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Correcting an Inaccurate Paradigm on Cellular Functions - Technical Version

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A Revolution in the Physiology of the Living Cell

by Gilbert N. Ling, Ph.D.
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Gilbert N. Ling, Ph.D.

Dr. Ling's Scientific Background

Dr. Ling graduated from National Central University in Chungking, China, also winning the biology slot of what is known as the Boxer scholarship, an important gift by America to China in the wake of the Boxer rebellion. Enrolling in National Tsing Hua University in Kunming, he roomed with C.N. Yang who, with T.D. Lee, was awarded the Nobel prize for physics in 1957.

Admiring the "holistic" approach taken by Ralph W. Gerard ("The Unresting Cell" Harper, 1940), Ling approached Dr. Gerard at the famous University of Chicago Department of Physiology to be accepted as a graduate student.

Later Ling received his first laboratory position at the Wilmer Institute of Ophthalmolgy, John Hopkins Medical School in Baltimore, where Ling was permitted to pursue his own direction of research.

When Gerard moved to the Neuropsychiatric Institute at the University of Illinois, Ling followed, now having available a well-designed research laboratory, also designed by Ling.

In 1957 Ling accepted a position at the newly inaugurated Department of Basic Research at the Eastern Pennsylvania Psychiatric Institute in Philadelphia. Here Ling continued his work on his growing Association-Induction hypothesis.

Through the efforts of Pennsylvania Hospital's neurologist, Frank Elliott, M.D., the John A. Hartford Foundation (founded by the A and P estate) provided funds for constructing on the ground of the Pennsylvania Hospital a new laboratory. During the next 27 years Ling's Association-Induction hypothesis of cellular function was put to extensive worldwide tests.

Termination of Ling's research grant resulted in another move, including Ling's entire laboratory, to Melville, New York, Damadian Foundation for Basic and Cancer Research, (% Fonar Corporation) a rescue performed by Ling's friend, Raymond Damadian.All physiological systems considered by the health professional, in small or large, must begin at the cellular level. It's a basic truism that the manner in which each cell functions and behaves under differing environments, including cooperative relationships between adjoining and remotely located cells, determines the functioning of each organ and each system.

Consequences of Faulty Cellular Models

A model that describes cellular functions and their relationships in error will cascade inoperative medical techniques throughout medical literature. Such accumulated paradigm errors often reach patients producing ill health and death. There are countless examples throughout medical history where faulty premises or theories have brought about drastic negative consequences.

In describing potential causes for rheumatoid disease and cancer, Roger Wyburn-Mason, M.D., Ph.D. listed two widespread, faulty medical paradigms.

Tuberculosis was once defined in terms of 100 different diseases, depending upon which part of the body symptoms appeared. Of course, treatments usually took on weird and obscure rationale when attempting to solve each of these different appearing symptoms. Then the tubercule bacillus was discovered, and all of those 100 names collapsed, now being named TB of the lung, TB of the spine, TB of the skin, and so on. Rational treatments took hold, and, for many years, reduced the tuberculosis problem.

As is now true for rheumatoid diseases, syphilis was once described as a classical auto-immune disease -- until discovery of the spirochete.

Let's consider our grand fight against cancer, whose 26th birthday was celebrated December 23, 1997. Legislation on that date 26 years ago created the unprecedented multi-billion dollar government-private sector alliance known as the "War on Cancer." It was signed by President Nixon in 1971, six months before the Watergate break-in.

I hate "everyone knows," but this is one time the generality is fully justified, for everyone knows that billions have been spent on faulty treatments based on faulty paradigms, and there seems no way to halt this powerful, destructive engine.

Professor Alfred Burger, University of Virginia, wrote in his monumental treatise, *Medicinal Chemistry*, (pg 19, 2nd ed.), "Almost all the problems of medicinal chemistry would become more amenable if we had even an inkling of the reaction of any drug with body chemicals. . . . "

Could this be so because the theory of the living cell -- seat of body chemicals -- taught in all medical schools to this day is wrong?

Dr. Gilbert Ling is a world-class scientist, who has spent a lifetime researching cellular functions, also collaborating with top-ranking scientists, and producing peer-reviewed literature that ranks among the highest.

His book, *A Revolution in the Physiology of the Living Cell*,

summarizes not his philosophy, but the results of definitively brilliant laboratory work on and about the living cell.

The book is exceptionally well-written and foot-noted, and is easily read by one versed in a course or two of chemistry and physics, although here and there it helps to have a broader range of knowledge of physiological mechanisms.

