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What Is Life Answered
in Terms of Properties and Activities of Auto-cooperative Assemblies of Molecules, Atoms, Ions and Electrons Called Nano-protoplasm

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KEY WORDS: AI cascade mechanism; Alan Chalmers; anti-science; association-induction hypothesis; atoms; ATP; auto-cooperative adsorption; c-value; c-value anlogue; Ca^{++}; cardinal adsorbent; cardinal site; cell; chlorine ion; cooperative phenomena; death; drugs; drug design; electrons; H^+; hemoglobin; hormones; hydrogen ion; K^+; Kinetic Theory of Gases; life; life activity; living; living cell; macroscopic protoplasm; microscopic protoplasm; molecules; Na^+; nano-protoplasm; ouabain; polarized-oriented-multilayer theory of cell and model water; PM theory; POM theory; potassium ion; proteins; protein folding; protoplasm; Queen Victoria's transistor radio; Salt-Linkage Hypothesis; Schrödinger; Science; science philosophers; Scientific Method; sodium ion; sodium pump; two-state model; What is this thing called science?; Yang-Ling Cooperative Adsorption Isotherm.

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Abstract of background and content

IN MID-17TH CENTURY English chemist, Robert Boyle regretted: "It is highly dishonorable for a reasonable soul to live in so Divinely built a mansion as the Body she resides in, altogether unacquainted with the exquisite structure of it." After the Lisbon earthquake in 1755 where many innocent men, women and children were killed for no faults of their own, French writer Voltaire lamented in his poem: "Man is a stranger to his own research…thinking atoms …have measured distant stars,…Ourselves we never see or come to know." In 1738 and thus seventeen years before Voltaire invoked thinking atoms, Swiss-Dutch mathematician, Daniel Bernoulli published his Kinetic Theory of Gases. In this theory gases are collections of rapidly and randomly moving corpuscles or molecules and their ceaseless bombardments on the wall of the container give rise to the pressure in all directions—a phenomenon unexplained until then. Notwithstanding, he and three other top-notch advocates of the Kinetic Theory of Gases were all rejected. Indeed, physicists as a whole did not believe that atoms (and molecules) are real until the beginning of the 20th century—some 170 years after Bernoulli's publication. The present paper mentions how the last rejected creator of the Kinetic Theory of Gas, the ill-fated Austrian mathematician-physicist, Ludwig Boltzmann also invented the modern branch of physics called Statistical Mechanics before taking his own life. And it was this invention and the new knowledge on protein structure uncovered by the German chemist, Emil Fischer that had provided the twin pillars, on which "A Physical Theory of the Living State" called "the Association-Induction Hypothesis" was launched in 1962. Another sixty years of continued theoretical and experimental studies later, I am ready to present here the most up-to-date and mature version of the only known definition of life. Thus, at long last Mankind has come to know what we are in our divinely built Mansion.

1 Introduction

In the title of a booklet he wrote, one of history's greatest physicists, Erwin Schrödinger (1887–1961) asked the question "*What is Life?*" (Schrödinger 1944.) That booklet has been reprinted by the Cambridge University Press at least 18 times and must have sold thousands upon thousands of copies. Notwithstanding, Schrödinger only asked but did not answer the question he posed. Francis Crick of the DNA fame was more straightforward. In his 1981 book, "*Life Itself, its Origin and Nature*," he wrote "that it is not easy to give a compact definition of either *life* or *living*" (Crick 1981 p. 49.)

To make certain that I did not overlook any existing cogent answer(s) to the question what is life, I quote below the definitions of the word, life, from six dictionaries, ranging from the elementary to the advanced, from the traditional to Wikipedia, which is being steadily updated.

Thorndike and Barnhart: High School Dictionary

(Life is the) Quality that people, animals and plants have and that rocks, dirt and metals lack.

Webster's New Collegiate Dictionary, 1977

1. (Life is) The quality that distinguishes a vital and functional being from a dead body.
2. (Life is) A principle or force that is considered to underlie the distinctive quality of animate beings.
3. (Life is) an organismic state characterized by capacity for metabolism, growth, reaction to stimuli, and reproduction.

Webster's New Twentieth Century Dictionary (unabridged), Second Edition, *1968*

1. (Life is) That property of plants and animals which makes it possible for them to take in food, get energy from it, grow, adapt themselves to their surroundings, and reproduce their kind: it is the quality that distinguishes a living animal or plant from inorganic matter or a dead organism.
2. (Life is) The state of possessing this property as we tried to bring the drowned child back to life.

The Oxford Universal Dictionary on Historical Principles. 3rd and Revised Ed. 1955

1. (Life is) Primarily, the condition, quality or fact for being a living person or animal.
2. More widely, (life is) the property which distinguishes a living animal or plant or a living portion of organic tissue, from dead or non-living matter; the assemblage of the functional activities by which the presence of this property is manifested.

Wikipedia, the Free Encyclopedia, July 15, 2012

Life is a characteristic that distinguishes objects that have signaling and self-sustaining processes from those that do not.

The American Heritage Dictionary of the English Language, 4th edition

1. (Life is) The property or quality that distinguishes living organisms from dead organisms and inanimate matter manifested in function such as metabolism, growth, reproduction and response to stimuli or adaptation to the environment originating from within the organism.
2. (Life is) The characteristic state or condition of a living organism.

The definitions given by the six dictionaries for the word, life, are most frequently expressed as *a property, a quality, or a state* and less frequently as a principle, a force, a characteristic, a condition, a fact or activities. However, none tells us even briefly what that property, quality, state etc. is in terms of the laws of physics and chemistry that govern the dead world.

Given the pervasive ignorance on what life is worldwide, how can a reader reconcile it with the title of this article *What Is Life Answered* and the content of its abstract to the same effect? When the reader reaches the end of this communication, he or she will know the full story of the cause of this apparent contradiction. But before that I offer a pointer.

Beginning in mid-20th century, the continued effort of a tiny minority of our species has succeeded in understanding in modern physico-chemical terms what distin-

guishes the vast amount of dead matter that makes up virtually the entire Universe—from the least amount of matter that makes up a bacterium, a rose, a nightingale as well as you and me: ***life****.*

Only the new truth found by this tiny minority is still unknown to the vast majority of our kind, nor taught in high school and college courses across the whole world with one lone exception—in Gifu University of Japan under the direction of Prof. Hirohisa Tamagawa. The main cause for this delayed response is not hard to imagine if one looks for it in the past history of science. For example, it took one hundred and seventy (170) years for the physicists to accept the (revolutionary) Kinetic Theory of Gases and the reality of atoms and molecules. Our delay has not reached that vintage yet. Nonetheless, a part of this communication is devoted to making this "dark age" as brief as possible. And to achieve that goal, I shall start with a question.

1.1 Principle of sequential invention

To assess the long-term outcome of a prolonged delay of the adoption of one of the most relevant of all basic knowledge to the long-term welfare of Mankind, I turn to the Website that I introduced earlier (Ling 1998.) It bears the title, "*Science Cannot Cure Cancer and AIDS without Your Help.*"

In this Website, I started with a question embedded in a parable: Could Queen Victoria of England at the height of her power and her entourage of brilliant minds repair a transistor radio, which I sent her by magic and it broke? My answer was an emphatic no—not even if she is willing to enlist all the capable scientists in the world and to spend every shilling in the Treasury of Great Britain. There was no way for anyone to fix the faulty operation of something at a time when no one knew that something existed (Ling 1974; Ling 1992 p. xxii; Ling 1998.)

Yet, once we understand how electrons work in a radio, it would cost next to nothing to have a broken one restored to normal function. Thus the invention of something epochal but complex can only follow the understanding of the underlying basic knowledge. For convenience of reference, I named the underlying principle, the ***principle of sequential inventions*** (Ling 1998 p. 3.)

Thus fifty years after Michael Faraday discovered magneto-electric induction (1831) the first electric power plant came into existence (1880.) Thirty years after Maxwell introduced his unified theory of electromagnetic waves, (1867–1873,) Marconi obtained a British patent for the future radio industry (1900.) Notice also that the discovery of one relevant basic truth spawns not just one useful product but an ever-expanding tree of other basic knowledge and their respective useful products. The universal rule is that the more relevant and the more basic a new discovery, the wider is its beneficial impacts on the current and future welfare of all Mankind. From the vantagepoint of humanity, no basic knowledge could be more relevant and more basic than what life is in terms of the laws that govern our Universe.

By the same token, we cannot cure deadly cancers for a similar reason that Queen Victoria could not fix her broken radio. Only in this case, it was not the absence of relevant basic knowledge but the reliance on an entrenched, *erroneous* basic theory of life, called the membrane theory and/or membrane pump theory, that have been road-blocking what could be unprecedented progress in biomedical research and education.

In both the membrane theory version and the membrane pump theory versions, the basic units of life called cells are membrane-enclosed tiny sacs of watery solutions comprising

ordinary liquid water, fully dissociated ions and so-called "native proteins" (see Ling 2006 for reasons why the so-called "native proteins" are not native.) And both versions have been thoroughly disproved. Thus the **membrane (pump) theory** has been disproved by (at least) three sets of independent evidence published between 1962 and 1980 (Ling 1998a): (1) 1500% to 3000% energy insufficiency to operate just one pump at the cell surface, the sodium pump (Ling 1962; Ling 1997), (2) squid axon membrane sacs with its cytoplasm replaced by a watery solution of the right composition do not exclude Na^+ or accumulate K^+ (Ling and Negendank 1980), (3) muscle cytoplasm without functional cell membrane and postulated pumps excludes Na^+ and accumulates K^+ (Ling 1978; Ling 1997.) With equal thoroughness, has the alternative **membrane theory** been disproved in theory and via a multitude of experimental testing (Ling 2011.)

If the disproof of the still widely taught membrane or membrane (pump) theory were all I could tell you, it would give little comfort to anyone. *After all, it might take decades if not centuries to invent and then prove valid an alternative new theory to replace the wrong one.* Ironically, the virtually unknown achievement alluded to above holds the key to a happier and more secure future for all Mankind. But as also mentioned briefly above, to reach that long term goal we must first find ways by which the new truthful knowledge (and other revolutionary truths yet to come in the future) can be taught widely and soon. To reach that objective, we need to unearth whatever unconcealed as well as concealed "Trojan horses" that threaten the spreading of newfound knowledge.

1.2 The buried knowledge

A man unexpectedly inherited a big bag of silver. Worried that others would steal it, he buried the bag underground. However, even that did not stop him from worrying. Maybe the site is too close to a busy street and that means danger. For what he thought would add more safety, he put up a big sign over the burial site, announcing that "***there is no buried treasure of 300 taels of pure silver at this location***." Not long after that, a passerby saw the sign. Distrusting the reliability of the one that put up such a sign, the passerby got a shovel and started digging. Soon he found the bag and walked away with the 300 taels of silver.

This is, of course, an old Chinese story. Believe it or not, it has a modern counterpart. Only it was not another Chinese character that did the repeat but the British corporation that publishes the ***Economist*** magazine (with its alleged global circulation of 1473937.) Thus its Technology Quarterly Section of its Dec. 5, 2003 issue contained an article entitled "MRI's Inside Story". In this article, the magazine announced to its worldwide readership: "*Following an obscure theory by Gilbert Ling, a physiologist…Most scientists consider Dr. Ling's ideas as wacky at best*." (*Wacky* is slang for irrational, crazy according to the Webster Dictionary.) A passerby saw this announcement. He too suspected the magazine had something to hide and began digging. What he found was a collection of scientific publications on or about life including:

> ***"A Physical Theory of the Living State: the Association-Induction Hypothesis***", a 680-page long monograph published in 1962 by the Blaisdell Publ. Co., a branch of Random House Publishing Co. of New York;
>
> ***"In Search of the Physical Basis of Life***", a 791-page long monograph published in 1984 by the Plenum Press of New York and London;

> ***"A Revolution in the Physiology of the Living Cell"***, a 378-page long monograph published in 1992 by the Krieger Publishing Co. of Melbourne, Florida;
>
> ***"Life at the Cell and Below-Cell Level: The Hidden History of a Fundamental Revolution in Biology***", a 373-page long monograph published in 2001 by the Pacific Press of New York.

Not to mention more than 200 scientific reviews and full-length articles on subjects related to life and living—published one after another in established scientific journals mostly in the US and UK over a long span of time. Thus a new unifying theory of living phenomena, called the *association-induction hypothesis*, was introduced half a century ago. And it has been extensively tested and confirmed worldwide—without a single major setback. *And imbedded in the four books and other documents published over half of a century, is an evolving theory of life in physico-chemical terms*.

What is more, 15 years after its introduction, the association-induction hypothesis had led Raymond Damadian to invent the medical technology known as *magnetic resonance imaging* or MRI. And here is how Dr. Damadian described that moment of history in a letter to me dated November 9, 1977 (Ling 1992 p. xxv; Ling 1984 p.vii.)

> "On the morning of July 3, at 4:45 A.M….we achieved with great jubilation the world's first MRI image of the live human body. The achievement originated in the modern concepts of salt water biophysics, on which you are the grand pioneer with your classic treatise, the association-induction hypothesis." (Ling 2001 p. 83.)

