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# Truth in Basic Biomedical Science Will Set Future Mankind Free

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*Abstract:* It is self-evident that continued wellbeing and prosperity of our species in time to come depends upon a steady supply of major scientific and technologic innovations. However, major scientific and technical innovations are rare. As a rule, they grow only in the exceptionally fertile minds of men and women, who have fully mastered the underlying basic sciences. To waken their interest in science at an early critical age and to nurture and enhance that interest afterward, good textbooks at all level of education that accurately portray the relevant up-to-date knowledge are vital. As of now, the field of science that offers by far the greatest promise for the future of humanity is the science of life at the most basic cell and below-cell level. Unfortunately, it is precisely this crucial part of the (standardized) biological textbooks for all high schools and colleges in the US and abroad that have become, so to speak, fossilized. As a result, generation after generation of (educated) young men and women have been and are still being force-fed as established scientific truth an obsolete membrane (pump) theory, which has been categorically disproved half a century ago (see Endnote 1.) To reveal this *Trojan horse* of a theory for what it really is demands the concerted efforts of many courageous individuals especially young biology teachers who take themselves and their career seriously. But even the most courageous and the most resourceful won't find the task easy. To begin with, they would find it hard to access the critical scientific knowledge, with which to convert the skeptic and to rally the friendly. For the wealth of mutually supportive evidence against the membrane (pump) theory are often hidden in inaccessible publications and/or in languages other than English. To overcome this seemingly trivial but in fact formidable obstacle and to reveal the beauty and coherence of the existing but untaught truth, I put together in this small package a collection of the major clenching theoretical and experimental findings. These findings will remove the last trace of uncertainty about the total disproof of the membrane theory. In addition, I have also included an introduction of the association-induction hypothesis, which is the *one and only* unifying theory of the living cell that has survived and unwaveringly grown more comprehensive and powerful after more than half of a century of worldwide testing.

(In Endnote 1 on page 43, I will show: (1) how (one of the postulated membrane pumps,) the sodium pump (alone) would consume at least 15 to 30 times the total available energy of muscle cells; and (2) that, pump or no pump, they are all variants of the membrane theory, and as such long dead.

IN DECEMBER 1910, the Irish physical chemist, Frederick G. Donnan presented at the London Physiological Society, a summary of what was to be known later as the Theory of Donnan Equilibrium. The full version of the theory was written in German, and published in the year following (Donnan 1911). Translated into English, the title of the article reads: *Theory of Membrane Equilibrium and Membrane Potential from the Presence of Non-dialyzable Electrolytes: A Contribution to the Physico-chemical Physiology*.

From this and others closely following publication(s) of Donnan and his coworkers, I summarize the Donnan theory as follows:

A membrane of the right kind separates two solutions named respectively solution 1 and solution 2. Both solutions contain one (or more) permeant (being able to permeate) ionic species, but solution 1 alone contains an ionic species, to which the membrane is impermeable (being unable to permeate). As a result, an electrical potential difference, $\psi$, would develop at equilibrium between the two solutions. Referred to as a *membrane potential,* $\psi$ equals in magnitude the difference between the electrical potential, $P_1$, in solution 1 and the electrical potential, $P_2$, in solution 2. This relationship is summarized in Equation 1, where *n* represents the valence of the permeant ion species and that $C_1$ and $C_2$ are respectively their concentrations in the two solutions. R, T and F are the gas constant, the absolute temperature and the Faraday constant respectively.

$$\psi = P_1 - P_2 = (RT/nF) \log (C_1/C_2). \quad (1)$$

Alternatively, we may write

$$\psi = P_1 - P_2 = (RT/F) \log r, \quad (2)$$

where

$$r = (C^+_1 / C^+_2)^{1/x} = (C^-_2 / C^-_1)^{1/y}. \quad (3)$$

In Equation 3, $C^+_1$ and $C^+_2$ are respectively the concentrations of the *permeant cation* of valency *x* in solution 1 and 2. $C^-_2$ and $C^-_1$ are respectively the concentrations of the *permeant anion* of valency *y* in the two solutions. A simplified version of Equation 3 is Equation 4:

$$(C^+_1 / C^+_2)^{1/x} = (C^-_2 / C^-_1)^{1/y}. \quad (4)$$

Equation 3 and 4 illustrate the pervasive *connectedness* of the Donnan membrane equilibrium. That is, a change in the concentration of one ionic species invariably alters the equilibrium concentrations of *all* the other ionic species present. However, to gain a deeper insight into the background of Donnan's theory, we need to know something about Donnan's personal history.

Donnan was born in Colombo, Ceylon but returned to Ireland at the age of 3. He was educated in Queen's College in Belfast. In 1893 he went to Leipzig to study under the renowned physical chemist, Wilhelm Ostwald (1853–1932.) In 1896–1897 he moved again — this time to study under another famous physical chemist, Jacobus van't Hoff (1852–1911) in Berlin. For reasons described below, I mention that in 1885 van't Hoff

(rather casually) introduced the concept of *semipermeability* to describe a membrane that is permeable to water but impermeable to (all) ions, molecules and other solutes dissolved in water (van't Hoff 1887).

However, to the best of my knowledge, semipermeable membrane as van't Hoff originally described in these words has not been observed in the real world. Instead, a membrane found permeable to water is invariably permeable also to some solutes. In time, the definition of the term, semipermeability has gradually loosened to denoting permeability to water and some solutes but not to others. As an example, Donnan' one-time teacher, Wilhelm Ostwald showed that a copper-ferrocyanide precipitation membrane is impermeable to sucrose but permeable to water and the positively charged potassium ion, $K^+$ (Ostwald 1890.)

As a whole, Donnan made an important contribution to general physiology (*algemein Physiologie* — a name first introduced by H. Dutrochet (1776–1847), see Rich 1926, p. 359,) by providing a quantitative physico-chemical framework, on which to erect and to test sundry subsidiary hypotheses of the properties and behaviors of the living cell. In the section immediately following, I shall demonstrate how, at that time, investigators came to believe that Donnan's theory can explain not only (1) cell **membrane permeability** *per se*, but also three other cardinal attributes of the living cell: (2) **solute distribution**, (3) **swelling and shrinkage** and (4) **electric potential**.

### *1. Membrane permeability*

Hugo deVries (1848–1935) was a highly productive and influential botanist. He introduced the term, *Plasmolysis* (and its reversal, *deplasmolysis*) to describe the stepwise shrinkage of water-filled protoplast of a large mature plant cell when immersed in a concentrated solution of sucrose or sodium chloride (NaCl) as illustrated in Figure 1.

deVries gained wide recognition for demonstrating that the protoplast of the root hair cells of red beet, when immersed in a concentrated NaCl solution, stayed shrunken for 7 days (deVries 1871.) This experimental observation convinced many contemporary investigators that the cell membrane of (at least some) plant cells is indeed impermeable to NaCl. Yet in undergoing shrinking, the root cell also clearly shows that the same cell membrane is fully permeable to water.

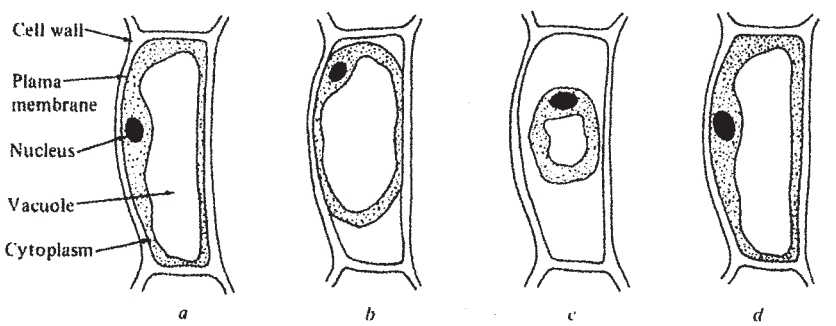


FIGURE 1. Diagrammatic illustration of successive stages in the plasmolysis (b,c) of a mature plant cell (a) and deplasmolysis (d). (From Dowben 1959)

deVries also introduced the so-called *osmotic method* to determine the permeability or impermeability of biological membranes (de Vries 1884.) To wit, solutes like NaCl that cause sustained protoplast shrinkage in large plant cells when added to the bathing solution are considered impermeant. Solutes that do not cause protoplast shrinkage at all are considered highly permeant. Solutes that cause transient protoplast shrinkage followed by recovery to its initial volume are considered moderately permeant (Ling 2007, p.26.)

Eighteen years after deVries's epochal finding on red beet hair cells, Emil Abderhalden announced another historic discovery of lasting importance (Abderhalden 1898.) He demonstrated that the human red blood cell contains no (or very little as proven later) sodium ion ($Na^+$) even though the blood plasma, in which the red blood cell spends its life, contains an abundance of $Na^+$.

Considered together, the discoveries of deVries and of Abderhalden support the belief that both *plant* and *animal* cells are permeable to water but impermeable to $Na^+$, which is, of course, the dissociated (or ionized) cationic component of NaCl. As such, these discoveries offered — or appeared to offer — experimental verification of the (loosened) definition of semipermeability, which provides the conceptual foundation of Donnan's theory of membrane equilibrium.

### *2. Swelling and shrinkage*

Shortly after Donnan introduced his membrane equilibrium theory, Höfler (1918) worked out a method of measuring the (changing) volume of the irregularly shaped protoplast during *plasmolysis* (Figure 1.) Using this method, Höfler showed that mature plant cells do indeed behave like a *perfect osmometer*. That is, the product of the equilibrium volume of the protoplast (v) and the osmotic pressure ($\pi$) of the surrounding medium is a constant — as van't Hoff had pointed out in his classical theoretical work (for details, see Ling 2007, pp. 20–21.) In their 1932 review, McCutcheon and Lucké hailed Höfler's finding as offering the ultimate proof of the membrane theory (McCutcheon and Lucké 1932 pp. 86–87.)