Autocratic medical paradigms have held back progress in medical science since the death of Hippocrates -- and probably earlier. Few of these faulty concepts can be more basic than the defective Membrane-Pump model which attempts to describe the workings of cellular mechanisms.

Sometimes it's not so much a suppressive medical school authority that has held back appropriate revolutionary concepts as it is the "everybody-knows" attitudes suffered by us all.

For many years the Membrane-Pump mechanism has been used to explain how Na^+ can reach a lower concentration level inside a cell when it is surrounded by a sea of higher Na^+ concentration. This Membrane-Pump model, although never adequately tested scientifically, ruled medical text-books -- as well as *Scientific American* -- for more than a generation. There were micro-molecular pumps, it was theorized, that functioned by permitting K^+ to enter the cell, but which kept out excess Na^+. This, it was said, kept the concentration of Na^+ within the cell lower than the concentration in the surrounding fluids.

During a life-time of astute laboratory observations, Ling not only totally demolishes this faulty paradigm, but seems to rely on electromagnetic mechanisms which cause cells to cooperate and communicate together, much as -- by metaphor -- the wheeling and darting of a flock of birds appears as though the flock moves together as a single organism without apparent signal to one another.

The Membrane-Pump Theory

Disorder in a system, such as the universe or a container of hot chocolate, always increases, even as the available energy for useful work decreases. This is known as the law of entropy.

Renown scientist Clerk Maxwell designed a thought experiment that would contradict the law of entropy. As I remember the story, a small demon was housed in a tube through which air of normal room temperature passed. The small demon would capture each molecule, and separate them into two directions, one for hot air and one for cold air, thus defying the universal law of entropy.

The original Boyle-Conway model of the membrane theory was derived from a single postulate known as "atomic sieve." The atomic sieve theory was introduced by Moritz Traube in the middle of the last century to explain semi-permeable behaviors of copper-ferrocyanide membranes. It was disproved in the early part of the century, reintroduced and disproved again in the thirties in connection with the downfall of the Boyle-Conway special version of the atomic-sieve theory, and then introduced again as a key concept in the construction of Na^+ -channel, K^+ -channel models now popular.

Since the disproof of this postulate, a patch-work of inconsistent explanations have held it together. Addition of the Membrane-Pump theory, as one of those ad hoc patchworks, has not been verified in inanimate model systems.

According to the Membrane-Pump theory the cell owes its continued existence and unique composition to a microscopically thin covering: the *plasma membrane*. Contained within this membrane are small structures which act as specific pumps, operating at the expense of energy stored in high-energy-phosphate bonds of ATP and other organic phosphates. This theory requires continual energy expenditure to maintain an uneven balance between K^+ inside the cell membrane, and Na^+ outside the cell membrane.

The celullar Membrane-Pump theory developed to explain the low level of Na+ in cells such as muscle cells, nerve cells, and erythocytes was a general theory attempting to deal with all solutes in living cells. According to Ling, "The Na pump theory has never attempted to offer more than an *ad hoc*, patchwork theory dealing with *one* solute, Na^+."

No one, says Ling, has yet given even a rough estimate of just how many pumps are required to keep afloat the cellular membrane-pump theory of "unifacial" cellular functions. There simply is no Maxwellian demon that can keep it afloat. (On the other hand, "bifacial" cells such as epithelial, frog skin, intestinal mucosa, kidney tubules, etc. have two different types of membranes. Active transport of Na^+ and other solutes across bifacial cells is not disputed.)

Ling's Association-Induction Hypothesis

All aspects of the association-induction hypothesis derive from a single set of deduced postulates which include the manifestation of closely associated, cooperatively linked protein-ion-water systems maintained at a high (negative) energy, low entropy living state.

In their order of abundance a cell interior consists of water, protein and K^+. Again in order of abundance, a cell exterior consists of water and Na^+. "Thus," Ling writes, "in the broadest sense, cell physiology is a story of assemblies of water-protein-K^+ in an environment of water and Na^+."

So, then, what keeps the cell from dispersing?

According to Ling, "The intuitively attractive idea that the preservation of cell contents is due to an enclosing membrane (with or without the help of membrane pumps) has not stood the test of time. . . . The major forces holding most of the atoms and molecules together are more like those holding together a school of fish swimming in the ocean. The cohesive forces are primarily interactions among individuals of the school. Not only do these interactions keep the school together, they also enable the entire school to alter the direction of motion swiftly and coherently."