Thus, Damadian's invention has confirmed once more the *principle of sequential inventions* I introduced and mentioned above. But that was not all. Damadian's comments on the association-induction hypothesis also shine light on a big medium's total disregard of the good name of people who have done them no harm. Indeed, the potential damage it has done by its reckless abusiveness is matched only by its inability to tell truth from lies.

Not long after I heard about the attack, I wrote to the top brass of the magazine. I asked them if they had actually interviewed the majority of the world's scientists and got everyone of them to divulge their assessment on my scientific ideas as irrational and crazy or *wacky at best*? And if so, where is their published evidence—when in fact I could not find a trace of such evidence in the literature? Though a separate set of my letter and supportive documents was sent respectively to the President, the Board Director, the Editor-in-Chief of the magazine, not one answered or told what they had actually done and not done, and apologized publicly.

A detailed account of this bizarre episode including a reasoned guess at its immediate motivation for mounting the attack is published in the same issue of the journal publishing this one (Ling 2012.)

Done with one example of how one wide-circulating magazine interferes with the normal spreading of newfound scientific knowledge by lying to the public about my scientific reputation, I now approach a broader and sustained attack on science and even the existence of truth.

1.3 The Scientific Method and what it can and cannot do

For a long time, most practicing scientists shared the belief that the set of step-by-step procedures to find truth and called the Scientific Method was invented in the West. In fact, this belief is totally wrong. The Scientific Method was invented by an Arab, Ibn al-Haytham or (its Latinized version) Alhacen, who lived in the Islamic Golden Age between

965 and 1040 (Alhacen 2013.) The West did not adopt the Scientific Method until the post-renaissance or early modern period. And then falsely attributed its invention to Galileo Galilee, René Descartes, Robert Boyle and others.

The more organized truth-seeking that followed the adoption of Scientific Method has been known as *modern science* (or simply science)—to be distinguished from the earlier disorganized intellectual effort called *natural philosophy*. A unique gift that the Scientific Method has given to modern science is a way of experimentally *falsifying* a hypothesis. Nothing like it had existed before.

By making it possible to determine if one's own hypothesis or that from others has validity, the Scientific Method had also transformed the search for truth from one of isolated individual activities to the cooperative activities of an open-ended group of individuals or groups of individuals worldwide.

However, to carry out communication far and wide was no easy task at that time. To answer this and related needs, the immensely wealthy Islamic caliphs had built the famous edifice called the *House of Wisdom* in Bagdad, a city itself then newly-erected on the bank of the Tigris River. Nominally referred to as a library, the House of Wisdom was much, much more. To begin with, the caliphs gathered needed "tools" from remote sources. Thus they learned how to make (cheap and foldable) paper as well as printing from China and "Hindu-Arabic numerals" from Hindu. They also bought or otherwise obtained recorded knowledge from diverse surviving or rapidly-vanishing old culture and had them all translated into a single language, Arabic. And then printed them on paper to be bound into books or pamphlets—made available at easily accessible at the library of the House of Wisdom and elsewhere in the Islamic Empire. Then just as quickly the Islamic Golden Age ended. Only then, modern science became the foster child of European West. Years passed.

1.3.1 Science philosophers and anti-science

Far down the time line, a small group of individuals who lived and died in the second half of the 20th century appeared on the scene and called themselves Science Philosophers. Often referred as a group, they are Karl Popper (1902–1994), Thomas Kuhn (1922–1996), Paul Feyerabend (1924–1994) and Imre Lakatos (1922–1974.) In my guess, their immense visibility and influence could be traced to the fact that each top-ranking university across the world usually had a Department of Philosophy. However, a main subject of philosophy had been the philosophy of Nature—a subject matter that had been taken over by Modern Science and taught in newly installed science departments. In consequence, the old philosophy departments were left with less and less subject matter for teaching and research. So when some individuals came along and proclaimed that they were masters of both science and philosophy, these individual were snatched up fast. In support of this explanation, I may mention that when Paul Feyerabend turned 46, he was offered professorships in no less than ten top universities of the world, including the Berlin University in Germany, Yale University of the US and Oxford University in Great Britain (Preston *et al* 2000.) Unfortunately, the overall legacy the quartet of science philosophers left behind is not what one would hope it to be—with the possible exception of the Hungarian mathematician, Imre Lakatos (Lakatos 2013.)

In what follows I shall comment on some specific ideas and pronouncements of Popper and Kuhn. As far as Feyerabend is concerned, when he proclaimed that science

cannot prove or disprove a hypothesis and that "everything goes," (Theocharis and Psimopoulos 1987 p. 596), my answer is simply that he was totally wrong as will be made clear below.

1.3.1.1 THOMAS KUHN

In 1962 Kuhn published "The Structure of Scientific Revolution" and became famous (Kuhn 1962, 1970.) Among many of his admirers was myself. (Ling 1992, p. 319.) The reason I thought so highly of his work at that time was that he stood apart from the other influential scientists who insisted that progress of science is linear. That is, new ideas all came directly from past science, thereby justifying the deployment of the *peer review system, in which establishment scientists or peers decide who get public support and who don't* (Ling 1998b.) To insist that scientific progress is linear, these scientists openly ignored historical facts. That is, science can progress smoothly but from time to time, it may also undergo drastic revolutionary changes (see Ling 1998c.) Unfortunately, Kuhn did not just highlight the existence of these revolutionary changes, he also separated scientists into two classes: revolutionary scientists are like eagles in the sky and normal scientists are like barnyard fowls. In consequence—in my view, he got so much flak that he overresponded by turning against revolutionary science—claiming successive revolutionary changes do not bring its investigator(s) closer to truth but only from one fad to another fad. It is this changing from talking about truth to talking about nonsense that had made his overall contribution to science and society anti-climatic and harmful (Theocharis and Psimopoulos 1987; Theocharis 1987. See also Horgan 1996.)

1.3.1.2 SIR KARL POPPER

Sir Karl Popper (1902–1994) was born in Austria, he later taught at the University of London and was knighted in England. It is widely known that according to him, science can only disprove a hypothesis but it cannot prove a hypothesis (Popper 2013.) On that I totally disagree. (See Section 1.3.2 below for reasons.)

1.3.1.3 ALAN CHALMERS

Unlike the quartet of science philosophers described above, Alan Chalmers is still alive and vigorously active. Born in Bristol, England in 1939, he got his Ph.D. from the University of London. While there he apparently came under the influence of Sir Karl Popper. In 1971, Chalmers went to Australia and began working at the University of Sydney. As of this time in 2013, he is working on the 4th edition of his enormously popular best-seller textbook, "*What Is This Thing Called Science*?" So far, this book has already been translated into fifteen languages.

"*What Is This Thing Called Science*?" first appeared in 1976. It was then sold as a textbook for an introductory university course on the philosophy of science. The heading of the last section of its second 1982 edition of the book reads "Why Bother?"

Dr. Chalmers also proclaimed that the most important function of his book is to combat the *Idealogy of Science,* which he saw as the insistence of *perpetrating the dubious concept of science and the equally dubious concept of truth.*

While I am not sure this was his original intention, he did write early on in the Introduction of the above-mentioned book the following passage: "We start off confused and end up confused at a higher level."

In their rebuttal of Chalmers's claims in the Nature magazine's Commentary, Theocharis and Psimopoulos together (1987) or Theocharis alone (1987) pointed out that it is only on true knowledge that the socially beneficial and economically profitable medical and technological applications can be firmly grounded. And true knowledge is often discovered by the judicious application of the Scientific Method. Indeed, this is a different way of expressing what is expressed in the *principle of sequential inventions* I presented earlier.

Notwithstanding, the anti-science movement has become so popular not only with the public but worse, it has become just as popular among the professional philosophers and scientists—not to mention teachers who buy and teach what is in such a highly popular textbook. Theocharis and Psimopoulos ended their commentary with the plea that scientists and philosophers stop running down their own profession and start fighting for their causes earnestly.

As a professional scientist all my life, I have always felt it an unexcelled privilege to have the opportunity to offer the foundation truths for more security and happiness of all members of our species in time to come. In my view, to deny that unexcelled privilege to our younger generations is bad beyond words. In direct opposition to Popper, I shall also demonstrate that a scientist can find old as well as new ways to prove a hypothesis with variations of the Scientific Method—as I try to do in the section immediately following.

Come to think about it, I suspect that different versions of what I am going to describe below could have been in practice all along. How else are you going to tell me that most serious-minded research scientists spend their time? Merely producing hypothesis that could only be disproved but never proved? If that were the case, before too long, the scientific laboratories worldwide would be filled with discarded scientific hypotheses and nothing else. That, of course, did not happen.

1.3.2 Proving a hypothesis via variations of the Scientific Method

To begin, I should remind the reader that the Scientific Method is limited in practice to deal with observations that lend themselves to experimental testing. You can do an experiment on rats but you cannot do an experiment on the Black Hole. Notwithstanding, scientific hypotheses that do not lend themselves to experimental testing can and have been proved with the aid of non-experimental methods. By variations of the Scientific Method, one can also prove observations that do lend themselves to experimental testing, as I shall also demonstrate below.

1.3.2.1 PROOF BY MATHEMATIC METHODS

That the square of the length of the longest side of a rectangular triangle equals the sum of the squares of the respective length of its two shorter sides is a scientific truth introduced independently by Pythagoras of ancient Greece and by an ancient Chinese scholar. And each had provided a distinctly different proof (Brownowski 1973.) The one often seen in Encyclopedia and easier to follow came from China (given in the treatise, Zhou Bei Suan Jing.) Thus both the ancient Greek and ancient Chinese have done what Popper (and Feyerabend) thought impossible: proving a hypothesis.

1.3.2.2 PROOF BY IMPROVING THE METHODOLOGY

The hypothesis is that the earth is round or better, spherical. Ferdinand Magellan unwittingly conducted an experiment proving that the earth is indeed round or spherical by sailing steadily westward until his surviving crew returned to the place where they started

from, Seville of Spain. Popper, however contended that this is a hypothesis that cannot be falsified (Theocharis and Psimopoulos 1987 p. 595, Col. 3.) I disagree. It would be falsified if Magellan and/or his crew sailed into empty space off the edge of the flat earth.

However, there is a valid and but different reason that Magellen and his crew might not have proved that the earth is spherical: for the returning crew could have proved the alternative hypothesis that the earth has the shape of a cone or a cylinder that is continual in less than three dimensions. But then all we need is to find a better experimental technique to conduct a different experiment. This time, it was a spacecraft rather than a ship sailing on the ocean waves.

Thus on December 11–12, 1990, the spacecraft Galileo took advantage of the solar eclipse ongoing on those days, and shot many pictures including a movie of the earth turning around and around revealing all sides of the sphere. We then have succeeded in proving that the Earth is spherical.

1.3.2.3 PROOF BY DISPROVING ALL OTHER ALTERNATIVE HYPOTHESIS

Suppose you were camping and found local mosquitoes were making your vacation intolerable. Think hard and you would probably reach the conclusion that there are only three ways to protect yourself: (1) install a mosquito-proof screened enclosure or net; (2) remove the pests by killing them or catch each pest that gets close and release it at a far-away location; (3) spray the near-by space with something that the mosquitoes do not like: a repellent. In fact the equivalent of each of these models has been proposed by cell physiologists to keep Na^+ level low inside the living cell. They are respectively:(1) the sieve theory; (2) the sodium pump theory and (3) the polarized-oriented multilayer (POM) of cell water (which partially excludes Na^+.)

Radioactive tracer studies have shown that Na^+ in fact traverses the cell membrane with ease, disproving model 1; energy inadequacy disproved model 2, leaving only the third choice still intact. In fact, the prior exclusion of the only existing alternative competing theories has proved the validity of the only known alternative, namely the POM theory of cell water as an integral part of the AI Hypothesis.

However, if someone claims to have found a fourth mechanism to get rid of mosquitoes or to partially exclude Na^+, it would most likely prove invalid because the POM theory has already many converging proofs—see Section 1.3.2.5 below. This is the beauty of the truth: there is only one.

1.3.2.4 PROOF ON A NON-ENDING LIST OF MODEL SYSTEMS

When the fragility and/or complexity of a living system forbids proving a hypothesis experimentally directly upon it, one can find or invent cogent models and test the hypothesis experimentally on these less fragile objects. Indeed, there is almost no limit on how far one can go in this general direction. For an example, see Section 5.2 below.

1.3.2.5 PROOF BY CONVERGING EVIDENCE

Confirmation of our hypothesis by our own laboratory or by other laboratories using similar or different techniques is an important step in proving a hypothesis on phenomena that are highly complicated like life. Two other variations would further enhance the validity: (i) retroactive confirmation from experimental studies carried out in the past by investigators who had no idea of the theory yet to evolve; (ii) experimental data that came as refutations of the theory but when carefully examined with or without new data turned out to be supportive evidence.

2 The great breaks that paved the way

As mentioned above, I take great pride in my role in the scientific accomplishment summarized in the title of this article. I also believe it an unexcelled privilege to do what I have been doing all my life.

