Terrain: Part II

Robert C. Greenberg, D.C., F.A.S.A.

The defining and usage of rH_2 was first noted in print by Dr. W. Clark. Dr. Clark's original intention for inventing this factor was to prove that E of a biological system was independent of pH. Unfortunately, this discovery did not prove his hypothesis, but instead further documented that a biological, or as he stated, 'an organic system' is fully dependent upon variances in the hydrogen ion concentration or pH.[32,33]

Therefore, when E of a biological fluid is electronically measured, this value alone does not fully represent the internal state of electron donors and electron acceptors. Instead, a true and accurate value must take into account the specific pH of the biological fluid in question.[34,35] Only by utilizing the Nernst equation and electronically measuring the E value and pH, can the most accurate representation of redox in an active biological solution, or rH_2, be derived. This derived value represents the standard by which all redox reactions existing within a biological solution must be measured.

In the advancing field of Biological Terrain Assessment™, the evaluation of rH_2 must be considered.[36,37,38] This factor serves as an excellent source of evaluating the relative condition of oxidative stress. When rH_2 is utilized in relationship to the biological fluids of blood, saliva, and urine, valuable scientific data can be ascertained in relationship to the degree and concentration of cellular oxidative stress. This objective information can aid in the assessment of many cellular and mitochondrial functions which have been directly associated with many diverse subpathological and pathological conditions.[39]

The optimal values of pH for biological solutions is a well documented and understood science. Guyton's *Textbook of Medical Physiology* clearly states and references the optimal ranges for blood, saliva, and urine.[40] However, until now the optimal values for rH_2 have not been as easily accessible and verifiable. Up to this time, the rH_2 values that were referenced as optimal were values compiled by the French hydrologist and professor Louis Claude Vincent.[41] Almost 40 years ago, Professor Vincent compiled the optimal values by testing a large base sample of athletes in the French Alps. He considered these individuals to be representative of optimally healthy individuals and therefore assumed that the mathematical average of their readings should represent the optimal factors. While testing a large sample population of subjects has a definitive place in determining laboratory values, the electrical and necessary mathematical calculations were not fully understood and known at that time. The required electrical equipment of that era to test E commonly experienced a phenomenon that is even present in today's advanced technologies. This phenomenon is known as electrode poisoning.[42]

Electrode poisoning is a generic, modern term for a deleterious alteration in the platinum surface of a redox electrode. When a redox electrode is placed in an oxidizing solution, chemical adsorption of oxygen occurs which causes a monomolecular oxide layer. This layer serves as an oxidation reserve which tends to maintain the electrode potential at an elevated level even when the redox potential of the sample solution has diminished, resulting in slow, sluggish response times. This effect is even more noticeable when dilute biological solutions are being tested. A similar scenario is created when the redox electrode is placed in a reducing solution. This time, however, chemical adsorption of hydrogen and not oxygen is encountered. The monomolecular reduced layer that is generated from a reducing solution creates the same ultimate effect on the electrode that the oxide layer created only the opposite. Whereby this newly formed layer creates slow sluggish responses, it now diminishes the electrode potential even when the redox potential of the sample solution has increased. Like the monomolecular oxide layer, the monomolecular reduced layer is sensitive to dilute biological solutions.[43,44] Since the phenomenon of electrode poisoning was not readily understood until recently, the probability that Professor Vincent encountered this factor or was even aware of its existence, is quite obtuse.

In addition to the electrode poisoning, additional factors need to be closely scrutinized before acceptance of Professor Vincent's values are generalized. The second factor that deserves investigation is temperature compensation of biological samples. While in its purest physical chemistry definition, E is not dependent upon variations in temperature, rH_2 is dependent.[45] A simple review of the Nernst equation clearly demonstrates the profound effects that varying temperature samples will have on the resultant rH_2 value. When testing biological fluids, it is almost impossible to guarantee that fluids that were drawn at different times and having varying durations of exposure to room temperature will have identical temperature readings. This fact dictates why it is essential to electronically and mathematically compensate for any variances that may occur in temperature from sample to sample during the actual testing process. While the overall significance that variances in temperature have on alterations in the resulting rH_2 factors are relatively small in comparison to the electrodes poisoning effect, they do nevertheless exist and should be considered. Not only do these temperature factors have a role to play in rH_2 derivation, but nowhere in the literature did Professor Vincent even acknowledge their existence. These factors create further credibility gaps which serve to allow additional query of Professor Vincent's rH_2 optimal values.

The final concern in relation to the precision and accuracy of the optimal rH_2 values is in the theoretical understanding of redox potential. By pure definition, "every redox reaction must have an oxidant and a reductant."[46] "There has to be oxidation to have reduction!" These simple but accurate scientific statements outline the parameters that must be adhered

to under 'ideal' situations. In other words, the variance in electron concentration from a given reaction should have a net gain or loss of zero. When one substance has donated a hydrogen atom, carrying with it the electron, another substance will accept the hydrogen atom and the corresponding carried electron.[47] Therefore, under 'ideal' parameters and viewing the rH_2 value as approaching perfect chemical laws, the current optimal values as determined from the works of Professor Vincent cannot be considered. When you solve the Nernst equation for the biological solutions of blood, saliva, and urine and consider all of the significant factual scientific information that is available today, a slight variance from Professor Vincent's values become apparent. The blood, which according to Professor Vincent has an ideal rH_2 of 22 should now more accurately be considered as 21.7. The saliva, which according to Professor Vincent also had a rH_2 of 22, now becomes 20.0. And the urine, which according to Professor Vincent had a rH_2 of 24, now becomes 20.6.