Cells exist largely as a result of the cohesive interaction among three major components: protein, ions, and water. Proteins provide the "scaffold" on which other proteins, ions, and water are anchored. While the plasma membrane is important to the cell's existence, as would be the skin to a cucumber or tomato, it is not at all of the same nature as the membrane is to a balloon.

Simply having the correct proportions of water, protein and ions is a necessary, but not a sufficient condition to produce a living cell. The relationship of each component to all the other components satisfies the necessary condition, and, even when placed in the correct positions, each must also exist in a discrete *state*, called the **living state**, with a high (negative) energy and low entropy.

Ling asks us to consider a collection of soft-iron nails tied end to end with bits of string, and scattered among them are iron filings. When a magnet is placed on the terminal end of a nail, the nail is magnetized, which then magnetizes successive nails all along the chain. Not only will the nails be locked together closer, but also the surrounding iron filings become organized into a definite pattern. Depending upon the strength of the magnet, the magnet causes the whole assembly to rise to a specific, discrete higher-(negative)-energy and less random, or lower entropy state.

Describing his Association-Induction (AI) hypothesis, Ling writes, "In living cells, according to the AI hypothesis, electrical polarization, or induction . . . takes the place of magnetic polarization in the model discussed."

Polypeptide chains of proteins are the equivalent of chains of nails. Water molecules and K^+ are the equivalent of the iron filings. The equivalent of magnets are "biologically potent substances of prime importance" called **cardinal adsorbents**, which include drugs, hormones, transmitters, and Ca^{++}, and other substances. The most unique and important cardinal adsorbent in maintaining the living cell in the specific and discrete high-(negative)-energy, low-entropy *living state* is the final product of cell metabolism -- ATP.

Being alive doesn't mean that a cell must continue with functional activity of one sort or another, but rather means that a cell exists in the specific, discrete high-(negative)-energy, low-entropy state called the *living state*.

"A functionally *active* (living) state and death represent two other discrete metastable equilibrium states of increasingly higher entropy and lower (negative) energy in the direction toward the ultimate random state."

The characteristic asymmetric distribution of K^+ and Na^+ is both a weathervane and the substance of the living state.

Proteins in the cell don't exist in their steric and electronic conformation known to exist after isolation and purification as special forms are, as a rule, required for cell proteins to serve their roles in the asymmetrical distribution of K^+ and Na^+ ions.

Ling writes that, "Polypeptide chains (of the same proteins or a variety of proteins), which are found pervasively throughout the entire cell, exist in a **fully-extended** conformation. By a fully-extended conformation, I do not mean that the proteins necessarily exist as perfectly straight chains, but rather that their backbone NHCO groups are not locked in α-helical, β-pleated sheets or in other intra- or intermacromolecular H bonds, and are therefore free to react with the bulk-phase water."

A fully-extended conformation is different from what conventionally has been called the extended conformation denoting the β-pleated sheet conformation. ". . . polypeptide chains in the fully-extended conformation polarize multiple layers of water molecules, and water existing in the state of polarized multilayers has reduced solvency for hydrated ions like Na^+, as well as K^+."

Amino acids joined together form polypeptides or proteins, and their ionized and electrically charged α-carboxyl and α-amino groups react, respectively with neighboring α-carboxyl and α-amino groups, forming neutral chains.

Trifunctional amino acids, aspartic and glutamic acids (i.e., β-carboxyl groups of aspartic-acid residue and γ-carboxyl groups of the glutamic-acid residue) remain as negatively charged functional groups which Ling shows as "Y-shaped" branches on the protein chains. Most of these Y-shaped branches are occupied by K^+ ions. "The mechanism that underlies the attachment of K^+ to the β- and γ-carboxyl groups is (localized) **adsorption**."

According to Ling, "**Adsorption is of great importance to the living phenomena. . . .**" It is electrostatic in nature, and represents an equilibrium phenomenon. "In a resting cell, the β- and γ-carboxyl groups preponderately prefer K^+ over Na^+. As a result, almost all cell K^+ is adsorbed on these fixed β- and γ-carboxyl groups and few of these groups are available for the adsorption of Na^+. However, they can become more available to Na^+ if, for example, K^+ is removed from the system."

About half of the cell Na^+ is adsorbed on β- and γ-carboxyl groups in resting frog muscle cells. The other half exists as free Na^+ in cell water. "The inability of Na^+ to compete successfully for K^+-preferring β- and γ-carboxyl groups and the low solubility of Na^+ in the cell water (virtually all existing in the state of polarized multilayers) explain the low concentration of Na^+ found in most resting living cells." [Underlining added.]