For a start, I mention one specific happy event of my life: I came to the United States to study cell physiology right after the end of WWII. I could do this because I had won shortly before a nationwide competitive examination in China. It was a competition for the (one) biology slot among 22 so-called Boxer Indemnity Scholarships (each on one specific subject) to continue advanced education in the US (see *Boxer Indemnity Scholarship Program* listed under Reference.) And it was also a time of forward-looking optimism in the US too, not unlike that underlying the government-funded pure scientific research in Germany of the 18th and 19th century. This pervasive self-confidence of America was also eloquently portrayed by the title of a report written by Vannevar Bush—, the science advisor to both President Franklin D. Roosevelt and President Harry S. Truman—, "***Science:—the Endless Frontiers***."

And for the first time in American history, the US federal government began funding basic scientific research with no strings attached (Atkinson and Blanpied 2008.) The GI Bill of Rights provided financial support for many returning veterans and many of them took up higher education and research in the biomedical fields. The increasing demands for research needs in turn stimulated the growth and creation of a great variety of scientific instruments, chemicals and radio-chemicals that had not existed before—or after. It was in a happy, proud and tolerant atmosphere I received generous supports from all the institutions that I attended in Chicago, in Baltimore and in Philadelphia from 1946 on.

Then suddenly the smooth-sailing American ship hit rock—in the form of the Vietnam War—a war that cost the lives of 60,000 young American and over one million young and not so young Vietnamese, Cambodian and others. As pointed out recently by Michael Keen, this war could very well have been prevented (Keen 2011.) However, we did go into the war and we did not win.

Worse, we also lost the future-oriented optimism on science—somewhere between the late 1960's and the early 1970's. In the dark mood of frustration and despair, which the faltering Vietnam War and the growing skepticism about the benefits of scientific research described in Sect. 1.3.1, cutback on research support followed. Added yet on the woes is that created by the ***peer review system*** installed for government fund allocation and it began to raise its ugly head. For who were they that advised the Chinese Emperor to dismiss the little village boy who told him that the elephant is not like a rope, a wall or a tree trunk but more like a big pig with a long nose. It was the panel of learned (but blind) scholarly peers that could not agree among themselves except in rejecting the little village boy. Who were they that advised Queen Isabelle of Spain to turn down Columbus's plan to reach China by going east? Again it was a panel of three navigator peers. Who were they that had delayed 170 years the acceptance of the Kinetic Theory of Gases and the reality of atoms and molecules? Again it was a collection of scholarly peers. (For yet more of the deadly track of peer review see Ling 1978a, 1998c.)

That I could in fact continue my work for some two more decades was to no small measure the gifts of a few courageous and dedicated scientists-administrators including Dr. Arthur B. Callahan of the Office of Naval Research (ONR) and Dr. Steven Schiaffino of

the National Institute of Health (NIH). But they eventually retired for one reason or another (Ling 2001 p. 367.)

Notwithstanding, I must not overlook my blessing. Thus, before the arrival of still harder times, the ***association-induction theory*** was already well on its feet in theory as well as in experimental verification (Ling 1962, 1965, 1969.) This is why I could announce on a prior page that Mankind now in fact understands what life is in modern physico-chemical terms.

For, in my belief, there cannot be a still more basic and more comprehensive theory in foundation biomedical science than the AI hypothesis. Accordingly, the ***principle of sequential invention*** (Ling 1952) tells us that like the incubation and hatching of a fertile egg, this proven new basic theory would one day begin to generate a world of mankind—benefiting products in areas closest to our long-term wellbeing. To mention just one, rationally designed drugs to combat incurable diseases already in existence and yet to come. *It is the AI Hypothesis that for the first time in history tells us what drugs do to life electronically in modern microscopic physico-chemical terms and its theoretical predictions have already been repeatedly confirmed* (Ling 1962 Chapter 6, pp 107–120; Ling 1984 Figure 7-10 and Figure 7-11 on pp. 204–205; Ling and Fu 1987, 1988.)

But to present the new definition of life and its underlying association-induction hypothesis, I must first update what had happened to biology since mid-19th century with the birth of the new science of cell physiology.

3 The beginning of cell physiology

3.1 The Berlin research university

The great Prussian philosopher, linguist and statesman, Wilhelm von Humboldt (1767–1835) played a key role in introducing the state-supported physiological (and other scientific) research in what came to be known as *research universities* (Humboldt, W. 2008.) Between 1830 and 1850, new physiological laboratories were springing up in the universities all over Germany. Its overall purpose was nothing else than the promotion and nurturing of the search for truth—labeled *Wiesenschaften* or pure science (McClelland 1980, Part III; Ling 2007 p.6.)

Among the research universities established, the first and foremost was the Berlin University. In 1949 what was originally called Berlin University changed its name for the last time to Humboldt Universität in honor of its founder, Wilhelm von Humboldt—and his naturalist brother, Alexander von Humboldt (Humboldt, A. 2010.) To show how well the von Humboldt brothers, but especially Wilhelm von Humboldt deserved this honor and to avoid giving the wrong impression that the former Berlin University only funded physiological research, I cite a list of its illustrious alumni and teachers below (Humboldt University 2010) in addition to those to be mentioned farther beyond in this article:

PHYSICISTS: Max Planck (1858–1947), Albert Einstein (1879–1955), Werner Heisenberg (1901–1976), Max von Laue (1879–1960), Erwin Schrödinger(1887–1961), Max Born (1882–1970), Heinrich Herz (1857–1894), Gustav Herz (1887–1975); **CHEMISTS:** Herman Emil Fischer (1852–1919), Fritz Haber (1868–1934), Jacobus Henricus van't Hoff (1852–1911); **PHYSICIANS:** Rudolf Virchow (1821–1902), Paul Ehrlich (1854–1915), Robert Koch (1843–1910); **PHILOSOPHERS:** Georg Wilhelm

Friedrich Hegel (1770–1831), Johann Gottlieb Fichte (1762–1814), Arthur Schopenhauer (1799–1860), Karl Marx (1818–1883), Friedrich Engels (1820–1895); **STATESMEN:** Otto von Bismark (1815–1898); **POET:** Heinrich Heine (1797–1856); **COMPOSER:** Felix Mendelssohn (1809–1847).

3.2 The cell theory and the membrane (pump) theory

Johannes Müller, a gifted scientist and popular teacher, headed the physiological research institute in the Berlin University. Among the large number of brilliant students he collected around him were the great physiologist-physicist, Herman Helmholtz (1821–1894) and two other members of the "Reductionist Four": Emil Dubois-Reymond (1818–1896) and Ernst Brücke (1812–1892.) The fourth member, Carl Ludwig (1816–1895) was in Leiden (Rothschuh 1973.) As if they were one, the *Reductionist Four* believed that the laws governing the dead world govern the living world too (Rothschuh 1973.) Their overall phenomenal success notwithstanding, the Reductionist Four were not able to resolve the central problem-what life is. The right time for that was yet to come.

First, the basic physical and chemical sciences were themselves still in their early stages of development. Secondly, it was organ physiology that the Reductionist Four were pursuing and organs are not the most basic unit of life. In fact, the cell was already on the way of being recognized as a more basic unit of life by another zoology student of Müller, Theordor Schwann (1819–1882.) In collaboration with botanist, Mathias Schleiden (1804–1881), Schwann introduced the "Cell Theory" in 1839 (Schwann 1839; Schwann 1847; Harris 1999 Chapters 10 and 11.) (For other earlier introductions of the cell theory, see Ling 2007 p. 5; also see Dutrochet 1837.)

Financial support from the government was one contributing factor to Schwann's success in formulating and publishing his "Cell Theory"; the availability of the microscope was another. The deployment of microscopes had also led to the discovery of an even more basic substance of life than the cell. Named ***sarcode*** in 1835 by the French zoologist, Felix Dujardin (1801–1960) who described this substance as a glutinous, translucent and water-insoluble living jelly (Dujardin 1835; Harris 1999 pp. 72–75; Ling 2007 pp. 10–17.)

The critical importance of the sarcode was dramatized by historian Thomas Hall in his treatise on "Life and Matter" (Hall 1969.) In the opening section of Chapter 14, Hall remarked that up to that point, the preceding history he presented in the first thirteen chapters could be regarded as preparation for what would be the subject matter of this Chapter 14. And what is the subject matter or title of that Chapter 14? It is nothing else than *Sarcode*, the living jelly from a protozoon. However, the name sarcode was later replaced by ***protoplasm***—introduced in 1846 by the German botanist, Hugo von Mohl (1805–1872) when he referred to a similar gelatinous substance in plant cells (von Mohl 1846; Harris 1999 p. 72.)

While I have always felt a sense of regret that an earlier name be replaced by a later one, especially since Dujardin went out of his way to give credit to still earlier workers (See Ling 2007 p. 113.) However, there is a defensible justification for this change. The word sarcode came from the Greek word, *sarkodes,* meaning fleshy, which is more appropriate to describe a substance of animal origin and protozoa are tiny animals. To cover both animal and plant materials, protoplasm is a better choice.

Figure 1 shows a viscous stream of plant protoplasm flowing slowly out the cut end of a giant cell of the alga, Nitella (Kuroda 1964.)

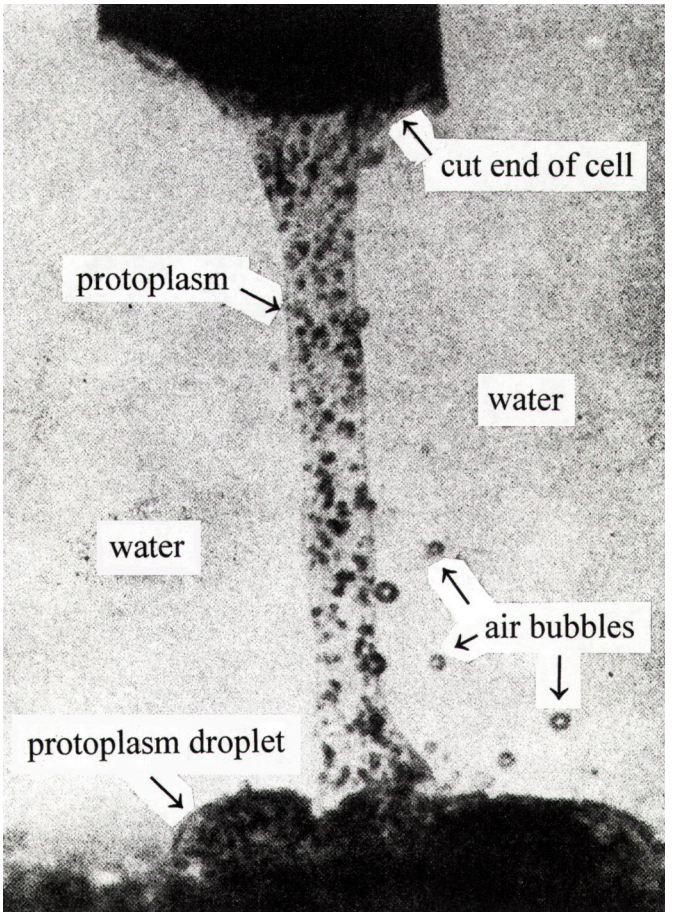

FIGURE 1. Outflow of protoplasm (endoplasm) from the cut end of a giant Nitella cell into a culture medium (labeled as water). The protoplasm collected as a flattened round droplet on the bottom of the cuvette. The photograph was taken 5 minutes after the cut was made. These protoplasmic droplets can survive 10-50 hours in the culture medium containing 80 mM KNO_3, 50 mM NaCl and 4 mM Ca $(NO_3)_2$. (From Kuroda 1964)

The invention of electron microscope and ancillary techniques enabled cytologists to demonstrate that the real cell membrane is only some 100 Angstrom units thick. As such, it is beyond the (ultimate) resolving power of the best light microscopes at 2000 Angstrom units (Davidson 2012.) What this tells us is that Schwann could not have seen and did not see the real cell membrane.

One sequence of Schwann's mistakes began with his erroneous assumption that the large mature plant cell (Figure 2), with its immense watery fluid-filled central vacuole is typical of all plant and animal cells. *He then called the outermost cellulose cell wall* (of a mature plant cell) *plus the layer of cytoplasm lying immediately beneath the cellulose cell wall plus the real cell membrane (together) as the* ***cell membrane*** *(or the* ***cell wall****.)* As a part of his Cell Theory, Schwann then postulated that imbedded in this thick "cell membrane" are microscopic devices (to be called pumps later by others) that control the chemical composition of the fluid inside and outside the cell (Schwann 1839 p. 197; Schwann 1847 p. 199.)