### *3. Cellular electrical potential as membrane potential*

Julius Bernstein (1839–1917) was a student of the great physicist-physiologist, Ludwig von Helmholtz (1821–1894) — a member of the *Reductionist Four*, who shared the belief that the laws governing the inanimate world govern the living too (Ling 2001, p. ii.)

Bernstein took seriously Ostwald's earlier suggestion (Ostwald 1890) that the electric potential he (Ostwald) observed across a copper ferrocyanide membrane (separating two $K^+$-containing solutions of different strength) might underlie not only the *resting (or injury) potential* of muscle and nerve but also the electric shocks delivered by electric eels.

However, it was not until the year 1902, when Bernstein was already 63 years old, that he finally published what he called the Membrane Theory of cellular electrical potential. He too called the cellular resting potential a *membrane potential* — ten years before Donnan made the same designation (Bernstein 1902, p. 54.)

Bernstein derived his equation for his membrane potential on the basis of an equation for diffusion introduced by W. Nernst (1889, see also Nernst 1892). Representing the membrane potential measured across the resting healthy cell surface as E, the Bernstein equation takes the following form:

$$E = (RT/F) \log ([K^+]_{in} / ([K^+]_{ex}), \quad (5)$$

where R, T and F are as defined for Equation 1 above. $[K^+]_{in}$ and $[K^+]_{ex}$ are the intracellular and extracellular $K^+$ concentrations respectively.

Donnan's theoretical model of membrane potential as shown in Equation 2 is in fact a more generalized version of what Bernstein suggested earlier in the form of Equation 5. However, there are significant differences between the two models. Bernstein's model requires two essential *a priori* assumptions. That is, the cell membrane must be impermeable to both the major anion in the bathing medium, $Cl^-$ as well as the major cation of the bathing medium (blood plasma), $Na^+$. In contrast, Donnan's model requires only one *a priori* assumption. Namely, the cell membrane is impermeable to $Na^+$.

The relationship between E (or $\psi$) and the absolute temperature T and that between E (or $\psi$) and log $[K^+]_{ex}$ in both Equation 1 and Equation 5 have been confirmed repeatedly, especially after new techniques were introduced to measure accurately the potential of individual living cells. These new techniques include that of impaling isolated (single) giant axons of squid and cuttlefish (Curtis and Cole 1942, Huxley and Stämpfli 1951.) And the technique of impaling single muscle fiber (and other large and small single cells) with the aid of the Gerard-Graham-Ling (*aka* Ling-Gerard) microelectrode (Ling and Gerard 1949, Ling 2007, pp. 46–49.)

### *4. Selective ionic accumulation*

Membrane permeability is a rate process. As such, it deals with a *transient* event. Abderhalden's discovery that red blood cells contain no $Na^+$ (or very little $Na^+$, as later work showed) was historically important for yet another reason. It shows that the asymmetric ionic composition of living cells persists throughout the entire life of the cell. As a result, a valid theory of the living cell must provide a mechanism for attributes that *endure* in time. On the surface at least, Donnan's theory answers this need. For it is an *equilibrium* theory — meaning it could in theory stay unchanging forever.

In harmony with this agreement, Hans Netter of the University of Kiel suggested that ionic distribution in living muscle, nerve and red blood cells could all be neatly explained in terms of the Donnan theory of membrane equilibrium (Netter 1928.) Mond and Amson from the same university also introduced a set of important ideas in that same year. That is, the permeability of the muscle cell membrane to $K^+$ and impermeability to $Na^+$ could be the result of the existence of a limiting membrane pore size that allows the passage of the smaller (hydrated) $K^+$ but not the larger (hydrated) $Na^+$ (Mond and Amson 1928.)

However, it was Boyle and Conway in their paper published in the 100$^{th}$ volume of the prestigious (English) Journal of Physiology that had taken Donnan's theory of membrane equilibrium to its full height — on all four of the cardinal attributes of cell physiology mentioned above.

> Regrettably, Boyle and Conway did not give due credit to Netter, nor to Mond/Amson, for ideas mentioned above, which these German scientists introduced first. (See Ling 1992, p. 4.) Perhaps it was the on-going War (WWI) with Germany that warped their judgement.

In the Boyle/Conway theory, the living cell membrane acts like an *atomic sieve,* an idea that was suggested 74 years before by Maurice Traube (1867) for his inanimate semipermeable copper-ferrocyanide membrane. Following Mond and Amson, Boyle and Conway

also postulate a limiting pore size permitting the passage of smaller (hydrated) $K^+$ but not the larger (hydrated) $Na^+$. Notwithstanding, Boyle and Conway's theory is far more advanced than the simple but seminal ideas Traube and Mond/Amson introduced respectively. For details, the reader must consult Boyle and Conway's original work. A few high lights are given below:

The Boyle-Conway version of the atomic sieve theory could apparently explain why muscle cell contains an abundance of $K^+$ at a high Donnan ratio (r) of 40. It could also explain why chloride ion, though proven fully permeant by Boyle and Conway (1941), exists at a very low level. That too could be construed as being equal to the Donnan theoretical value of 1/40 (Equation 3 and 4.)

On a still broader perspective, Boyle and Conway's theory also offers reasonable explanations of cell swelling and shrinkage (see also Proctor and Wilson 1916) and the cellular electric potential as described by Equation 5 (see Ling 1984, p. 34.)

In summary, Boyle and Conway's theory published in 1941 represents the pinnacle in the application of Donnan's theory of membrane equilibrium to the living cell. As such, it took on the shape of a unifying, all-encompassing membrane theory. For his work on the (Donnan) membrane equilibrium, Donnan received the Davy Medal of the Royal Society of London in 1928.

Then suddenly a chain of unexpected events that would turn the world of cell physiology upside-down made their appearances — even though at that time, many researchers in cell physiology, whose work would be profoundly affected, did not know. In that I was no exception.

## A set of dramatic top-to-bottom "turn-arounds" (with their coherence seen only in hind sight many years later)

### *1. A decisive new tool of unprecedented importance*

In the late 1930's, a tool for accurately measuring membrane permeability emerged for the *first time* in history: *radioactive tracer technology*. Almost overnight, studies with the aid of radioactive tracers like $^{24}Na$ have falsified the widely if not universally-held belief that the cell membrane is impermeable to $Na^+$. But that was not all.

In fact, as more and more radioactive tracer studies were performed and reported, it became clear that most if not all the ions and molecules hitherto considered impermeant are in fact permeant (Ling 2007, pp. 43–45, pp. 58–59.)

### *2. Disproof of the validity of the Osmotic Method itself*

In 1937 the Soviet scientists, Nasonov and Aizenberg published an exceedingly simple but critical experiment.

They immersed frog muscles in a concentrated sucrose solution, and observed that the muscle steadily shrank until it reached and stayed at a new constant volume equal to 92% of its initial weight. There is nothing surprising in that. What is surprising is the fact that while the muscle cells were shrinking, they were also steadily taking up sucrose until it too reached a constant level *inside* the cells. The authors concluded that membrane impermeability was not the cause of cell shrinkage caused by sucrose, nor is it the cause of

the low concentration of sucrose in normal resting muscle cells. The essence of this piece of work was repeated and confirmed by Kamnev (1938.)

Now, muscle tissues are, as a rule, made of bundles of parallel-running fiber-like muscle cells. The space between the individual muscle fibers is called the *extracellular space* (ecs), which is filled with whatever the bathing solution contains. In the experiments described above, it is mostly sucrose. Clearly, the validity of their conclusion that sucrose had in fact penetrated and accumulated *within* the muscle cells, depends critically on the correct choice of the volume percentage of the ecs of the frog muscle they used. Kamnev adopted an ecs value of 9%, a figure which is lower than earlier estimates. Thus, Boyle and Conway in the work described earlier used an ecs value of 13%. (For still other high estimated values, see Troshin 1966, Table 56 on p. 224.) Which one is right?

It is therefore fortunate that my coworkers and I, using a total of five independent methods, four of which are totally new, had extensively investigated this problem and reached what seemed to be a reasonable conclusion. That is, the ecs of the most widely used frog muscle (the sartorius muscle) averaged 9.2% ± 0.69%, which agrees well with Kamnev's figure of 9% (Ling 2001, p.44.)

The three Soviet scientists's simple experiment described above was the first to invalidate incisively the Osmotic Method for determining the membrane permeability of solutes, which deVries introduced and was used in a major share of the experiments that lent support for the membrane theory up to that time.

### *3. Höfler reversed his earlier stand*

On further investigating the protoplasm of mature *Tradescantia* cells, Höfler discovered that his earlier conclusion on the semipermeability of the cell membrane was wrong — and, to his credit, he made his new conclusion known without delay (Höfler 1926, 1932.) That is, it was the inner vesicular membrane (also called tonoplast) in immediate contact with the watery fluid in the central vacuole of the mature plant cell that is semipermeable (See Figure 2.) The outer cell (or plasma) membrane of the protoplast directly in contact with the external bathing solution is freely permeable to sucrose — thereby once more changing an earlier strong support for the impermeability of the living cell membrane to sucrose to evidence against it.