The concentration (in units of equivalents) of *adsorbed cations* and *adsorbed anions* should be roughly equal, since most cell proteins carry approximately an equal number of fixed cationic charges and fixed anionic charges.

Ions and other solutes usually fall into two categories: ions and other solutes are adsorbed on cellular macromolecules (primarily proteins) or freely dissolved in cell water. Virtually all cell water exists in states of polarized multilayers. (This **dynamic** structure results from the interactions of most of the cell water with exposed NHCO groups of some cell proteins, which, Ling says, exist in the *fully-extended conformation*. Solubility in this polarized multilayered water follows the *size rule*: decreasing solubility with increasing size of molecules and hydrated ions.)

High levels of K^+ in the resting cells is due to adsorption of almost all K^+ in the cell of the β- and γ-carboxyl groups of aspartic and glutamic residues of cell proteins.

Whereas, the low level of (hydrated) Na^+ is partly due to its weaker adsorption energy which prevents it from competing successfully against K^+ for the greater share of β- and γ-carboxyl groups, and also in part due to the low solubility of hydrated Na^+ in the cell water due to its large size and incompatibility of the surface structure of the hydrated ion with the dynamic cell water structure.

The cell, Ling says, exists in a (metastable) equilibrium state, requiring no continual energy expenditure. The protein-ion-water system exists in a high (negative) energy, low-entropy *living state*.

Though richer in lipids, the cell membrane shares the same kind of physico-chemical makeup as the cytoplasm; that is, it is maintained in a high (negative) energy, low-entropy *living state*, although it is more intensely polarized than cytoplasmic water. Cellular membranes are often dominated by anionic β- and γ-carboxyl groups or fixed cations. "Solute penetration is via either the polarized water (saltatory route) or by a process of association with, then libration around, followed by dissociation from, the cell surface fixed ions of opposite electric sign (adsorption-desorption route)."

Cell volume and its regulation reflect a balance between (1) tendency of the cell to gain more water formed into polarized multilayers, and (2) tendency to lose water because of a lower equilibrium intracellular concentration (lower than extracellular) of osmotically active solutes, and (3) restraining forces against swelling caused by salt linkages formed between fixed anions (e.g. β- and γ-carboxyl groups) and fixed cations (e.g, ε-amino groups), which lie on the same and neighboring protein chains.

According to Ling, only the outside surface of cell membranes faces a dilute aqueous solution, and the inside surface of the membrane is continuous with the cytoplasm in the sense that both represent fixed-charge systems consisting of associated, cooperatively linked proteins, ions, and water.

Cellular resting potential is a *surface-adsorption potential*, with its magnitude depending upon the nature, density, and polarity of fixed ions at the cell surface (e.g., anionic β- and γ-carboxyl groups), and on the nature and concentrations in the external medium of free

ions bearing the opposite electric charge as that of the fixed ion.

Cellular resting potential does not depend on the concentration of the bulk of intracellular K^+ and other ions.

Ling's Criteria for Satisfying a Scientific Revolution

For successful completion of a scientific or medical revolution in the establishment of a model for the living state cell, five satisfied criteria are supported by Ling's comprehensive laboratory experimentation and are summarized as follows:

Criterion 1. Complete one or more crucial experiments disproving the old theory, and which stands the test of time. (Proofs published 1962.)

A. Crucial experimental disproofs of the Membrane-Pump Hypothesis

1. Excessive energy need of the Na pump at the plasma membrane.

The Na pump alone would require 15 to 30 times the energy available in order to maintain the steady low level of the Na^+ ions found in living cells. The faulty presumption is based on the assumption that ATP, ADP, and creatnine phosphate all contain a large quantity of utilizable energy, an assumption long proven incorrect. Three remedial postulates were introduced to lower energy requirements, and, when each were put to test, all were disproven.

2. The Na pump is only one of the many pumps required at the plasma membrane.

Countless types of ions and solute molecules found within the cells' natural environment would require pumps.

3. More pumps are required at the membrane of subcellular particles.

Due to their much larger surface area, subcellular particles would require even more energy than similar pumps at the plasma membrane.

B. Additional experimental disproofs of the Membrane-Pump theory.

1. Cytoplasm-free membrane sac does not pump Na^+ against concentration gradients.

Cytoplasm-free squid-axon-membrane sacs, with both ends tied, filled with sea water and ATP, demonstrated inability to concentrate K^+ against Na^+ concentration gradients. (Experiments performed more than 25 years ago.)