Now, Johannes Müller—who was a full professor as well as the Director of the Physiological Research Institute in the Berlin University—shared a single small room with Schwann; the two also shared the use of a single microscope (Rothschuh 1973.) Both facts testified to the limited financial support even the top ranking Berlin University was

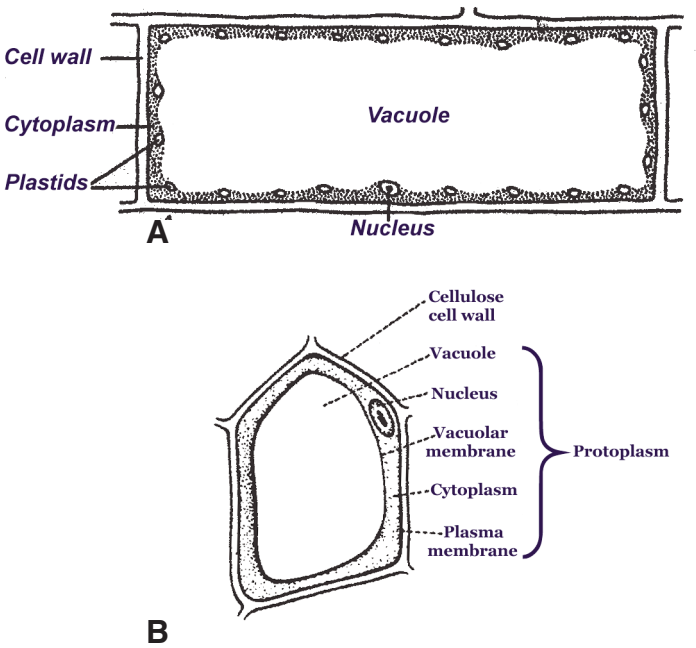

FIGURE 2. Two diagrammatic illustrations of mature plant cells. A. From Heilbrun (1937) who reproduced it from Miller's book of Plant Physiology; B. From Glasstone (1946.)

receiving from the government. Knowing what fantastic achievements these research universities but especially what the Berlin or Humboldt University had accomplished, its limited financial support carries a highly important lesson for future decision-makers on the financial support of science.

It suggests that a modest amount of money used wisely can produce far greater results than a lot of money spent thoughtlessly, an extravagance that not only wastes money but tends to bring into the search for truth, competing alternative motivations like search for power, which money is.

Beyond the small room and the microscope, Müller and Schwann also shared a belief in *vitalism*. Schwann's belief in vitalism was explicitly described in his Magnus Opus (Schwann 1839 p.184; Schwann 1847 (English transl.) pp.186–187.) Since all European universities began as religious institutions to educate future clergies, one is not surprised that even though Schwann's theory contained many serious errors known already at the time, the German textbook producers adopted it without question or dispute (Harris 1999 p. 106.) And as mentioned earlier, Schwann's theory of cells and what came to be known as membrane pumps has remained in textbooks worldwide to this very day, long after it had been disproved (see p. 6.) Again, my conviction is that if we work hard and intelligently on the subject, we will replace it with the right one not too long from now.

3.3 Protoplasmic theory

Ironically, Schwann's membrane-pump notion was far from being universally accepted in Germany at his time. Thus in 1861, thirteen years after Schwann introduced the membrane-

pump concept as a part of the **cell theory,** Max Schultze (1825–1874), Professor of Botany in Bonn, pronounced his *Protoplasmic Doctrine*, according to which, the living cell is a lump of protoplasm with a nucleus but **without a cell membrane** (Schultze 1861; Hall 1951.)

Seven more years later, another historical event took place. Thomas Huxley (1825–1895), described by the straight-shooting Baltimore Sun reporter, H. L. Mencken (1880–1956) as the greatest of English scientists (Mencken 1925)—mesmerized a lay audience in an Edinburgh Presbyterian Church by proclaiming that *protoplasm is the physical basis of life*. The issue of the journal that printed his talk was reprinted an unprecedented seven times (Huxley 1853.) But I must also make clear that it was not bed of roses for Huxley either.

One detractor close by was the Scottish philosopher, James Huchison Stirling (1820–1909.) Stirling pooh-poohed the existence of protoplasm by stating that the same substance could not be at once beef, lobster and the man who eats them (Hall 1969, vol. 2, p. 308.) Another question that could have been asked but was not asked at that time can be put this way: Is the darker nucleus that looks quite different from the surrounding protoplasm also a part of the physical basis of life?" We will return to this subject shortly below on page 18. Answers to Stirling's comment touches on the central role of ATP in life and death, for which the answer will come in the concluding Section 5.1.) For the moment, let us return to the time of Thomas Huxley, his friends and foes.

Far away on the opposite side of the planet, a tavern-owner in Melbourne publicly advertised a well-cooked physical basis of life. Taking offense at this flippancy, Sir Joseph Lockyer came to Huxley's defense in the very first issue of the magazine, **Nature** he had just founded. Lockyer pointed out with vigor and conviction that Huxley took risk in addressing a lay audience not for his own glory but for the common good of all Mankinds (Lockyer 1870.) Yet, by profession, Lockyer was an astronomer, not a cell physiologist. His vigorous defense of Huxley bespoke of a time that leading scientists took themselves seriously of their global responsibility in educating and caring for the future inhabitants of this small planet we share.

In the wake of the historic contributions of Max Schultze and Thomas Huxley, the protoplasmic approach flourished in the remaining decades of the 19th century. Indeed, as late as 1908, William A. Locy, Professor of Biology at the Northwestern University of Illinois in the United States wrote these words in his "*Biology and its Makers*": "All future progress will be made by studying this living substance (protoplasm)—the seat of vital activity. This was the beginning of modern biology." (Locy 1908.)

Locy too would have turned in his grave if someone were to tell him what happened to protoplasm after his optimistic forecast. It was quietly removed from textbooks and what we teach to the younger generation, ostensibly as a new addition to the list of once prominent but ***genuinely*** erroneous concepts—like Lavoisier's *caloric* and Georg Stahl's *phlogiston*.

Thus, advances in microscopy and related fixing and staining techniques have revealed in cells more and more sub-cellular structures and organelles. Like the nucleus, they too looked different from what Dujardin and von Mohl once described as sarcode or protoplasm. Summarizing this anticlimactic ending, the Encyclopedia Britanica Online stated in 2011: "*As the cell has become fractionated into its component parts, protoplasm, a term no longer has meaning.*" In truth, Encyclopedia Britanica Online and like-minded instigators of the disappearing protoplasm are all wrong.

And, they are not just wrong but wrong in a strange way—repeating an error made in an earlier attempt to erase the concept of protoplasm in the 1930's. In both cases, what were disproved were not the existence of protoplasm but two sets of mistaken theoretical concepts on the nature of protoplasm. (Details of the earlier case will be found on page 30 below.)

In this, the second suggested trashing, the wrong theoretical concept was not even explicitly pronounced but was assumed to be true by others. That concept is, Dujardin and von Mohl had considered all protoplasms to be in texture and appearance like the translucent, glutinous, water-insoluble living jelly that flows out a broken protozoa or large plant cell as shown in Figure 1. Repeating what was mentioned briefly above, I shall discuss the details of the wrong theory that led to the first false abandonment of the concept of protoplasm in the section on cell-water in Section 4.3 below.

Happily, in both cases, the AI Hypothesis was able to come to the rescue (see below.) As a result, the abandoned protoplasm was resurrected again and Locy's optimistic outlook re-established a second time.

4 The association-induction hypothesis

It took me all told 15 years to complete the new unifying theory of the living cell called the **association-induction hypothesis** or AI Hypothesis for short and AIH for shorter. The theory arrived in three parts. The first part is an embryonic version of the AI Hypothesis known as Ling's Fixed Charge Hypothesis centered on the selective K^+ accumulation in living ells (Ling 1952.) The second part marked the development of the AI hypothesis proper (Ling 1962.) The third part was first introduced under the name, the Polarized Multilayer (PM) theory of cell water and model systems (Ling 1965), only to be replaced later by the name, the Polarized-Oriented Multilayer (POM) theory of cell water and model systems (Ling 2003.) However, it was the introduction of the PM theory in 1965 that completed the presentation of the unifying theory.

In order to present the answer in a readily understandable manner to the question, What Is Life?, the relevant parts of the entire AI Hypothesis will be reviewed beforehand. But to make the presentation of the relevant parts of the AI Hypothesis themselves easily readable, I will present its two intellectual foundations or supporting pillars first—as I had done once in 1962 and I am doing it here once again.

4.1 Foundation pillars

The twin pillars on which the ***association-induction hypothesis*** is built are (1) the science (physics) of Statistical Mechanics and (2) the (correct part of) protein and polypeptide chemistry and physics. Accordingly, the first chapter of the monograph "*A Physical Theory of the Living State*" presents the fundamentals of *Statistical Mechanics*, which as pointed out earlier, the ill-fated Austrian mathematician-physicist, Ludwig Boltzmann invented almost single-handedly. In the 7th Chapter of the same monograph, I also introduced a new theory of proteins founded on the basic knowledge on proteins, which the great German chemist (Herman) Emil Fischer uncovered largely in the late 19th and early 20th century (Fischer 1906.)

4.1.1 Basic protein chemistry

Long before Locy wrote his prophetic comment on the living matter, Emil Fisher (1852-1919) had worked out the complex structure of proteins, which form the distinctive components of all living matter. Fischer also introduced the name, ***polypeptide***, which is a unique kind of organic macromolecules made only by living organisms (including synthetic organic chemists and engineers.) (Fischer 1906)

The chemical reaction that produces a polypeptide from free individual α-amino acids (or simply amino acids) is illustrated as follows:

$$NH_2\text{-}CH\text{-}(R_i)\text{-}COOH + NH_2\text{-}CH\text{-}(R_i)\text{-}COOH + NH_2\text{-}CH\text{ -}(R_i)\text{-}COOH \rightarrow$$

$$NH_2\text{-}CH\text{ -}(R_i)\text{-}CONH\text{-}CH\text{-}(R_i)\text{-}CONH\text{-}CH\text{-}(R_i)\text{-}COOH + 2H_2O. \quad (1)$$

Note that two *peptide linkages* (CONH) are formed from three free amino acids. Between each pair of amino acids that form a peptide linkage, two hydrogen atoms and one oxygen atom are lost in the form of two water molecules. The remainder of each amino acid in the protein formed is called an *amino acid residue*.

As a rule, each amino acid residue endows the protein a different side chain shown as R_i in the formula for the amino acids given above. As an example, R_i is a single H atom for the amino acid residue glycine; it is a methyl group for the alanine residue. R_i for aspartic acid residue is CH_2COOH, carrying at its end a β-carboxyl group. R_i for glutamic acid residue is CH_2CH_2COOH, carrying at its end a γ-carboxyl group. R_i for lysine residue is $(NH_2)CH_2\ CH_2\ CH_2$, carrying at its end an ε-amino group. R_i for arginine is $NH_2C(NH)_2\ CH_2CH_2CH_2$, carrying at its end a guanidyl group. In a neutral aqueous medium, the β-, and γ-carboxyl group are ionized and each carries a net negative charge and thus functioning as a mono-valent anion. In contrast, the ε-amino group and the guanidyl group each carries a net positive charge and as such, it functions as a mono-valent cation. Immobilized by their anchorage onto the lengthy protein chains, these anions and cations are referred to respectively as *fixed anions* and *fixed cations*. When a fixed cation joins a fixed anion and forms an electrostatic bond, a ***salt linkage*** is formed (Speakman and Hirst 1931.) Salt linkages, like its counterpart ***α-helical H bonds***, determine mostly the folding patterns known respectively as the tertiary and secondary structures of a protein. (For strong evidence of the key role of salt-linkages in the maintenance of the tertiary structure—contrary to belief of some protein chemists—, see p. 24 in Sect. 4.2 below.)

A distinctive feature of all life forms is its pervasive *connectedness*. The underlying long-distance information and energy transfer has been compared to that of a chain of falling dominos, (which goes only one way and thus irreversible) or a chain of tethered frictionless see-saws. In the latter case, a tiny perturbation (like that brought about by a curious visiting mouse) at one of the terminal seats of the chain can cause the entire chain to flip from one stable conformation to the only other alternative stable conformation as illustrated in Figure 3. (See also Ling 1962 pp. 145–146.)

The basic mechanism in both the falling domino chain and the chain of frictionless seasaws is mechanical and relies on gravity. In contrast, the long distance information and energy transfer in proteins is, according to the AI Hypothesis, fundamentally electronic as

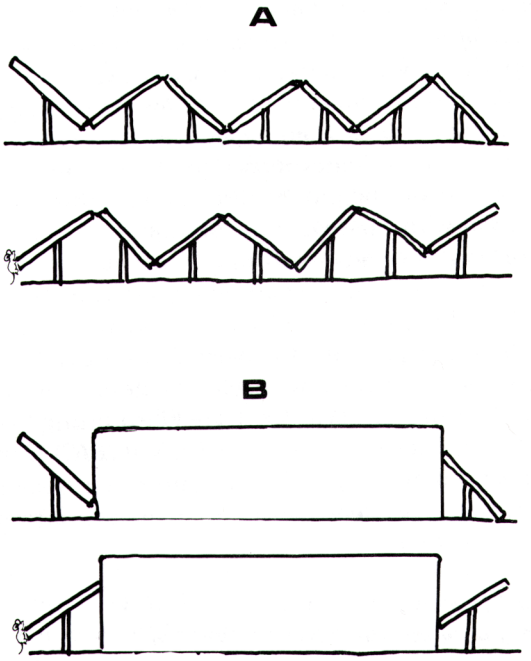

FIGURE 3. Mouse and Seesaw Chain Model. Figure A demonstrates how extensive changes over long distance can be achieved in a delicately balanced system in response to a minute energy input at a suitable site. Partial perception of the system (Figure B) produces the impression of a "magical happening". (From Ling 1992)

the word, *induction* indicates (Ling 1962 pp. xxxii–xxxvi.) And yet, protein chains are not electronic conductors. Quite the contrary, silk fibers, comprising a single pure protein, called silk fibroin, have been traditionally used to suspend and insulate pith balls in early demonstration of electric attraction and repulsion.