### *4. $Mg^{++}$ and $K^{+}$ distribution in the same living cells are shown to be completely independent, refuting the predicted constancy of the Donnan ratio, r*

When the rare radioactive tracer $^{28}Mg^{++}$ became available momentarily in the United States, Ling, Walton and Ling (1979) were in a position to take full advantage and conducted a detailed study of the equilibrium distribution in frog muscle cells of this divalent ion side by side with that of the mono-valent $K^{+}$. The main conclusion from this study is that *the distribution of these two types of ions, one mono-valent and the other divalent, are entirely independent of each other*. In other words, changing the concentration of one kind of ion in the external environment had no detectable influence on the distribution of the other kind.

This finding again irreconcilably contradicts Donnan's theory of membrane equilibrium. As illustrated in Equation 3 and 4, the theory predicts that all ions present in the

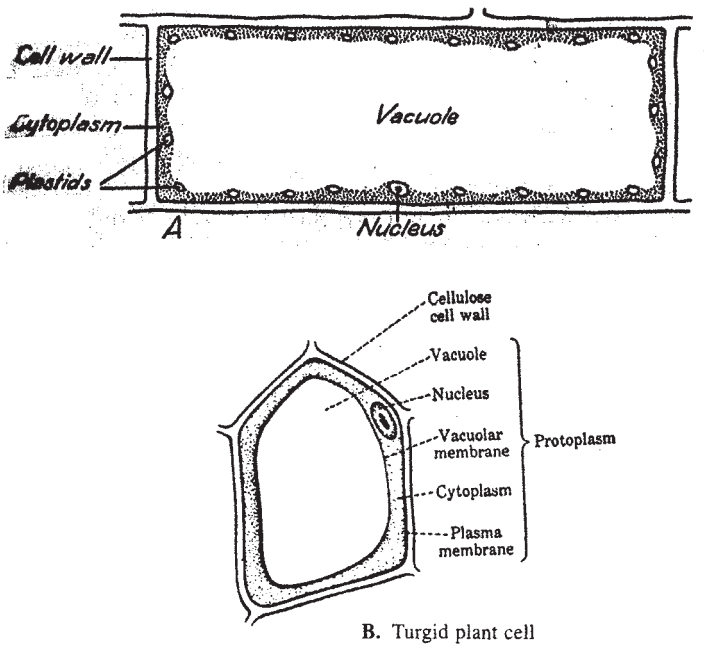


FIGURE 2. Diagrammatic illustration of a mature plant cell. (From Glasstone's Textbook of Physical Chemistry, van Nostrand, 1946.)

same system are "connected". In other words, all ions present in the system, whatever their valency and charge, should distribute in such a manner as to yield the same Donnan ratio, r (see Equation 4 on p. 20.) When the Donnan ratio of one ion changes, the Donnan ratio of all ions within the whole system change with it to assume the same new value. Our experiments showed just the opposite.

(The equilibrium distribution of both $Mg^{++}$ and of $K^{+}$ in fact follow rigorously the two-term Troshin equation, see p. 115 in Troshin 1966 and also p. 163 in Ling 2007 for this equation. See also the puzzling question, Why the two-term equation should contain only two terms at all in such a complex system as a living muscle cell? The answer given on page 220, paragraph 5 in Ling 2007 marks an exciting high point in the evolving history of the AI Hypothesis.)

## A simple but fatal mistake made 100 years ago in the original derivation of Donnan's theory of membrane equilibrium — until now, undisclosed

In his admirable Textbook of General Physiology, Sir William Maddox Bayliss pointed out in its Preface that the greatness of a scientist does not lie in his never making a mistake. Rather, it lies in his willingness to admit having made a mistake and promptly publicly correcting it. (Bayliss 1924) For without this willingness of all participating scientists to admit and correct his or her own now-proven mistakes, science as a continuing effort of all Mankind to seek the truth would be paralyzed — as is the case of the fossilization of biology textbooks mentioned in the Abstract of this paper.

By the same token, it is vital for the continued survival and prosperity of science that each member of the scientific community shoulders the duty, no matter how hard, of correcting mistakes made by other scientists as well. In agreement with the spirit of this belief, I want to report something unusual here. That is, in deriving the basic concept of Donnan's theory of membrane equilibrium, a serious mistake had been made and to the best of my knowledge not explicitly described and corrected until now. (But, see below.)

***This serious mistake lies in the assumption that there is an electrical potential $P_1$ or $P_2$ in each of the two solutions which the semipermeable membrane separates and hence the existence of a membrane potential, $\Psi$ (or E) as their algebraic sum. This assumption is a serious mistake because this it violates the Law of Macroscopic Electroneutrality.*** (see Nernst 1892, Encyclopedia Britanica 2010; Morikawa 2001, Guggenheim 1950.)

Having made this clear, I ask why someone has not explicitly pointed out this glaring error earlier. After all, 100 years is a long time. In fact, someone else did write about the underlying problem — but not in a straightforward way. That someone was no one else than Edward A. Guggenheim, one of the world's foremost thermodynamicist. And, alas, also a co-author of Donnan in the paper Donnan and Guggenheim published conjointly in 1932 in Zeitschrift für physikalische Chemie, vol. 162.

The inclusion of the term, $z_iF\Psi$, in Equation 4.2 and 7.2 in this 1932 article and their explanation, "*Ψ…hat denselben Wert für jede Ionenart*" (*Ψ*…the symbol for electrical potential…has the same value for each ion species,) left no doubt that the mistake was made (by Donnan alone in 1911; by Guggenheim alone in 1929 and by Donnan and Guggenheim conjointly in 1932.)

My guess is that Guggenheim was not fully aware or certain of this mistake until some time between 1932 and 1950 — then he made great effort in his 1950 advanced treatise on thermodynamics, stressing the importance of not violating the Law of Macroscopic Electric Neutrality (on page 330 to page 331.) Notwithstanding, Guggenheim never did come out and admit that he and Donnan had made the mistake.

This is regrettable. It is relevant to remember that Donnan had earlier written a preface for Guggenheim's 1933 book, *Modern Thermodynamics by the Method of Willard Gibbs*, when Guggenheim was still in his twenties. This fact suggests that Donnan might have been a kind and highly respected scientist to young Guggenheim for Guggenheim to come out and announce that Donnan and Guggenheim together had made a serious mistake and it was Guggenheim and not Donnan that had made this discovery.

Well, so much conjecture on a strange mystery that unfortunately could never be fully explained. In the following section, I turn to experimental discoveries that prove once and for all that no measurable electric potentials exist in (macroscopic) solution 1 and 2. Accordingly, no membrane potential Ψ (or E) could exist in the real world or even in theory. Wide-ranging experimental studies described next bears this out.

## Converging disproof of the theory of membrane potential Ψ (or E) in each of all four types of membrane models examined

The following are summaries and conclusions of the experimental findings — as a rule unexpected by the authors who made the observations — on four types of membrane

models. They are the glass membrane, the collodion membrane, the oil membrane and the phospholipid membrane. The glass membrane was the first extensively studied and it was also the first cited by a physiologist (Cremer) as a model of the semipermeable cell membrane.

*1. Glass membranes.*

In 1881, Ludwig von Helmholtz published his study of the electric potentials measured across two aqueous solutions separated by a thin glass membrane (Helmholtz 1881.) Max Cremer (1865–1935), a professor of physiology at the University of Berlin, cited this work and suggested that the glass membrane is a suitable model for the semipermeable membrane of the living cell (Cremer 1906.) The high sensitivity of the glass membrane electrode to $H^+$ but not to other mono-valent cations was attributed at the time to the (selective) permeability of the hydrated glass channels in the glass membrane to this very small $H^+$ ion. Then an unusual discovery was announced, to the astonishment of just about everyone in this field of investigation.

As a rule, a glass electrode has no sensitivity to silver ion ($Ag^+$.) Yet, it acquired full sensitivity to this ion after soaking overnight in a solution of silver nitrate (Horovitz 1923, aka Lark-Horovitz 1931.) The author Horovitz believed that it is *surface adsorption* of the ion involved rather than bulk phase *permeability* that determines ion specificity of the electric potential measured. In years following, Horovitz's experimental discovery was repeatedly confirmed by Nicolsky (1937), by Haugaard (1941) and by Ling and Kushnir (in Ling1960 and in Ling 1967.)

*2. Oil membrane*

In 1892 Nernst pointed out that in two ion-containing phases in contact, the Law of Electric Neutrality does not allow the accumulation of significant electric charges inside each phases. Accordingly it is only at the phase boundaries that ions accumulate and generate electric potential differences (Nernst 1892.) (The question arose, Would Donnan go ahead with publishing his theory if he had discovered and fully understood before 1911 what Nernst wrote in 1892.)

In 1913 Baur again pointed out that the electric potential measured across two aqueous solutions separated by a thin layer of oil, does not arise from the permeability of ions through the oil layer but is due to the adsorption of these ions on the two oil surfaces (Baur 1913.) Four years later, Baur and Kronmann provided additional evidence in support of Baur's adsortion theory. In still later years, Ehrensvard and Sillen (1938) and Colacicco (1965) further confirmed the work of Baur and of Baur and Kronmann.

Colacicco's study was particularly interesting and enlightening. He introduced different concentrations of KCl into the two aqueous solutions that an oil layer separates but found no measurable electric potential difference across the oil layer if the oil layer is just that, a plain layer of neutral oil.

However, if a negatively charged anionic detergent, say, sodium dodecyl sulfate is added to one solution, a dramatic change follows. Now, the side of the oil layer containing the anionic detergent becomes a cation-sensitive electrode. As such, this side of the oil layer gives rise to an electric potential sensitive only to the concentration of the cation,

$K^+$ in the bathing medium but not to that of the anion, $Cl^-$ also present. However, if instead an anionic detergent, a cationic detergent like cetyltrimethylammonium bromide is added, that side of the oil layer becomes an anion-sensitive electrode responsive only to $Cl^-$ but not to $K^+$. Colacicco's finding was in turn confirmed and extended recently by Tamagawa and Nogata (2004.)