2. Cells without a functional cell membrane and (postulated) pumps accumulate K^+ and exclude Na^+ as do normal cells.

Frog sartorious muscle was exposed to Ringer solution containing labeled ions, while the region of the muscle cells away from the cut was suspended in vaseline or air, thus making the intact part of the cell membrane and its postulated pumps nonfunctional, because air (or vaseline) cannot serve as a "source" of K^+ for the postulated inward K^+ pump, nor as a "sink" for Na^+ for the postulated outward Na^+ pump. Known as "effectively membrane-pumpless, open-ended cell (EMOC) preparation." (Proof published in 1978.)

Criterion 2. Demonstrate that all of the key evidence used to support the old theory is incorrect or equivocal; that is, capable of a different interpretation.

A. Reversal of evidence for free cell K^+ from K^+-mobility measurements.

The high mobility of K^+ measured in squid axons and frog muscle cell segments was once widely believed to prove the free state of K^+ in living cells. Later work showed that continued normal electrical activity used in monitoring the health of the squid axon studied did not insure the health of the cell's cytoplasm. Ling and Ocsenfeld showed that the high K^+-mobility reported earlier occurred only in cytoplasm inadvertantly injured or deliberately killed. In healthy cytoplasm, K^+-mobility is only 1/8th of that in an isotonic salt solution. These data contradict the membrane-pump theory, but are in harmony with Ling's association-induction hypothesis.

B. Reversal of evidence for free K^+ from K^+-activity measurements with intraceullular microelectrodes.

The use of sensitive microelectrodes to measure K^+ activity in large cells found that K^+-activity varied from 26% to 120% of the average concentration of K^+ measured by chemical analysis. These data contradict the membrane-pump theory according to which K^+ activity should uniformly equal that of a free solution containing the same K^+ concentration, but on the other hand supports the association-induction hypothesis.

C. Reversal of alleged proof for free water in living cells from the equal partition of urea and ethylene glycol between cell water and the external medium.

Equal partitions of urea and ethylene glycol between cells and their environments was once widely believed to have provided crucial evidence of (only) normal free liquid water in living cells. Extensive model studies by Ling and coworkers fail to find evidence for normal liquid cell water. In accordance with the *size rule* (and others) model polarized water which (partially) excludes Na^+, sugars, etc., does not exclude urea or ethylene glycol.

D. Alleged proof of Na pump made equivocal.

Labeled Na^+ in a globule of gelatin injected into amphibian eggs existed at levels lower than in the external medium, after the Na^+ in the gelatin globule had reached diffusion equilibrium with K^+ in the external medium, according to 1979 experiments by Horowitz and Paine. The sustained low level of Na^+ was believed to have proven continual outward pumping of Na^+ at the plasma membrane of the egg. Ling and associates demonstrated that the trapped K^+ exchanged very slowly, functioning as an effective *impermeant* cation. This reduced the steady level of other *permeant* cations like Na^+, as demanded by the Donnan equilibrium ratio equations.

E. Evidence of solute pumping in hollow membrane vesicles reversed or made equivocal.

1. Natural blood cell ghosts.

(a) Red blood cell ghosts

Active transport of K^+ and Na^+ has been demonstrated in red blood cells that have lost a considerable portion of cytoplasmic proteins following hypotonic lysis. Later studies revealed that the reuptake of K^+ and extrusion of Na^+ of these ghosts quantitatively depend on the concentration of the cytoplasmic proteins (primarily hemoglobin) remaining in the ghosts. In "white ghosts" having intact membranes and K, Na-activated ATPase, but containing no hemoglobin or other cytoplasmic proteins, no active transport takes place.

(b) Other natural vesicles

The solid contents of bacterial vesicles, sarcoplasmic-reticulum vesicles, and other vesicles are not those of empty vesicles, but equal or exceed those of intact cells Therefore they cannot be used to support the membrane-pump theory, and are equivocal.

2. Artificial membrane vesicles

Although claims have been made and widely cited that pure phospholipid-membrane vesicles, containing isolated K, Na-activated ATPase, actively transport Na^+, the data lead to a different

conclusion. It is the *leakage* of labeled Na^+ from the vesicles during their passage through an experimental step introduced to separate the fragile labeled Na^+ -loaded vesicles from the labeled Na^+ in the radioactive Na^+ -loading solution (Sephadex column) that gave the false impression of active transport.

Criterion 3. Disprove fundamental postulates of the old theory and verify postulates of the new theory.