While all proteins share *connectedness*, each protein is distinct from all other proteins. In their diversity, proteins resemble words in some languages. Thus, the diversity of words in the English language comes from linear arrays of assortments of the 26 letters of the alphabet. The diversity of a protein, on the other hand, comes from a linear array of assortments of some 20 amino acid residues. That the words, *eat* and *ate* are different in meaning is entirely arbitrary. That the small polypeptide A (glu-gly-lys) differs from the small polypeptide B (glu-lys-gly) has an ***electronic*** basis.

Thus, the γ-carboxyl group carried on the side chain of the glutamic acid residue (glu) in polypeptide B is a stronger acid than the γ-carboxyl group in the polypeptide A. This difference arises from a combination of two causes. First, the γ-carboxyl group is closer to the positively charged ε-amino group of the lysine residue (lys) in polypeptide B than in peptide A. Second, the neutralizing influence produced by the positively charged ε-amino group diminishes with the distance separating the *effector* group and the *target* group of a large molecule. Weakening of the fixed anion for the positively charged hydronium ion, H^+, means that more free H^+ exists in the medium and that, in turn, reveals that its source

acid is a stronger acid—in a sequence of elementary events that will be further elaborated upon below.

An electronic effect that is transmitted through intervening space is called a direct or D-effect. On the other hand, electronic effect transmitted through the intervening linked atoms is called an inductive or I-effect. I-effect and D-effect acting together are known as F-effect (Hermans 1954.) Next, we discuss one kind of the *target group* of these electronic effects in the form of the carboxyl group of acetic acid or HAc.

In neutral water, part of the acetic acid dissociates into a positively charged hydronium cation, H^+ and a negatively charged anion, Ac^-:

$$HAc \leftrightarrow H^+ + Ac^-. \tag{2}$$

When equilibrium is reached, there will be a quantitative relationship among the concentrations of the individual ions and molecule as shown next.

$$K_a = ([Ac^-] [H^+]) / [HAc] , \tag{3}$$

where $[Ac^-]$, $[H^+]$ and [HAc] are the molar concentration of the dissociated acetate anion, the dissociated hydronium ion and the undissociated acetic acid respectively and K_a *is* the *acid dissociation constant*. Shown in the following Equation 4 is the pK_a of this acid (HA) equal to the negative logarithm of the acid dissociation constant, K_a to the base 10:

$$pK_a = -\log_{10} K_a . \tag{4}$$

Having made clear what pK_a stands for, we proceed to examine how it is determined by the molecular structure of the acid. Consider as an example acetic acid, CH_3COOH. It is an acid that makes our salad pleasantly sour but not too sour. Accordingly, it is a weak acid with a high pK_a equal to 4.756. In contrast, trichloroacetic acid (TCA), CCl_3COOH is a chemical agent we use in the laboratory to denature proteins and make them insoluble in water and thus easily separable from other components of an aqueous mixture. Though sharing the same carboxyl group with acetic acid, trichloroacetic acid or TCA is a very strong acid. Its pK_a is below unity at 0.66.

The profound difference in pK_a of acetic acid and TCA tells us that the negatively charged oxygen atom in the dissociated carboxyl group attracts and thus holds onto the hydronium ion much more tightly in acetic acid than in TCA. This difference in turn reflects the profoundly different impact the attachment (onto the α-carbon atom of the acid) of a chlorine atom has than that of a hydrogen atom on the pK_a of the adjacent carboxyl group. The reason is as follows.

The atomic weight of the chlorine atom is 35.5 and its atomic number is 17. This number indicates that there are 17 protons in the nucleus of each chlorine atom, whereas there is only one proton in the nucleus of a hydrogen atom. Since each proton carries a positive charge, the orbiting negatively charged electrons in a chlorine atom are much more strongly attracted to the nucleus of a chlorine atom than the orbiting electron in a hydrogen atom is attracted by the single proton in the nucleus of a hydrogen atom. When three H atoms on the α-carbon atom of acetic acid are replaced by three chlorine atoms, electrons in the vicinity are drawn toward the chlorine atoms and their aggregate impact is

passed on to reach the distant singly charged oxygen atom of the carboxyl group. The net result is a reduction of the effective negative charge of the oxygen atom and a lowering of the attraction between that oxygen atom and (free) hydronium ion, thereby making TCA a much stronger acid than acetic acid. Indeed, it is precisely with this example of acidity change from acetic acid to TCA that G. N. Lewis introduced his Induction Theory in 1923 (Lewis 1923.)

The development of Quantum Mechanics revolutionized physics and chemistry. In 1933 James and Coolidge using elaborate wave mechanical methods, were able to derive quantitative attributes of the hydrogen molecule with accuracy to the sixth decimal place (James and Coolidge 1933.) For a while, many felt that it was a matter of time before physicists would be able to solve all chemists's problems with immense accuracy. However, this optimistic outlook was less than realistic. Indeed, in the ensuing years to this very day, neither James, nor Coolidge nor anyone else has succeeded in explaining in wave-mechanical or other terms, the striking difference in the pK_a of acetic acid and TCA. Indeed, we are back at the roots again. In dealing with isolated single bodies like a distant star or even a single hydrogen molecule, physicists can do marvels. Again in dealing with vast number of items, statistical mechanics can provide equally accurate computations. It is in dealing with entities somewhere between, like an acetic acid, that it is hopeless to try to achieve the kind of accuracy physicists are used to in their chosen models of utter simplicity.

Lewis's Induction theory and its follow-ups have provided the backbone of theoretical organic chemistry in the hands of Hammett (1940), Branch and Calvin (1941), Ingold (1953) and Taft and Lewis (1958.) Both Hammett and Taft have provided useful empirical constants for chemical groups that can be used to predict quantitative data of new chemicals. However, it was Chiang and Tai who have profoundly improved the theory by liberating it from the restriction imposed by the limited empirical constants available. They did it by introducing a method of calculating what they call ***inductive indices***—from known and accessible parameters of molecular structure, atomic electronegativity and bond length (Chiang and Tai 1963. For brief description of key ideas, see Ling 1984 pp. 185–188; Ling 1992 p. 113.)

4.1.2 Statistical Mechanics

When you flip a coin, there is no way to foretell whether tail or head would come up. However, if you flip the coin a million times, you can foretell with great accuracy that half would be heads, the other half would be tails. The mathematical science that deals with laws governing large numbers is, of course, *statistics*.

In the 17th century, Boyle's Law was discovered. It tells us that the volume (v) of a body of gas is inversely proportional to its pressure (p) so that the product pv is a constant. However, the foundation of this law was entirely empirical. For that reason, it could not explain why pressure is exerted on the container wall in all directions. In 1738, Dutch-Swiss mathematician Daniel Bernoulli (1700–1782) first introduced the ***Kinetic Theory of Gases***, in which air or gas represents a vast number of rapidly moving, extremely small corpuscles or gas molecules, their bombardment on the container wall producing the all-directional pressure. However, Bernoulli's theory was rejected. So were three other independent physicists each presenting an improving but basically similar theory. They

include English physicist, John Herapath and a Scottish scientist employed in India, John James Waterston and the Austrian mathematician-physicist, Ludwig Boltzmann. All told it took one hundred and seventy (170) years before physicists finally accepted atoms (and molecules) as real—unbelievable as it appears to me today (Brush, S.G. 2003.) Of more direct relevance to my work, however, was the fact that Boltzmann did not just present the most advanced version of the kinetic theory of gases. He also introduced the new science of ***statistical mechanics***, *which explains macroscopic natural phenomena in terms of the vast number of microscopic atoms and molecules in precise quantitative terms* (Cohen 1997.)

Both the membrane (pump) theory (of Schleiden and Schwann) and the *original* protoplasmic theory (of Dujardin, von Mohl, Dutrochet and Huxley) were attempts to explain natural phenomena in terms of ***macroscopic*** concepts like membranes, semipermeability for the membrane (pump) theory and glutinous, diaphanous and water-insoluble traits for the (original) protoplasmic theory. Like the Kinetic Theory of Gases, the association induction hypothesis is a theory that attempts to explain macroscopic (living) phenomena in terms of vast numbers of **microscopic** molecules, atoms, ions and electrons—as made clear in the title of this communication.

As an example, in the immediately following section is a very useful statistical mechanical formulation on the distribution of molecules in space called the *Boltzmann distribution law*. (See *Luke, BT & Assoc*. in the Reference List.) With its help, one can, for example, determine the density of dust particles in the atmosphere in locations not too far from the surface of the earth. More precisely, the kinetic energy of the dust particles tends to move the dust particles farther away from the earth while gravity restrains that movement. The relative density of dust particles is then expressed as an exponential function of the ratio of gravitational attraction energy divided by the average kinetic energy equal to kT, where T is the absolute temperature and k is the Boltzmann constant. A similar formula will be used in the new theory of selective accumulation of one species of cation, K^+ over another one, Na^+—in the embryonic version of the AIH introduced in 1952 and known as Ling's Fixed Charge Hypothesis (Ling 1952.)

In pages immediately following, I shall begin with a simple account of the key features of Ling's Fixed Charge Hypothesis. It will be followed by a simple account of the Polarized-Oriented Multilayer theory of cell water (Ling 1965.) After that, there will be a brief presentation of the association-induction hypothesis proper (Ling 1962.)

4.2 Ling's fixed charge hypothesis

Some time in the year 1950 I was, as usual, reading and thinking in the Welsh Library of the Johns Hopkins Medical School. Suddenly, a new idea struck my mind. It was a theoretical mechanism for the selective accumulation of K^+ over Na^+ in living cells (and in inanimate model systems) that would not require a continual supply of energy as in the membrane (pump) theory. For a moment at least, I was ecstatic.

The new idea arose from a synthesis of several basic facts of physics. First, properties of matter can be roughly sorted into two categories. Long-range attributes include sight and sound that can be perceived at different distances away from their origin. Short-range attributes include textures and taste of an object, which can be perceived only by direct contact. Now, the long-range attributes of the pair of mono-valent cations, K^+ and Na^+ are indistinguishable. In contrast, the short-range attributes of size are different in these two ions with a twist. The (naked) K^+ is larger than the (naked) Na^+. However, when brought

into contact with water, the smaller (naked) Na^+ takes on a more or less permanent coat of hydration water thicker than that taken up by the larger (naked) K^+. As a result, the hydrated Na^+ is substantially larger than the hydrated K^+. However, to experience this size difference, these cations have to be brought into close contact with a sensing device such as a set of fixed negative charges or fixed anions.

Fixed charges have been on the menu for a long time. Only those fixed charges widely considered up to that time are ***fully dissociated*** from their oppositely-charged free ionic partners or counter-ions (For illustrations, see Figures 2 and 3 on p.6 of Ling 2005.) This concept of full dissociation between the fixed charges and their counter-ions reflects the widely adopted principle of full ionic dissociation in aqueous media, often associated with names of scientists including physical chemists Jacobus van't Hoff, Wilhelm Ostwald, Sven Arrhenius and physicist, Peter Debye. It was therefore against the popular belief that I introduced in 1952 the new idea of enhanced ionic association when one of the reacting charges or ion is fixed in space in these words (Ling 1952 p. 769):

> " (i) The force of attraction between ions of opposite signs 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 when the kinetic energy of both reacting charged particles is made negligible, as for example in the macroscopic model of oppositely charged pith balls.
>
> (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. The individual fields thus overlap, and sum with respect to the force exerted collectively upon a free ion of opposite sign."

These simple statements referred to, as "*the Principle of Enhanced Ionic Association by Site Fixation*" was valid then, as it is valid today. And for what we try to do here, it was adequate then, as it is adequate now.

Notwithstanding, fifty-three (53) years after the 1952 publication, I further developed and presented the more detailed underlying theory in a paper entitled: "An Updated and Further Developed Theory and Evidence for the Close-contact, One-on-one Association of Nearly All Cell K^+ with β- and γ-Carboxyl Groups of Intracellular Proteins" (Ling 2005.) For those interested in this more advanced version, he or she can download a free pdf version online. (For direction, see Ling 2005 in Ref. List)

In addition, I want to add that the misguided adoption of the ionic dissociation theory described in Ling (2005, Appendix A on pp. 50–53) also made many protein chemists reluctant to recognize even today the idea that salt linkages formed between pairs of fixed cations and fixed anions are the most important part of the tertiary structure of many folded protein as I had suggested in 1962 (Ling 1962 p. 249) and repeated again and again ever since.