*3. Collodion membrane*

In the commercially available form called collodion, nitrocellulose is soluble in mixtures of ether and ethyl alcohol. When the closed end of a glass test tube is dipped into such a collodion solution and allowed to dry in a moist environment, a collodion thimble electrode could be slipped off the test tube and used as model for electric potential studies. As an example, if different concentrations of KCl are added to the solutions bathing two sides of the thimble, a $K^+$-sensitive electric potential difference — following more or less the dictate of Equation 5 — will be measured.

In the 1920's, Leonor Michaelis and his coworkers conducted extensive investigations of the collodion membrane electrode as a model of the cell membrane. When Horovitz's iconoclastic discovery became known, the question was rasied, Could there be electric charges on the surface of the colloidion membrane too? This, Michaelis and Perlzweig (1927) categorically rejected on the ground that nitrocellulose is a neutral substance and devoid of net electric charges. Little could they imagine how the advent of World War II could profoundly change the foundation of their reasoning at the time.

World War II cut off the import from Germany of the Schering brand of collodion, which up to that time, Michaelis's laboratory routinely purchased and used. As a result, Michaelis's students, most importantly, Carl Sollner, had to prepare their own collodion from scratch. To their amazement, the purer the collodion they manufactured, the worse it became as building material for their cell membrane model. In fact, the purest colloidion they made generated no electric potential at all.

Then they discovered something few expected. It was an impurity in the Schering brand of collodion that made it a good model-building material. And that impurity was soon identified as carboxyl groups, which can be added at will onto the collodion by merely exposing it to an oxidizing agent like sodium hydroxide or sodium bromide. The oxidized collodion thimble electrode thus prepared behaves very well as a model of the cell membrane: it is fully sensitive to $K^+$ like the living cell in accordance with the prediction of Equation 5. (For additional evidence that it is the carboxyl groups on the two surfaces of the collodion membrane electrode, see below.)

*4. Phospholipid bilayer membrane*

Measurement of the electric potential difference across phospholipid bilayer membranes yielded equally convincing evidence that it is not the ionic permeability through the membrane but the algebraic sum of two surface potentials on each side of the phospholipid bilayer. Thus, when negatively charged phosphotidic acid was used to make the bilayer, the potential measured is cation-sensitive. When positively charged lysyl phosphotidyl glycerol was used, the bilayer is anion-sensitive. When electrically neutral phosphotidyl choline or diglycolsyl diglyceride was used, the bilayer demonstrates no (or very weak)

sensitivity to cations or anions (Colacicco 1965; Hopfer *et al* 1970; Ohki 1972; McDonald and Bangham 1972.)

Not one of the four types of inanimate models generates an electric potential difference in the way that Donnan's theory predicts. In retrospect, the perfection of this (and other similar) kind of converging experimental evidence provide insight on what a scientific truth really portends.

We now conclude that Donnan's theory of membrane equilibrium and membrane potential has been invalidated because in its theoretical derivation, a key assumption violating the Law of Macroscopic Electric Neutrality has been made. Experimentally, the theory has also been thoroughly disproved. In both inanimate model studies and in the studies of living cells, the predicted behaviors of ions have all been unequivocally falsified. *It is entirely safe to say that Donnan's theory of membrane equilibrium and membrane potential have proven totally erroneous.*

**Historically speaking, Donnan's theory of membrane equilibrium though eventually proven erroneous, nonetheless played a positive role in the development of cell physiological science — like Stahl's phlogiston theory did in the development of the modern theory of chemistry**

In the late seventeenth and early eighteenth century, there lived in Germany a talented and famous scientist-physician by the name of Georg Ernst Stahl (1660–1734.) Stahl inherited from his teacher, Johann Becher, the idea that combustible materials contain an ignitable matter, which Stahl gave the name, *phlogiston*. Although the phlogiston theory eventually was found erroneous, it has, nonetheless, provided a basis for the many experiments that contributed to the birth of the new chemistry under the influence of Antoine Lavasier (1743–1794.) (For another idea of Stahl, see Endnote 1 on p. 43.)

In my opinion, Donnan's theory of membrane equilibrium and membrane potential also provided the basis for the many experiments that contribute to the birth and development of a new theory of the living cell like the *association-induction (AI) hypothesis* — to be described below after a brief introduction to a revolutionary change in the development of physics itself.

**From the world one can see to the microscopic world of atoms, electrons one cannot see**

Before entering into the history and substance of the association-induction (AI) Hypothesis itself, I would like to describe the background of another more subtle historic relationship between Donnan's theory and the AI Hypothesis. Namely, the transition in physics from the investigation of the *macroscopic* world, which one can see to the new *microscopic* world of atoms, electrons etc., which as a rule, one cannot see (even with the best light microscopes.)

As pointed out earlier, Donnan's theory of membrane equilibrium is by and large a play on van't Hoff's concept of semipermeability. In introducing this concept, van't Hoff had in fact divided the world of solutes into two categories. Permeant solutes can traverse these semipermeable membranes, whereas impermeant solutes are unable to do so, not in hours, nor in days nor even in years, but forever.

The question arose, is such an idea of eternal impermeability compatible with our more modern knowledge of the world we know today? The answer is No. That being the case, Why did an exceptionally gifted scientist like van't Hoff make such a terrible blunder?

The answer is, van't Hoff, like his student, Donnan, lived in a world before physicists and chemists finally understood and accepted the new *microscopic* physics that the Austrian mathematician-physicist, Ludwig Boltzmann (1844–1906) had almost single-handedly invented. It is a new way of interpreting *macroscopic* phenomena and objects (measured in micra or $10^{-4}$ cm or larger) in terms of the properties and behaviors of *microscopic* atoms, electrons etc., (measured in Ångstrom units or $10^{-8}$ cm *and/or* in nano-meters or $10^{-6}$ cm) — known now as *Statistical Mechanics*.

Similarly, *permeability* and *impermeability* spoken at van't Hoff's and Donnan's time were ***macroscopic*** properties. How they can be re-interpreted in ***microscopic*** statistical mechanical terms is a central theme of the association-induction hypothesis to be briefly summarized below.

## The Association-Induction (AI) Hypothesis

More than half of a century has passed since the embryonic version of the association-induction hypothesis was published in 1952 and known as Ling's Fixed Charge Hypothesis (Ling 1952.) The main theme of the AI hypothesis was presented in 1962 in a 680-page volume entitled *A Physical Theory of the Living State; the Association-Induction Hypothesis* (Ling 1962.) However, it was not until 1965 when the *Polarized (Oriented) Multilayer (PM or POM) Theory* of cell water was added (Ling 1965) that the association-induction hypothesis became complete.

Since 1952 and even before, the hypothesis has been extensively tested here and abroad. Over 200 original articles and reviews have been published from my laboratory alone. Throughout this whole lengthy period of time, there has been no major reverse in the steady growth and evolvement of the AI Hypothesis. Notwithstanding, the association-induction hypothesis is virtually unknown and just as bad, untaught.

### Historic background of the association-induction hypothesis

So far, I have discussed how the AI Hypothesis may be seen as an (opposing) sequel of the membrane theory in the same way that Lavoirsier's modern chemistry is an opposing sequel to Stahl's Phlogiston theory. I must now point out the more obvious. That is, the AI Hypothesis is the heir to a theory that was introduced in 1835 by the French zoologist, Felix Dujardin (1801–1860.)

Dujardin described in 1835 a water-insoluble, translucent and gelatinous material that oozes out from within a crushed protozoon — called infusoria then — and gave this "living jelly" the name of *sarcode* (meaning fleshy) (Dujardin 1835.) Later this name was replaced by the term, protoplasm, a name introduced by the German botanist, Hugo von Mohl (1846) who described a similar material that lies under the cell wall of plant cells.

The protoplasmic concept became widely adopted for decades, highlighted by two historic events that occurred in the 1860's. They are respectively Max Schultze's pronouncement in 1861 of the "protoplasmic doctrine" that "living cells are lumps of protoplasm with a nucleus" (Schultze 1861) and second, Thomas Huxley's 1868 Sunday

evening lecture in an Edinburgh church, in which he announced that *protoplasm is the physical basis of life* (Huxley 1869.)

Unfortunately but perhaps inevitably, the protoplasmic theory steadily lost ground as time wore on. The main reason for this decline of popular interest was the discoveries of more and more intracellular structures or organelles in addition to the cell nucleus. None of these inclusions could be seen as being made of the same colorless, translucent material that Dujardin and von Mohl saw and described as sarcode or protoplasm.

This decline in popularity can be put in the words of Encyclopedia Britannica Online, "*as the cell has been fractionated into its component parts, protoplasm as a term no longer has meaning*." ("Cell" Encyclopedia Britannica 2009.)

The truth is, once more, the protoplasm of Dujardin, von Mohl, Schultze and Huxley is like van't Hoff's semipermeability, and Donnan's theory of membrane equilibrium, an example of *macroscopic* objects or concepts. And to move forward again, it too must await for the arrival of *microscopic* interpretations like that introduced by the AI Hypothesis.

As I have pointed out in preceding pages, the AI Hypothesis has been described in four full-length books (Ling 1962, 1984, 1992 and 2001.). To these volumes, the reader will have to turn to as the sources of information on the history of the evolving theory and results of experimental testing up to the current year. However, for those looking for a bird's eye view of the theory (up to the year 1998), I would recommend the two-tier summary given online, one very brief and the other somewhat longer in www.gilbertling.org/lp6c.html.