A. Disproof of key postulates of the Membrane-Pump theory.

1. Disproofs of the free-K^+ postulate of the Membrane-Pump theory.

Extensive evidence exists, discussed in B.1. below.

2. Disproof of the free-water postulate of the Membrane-Pump theory.

Extensive evidence exists, discussed in B.2. below.

3. Disproof of the Membrane-Pump concept as the basis for the selective K^+ and Na^+ distribution of the unifacial living cells.

See pragraph A.1 under Criterion 1 above.

B. Verification of key postulates of the Association-Induction hypothesis.

1. Verification of the adsorbed state of cell K^+ .

(a) Verification of deduced postulate in inanimate model systems.

(1) The postulate of enhanced counterion association with charge fixation:

Confirmed in the demonstration of enhanced association with countercations following the joining together of isobutyric acid into polyacrylic acid and in the enhanced counterion association observed in non-cross-linked and cross-linked polystyrene sulfonate.

(2) The postulate of stoichiometric binding of alkali-metal ions on β- and γ-carboxyl groups of proteins.

After elimination of competing fixed cations, confirmation was achieved in hemoglobin.

(b) Verification of the localized adsorption of and K^+ and other alkali-metal ions on β- and γ-carboxyl groups of proteins at the A bands of frog muscle cells.

(1) Confirmation of the predicted localized distribution.

Since about half of the β- and γ-carboxyl groups of voluntary-muscle proteins are carried by myosin, and since myosin is found only in the A bands, the AI hypothesis predicts that the majority of the muscle K^+ and other ions that can reversibly and stoichiometrically replace K^+ (e.g., Cs^+ , Tl^+, Rb^+) will be found in the A bands. Confirmation is extensive, by the following:

i. autoradiographic demonstration of localized distribution of radioactive Cs^+ and Tl^+ in A bands in air-dried and in frozen-hydrated muscle fibers.

ii. transmission-electron-microscopic demonstration of localization of electron-dense Cs^+ and Tl^+ at the A bands in frozen dried as well as frozen hydrated muscle cell preparations.

iii. dispersive x-ray microphobe analysis revealing high density of K^+ at the A bands.

(2) Confirmation of the adsorption of K^+ and other alkali-metal ions on β- and γ-carboxyl groups at the A bands.

i. Pinpointing the K^+-adsorbing sites on the A bands: The same sequential order of selective accumulation (e.g., Cs^+ , Tl^+, Rb^+) seen in normal cells is preserved in muscle cells without a functional membrane and therefore without the postulated pumps. Differences among Cs^+ , Tl^+, and Na^+ only short-range attributes, and can be recognized only in consequence of close-contact adsorption. Since this close-contact adsorption does not occur at the cell membrane, it must occur primarily in the cytoplasm at the A bands.

ii. The identification of the close-contact-adsorption sites at the A bands as β- and γ-carboxyl groups: acid titration yielded pK_a of the alkali-metal ions binding acidic groups characteristic of β- and γ-carboxyl groups; confirmation of predicted pH-dependent reduction of alkali-metal accumulation in 2-mm muscle segments by specific carboxyl reagent,(1-ethyl-3-(3-dimethylamino-propyl)carbodiimide), (abbreviated as EDC).

2. Verification of the existence of the bulk cell water in the adsorbed state of polarized multilayers.

Rotational-motional restriction of the bulk-phase water in model systems and in living cells is a fundamental derived postulate (or prediction) of the polarized-multilayer theory of cell water. Another important derived postulate is size-dependent solvency reduction for solutes in polarized water (the *size rule*) Other major derived postulates concern increased osmotic activity, enhanced vapor sorption, and freezing-point depression. Confirmations of these derived postulates in inanimate model systems and in living cells are presented together.

(a) Verification of the primary postulate of reduced rotational and other motional freedom of water in model NP-NP-NP systems (or modifications) and in living cells. [Two juxtaposed surfaces each containing a checkerboard of positive and negative sites at proper distances apart (NP-NP systems), or a matrix of linear chains containing alternatingly positive and negative sites also at proper distances apart (NP-NP-NP systems) cause multilayer polarization of water.]

(1) Quasi-elastic neutron scattering (QENS)

QENS confirmation of motional restriction offers the most important evidence for this postulate, because QENS measures the motional freedom of the average, hence the majority, of water molecules in the system.

(2) In a solution of polyethylene oxide (PEO), free of both paramagnetic and diamagnetic "impurities," nuclear magnetic resonance can also measure rotational-motional freedom of bulk-phase water. Nuclear magnetic resonance time (τ) observed indicates a modest reduction of rotational freedom (e.g., the τ of polarized water is 3 to 19 times longer than the τ of normal liquid water). In living cells, τ is also reduced although the reduction is obscured by the presence of paramagnetic ions and proteins.