Referring to it as the "salt-linkage hypothesis", Ling and Zhang then published in 1984 a set of strong experimental evidence of the dominant role of the salt linkages in the maintenance of what has been referred to as the tertiary structure of many globular proteins (Ling and Zhang 1984; Ling 1992 p. 44; Ling 2001 p. 55, 238, 323.)

Having established the high propensity of the K^+ (or Na^+) to associate with the fixed negative charges, I then pointed out in the same 1952 article that in living cells, most of the fixed negative sites exist in the form of β- and γ-carboxyl groups carried respectively on the aspartic-acid and glutamic-acid residues of intracellular proteins. In frog muscle, I showed that the protein myosin alone carries enough β- and γ-carboxyl groups to associate

with all the K^+ and Na^+ found in the cell. The next step was to invent a mechanism of selective accumulation of K^+ over Na^+—as found in most living cells that have been carefully studied. And as made clear on a preceding page, I succeeded.

The Coulomb Law dictates that the electrostatic attraction between a positive electric charge and a negative electric charge varies directly with the product of the sign and magnitudes of the two charges and inversely with the square of the distance between the charges. However, when the ions are in water, the interaction is severely reduced by a familiar number called the *dielectric constant*, usually given a value of 81. But when the ionic interaction takes place at very close range in water, the phenomenon of *dielectric saturation* kicks in, lowering sharply the value of the dielectric constant according to the distance of separation as shown in the inset of Figure 4 (Hückel 1925; Debye and Pauling 1925; Hasted *et al* 1948; Grahame 1950; Ling 1952.) With these background information on hand, I then presented a mechanism of selective K^+ accumulation over Na^+ in living cells and model systems.

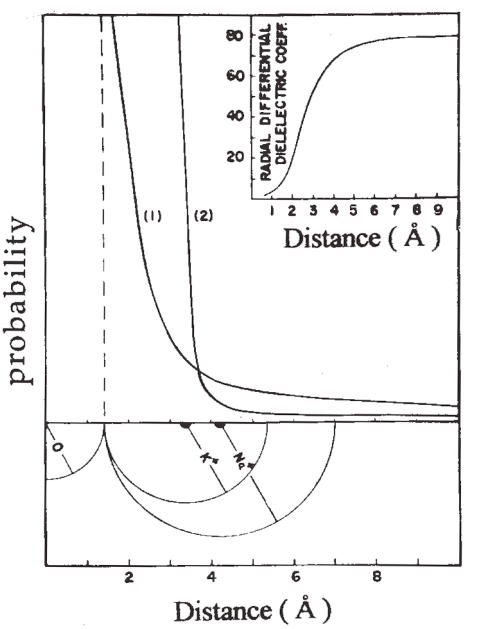

FIGURE 4. A theoretical model for the selective accumulation of K^+ over Na^+ in a fixed oxyacid site presented in 1952 as a part of Ling's Fixed Charge Hypothesis (LFCH). The computation takes into account the decrease in the dielectric constant of water (referred to in the Inset as "radial differential dielectric constant") when approaching a fixed ion as illustrated in the inset. Theoretical curve (2) shows the probability of finding a mono-valent cation (e.g., K^+, Na^+) associated with the fixed oxyacid anion—partially represented at the extreme left of bottom section of the figure—at a distance away from the center of the oxygen atom of the oxyacid group indicted on the abscissa in Angstrom units. Note that only the hydrated K^+ with its smaller radius (shown in the bottom figure) can enter the "shell of high probability of association" around the negatively charged oxygen atom of the oxyacid group and becomes preferentially adsorbed over the larger hydrated Na^+, the center of which stays largely out of the shell of high probability) also shown in the bottom part of the figure. (From Ling 1952)

Let us begin by focusing our attention on a ***single*** anionic oxygen atom of an oxyacid group like a β-, or γ-carboxyl group—among the vast number of similar anionic oxygen atoms inside a single muscle cell. The impact of dielectric saturation as illustrated in the inset of Figure 4 is to create around the anionic oxygen atom a shell of elevated probability for finding a free (mono-valent) cation. However, the main picture of Figure 4 shows that only the hydrated K^+ is small enough to enter into the space or "shell" of high probability and become accumulated therein. On the other hand, the larger hydrated Na^+ could not come that close to the center of the oxygen atom of the oxyacid group and must settle for a different space or "shell" of lower probability farther away. Since only one mono-valent cation accompanies each fixed mono-valent oxyacid anion, the larger hydrated Na^+ becomes thus selectively excluded by the competing K^+. This theoretical model yields a 7 to 1 preference of the smaller hydrated K^+ over the larger hydrated Na^+—, which is lower than found in many types of living cells, which could be as high as 40 (see below)— but in the right direction.

When I first discovered this new idea I was very excited and thus in a mood probably not unlike that of Archimedes (287–212 BC), when he found a new way to estimate the volume of irregularly shaped golden crown of unknown purity. But I did not go on the street naked shouting "Eureka!"—as legend tells us that our illustrious predecessor did. Yet I must have told a few friends excitedly about it and some of them remembered.

One early afternoon I was walking on the boardwalk of the Johns Hopkins Medical School in the general direction of the Welsh Library, when I saw an overflowing crowd at the entrance to the main (sloping) auditorium. Just after I had found out that it was Professor A. B. Hastings from Yale University giving a talk on his expertise subject, K^+ in living systems, I heard a cry in the audience "Is Dr. Ling here?"

For a moment I hesitated. But with encouragement from the audience, I ended up standing on the podium and trying to draw on the blackboard a picture like that shown in Figure 4—to illustrate how selective K^+ accumulation over Na^+ could be achieved without continual energy consumption. After I finished, Prof. Hastings, the honored guest speaker, walked over to me and shook my hand, saying at the same time that all his life he has suspected the selective K^+ accumulation in the living cell had something to do with the hydrated ionic diameters. And then he added: "Now you got it."

In years following, recollection of this moment keeps on returning to my mind. It is great man like Prof. A. B. Hastings that has made scientific research uniquely rewarding. And for this reason, I have been telling this story every time I had a chance and will continue to do so in the future—if only to show the young generation that the road to scientific discoveries is not all paved with jagged and dangerous rocks. It also has its softer and kinder moments.

In the six decades following the introduction of Ling's fixed charge hypothesis (FCH), many studies have been carried out in our laboratory and elsewhere. And they have consistently affirmed experimentally the validity of the theory that almost all K^+ in frog muscle cells are indeed (electrostatically) adsorbed one-on-one, in close contact on the β-, and γ-carboxyl groups of myosin and other cell proteins. The following are examples:

(1) According to Ling's FCH (and its later version the association-induction hypothesis [AIH]) virtually all cell K^+ compete for the same binding sites and the effectiveness of a specific kind of mono-valent cation in displacing other ions from these binding sites should vary with their respective short-range attributes. In contrast, if the K^+ is free as in the membrane or membrane (pump) theory, all mono-valent cations should behave and act entirely alike—qualitatively and quantitatively. Using radioactive

isotope-labeled K^+ and other alkali- metal ions, Ling and Ochsenfeld (1966) showed that the effectiveness of the same concentration of one hydrated ion in displacing two different mono-valent ions differs sharply.

(2) According to Ling's FCH (and its later version the AIH), in voluntary muscle cells, much of its cell K^+ are engaged in one-on-one, close contact adsorption on the bountiful β-, and γ-carboxyl groups of myosin found on the two edges of the A-bands (Engelmann 1873; Hanson and Huxley 1953.) The FCH (and AIH) predicts that cell K^+ or its surrogates should be found also on the two edges of the A-bands. In contrast, according to the membrane theory or membrane (pump) theory, K^+ and its strongly adsorbed surrogate mono-valent cations should be found wherever there is (cell) water. And accordingly, a more or less even distribution of K^+ throughout the cell is the expectation. However, since the I-band has a somewhat higher water content than the A band (Huxley and Niedergerke 1958), the even density of surrogate ions in the I bands should be somewhat higher than in the A bands.

Using a variety of sophisticated technologies, Ludwig Edelmann from Germany has developed one innovative technique after another, steadily and consistently refuting the prediction of the membrane or membrane (pump) theory. At the same time, these studies have provided some of the most convincing visual proofs that the surrogates of K^+ like Cs^+ and thalium ion (Tl^+) are like K^+ engaged in one-on-one, close-contact adsorption on the two edges of the A-bands in living (fully-hydrated) frog muscle cells as predicted in Ling's FCH (Edelmann 1988; Edelmann 1989; for review, see Ling 1992; see also Ling and Ochsenfeld 1991.)

Figure 5 from Edelmann (2001) provides a shining example. In (a) a 0.2 μ (or 200 nm)-thick section of a freeze-dried and embedded frog muscle was exposed to an aqueous solution of 100 mM LiCl and 10 mM CsCl. Viewed under an electron microscope, it shows that the electron-dense Cs^+ ions (atomic weight 132.9) adsorbed onto the two edges of the (dark) A bands (and the Z-lines in the middle of the (light) I bands. In (b) similar exposure to the Li-Cs solution of a muscle killed by prior exposure to glutaraldehyde produced no localized adsorption of Cs^+ as shown in (a).

4.3 The polarized-oriented-multilayer theory of cell water

As an integral part of the association-induction (AI) hypothesis, the polarized-oriented multilayer (POM) theory was first presented in 1965 at a symposium under a somewhat different title: polarized multilayer (PM) theory (Ling 1965)—three years after the publication of the association-induction hypothesis proper (Ling 1962.) The title of the Symposium was "*Forms of Water in Biological Systems*." It was sponsored conjointly by the New York Academy of Science and the Office of Naval Research.

4.3.1 Exclusion of Na^+ /other large solutes and other physical-chemical attributes of polarized-oriented water

A main point made in this 1965 presentation is that *all or virtually all the water in the living cell assumes the dynamic structure of polarized-oriented multilayers*. Figure 6 is a reproduction of the key original figure I presented at that conference demonstrating the suggested mechanism.

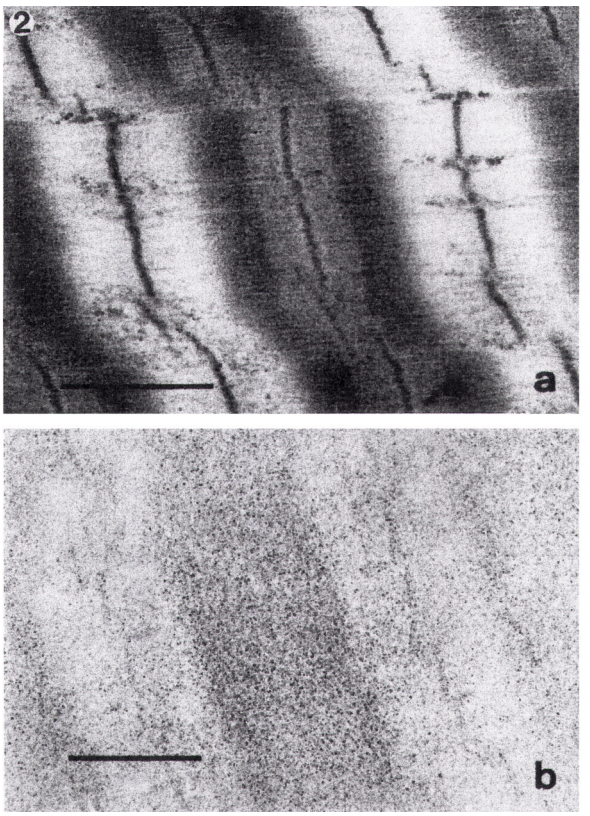

FIGURE 5. 0.2 μm-thick section of frog sartorius muscle stained with a solution containing 100 mM LiCl and 10 mM CsCl as described by Edelmann (1984.) (a) Freeze-dried and embedded sections without chemical fixation. (b) Glutaraldehyde fixed (and thus killed) and conventionally embedded muscle. Bars: 1 μm. (Edelmann 1988, 2001)

In a preceding section, I have already described the one-on-one, close contact association of virtually all the intracellular K^+ on the β-, and γ-carboxyl groups. Since water, K^+ and proteins make up the bulk of the osmotically active substances of all living cells, the association aspect of the AI Hypothesis in this brief narrative is now complete.

The second main point made in the 1965 presentation is that water assuming such a dynamic structure excludes (incompletely by a small margin) larger solutes like sucrose and hydrated Na^+. Hence what is known as the "**size rule**": the larger the solute molecule, the lower is its equilibrium concentration in a polarized, oriented cell (or model) water (Ling 1993; Ling and Hu 1988; Ling, Niu and Ochsenfeld 1993.)

There are two basic mechanisms for this (incomplete) exclusion of larger solutes: an *energetic* mechanism and an *entropic* mechanism. We will begin with the energetic mechanism.

Since the water-to-water interaction energy is higher in the dynamically structured cell water than in the bathing normal liquid water, it would need to spend extra energy in excavating a hole in the cell water to accommodate sucrose or hydrated Na^+ than the energy recovered from filling the holes left behind by these solutes in the surrounding normal water. And the net energy difference in energy expenditure and recovery is the larger, the larger the solute molecule or hydrated ion involved. The Boltzmann distribution law then dictates a lower concentration for the larger solute in the dynamically structured water.