For those interested in more detailed accounts of the progress made mostly in the last two decades, he and she can go to my website: www.gilbertling.org "*Science Cannot Conquer Cancer and AID without Your Help*"(Ling 1998) and find on its front page a list of ten titles. Each title introduces a detailed review on one of the special subdivision of cell physiology, including the (1) sodium pump, (2) selective $K^+$ accumulation, (3) oxidative phosphorylation, (4) multilayer polarization-orientation of cell water etc. Each is written in pdf format and can be downloaded free (and printed out) by merely clicking the title on the front page.

The remainder of the present article briefly describes the concept of "nano-protoplasm," a term I first introduced only two years ago in 2008 as the ultimate unit of life. And how *association* and *induction* play in not only determining the chemical makeup of nano-protoplasm but also in how they work in making nano-protoplasm *alive*. Then, in the briefest manner, I will also describe how the nano-protoplasms and the cells and organ they compose, perform the four cardinal cell physiological *attributes:* (1) selective solute distribution, (2) permeability, (3) cellular resting potential and (4) cell swelling and shrinkage.

**Nano-protoplasm defined by what it is and what it does**

The red blood cell is the first animal cell discovered and recognized as a living cell. Finding the low level of $Na^+$ in human red cells was another landmark event in cell physiology. Indeed, as I have pointed out recently (Ling and Ochsenfeld 2008, abstract) all the major theories of the living cell are built around this discovery of Abderhalden reported over a century ago. For this and other reasons, we will continue to use the human red blood cell to illustrate what is the chemical makeup of nano-protoplasm and what nano-protoplasm does in making *life* and *life activity* what they are.

(I hasten to add that although the underlying concept has been the central theme of the AI Hypothesis from its beginning, the name, nano-protoplasm was a relatively new addition. Indeed, it was first introduced in the year 2008 in the publication bearing its name in the title: *Nano-protoplasm, the Ultimate Unit of Life.* (Ling 2007b, I regret that the publication of the journal, Physiological Chemistry Physics and Medical NMR has fallen behind schedule, hence the year disparity.)

Now, mature human red blood cells — to be subsequently referred to as rbc — have no nucleus nor any other sub-cellular organelle or structures. Indeed, seen through the best light microscope or even electron microscope (see Figure 3) the entire cell is a homogeneous biconcave disk.

64% of the weight of a rbc is water. 97% of the remaining (36%) solid belongs to a single protein, hemoglobin. Therefore water and hemoglobin together make up more than 98% of the rbc. Note that all the non-hemoglobin solids including non-hemoglobin proteins (e.g., enzymes) and phospholipids that partially make up the enclosing cell membrane are, according to the best source available, less than 2% of the weight of the rbc (Ponder 1948, 1971.) Indeed, that less than 2% weight also includes 100 mmoles per kg of $K^+$ and 2 mM of ATP, which is, of course, the end-product of all energy metabolism.

Thus, in theory we can use a magic scalpel to cut up a rbc into smaller and smaller pieces until each piece contains just one hemoglobin molecule. And with this hemoglobin molecule also are about 7000 water molecules, 20 $K^+$ and one ATP molecule. Through specific *association* mechanisms of one kind or another, all these entities are bound together into one coherent nano-protoplasmic unit described by the formula:

$$\mathbf{(Hb)_1\ (H_2O)_{7000}(K^+)_{20}\ (ATP)_1}.$$

(For a more general formula for all kinds of nano-protoplasms, see Ling 2007b, p.121.) Assumed spherical in shape, ***this specific nano-protoplasmic unit would measure*** **6.8 nano-meters** ***in diameter***.

Having completed a short description of what a nano-protoplasm unit is, we now proceed to describe what nano-protoplasm can do in *life* and *life activity*.

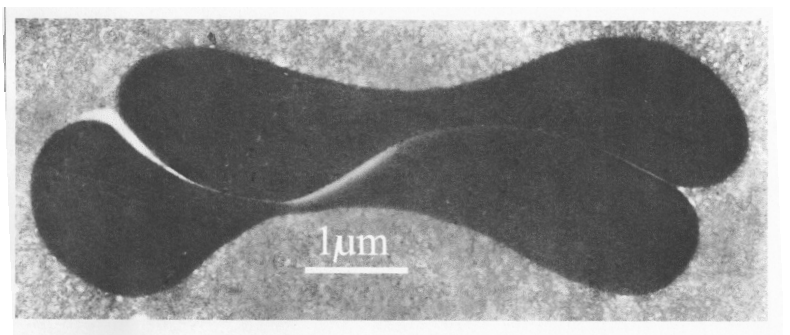


FIGURE 3. Electron micrograph of the cross-section of two mature human red blood cells in blood plasma. (Cryofixed, freeze-dried and embedded in Lowicryl.) (Gift of Dr. Ludwig Edelmann.)

As a rule, each nano-protoplasm existing in its natural environment can exist in just two alternative conformations as illustrated in Figure 4. They are respectively the α-helical state (left) and the fully extended state (right.)

A nano-protoplasm unit would exist in the fully extended state when ATP is adsorbed on the ATP-specific *cardinal site* also shown in the diagram of Figure 4. There, *all the components shown in the molecular formula are in the associated state*. In this (auto-cooperative) assembly, ATP functions as an *electron-withdrawing cardinal adsorbent* (EWC); its adsorption brings about a falling-domino like chain reaction that was once called *Indirect F-effect* (Ling 1962, pp. 92–102.) Since 2007, this term has been replaced by the new name, *AI-cascade mechanism.* (For a detailed step-by-step description of the AI cascade mechanism, see Ling 2001, pp. 170–178 or Ling 2007b, pp. 137–140.)

The adsorption of ATP on its cardinal site induces an *AI-cascade mechanism*. As a result, all the $K^+$, as shown in the illustration, become engaged in *close-contact, one-on-one adsorption* on the β-, and γ-carboxyl groups of the hemoglobin molecule. And in addition, the bulk-phase water molecules are adsorbed (directly or indirectly) as *polarized-oriented multilayers* on the exposed imino (NH) and carbonyl (CO) groups of the fully extended polypeptide chains of the hemoglobin molecules.

Removal of ATP from its specific cardinal site on the hemoglobin molecule causes the reverse change. The operation of the *AI-cascade mechanism* would now lead to an all-or-none (auto-cooperative) desorption of both the $K^+$ and water molecules and the assumption of the hemoglobin molecule in its folded state, in which the backbone NH and CO groups are now locked in H-bonds with other backbone CO and NH groups and the β-, and γ-carboxyl groups form salt-linkages with fixed cations in the form of ε-amino groups on lysine residues and guanidyl groups of arginine residues.

The nano-protoplasm containing the fully extended protein as shown on the right-hand side of the Figure 4 represents the *resting living state.* On the other hand, the nano-protoplasm containing the protein in the folded state shown on the left-hand side of the figure corresponds to either the *active living state* (if the change is reversible) or the *death state* (if the change is irreversible.)

To illustrate how the *dead state* of nano-protoplasm differs from one in the *active living state*, let us consider muscle contraction. A normal muscle at rest is in the *resting living*

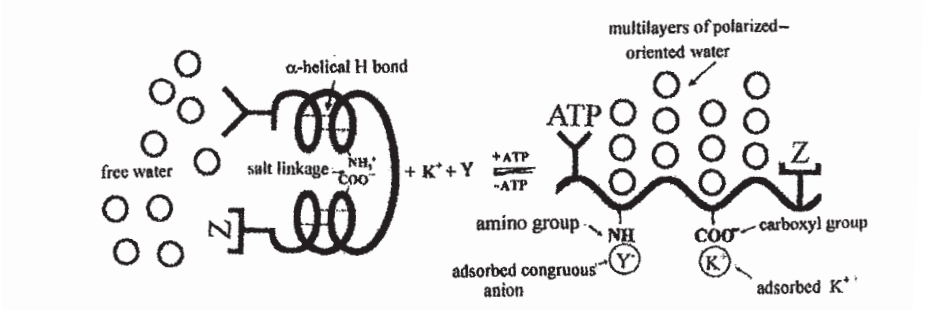


FIGURE 4. Diagrammatic illustration of the all-or-none auto-cooperative transition between the folded (left) and fully-extended (right) state of the a nano-protoplasm unit.

*state*. When it is stimulated say by a single electric shock, it would momentarily lose its ATP and transiently go into a shortened *active living state*. But with replenishment of ATP, it will return to its *relaxed resting living state*. However, if the muscle were poisoned say with the drug iodoacetate, it would not be able fully to regenerate its lost ATP after each contraction. Eventually, it would have lost all its ATP and with that, the muscle would enter a permanently shortened *death state*, which in conventional terms is also known as *rigor mortis.*

In summary, *being alive* means the existence of the nano-protoplasm in the resting living state. Reversible shifts between the resting and active living state constitutes *life activities.*

Having understood what is alive and what is dead in general terms, we are now in a position to discuss two sets of highly important quantitative parameters that play key roles in the all-or-none auto-cooperative transitions between the resting and active living (or dead) state we just mentioned. These parameters are called respectively c-value (and c'-value) and c-value analogue (and c'-value analogue.)

These values are all electronic parameters and hence broadly speaking, *inductive* — of the association-*induction* thesis — in their functions. Rigorously defined elsewhere (Ling 1962, pp. 57–60, Ling 2006a, Appendix I on p.118) the c-value and c'-value may be regarded as describing the effective electron and positive charge density of the β-, and γ-carboxyl groups and fixed cations respectively. On the other hand, c-value analogue and c'-value analogue measure the electron and positive-charge density of dipolar backbone carbonyl (CO) and imino (NH) groups respectively.