It is therefore imperative for all students evaluating Biological Terrain to use these values as ideal parameters and not to anticipate the return of the patients' values to these exact points. These specific factors are intended to serve as reference points and must be utilized as such. They clearly represent 'ideal' optimal factors that unlike most standardized laboratory values have not been mathematically adjusted to compensate for the deteriorating health of the population. It is certainly possible, if deemed appropriate, to accurately and scientifically analyze with precise electronic equipment, a mass population and mathematically compute the mean rH_2 values. These new derived values would then become the new optimal values that all biological standards would be referenced against. But truthfully, why we would want to have those values that represent the true state of unwellness of our population?

We must remember that the assessment of the rH_2 factor in the Biological Terrain does not actually diagnose a condition.[48] Its truest wealth of value is attained from the scientific determination of relative and comparative states of oxidative stress only. Therefore, it makes more sense to understand that the statistical mean is insignificant when we consider the concept of scientific optimal value. The true evaluation of the rH_2 factor should therefore center around the degree of variance that the sample displays from the optimal value and the direction that the chosen therapy moves the biological fluid. These simple but profound examples exemplify the raw, easily attainable, purely scientific data that the assessment of the Biological Terrain can offer.

Upon reviewing the current literature, it becomes apparent that through the observation and assessment of variations in the rH_2 factor, the degree and extent of cellular oxidative stress could be determined. Within these factors lie the potential key in regulating biological tissue aging as well as solving the mystery of countless disease and pathological conditions. Many authors, scientists, and practicing physicians already recognize the significant inherent role that oxidative stress has in the realm of current medicine. However, it is time to unveil the core definition and understanding of this paradigm and fully grasp its most basic meaning. Oxidative stress is related to a shift in the redox potential toward increased oxidation of cellular macromolecules. By fully embracing this current awareness, testing for the body's E value and solving the rH_2 factor, it becomes evident that a simple, accurate assessment of the cellular oxidative stress level is readily accessible. The utilization of this technology is freely available to every practitioner today. However, one must first have a thorough comprehension of redox potential to fully understand the premise of Biological Terrain and its significance. The study of Biological Terrain is not only a gift from the past, but also represents a key to the successful health of our present and our future.

The precision and accuracy afforded us by modern-day technological advancements is enabling us to obtain more scientific research data than ever before in history. It is our responsibility as scientists and practitioners to embrace these expanded discoveries and shift our old health paradigms. This will enable us to accurately access and monitor the expanding health needs of our patients. Given the overall circumstances of his era and the relative state of biochemistry and electronics, the work that Professor Vincent accomplished was truly nothing short of extraordinary. Today, with the aid of modern technology and current perspectives on physics and chemistry, I have been able to not only improve on his foundation, but document the entire process as well. My truest heart felt thank-you is therefore extended to a mentor that I have never had the opportunity to meet, Professor Louis Claude Vincent!

References

(32) Clark WM. *Oxidation-Reduction Potentials of Organic Systems.* Williams & Wilkins. 1960.

(33) Clark WM. *ibid.* 1960.

(34) Carter D, Phillips A, Silver J. Measurement of oxidation-reduction potentials and Ph of tissues. *J Physiol* 129:33. 1955.

(35) Hesselink TL. *Free Radical Chemistry.*

(36) Elmau H. *Bioelectronic According to Vincent and Acid-Base Household in Theory and Practice.* Haug Verlag, Heidelburg. 1985.

(37) Kollath W. *Regulators of Life – from the Nature of the Redox Systems.* Haug Verlag, Heidelburg. 1968.

(38) Roujon L. *Theory and Practice of the Bio-Electronic "Vincent."* SIBEV, 1975.

(39) Elmau H. *ibid.*

(40) Guyton A. *Textbook of Medical Physiology.* WB Saunders Co. Philadelphia 1991.

(41) Roujon L. *ibid.*

(42) Anson FC, Lingane JJ. Chemical evidence for oxide films on platinum electrometric electrodes. *J Am Chem Soc.* 79, 4901.

(43) Clark WM. *ibid.*

(44) Ingold W. Redox measurement principles and problems. *Redox Measurement.* Urdorf, Switzerland, 1982.

(45) Clark WM. *ibid.*

(46) Stryer L. *Biochemistry.* 3rd Edition. WH Freeman and Co., New York, 1981.

(47) Levine SA, Parris MK. *Antioxidant Adaptation-Its Role in Free Radical Pathology.* Allergy Research Group, 1994.

(48) Greenberg, RC. Biological terrain assessment™. *Townsend Letter.* Oct/Nov 1996.

Address of the author:

Robert C. Greenberg, D.C., F.A.S.A
P.O. Box 560
Payson , AZ 85547