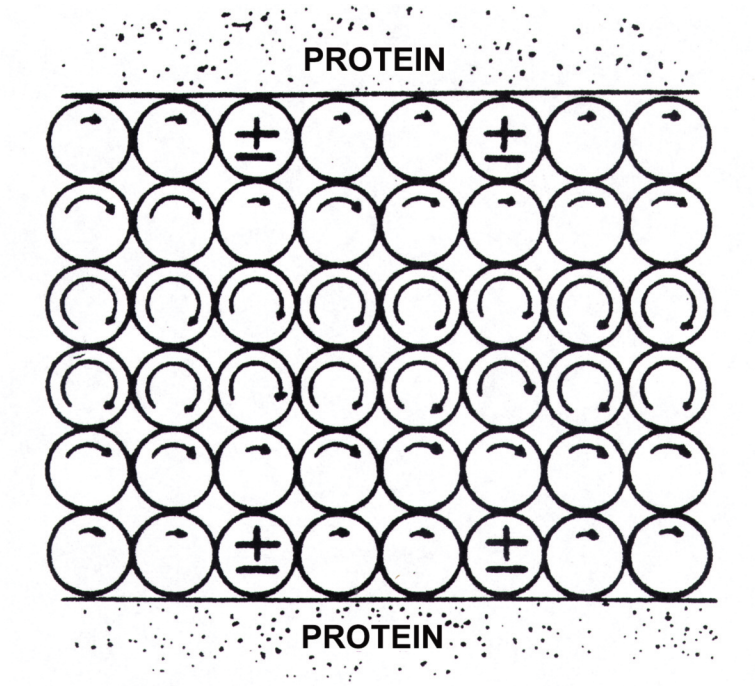

FIGURE 6. The original illustration of the polarized (oriented) multilayer theory of cell water, in which each water molecule is represented as a circle containing a curved arrow. The length of the arrow indicates the assumed degree of motional freedom. Later work shows that the degree of motional restriction is much more uniform rather than showing a steep gradient as suggested in this original illustration. (From Ling 1965)

The excess water-to-water interaction energy is given the name, *exclusion intensity* and represented by the symbol, U_{vp}. We soon found out that this parameter has at first sight, an unusual feature. That is, the absolute magnitude of the excess energy in cell or model water capable of producing a striking size-dependent solute exclusion is very small—when compared to the total water-to-water interaction energy. The outcome is the consequence of a balancing act—like that operating in an elevator or an analytical balance. As an example, the *exclusion intensity (U_{vp}) is only 126 cal/mole in the dynamically structured bulk phase water of living frog muscle cells*—and thus orders of magnitudes lower than the vaporization energy of water equal to about 10,000 cal/mole. U_{vp} of all the various *extrovert* model systems that we had studied and had demonstrated size-sensitive solute exclusion are even lower than that found in frog muscle cells (See Ling, Niu and Ochsenfeld 1993.)

The *entropic* mechanism of solute exclusion also shows a variation with the size of the excluded solute. Here too, the larger the solute molecule (or hydrated ion), the larger the degree of exclusion. First, the larger molecules are more likely to have more varieties of motional freedoms. As an example, a simple mono-atomic solute has only one degree of motional freedom, namely, the *translational*. Big and complex molecules like sucrose, on the other hand, have one to more *rotational* motional freedom in addition to the translational freedom. Furthermore, these rotational motions are more likely to be restricted by the less mobile polarized-oriented bulk-phase water molecules, further reducing their entropy.

Beyond the bulk-phase energetic and entropic mechanism described above, there is a third factor that may come into play and it concerns the surface of the excluded solute (Ling 1993.) Thus, the surface structure of some molecules or assemblies of molecules may fit the surrounding dynamic water structure and thus creates a favorable energy for the retention of that molecule or assembly of molecules in the cell (or model) water. Urea, ethylene glycol belong to this category and like ethylene glycol, so are a number of the so-called *cryoprotective agents* that prevent living cells from being damaged when cooled to and stored in liquid nitrogen at a temperature of −180°C as given by the authors (Luyet and Hartung 1941; Polge, Smith and Parkes 1949; Rall 1987) or even lower at close to absolute zero (Ling and Ochsenfeld 2008.)

In one extensive study reported by Ling and Ochsenfeld (1989), it was shown that the same solution containing a high concentration of polyvinylpyrrolidone or PVP (which polarizes and orients the bulk phase water) partially excludes sucrose but at the same time demonstrates an equal distribution for urea—in full accord with the PM theory of cell and model water solvency summarized above. This demonstration of equal solvency for some solutes and unequal solvency for other solutes refutes the first rejection of the concept of protoplasm for a wrong reason, namely bound or non-solvent water is present in all protoplasm and bound or non-solvent water excludes 100% all solutes big, small or in-between. A.V. Hill's discovery that urea distributes equally between muscle cell water and external medium, was seen widely then as an incisive disproof of the concept of bound water and that of protoplasm defined then by its possession of bound water (Hill 1930; Hill and Kupalov 1930; Blanchard 1940.) Hill's conclusion was wrong because he only disproved an erroneous theory.

We now introduce a quantitative parameter called the (true) ***equilbrium distribution coefficient*** or **q-value** to represent the equilibrium distribution ratio of a solute between two phases like the total cell water and the external bathing solution. In addition, I also introduced a **ρ-value** (Greek letter, rho, not English letter p) called the *apparent equilibrium distribution coefficient*. The q-value as a rule does not exceed unity (but there are exceptional cases of small excess beyond unity.) There is no limit on the magnitude of a ρ-value. However, if a ρ-value of a solute is substantially higher than unity, most of the solutes involved must be adsorbed—according to what is called the *surplus adsorption rule* (Ling 1992 p. 426.) Thus K^+ in living muscle cells may exhibit a ρ-value of 40. Since its q-value of K^+ is way below unity, all but 1% of the cell K^+ is adsorbed on β- and γ-carboxyl groups in frog voluntary muscle cells.

Since then, this area of research has really blossomed both in theory and in the variety and depth of worldwide experimental confirmation. As examples, Table 5.5 in my 1992 book (Ling 1992 p. 108–109) summarizes the work on the state of water in living cell and model systems in terms of: 1, solute distribution, 2, osmotic activity, 3, swelling and shrinkage, 4, freezing point depression, 5, vapor sorption at near saturation, 6, NMR rotational correlation time, τ_i, 7, Debye reorientation time, τ_D, 8, quasi-elastic neutron scattering. Without exception, all subjects studied have yielded support for the POM theory of cell water. Progress continued during and after 1992, including something extraordinary.

4.3.2 A new theoretical foundation for the polarized-oriented multilayer theory

Up to the turn of the century, none of the existing theories of multilayer polarization of water provided precise *quantitative* insight into how far the (effective) polarization can

reach. For this reason, I am very happy to have discovered a short cut and as a result introduced a new theoretical foundation for the POM theory in 2003 (Ling 2003.)

It was demonstrated theoretically that under ideal conditions, a checkerboard of alternatingly positively-charged P sites and negatively-charged N site at the precisely defined distance of 3.1 Å apart and called an ***Idealized NP Surface*** as illustrated in Figure 7, can polarize and orient multilayers of water molecules ***ad infinitum***. It should be recognized that electrical *polarization* or what Debye called *distortion polarization* plays a key role only at the first one (or perhaps an additional) layer(s) of water molecules; the *ad infinitum* long-range effect is due to self-propagating *orientation* or what Debye called *orientation polarization* (Debye 1929.)

Moreover, the theory also shows that water so (polarized) and oriented under ideal conditions, cannot be frozen at any *attainable* low temperature, which, as dictated by the Third Law of Thermodynamics, cannot go below absolute zero (Fowler and Guggenheim 1960 p. 224.) The prediction of non-freezable water was confirmed retroactively by work published half a century ago. Its authors, Canadian chemists, P.A.Giguère and K.B. Harvey (1956) were puzzled and could not explain the continued existence of water in its liquid state—as witnessed by its characteristic infrared absorption spectrum—in a 10 micra-thick layer of water held between polished silver chloride prisms at the temperature of liquid nitrogen and given by the authors as –176° C. I then discovered that AgCl crystals possess structures very close to that of the ***Idealized NP Surface*** (For AgCl crystalline structure given by Glaus and Calzaferri (1999), see Figure 9 in Ling 2003 on p. 118.) In

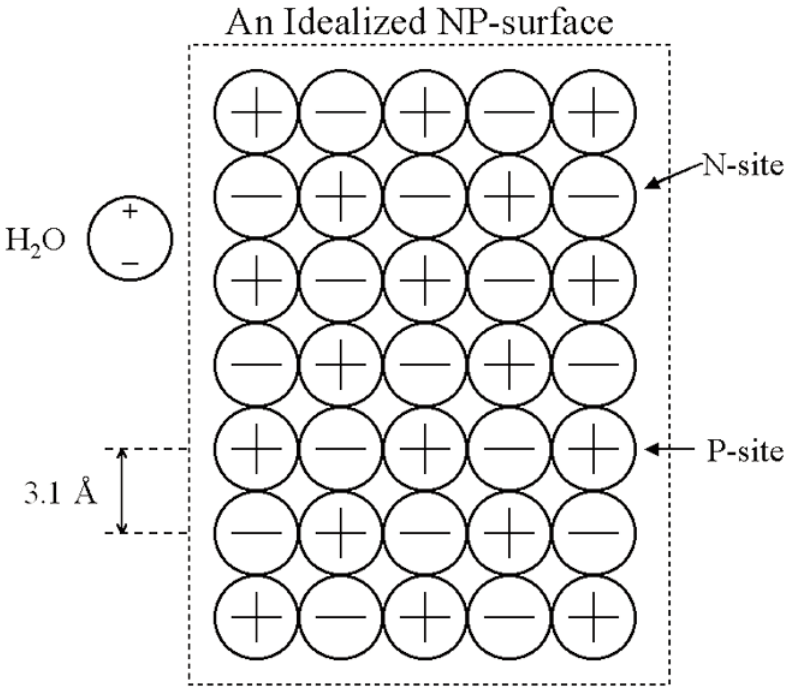

FIGURE 7. An Idealized NP surface. The distance between a pair of the nearest-neighboring N and P site is equal to the distance, r, between neighboring water molecules in the normal liquid state and equal to 3.1 Å.(From Ling 2003)

sharp contrast, no signs of non-freezable water were found in similar infrared spectrum of water held between (transparent) fluorite (CaF_2) plates with a different crystalline structure (Fox and Martin 1940.)

The prediction of long-range water (polarization) orientation was further dramatically affirmed experimentally by Zheng and Pollock in their publication on the "marching particles" near an NP surface of polyvinyl alcohol surface in the Phys. Rev. (Zheng and Pollock 2003.) To my surprise and disappointment, these authors did not refer to my paper on the *ad infinitum* water polarization (Ling 2003), nor the fact that a long list of linear polymers (including polyvinyl alcohol) are effective water dynamic structure inducers (Ling, Walton and Bersinger 1980.) (See also Pollack 2012.)

At the conclusion of this subsection, I would strongly recommend to the reader to download my 2003 article on "A New Theoretical Foundation for the Polarized-Oriented Multilayer Theory ..." by clicking Article No. 2 on the front page of my Website <www.gilbertling.org>. This article covers some of the most fascinating stories in the history of science.

4.3.3 The difference between freezing and vitrification of cell water

The dictum that under idealized condition, the dynamically polarized-oriented water cannot be frozen at any attainable temperature may appear in conflict with the phenomena of cryoprotection—where the addition of glycerol, ethylene glycol or other cryoprotectants keeps frozen living tissues alive at liquid nitrogen or helium temperature. Actually there is no conflict at all.

The word, frozen (living tissues) used here is not the freezing in the above-mentioned dictum, which means conversion of (normal or modified) liquid water into crystalline ice. The function of cryoprotectants is to prevent H_2O in the "frozen" tissue from turning into crystalline ice. So what is the physical state of water in the well-protected but solid living tissues in liquid nitrogen or liquid helium?

In 1937, Father (B. J.) Luyet first referred to this water as *vitrified* water. It was then thought to be amorphous and homogeneous. However, later work led to the idea of vitrified water as polymorphous. Based on the POM theory and the experimental findings of Ling and Zhang published in the early 1980's, I suggested that the vitreous state of living tissue cells kept at liquid nitrogen or liquid helium temperature is *vitrified polarized-oriented multilayer state* (Ling and Zhang 1983; Ling 1992 p.102–106; Ling 1992a pp.427–432; Zhang and Ling 1983.)

4.4 The association-induction hypothesis proper

The association–induction hypothesis is the one and only unifying theory of life at the cell and below-cell levels. It was published ten years after its embryonic prelude, Ling's Fixed Charge Hypothesis appeared in print in 1952. As pointed out earlier, for well over one half of a century, the association-induction (AI) hypothesis has successfully stood all the extensive testing here and abroad with no major setback. In fact, all criticisms of the theory known to me at the time (1998) have been answered (see Ling 1998d and Ling 1998e.) And with no known exception, what came as criticisms turned around and became additional supports for the theory (Ling 1998d and Ling 1998e.). I do not know of any additional criticism of the AI hypothesis published after 1998.