(3) Ultra-high frequency dielectric studies

Dielectric studies at ultra-high frequency (up to 75 GHz) demonstrate complete lack of free normal liquid water in brine shrimp cyst cells. Bulk of water in this and other types of living cells studied reveal lower characteristic frequency and longer correlation time, τ_l, in agreement with QENS and NMR findings.

(b) Verification of the requirements of NP-NP-NP systems (or equivalents) for water solvency reduction.

A protein matrix in the fully-extended conformation constitutes an NP-NP-NP system, polarizing water in multilayers on the exposed poly-peptide NHCO groups. Water so polarized has reduced solubility for Na^+, sugars, free amino acids and other large solutes according to the *size rule*, and exhibits other properties seen in living cells. Native proteins, with most NHCO groups locked in α-helical or other inter- or intramacromolecular H bonds, have been shown to have less or no effect on water solvency, in agreement with Ling's AI hypothesis.

(c) Verification of other postulated properties of water existing in the state of polarized multilayers.

(1) High osmotic activity: Osmotic acitivty of polarized water far exceeds that expected from the molar concentration of the fully-extended proteins. This excessive osmotic activity agrees with Ling's AI theory, which requires the interaction of water with fully-extended cell proteins to account for most of the cells' osmotic activity (since the major cell ion, K^+, is adsorbed and thus osmotically inactive.)

(2) Lowered freezing point and reduced rate of freezing: Water in solutions of native proteins at concentrations as high as 50% exhibits no freezing-point depression. Water existing in state of polarized multilayers has a lower freezing temperature (and reduced rate of freezing); the degree of lowering follows the protein (e.g., gelatin) or polymer (e.g. polyethylene oxide) concentration. At very high concrentrations (e.g. 60%), the water might not be freezable at liquid-nitrogen temperature. Data agree with the observed unusual freezing pattern seen in living cells.

(3) High vapor sorption: Water in model systems containing fully-extended proteins or polymers, and water in living cells, follows Bradley's multilayer adsorption isotherm. At physiological vapor pressure, polymers and extended proteins as well as living cells take up a comparably greater amount of water, while solutions of native proteins take up much less.

(4) The pattern of nonelectrolyte distribution: Linear distribution and the *size rule* are followed by both model polarized water and living cells.

Criterion 4. Demonstrate that the new theory, cast into rigorous form of equations, can quantitatively explain experimental findings that can also be quantitatively explained by the old theory, *as well as* those that *cannot* be quantitatively explained by the old theory.

There are no known major phenomenon that can be explained only by the membrane-pump theory. Regarding Ling's AI hypothesis, the following phenomenon can be explained.

A. Solute Distribution

1. AI hypothesis explains the unequal distribution between cell water and its environment of *any* water-soluble compound in existence, regardless of their total number. Membrane-pump theory cannot explain unequal distribution of *any* water soluble compound.

2. AI hypothesis can explain why nonelectrolyte distribution in living cells follows the *size rule*. The membrane-pump theory does not.

3. AI hypothesis readily explains the rectilinear distribution of many solutes found in concentrations lower than those of the bathing medium. Membrane-pump theory cannot.

B. Permeability

1. AI hypothesis can easily explain the bulk-phase-limited diffusion of water into and from frog ovarian eggs and giant barnacle muscle fibers. Membrane-pump theory cannot.

2. AI hypothesis can explain permeability of living cells to very large molecules and hydrated ions, while the model of lipid bilayer cell membranes with rigid pores of small dimensions to fit K^+ and Na^+ (membrane-pump theory) cannot.

3. AI hypothesis explains why, after extraction of 95% of the lipids from the inner membrane of liver mitochondria, the trilaminar structure of the cell membrane remains largely unchanged. Membrane-pump theory cannot.

C. Cell Volume and Shape

1. AI hypotheis can explain the invariance of cell volume (or water content) per unit dry weight of each cell type. Membrane-pump theory cannot.

2. AI hypothesis can explain why the swelling and shrinkage of frog muscle does not follow the prediction of van't Hoff's law (law of osmosis). Membrane-pump theory cannot.