Results of theoretical computations, shown in Figure 4.11 on p. 77 in Ling (1962) or in Figure 7 on p. 131 in Ling (2007b) affirmed by experimental data, revealed that at low c-value, the β-, and γ-carboxyl groups prefer to adsorb $K^+$ over both $Na^+$ and fixed cations. In contrast, at high c-value, the converse is true. They then prefer $Na^+$ and fixed cations over $K^+$. In a parallel manner, at low *c-value analogue*, the backbone carbonyl groups prefer to adsorb water molecules (as polarized-oriented multilayers) while at high *c-value analogue*, they prefer to form α-helical bonds with other backbone sites.

The amazing thing about the AI cascade mechanism is that it allows the binding of an *electron-withdrawing cardinal adsorbent* (EWC) like ATP to bring about a uniform change of both the c- and c'-value as well as the c- and c'-value analogue of all the sites far and near in the nano-protoplasm at once. To understand how this is done, you must consult the fuller description given earlier (Ling 2001, pp. 170–78 and Ling 2007b, pp. 137–40.)

Indeed, it is on this keyboard of c-value, c-value analogue etc. that *electron-withdrawing cardinal adsorbents* (EWC) like ATP and *electron-donating cardinal adsorbents* (EDC) like the cardiac glycoside drug, ouabain play their living melodies.

Having disclosed how electronic polarization or *induction* plays a key role in all life activities, we are now in a position to look deeper into the concept of *association* (of the *association*-induction hypothesis) and how it plays its key part in life phenomena.

First, let us begin with the opposite. The Donnan theory of membrane equilibrium is based on the concept of ionic *dissociation*. Van't Hoff's famous equation, $\pi v = inRT$, where *n* is the number or moles of the osmotically active electrolyte in the system of volume v. "i" is the dimensionless *van't Hoff factor*. For a dilute solution of an electrolyte like NaCl, van't Hoff demonstrated that i is not unity but close to 2, showing that the $Na^+$

and $Cl^-$ are not associated but are *nearly fully dissociated*. Van't Hoff's Swedish student, S. Arrhenius—and later, Paul Debye all become famous elaborating on the *dissociated state of ions* in dilute solutions (For more details on this subject, see Ling 2005.)

By this time, the reader must be fully aware of the great importance of the pair of alkali metal ions, $K^+$ and $Na^+$ in living phenomena.Yet as a singly-charged cation, this pair of chemically highly similar alkali metal ions are identical as far as long range attributes are concerned. There is no secret here. We all know that sight and sound, for example, are long-range attributes. One can see and hear from close and far. However, touch is a short-range attribute. To find out how an object feels, you have to touch and thus be in direct contact with the object.

Now, the only difference between $K^+$ and $Na^+$ are in their short-range attributes, notably their size. Naked $K^+$ is larger than naked $Na^+$. But dissolved in water, each ion takes on a more or less permanent coat of water of hydration. The hydrated $K^+$ is now smaller than the hydrated $Na^+$. Indeed, the atomic sieve theory of Mond and Amson and that of Boyle and Conway had utilized these short-range attributes already to sort them apart. But as the reader knows by now, that theory does not work.

In contrast, the AI Hypothesis also used the short range attributes to set this pair of ions apart — when they are engaged in *close-contact*, *one-on-one* adsorption on β-, and γ-carboxyl groups. But that is not all. It is this *close-contact, one-on-one adsorption* that creates the reversal of preference with c-value changes of the β-, and γ-carboxyl groups. Indeed, it is no exaggeration to say that without this close contact, one-on-one adsorption, life and living activity, as we know them, would not be possible.

This conjecture will become clearer by seeing how the AI Hypothesis offers its interpretations on the four cardinal cell physiological attributes to be discussed next in the order: (1) solute distribution, (2) solute permeability, (3) electric potential and (4) swelling-shrinkage.

One recalls that at one time, Boyle and Conway felt that their atomic sieve theory based on Donnan's theory of membrane equilibrium could offer a similar unifying interpretation — before their theory collapsed.

## The new unifying interpretations of the quartet of cardinal cell physiological attributes

### *1. Distribution of ions and other solutes in intact living cells (and in isolated parts thereof)*

The general principle that a solute can exist as a free solute dissolved in cell water and also bound to some fixed element like intracellular proteins can be traced to Martin Fischer (1921) and others like Moore and Roaf (1908.) (See also Ling 2005.) Later, it was elaborated further by Troshin (1966), who also introduced a two-term (Troshin) equation (see Ling 2001, p. 163.) But it was not until 1952, that Ling offered a *microscopic* (or *statistical mechanical)* mechanism as to why $K^+$ and $Cl^-$ are almost fully *dissociated* in an aqueous solution of similar strength, while $K^+$ and fixed anions like the β-, and γ-carboxyl groups are almost completely *associated*.

The underlying cause of full association was given the name, the *Principle of Enhanced Ionic Association due to Site Fixation.* The more up-to-date and complete statistical mechanical derivation was given in detail 43 years later (Ling 2005 pp. 12–18) but in 1952,

Ling gave the following explanation, which is a lot simpler but informative enough for our present purpose today as in 1952.):

> (i) "The force of attraction between ions of opposite sign in solution is opposed by the kinetic energy of the ions themselves. If one of the ions is rigidly fixed, half of this energy is abolished, so that the ions stay on the average closer together than they would be able to do when both are free…"
> (ii) "Fixation allows the close juxtaposition of a number of similarly charged ions, for the repulsive forces between them are less strong than the covalent bonds of fixation. Their individual fields thus overlap, and sum with respect to the force exerted collectively upon a free ion of opposite sign." (Ling 1952, p. 769.)

It was then shown how the *Boltzmann distribution law* (see Boltzmann 1904) would predict theoretically a 7-to-1 preferential adsorption of $K^+$ over $Na^+$ on the β-, and γ-carboxyl groups of mostly myosin in frog muscle cells. It is true that a 7-to-1 preference was not as high as observed (40-to-1) but it is also without question headed in the right direction. This then offered a partial semi-quantitative explanation how $K^+$ is preferentially accumulated over $Na^+$ in almost all living cells but it is not the full answer.

The full answer would have to address why the concentration of $Na^+$ does not match that in the external bathing solution. A qualitative theoretical explanation was provided for the first time in 1965 with the introduction of the polarized/oriented multilayer theory of cell water (Ling 1965.)

Again using basic statistical mechanical theory, Ling introduced in 1993 a quantitative equation for the (true) equilibrium distribution coefficient or *q-value* of a solute (entirely) in the cell water, comprising a linear combination of two terms, a *volume factor* and a *surface factor* (Ling 1993.) In contrast, a *ρ-value* or *apparent equilibrium distribution coefficient* owes an additional part of its origin to adsorbed solute also present inside the cell. q-values never exceed unity. ρ-value can exceed unity by a large factor sometimes. When it does that, most of the intracellular solute involved is in an adsorbed state.

And it is largely the volume factor of the q-value mentioned above that gives rise to the so-called "size rule" (Ling and Hu 1988.) Namely, the larger the solute molecule or ion, the lower is the q-value. As an example, the q-value of sucrose in frog muscle is about 0.15 due largely to the large size of the sucrose molecule. $Na^+$ (as chloride), on the other hand, has a ρ-value of between 0.3-0.4 when a small portion of the intracellular $Na^+$ is adsorbed on the β-, and γ-carboxyl groups that are not occupied by $K^+$ and fixed cations. The true equilibrium distribution coefficient or q-value of the $Na^+$ is in the range 0.2 to 0.3 due to its large hydrated size. (As mentioned in the *Abstract*, see Ling 1997, also Endnote 1 of this article on the fate of the postulated sodium and other membrane pumps.)

### *2. Cell permeability*

While the large aggregates of nano-protoplasm that make up the cytoplasm provide both the β-, and γ-carboxyl groups that selectively adsorb $K^+$ (over $Na^+$) and the polarized-oriented multilayers of cell water that effectively exclude $Na^+$ (as $Cl^-$), I pointed out that a similar collection of various nano-protoplasm at the cell surface determines the ionic permeability of the cell (Ling 1960.) A diagram by Ling and Ochsenfeld of a portion of a nano-protoplasmic unit containing just four β-, and γ-carboxyl groups is illustrated in Figure 5. The cell surface nano-protoplasmic water provides the pathway for the entry (or

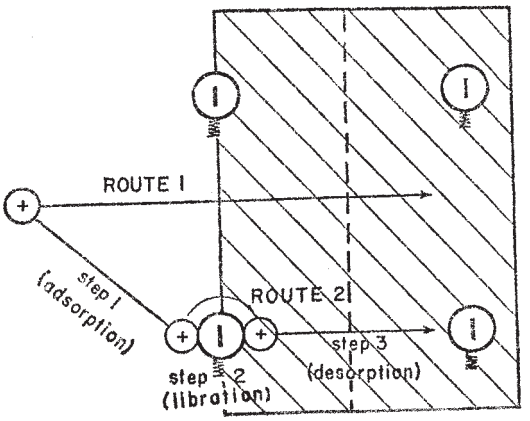


FIGURE 5. Alternative routes of permeation for an external cation into the surface nano-protoplasmic units carrying exclusively fixed anionic sites. Saltatory route (route 1) entails leaping through multilayers of polarized-oriented water molecules. Adsorption-desorption route (route 2) involves three consequent steps shown in the figure. (Ling and Ochsenfeld 1965.)

exit) of a neutral solute molecule like sucrose *via* the so-called *saltatory route* (labeled as Route 1 in the figure.) On the other hand, the β-, and γ-carboxyl groups carried on surface nano-protoplamic units provide a second pathway for cation entry (or exit). Known as the *adsorption-desorption route,* this route is labeled Route 2 in the figure. The low c-value of these β-, and γ-carboxyl groups maintained by an adequate amount of adsorbed ATP on the controlling cardinal sites, makes $K^+$ entry much preferred over that of $Na^+$ by the adsorption-desorption route.

However, as a rule, due to its high concentration in the bathing medium, there would always be some highly energized $Na^+$ that would be able to enter *via* the polarized-oriented surface water. This would then account for the much faster rate of $Na^+$ exchange than $K^+$, notwithstanding that the total number of $K^+$ entering a cell during a given time period is much higher than that of $Na^+$.

This relationship bears resemblance to what happens at a tollgate. Like the highly energized $Na^+$ at the cell surface, the tag-carrying cars going through the tollgate in a given time is faster but also much smaller in number than the total number of non-tag carrying cars (like $K^+$) which enter or exit *via* the more abundant but slower non-EZPASS gates.

Theory also shows that the degree of electric polarization-orientation of the surface water can exercise powerful influence on the rate of solute permeation (Ling 1993.) An effective control of the state of surface water polarization-orientation thus provides the means for a highly important physiological dimension that was as a rule completely overlooked in conventional cell physiological discourses: *diversity in the rate of permeation for a given s*olute.

Thus in order to survive, a frog cannot have just a single fixed rate of permeability for glucose. It needs the glucose to be *rapidly transported* across the cell barriers of the digestive tract as well as the muscle cells in order to provide energy speedily to rapidly contracting muscle cells of a frog escaping from danger. Yet at the same time, the frog must

also offer a leak-proof surface skin cell barrier to prevent a steady loss of glucose to the external pond water. In fact, cell (membrane) barriers to glucose *three orders of magnitude apart*, have been demonstrated in the same North-American leopard frogs, Rana *pipiens pipiens*, Shreber. (See Ling 2007, pp. 187–90.)

I conclude this section by raising another critical question. Does the cell surface contain only *fixed anion*s in the form of β-, and γ-carboxyl groups? Or does it also have *fixed cations* as well? According to the AI hypothesis, the surface of excitable cells like muscle and nerve is populated only by β-, and γ-carboxyl groups. In a way, this is to be expected from what physicists call the Faraday Cage Effect. That is, excess electrical charges of a macroscopic object collect at the surface only. In the following section on cellular resting potentials, we offer additional evidence in support of this conclusion. (For examples of cells that do not follow this rule, see Ling 1984, section 14.4.4 on p. 488.)

### *3. The cellular resting (and action) potential*

This subject is of particular interest to me because I started my career in cell physiology on this subject. Indeed, both my Ph.D. thesis at the University of Chicago (Ling 1948) and the first four full-length papers I co-authored with my beloved professor and teacher, Prof. Ralph W. Gerard (Ling and Gerard 1949) bear the words, *membrane potential* — which I later found out to be wrong.

In 1955 and again in 1959 I first suggested briefly that the resting potentials of living cells is not a membrane potential at all but essentially similar to the electric potentials of glass electrodes (Ling 1955, 1959.) At that time, I did not know that Cremer already had made a similar suggestion many years before (Cremer 1906.) Indeed, I did not find out about this page in history until the 1980's when I was gathering materials for writing my second book, "In Search of the Physical Basis of Life" (Ling 1984 p. 22.)

However, I did more than just point out an analogy between the electrical potential of glass electrode and of the living cell in 1955 and 1959. I was also presenting a new theoretical fundamental mechanism that I suspected to underlie the electrical potentials in both systems. Since the history of this search has been described repeatedly before (Ling 1992, Chapter 11; Ling 2001 pp. 209–24), I shall go straight to our final conclusion here.

That is, the cellular resting potential is a *close-contact, surface adsorption potential* on the β-, and γ-carboxyl groups of nano-protoplasm occupying the cell surface. It is in this research conducted by myself (and my associate, Leo Kushnir) that I reached the conclusion that for typical living cells like muscle and nerve, the cell surface is essentially *anionic* due to the presence of β-, and γ-carboxyl groups only.

The reader will recall that when carboxyl groups are attached to (electrically neutral) nitrocellulose of the collodion used to make collodion thimble electrodes, these carboxyl groups endow the thimble electrode the ability to respond electrically to external $K^+$ concentration like the living cell. Glass tubing made with Corning 015 glass is designed for making good pH electrodes as it does not show sensitivity to "interfering ions" like $K^+$ for example. Ling and Kushnir showed that if such a Corning 015 glass electrode is coated with a very thin layer of oxidized collodion, it then acquires full sensitivity to $K^+$ as the oxidized collodion thimble electrode does. The characteristic *insensivity* of the underlying glass electrode to $K^+$ has now entirely vanished (see Ling 1960 and Ling 1967.)

This specific experiment was undertaken to test the theory that it is something on a very thin surface layer of the electrode that exclusively determines the specificity of ion sensitivity (See earlier theories of Nernst and Baur mentioned on p. 28 above.) Our finding that the collodion-coated glass (CG) electrode behaves indeed like an oxidized colloidion thimble electrode has fully confirmed our expectation (Ling 1967.) Then we went one step further.

We exposed the (oxidized) collodion coated glass electrode to a solution of poly-lysine, which carries a great abundance of positive electric charges on the ε-amino groups at the end of the lysine side chains. Whereas the oxidized collodion-coated glass electrode shows no sensitivity to chloride ions in the test solution, this poly-lysine-treated, collodion-coated glass (PCG) electrode develops anion sensitivity at low pH — when the carboxyl groups are neutralized and thus have lost its sensitivity to $K^+$ as illustrated in Figure 6. Since living cells like frog muscle also show no sensitivity to chloride ion and their sensitivity to $K^+$ is insensitive to pH between pH 5 and 10 (Ling and Gerard 1949), one can conclude that muscle and nerve cell surfaces are primarily anionic. This exclusively anionic surface of excitable cells like muscle and nerve also plays a key role in the creation of what is known as the *action potential*. For full details, the interested reader can have a full review of this in Ling 2007, p. 205. Here, it suffices to give a very short description.

If the muscle cell surface contains both anionic (β-, and γ-carboxyl groups) and cationic (ε-amino and guanidyl) groups like that in the cytoplasmic nano-protoplasm described earlier, a rise of the c-value of the surface β-, and γ-carboxyl groups would cause the formation of salt linkages as illustrated in Figure 4. Instead, the absence of fixed cationic groups on the cell surface leaves the surface β-, and γ-carboxyl groups no alternative but to adsorb $Na^+$, thereby creating one major component of the action potential called the "*overshoot*" (Ling 1984, p. 73, Ling 2007, pp. 204–07.)

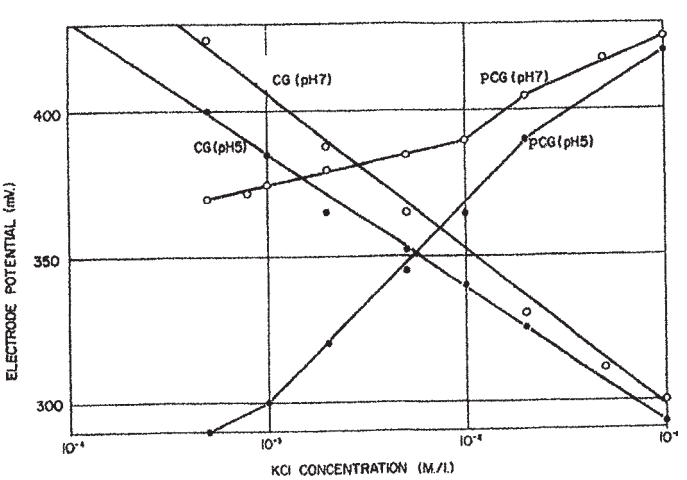


FIGURE 6. Cation and anion sensitivity of a collodion-coated glass (CG) electrode and of a poly-lysine treated collodion-coated glass (PCG) electrode at two different pH's as indicated. (From Ling 1967.)

I finish this section with a reference to the uncannily close correspondence of the electrode behavior of the CG electrode and the living frog muscle: (i) They both exhibit the same rank order of relative preference for the alkali-metal ions: $Rb^+ > K^+$ , $Cs^+ > Na^+$. (ii) Both are indifferent to $Cl^-$. (iii) Both demonstrate a $H^+$ over $K^+$ selectivity of about 150. (iv) Both are totally indifferent to external $Mg^{++}$ (Ling 1992, pp. 287–89; Ling 2001, pp. 218–19; Ling 2007, pp. 198–200.)

And this close resemblance is no accident. There is a very good and enlightening reason behind it. Cellulose, like proteins has the inherent capability of existing in two conformations: folded and fully-extended (see Ling 2007, p.189.) In preparing collodion, electron-withdrawing nitro groups are introduced, thereby lowering by the inductive effect (or more precisely, the *direct F-effect*) the c-value of the carboxyl groups introduced. This c-value lowering makes these carboxyl groups not only qualitatively, but quantitatively like the β-, and γ-carboxyl groups of muscle and nerve cell surface nano-protoplasm under the domination of the *electron-withdrawing (cardinal adsorbent*) ATP (For the most recent dramatic verification of this theoretical prediction, see Ling and Ochsenfeld 2008.)