3. AI hypothesis explains why, in isotonic solutions of various salts, only salts of certain cations and anions (e.g., K^+ and Cl^-) cause extensive sustained swelling, while much less swelling occurs in isotonic solutions of other salts (e.g., K^+ and $SO_4^=$) -- even though K^+, Cl^-, K^+, $SO_4^=$ and Mg^{++} are all permeant. AI hypothesis also explains why these swelling and shrinkage responses persist when the (long) muscle cells are cut into 2-mm- and 4-mm long segments with both ends open (and no membrane regeneration). The membrane-pump theory does not.

D. Cellular Electrical Potential

1. The AI hypothesis explains why the resting potential (ψ) of muscle and nerve is indifferent to the concentration of external (highly permeant) Cl^- and Mg^{++} concentration. Membrane-pump theory cannot.

2. AI hypothesis can explain the existence of a resting potential in the face of the now-proven adsorbed state of the bulk of intraceullular K^+. Membrane-pump theory cannot.

3. AI hypothesis can explain why it is the relative *adsorption constants*, rather than their *permeability constants* of external monovalent ions, that determine this relative depolarizing action on ψ, resting potential. Membrane-pump theory cannot.

Criterion 5. Establish that the new theory, cast into rigorous form of equations, can quantitatively explain experimental findings that can also be quantitatively explained by the old theory, *as well as* those that *cannot* be quantitatively explained by the old theory.

A. Solute Distribution

1. Membrane-Pump Theory

The only equations proposed for the steady levels of solutes in livng cells is for cases where the intracellular solute concentration exceeds the extracellular concentrations. No equation, for example, has been proposed for the distribution of cell Na^+ which exists at lower concentration than in the external medium, despite immense efforts spent in research on this subject.

2. Ling's AI Hypothesis

So far as can be determined to date, all equilibrium distribution of solutes in living cells, including the equilibrium distribution of Na^+ in living cells, can be quantitatively described.

b. Permeability

1. Membrane-Pump Theory

Equations for the rate of permeation into living cells (e.g. labeled Rb^+ into barley roots) have been introduced based on the assumption that combination of the permeant solute with a carrier is required before the solute's dissociation from the carrier and entry into the cells. Since this carrier concept has been disproven, equations based on this assumption are no longer on solid ground. Equations based on the AI hypothesis, however, are on sound foundation.

2. The AI Hypothesis

Solute permeation rates into living cells and various model systems have been successfully described by a general equation (not given here) for membrane-limited permeation. Other equations can describe simple and more complex bulk-phase-limited diffusion of water into living cells (and variations), which cannot be predicted by the membrane-pump model and its equations.

C. Cell-Volume Regulation

In terms of the membrane-pump theory, the van't Hoff (law of osmosis) equation is valid only in describing the volume changes of tonoplast-enclosed, and dilute-aqueous-solution filled central vacuoles of old plant cells, but not the plasma-membrane-enclosed cytoplasm of plant or animal cells. Cell-volume regulation is a function of cell water (plus a constant), and the AI derived equations are capable of explaining the different cell volumes maintained in solution containing different concentrations of the *permeant* as well as the *impermeant* nonelectrolytes in the external medium, depending on the molecular weights of the nonelectrolytes.

D. Cellular Resting Potentials

Only a portion of the Hodgkin-Katz-Goldman equation for cellular resting potential as derived for the basis of the membrane-pump theory has been experimentally verified. The remainder of the equation has been falsified or is in doubt.

A more general equation for the surface-adsorption theory is presented, consistent with the AI hypothesis.

Completed Revolution

According to Ling, "The foregoing comparison between the five criteria necessary for a scientific revolution and the current status of the membrane-pump theory and of the association-induction hypothesis lead to a clear-cut conclusion: a revolution is now complete."

Point by point, and in great scientific detail, Dr. Ling has demonstrated experimental studies that have satisfied all of the above five criteria for bringing about a revolution in thought in the way that cellular mechanisms are viewed.

Ling's notes at the end of each chapter are both entertaining and highly educational. It's clear that Ling is a learned and objective scientist with a long history of important research grants and peer reviewed literature to his credit. His past publications include *A Physical Theory of the Living State* (1962) and *In Search of the Physical Basis of Life* (1984).

Conclusion

Dr. Ling, a creditable biological research scientist, has presented well-written documentation of a life-time accumulation of his scientific experimental evidence which clearly substantiates his Association-Induction hypothesis, and virtually demolishes the Membrane-Pump theory, showing it to be, at best, an inaccurate metaphor without good scientific underpinnings. He's published more than 200 peer-reviewed scientific papers.

May I take this opportunity to most highly recommend reading Dr. Gilbert N. Ling's book.