### *4. Cellular swelling and shrinkage*

There are very few scientists as admirable to many including myself as Jacobus van't Hoff — not only for his science but also for his love of classical music and of Nature. But he was a scientist of his time. Although a contemporary of Ludwig Boltzmann, the microscopic interpretation of macroscopic phenomena introduced by Boltzmann was still not yet widely accepted. Indeed, van't Hoff was much closer intellectually to Wilhelm Ostwald, who was a major opponent of Boltzmann and Boltzmann's belief that atoms exist as part of the microscopic units of which macroscopic objects are made.

Along with his concept of semipermeability, van't Hoff also introduced his *bombardment theory of the osmotic pressure*, in which the impermeant solutes produce the (osmotic) pressure in the same way gas molecules produce pressure by bombarding the container wall. That the bombardment theory is wrong has been widely recognized for quite some time now. In 1970 Ling and Negendank published further evidence against the bombardment concept (Ling and Negendank 1970.) They demonstrated that sucrose can cause shrinkage of surviving muscle cells without being in direct contact with (and bombarding) the muscle cell surfaces — but by vapor sorption from a solution containing water at a reduced activity or vapor pressure.

Next, I shall answer the question why a muscle cell swells intensively in isotonic KCl solution but not in isotonic NaCl solution (von Korösy 1914.) The once popular answer was based on the then-widely held but incorrect belief that the cell membrane is permeable to KCl but not to NaCl and the dictate of de Vries's osmotic method working in reverse: permeant solutes cause swelling; impermeant solutes cause shrinkage. As mentioned earlier, radioactive tracer studies completely disproved that once popular interpretation.

Then the pump-leak model was introduced in which the incessant activity of countless postulated membrane pumps located in the cell membrane renders the cell membrane *effectively impermeable to* $Na^+$ (Wilson 1954, Leaf 1956.) However, extensive evidence cited in Endnote 1 rules that out too.

An altogether different mechanism for the ability of isotonic KCl induced swelling of frog muscle cells is the offspring of the marriage of two sets of theoretical concepts. One

set was published in 1969 and already illustrated in Figure 4 (Ling 1969, p. 47) and a new set of theoretical concept 34 years later in a paper entitled: "A new theoretical foundation for polarized-oriented multilayer theory of cell water and for inanimate systems, demonstrating long-range dynamic water structuring of water molecules" (Ling 2003.)

In this 2003 paper, I have shown on theoretical grounds that, under ideal conditions, water molecules can be polarized and oriented *ad infinitum* by an idealized checkerboard of alternatingly positive and negative sites called an *idealized NP system*. And that a matrix of fully-extended protein chains can function in a way resembling the idealized NP system and produce far deeper layers of water molecules than the average distance maintained primarily by salt linkages between nearest neighboring protein chains in normal living cells (see Figure 4.) It follows that when the size-limiting salt linkages are somehow disengaged, more water molecules will enter the muscle cells and assume the state of polarized-oriented multilayers like water molecules found in normal living cells.

Next I shall demonstrate why an isotonic KCl solution would break open the size-limiting salt linkages as first briefly suggested in 1962 (Ling 1962 pp. 247–50.)

Maintained by an adequate supply of the *electron withdrawing cardinal adsorbent* (EWC), ATP, the β-, and γ-carboxyl groups in a frog muscle that are normally engaged in the size-maintaining salt linkages (see left-hand side figure in Figure 4) assume a low c-value. As a result, these β-, and γ-carboxyl groups show a strong preference for $K^+$ over ($Na^+$ and) fixed cations. Since the normal frog blood plasma and tissue fluids contain only a very low level of $K^+$ (2.5 mM), the normal muscle size is maintained.

However, when the muscle is artificially immersed in a solution containing a much higher concentration of KCl (e.g., 118 mM), many of these size-maintaining salt-linkages would be split apart. More water molecules then rush into the muscle cells causing swelling.

I shall next describe a pathological phenomenon that is at once a theoretical variant of the phenomenon of KCl-induced cell swelling but also a familiar subject to all of us since our childhood. Namely, a bump on a hard object may cause your damaged arm or leg to swell up.

As just mentioned, isotonic KCl causes tissue swelling. Yet, the near-isotonic (100 mM) NaCl normally present in the blood plasma and tissue fluid does not cause tissue swelling. According to the AI Hypothesis, this seeming contradiction arises from the theoretically deduced fact that the β-, and γ-carboxyl groups engaged in the size-maintaining salt linkages have very low affinity for $Na^+$.

However, this low affinity for $Na^+$ is true as long as a normal level of adsorbed ATP is maintained — as it is the case for most frog or human muscle and other cells most of the time.

However, when an injury strikes, it may cause local loss of ATP by interrupting its regeneration. Such a loss of ATP would in theory bring about a rise of the c-value of the β-, and γ-carboxyl groups engaged in the size-maintaining salt linkages, and cause them to increase their preference for $Na^+$. That injury-induced increase of $Na^+$ preference then ushers in the salt-linkage splitting and water entry sequence. Tissue swelling follows as a result.

Thus, in theory it is the injury-induced loss of ATP that makes the normally harmless, indeed indispensable 100 mM NaCl in the blood plasma to become as harmful as 118 mM KCl and the local damaged tissue now undergoes strong swelling.

## Conclusion

In the better part of this communication, I have presented a broad collection of widely different and yet mutually supportive clenching evidence that further enhance the cardinal conclusion presented 49 years ago disproving the membrane (pump) theory (Ling 1962, Chapter 8.) Indeed, at this moment, one can unhesitatingly conclude that there is not even a trace of theoretical and experimental evidence that supports this theory in a positive way uniquely. In contrast and just as sweepingly, one cannot find any evidence that significantly contradicts the alternative association-induction (AI) hypothesis. Indeed, with each passing day, the AI Hypothesis has grown ever simpler (see Ling and Ochsenfeld 2008) while at the same time, it can explain an ever widening range of the living phenomenon (see Ling 2007b.)

The separation of the two contending theories is razor-sharp with virtually no shared common ground. Yet to this very day it is the membrane (pump) theory that is taught as established scientific truth in all high-school and college biology textbooks — not only in the United States but in all other countries worldwide.

Since the 1970's I have been trying everything I could think of to help in correcting this absurd situation (Ling 1997, 1998, 2007 p.62, 2007a) but, so far, to no avail. Nor is this surprising — in retrospect, I have been asking the older generation of people in power to make the necessary change and they refuse to do so.

Now I realize that I should address myself to the young generation of men and women, especially young biology teachers who take themselves and their work seriously. This is indeed why I wrote this article. After all, it is these young teachers that have the power to teach their students the truth rather than a disproved theory masquerading as truth. And by teaching the coming generations truth, they will unshackle and free the future Mankind to innovate, to survive and to prosper in the countless years to come.

*Endnote 1.*

Georg Stahl famous for his introduction of the concept of *phlogiston*, was also the author of another idea known as Stahl's *animism*. In this doctrine, an immaterial soul or vital principle distinct from matter, produces all phenomena peculiar to the animal world.

In the *Abstract* at the beginning of this communication, I mentioned that to this day, high school and colleges still teach as established truth the membrane pump theory. Who really introduced this membrane pump theory was itself an intriguing story. Many teachers and investigators especially in the botanical fields believe that it was Wilhelm Pfeffer who introduced the membrane theory. This is not true. In his "Osmotic Untersuchungen." (Pfeffer 1877), he did not even mention once the words, membrane theory. Others attributed the introduction of the sodium pump to Robert Dean. This too was a mistake (Ling 1997 p.123.) After a great deal of searching I finally realized that the introduction of the *membrane pump theory* actually predated the introduction of the simpler membrane theory.

The reader knows well by now that the first paper bearing the term, membrane theory was written by Julius Bernstein. But this was only a theory of limited scope, dealing with a specific subject, the cell electric potential. The membrane theory that covers the entire

field of cell physiology was in fact first introduced by no other than Frederick Donnan under the title of membrane equilibrium and membrane potential. And that was in the year 1911.

In contrast, the membrane pump theory was introduced inostensibly by two investigators: Dutrochet and Schwann. Though a few years earlier, Dutrochet's membrane pump theory was an extrapolation from macroscopic membranes like pig's bladder and rabbit intestine. He never claimed to have seen the cell membrane. Theodor Schwann, on the other hand, believed that he actually saw the cell membrane and he also believed that there were what we now call membrane pumps in his cell membrane. True, Schwann did not explicitly introduce the membrane pump theory as such; it came as an integral part of his Cell Theory, which he published in 1839 and thus 72 years ahead of the publication of Donnan's membrane theory.

Theodor Schwann in his Cell Theory (Schwann 1839, p. 184) adopted explicitly Stahl's *animism* in explaining the working of what became known later as the membrane-pump (theory.) This is not surprising because Schwann's professor and sponsor, Johannes Müller was also an outspoken believer in vitalism (Ling 2007, p. 6.)

My first contact with the membrane pump theory, or more specifically, the sodium pump hypothesis began with a departmental seminar on the subject of "The Sodium Pump" based entirely on what I found in my library research (Ling 1997, p. 124.) It culminated in my publication as Chapter 8 of my first book (Ling 1962,) in which I presented the details of the definitive critical experimental data disproving the sodium pump hypothesis. (*In the bulk of the remainder of the book, I also introduced the main theme of the association-induction hypothesis*.) The result of my study shows that in frog muscle, the minimum energy need of the sodium pump is at least 15 to 30 times the total energy available — on the assumption that the frog muscle needs energy to do just one thing, pump sodium. The last paper I wrote on the subject was a 75-page long review I published in 1997 under the title "Debunking the Alleged Resurrection of the Sodium Pump Hypothesis." (Ling 1997.) A pdf version of this article can be downloaded and printed by clicking the title listed on the front page of my website, www.gilbertling.org.

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