Notwithstanding, and as pointed out earlier, it is the alternative membrane theory or the membrane pump theory that is taught as truth worldwide at all levels of education—long after it has been soundly and unequivocally disproved (see p. 6 above.) Yet, among the advocates of the membrane (pump) theory could be counted some of the ablest scientists in history, including notably J. van't Hoff and A.V. Hill. In retrospect, I now see that they—like the *Reductionist Four*—also came on the scene before the ***microscopic*** approach in physics and chemistry became widely taught and practiced. As a result, they had no choice but to join the movement of interpreting life phenomena in terms of membranes, pumps, semi-permeability, channels, gates and other ***macroscopic*** concepts. It is no surprise that their respective talents notwithstanding, they failed.

Luckily, my generation of investigators arrived on the scene much later and thus in time to access the new science of *statistical mechanics*—invented primarily by Ludwig Boltzmann (Gurney 1949; Cohen 1997.) Thus privileged, I was able to construct a theory of life phenomena in terms of *microscopic entities* and named it the ***association-induction hypothesis***.

In the two preceding sections, we have gone to some details describing how some sites (β-, and γ-carboxyl groups) of the cell proteins interact with K^+ and other sites on the cell proteins (backbone NHCO groups) interact with the bulk-phase water. Based on the outline of protein chemistry and behavior, I shall describe how **the AI Hypothesis offers a self-consistent set of molecular mechanisms for the cell proteins to function as a coherent unit.** And that in turn enables the cell and its parts to stay alive and engage in life activities. We begin with the subject of the *target* groups and *effector* groups on cell proteins.

4.4.1 Target and effector groups

As in the case of chlorine atom-for-hydrogen atom substitution in acetic acid, a substitution of one atom or group of atoms linked to a molecule by ***covalent bonds*** produces the classic inductive effect. It is now well established that the substitution of one chemical group for another attached to the parent molecule by ***H-bond*** as well as by ***ionic bond*** also produces parallel inductive effects. The variety of the target groups for the inductive effect has also been broadened to include not only the pK_a of acidic groups, but also the pK_b of the basic groups, the strength of H-bonds and oxidation-reduction potentials (Ling 1984 pp. 183–198; Ling 1992 pp. 111–134.)

Figure 8 illustrates the influence of induction effect expressed by Taft's inductive constants of a list of effector groups on the pK_a of target carboxyl groups and pK_b of target amino groups close by and farther away. Figure 9 shows the inductive influence of similar effector group substitutions as expressed by Hammett's inductive constants σ on the strength of H-bonds as target groups. In both cases the effector groups are linked to the protein molecule by covalent bonds.

4.4.2 The transmission factor; the reach of Direct F-effect

The transmission of the combined inductive or I-effect (through intervening atoms) plus direct or D-effect (through intervening space) produces the F-effect. However, as time progressed, there have been more and more emphasis on the I-effect and less and less on the D-effect. Thus, in fact when I use the term Direct F-effect, it would be referring

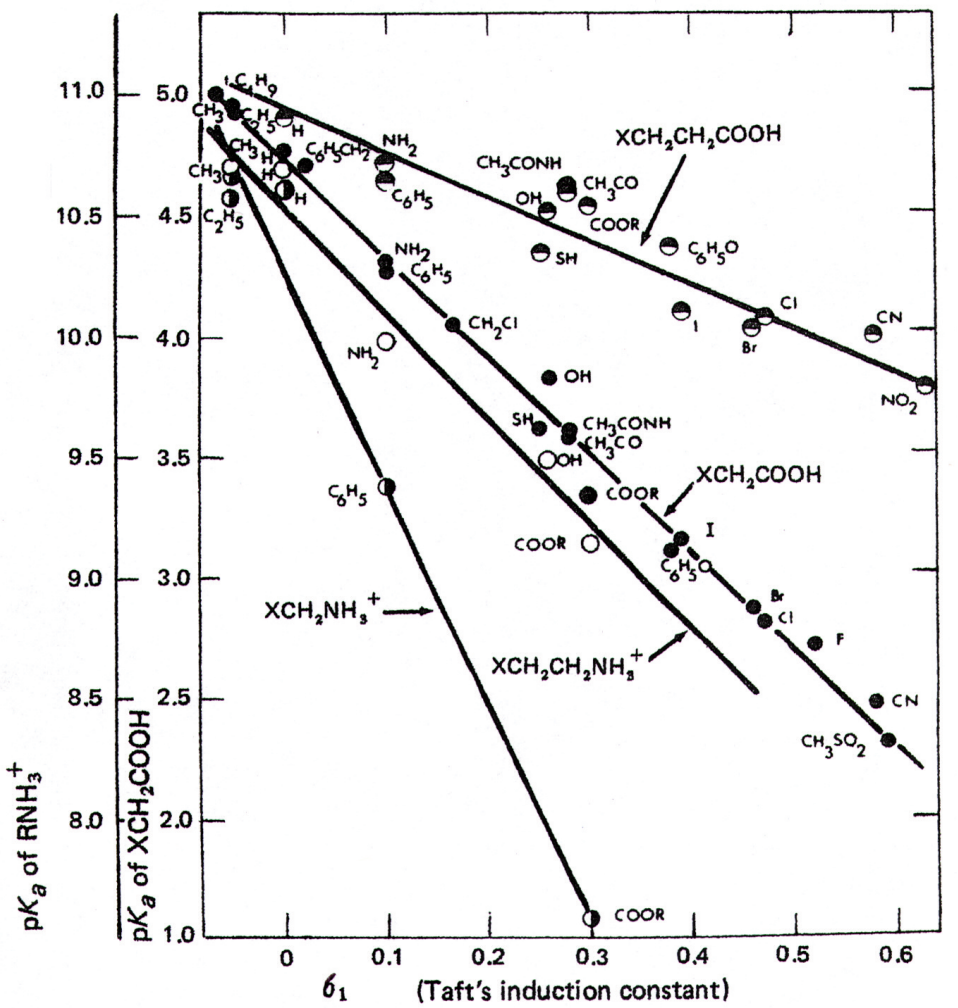

FIGURE 8. Linear relationships between the pK_a's of two sets of substituted carboxylic acids (acetic acid and n-propionic acid) and the pK_b's of two sets of substituted amines (methylamine and ethylamine) shown respectively on the double ordinates **and** Taft's induction constants, σ_1 of each of the substituents (marked as X in the structural formulae of the acids and amines) shown on the abscissa. (From Ling 1964)

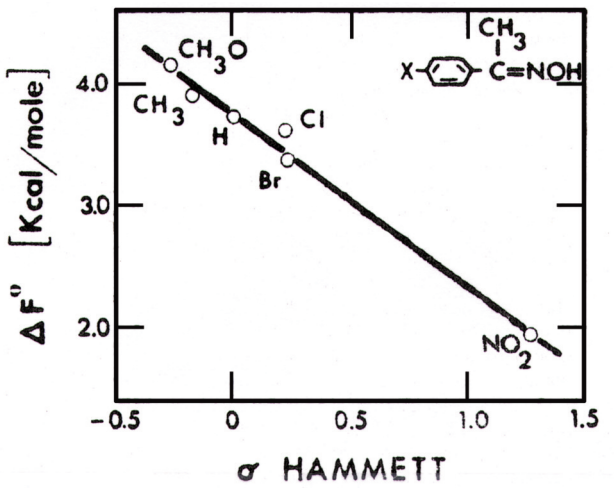

FIGURE 9. Relationship between Hammett's induction constants, σ's of the substitute X, on the free energy of dimerization of para-substituted acetophenone oximes. The structural formula of acetophenone oximes is shown in the upper right corner of the figure, where X represents the substituent. The free energy of dimerization shown as the ordinate was calculated from Reiser's data (Reiser 1959.) The fact that powerful electron donating substituents like the methyl group (CH_3) strongly enhances the strength of dimerization suggests that it is primarily the electron donating nitrogen atom (rather than the electron accepting OH group) that determines the strength of the H-bonds formed between a pair of the molecules (From Ling 1964)

largely if not exclusively to the I-effect mediated through intervening atoms. The ease or difficulty of the transmission of the inductive effect depends on what part of the protein molecule the transmission takes place.

The transmissivity for the passage of the I-effect through each saturated carbon atom is 0.333 according to Chiang and Tai (Ling 1984 p. 187.) However, others including Taft and Ling gave values as high as 0.48 (Ling 1964a; Ling 1984 p. 189.) Transmissivity through the peptide bonds is even more efficient by far.

Indeed, as shown in Figure 10, there are at least three sets of independent experimental data, which indicate that the Direct F-effect can be transmitted through three peptide linkages to reach a functional group directly or a functional group at the end of a short side chain. The details of the figure have been rewritten from those published earlier (Ling 2001 p. 161; Ling 1992 p. 125 and p. 132.) Why is the inductive or Direct F-effect transmitted so much more effectively through the polypeptide chain? The answer comes in subsection 4.4.3.1 below.

4.4.3 Transformation of proteins between two stable states

As pointed out repeatedly above, a unique feature of life is its connectedness. To achieve that end, I cited two models: the falling domino chain and the tethered frictionless seesaws. Both models can exist in two stable (or meta-stable) states only. In this subsection, I examine three different types of evidence to demonstrate the existence of physiologically active proteins in two stable discrete states also.

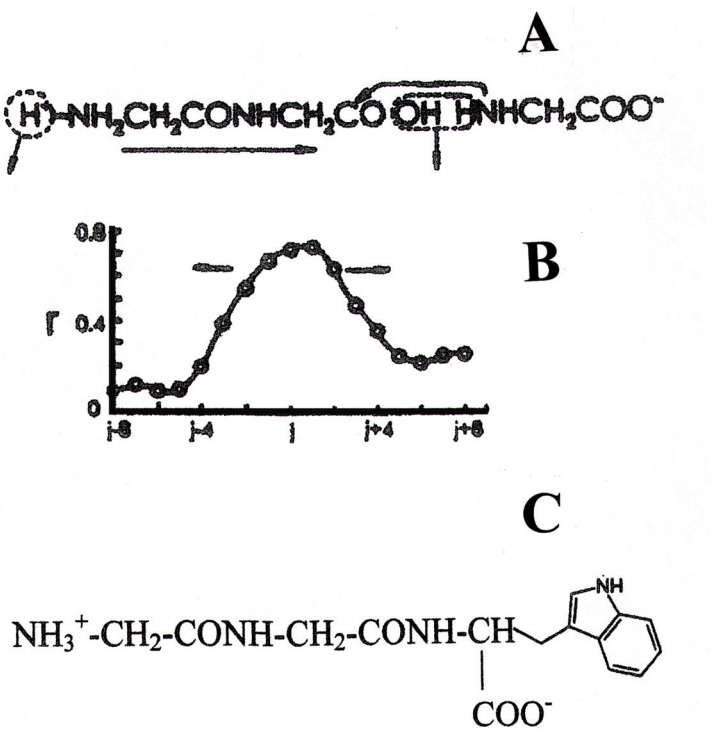

FIGURE 10. Three sets of experimental evidence for the effective transmission of the inductive effect through a length of the polypeptide chain.

(**A**) Demonstration of significant change of the affinity of the terminal amino groups of glycine and glycine peptides for H^+ as indicated by a significant decrease of its pK_a in response to a distant substitution of a carboxyl OH group by a stronger electron-withdrawing glycyl group at a minimum of ***three*** peptide linkages away (Stiasny and Scotti 1930; Czarnetzky and Schmitt 1931; Ling 1962 p. 94.)

(**B**) Linear correlation studies between the propensity of the peptide group of each of the 19 α-amino acid residues to form α-helical structure in proteins (i.e., *α-helical potential*) and the electron-donating power of their respective side chains (expressed as the acid dissociation constants of their α-carboxylic acid analogues) provided the data given in Table 1 on p. 46. Additional data given by Garnier *et al* made it possible to study the linear correlation (given as ordinate, r in Figure B) between the electron-donating strength of a specific amino acid residue referred to as the jth on the α-helical potential of the peptide linkage at different distance away from the jth residue. Thus as shown in the illustration, the positions labeled j-4 represents the peptide linkage of the 4th residue on the N-terminal side of the jth residue. On the other hand, the position labeled j+4 represents the peptide linkage of the 4th residue on the C-terminal side of the jth residue. The results as plotted in the figure show that the effect of each amino acid residue extends to at least the ***third*** peptide groups both upstream and downstream (Garnier *et al* 1978; Ling 1986; Ling 1992 p. 120.)

(**C**) Quenching of fluorescence of the indole group of the L-tryptophan residue (illustrated at the extreme right of the polypeptide formula shown) in a series of synthetic polypeptides of the general formula, $(glycine)_n$-L-tryptophan in consequence of the increasing distance created by the stepwise increase of the value of number of glycine residues, n separating the target indole group and the electron-withdrawing effector NH_3^+group, shown at the extreme left of the $(glycine)_2$-L-tryptophan formula. Best-fitting theoretical pH titration curves of the experimental titration data collected (not shown here) were based on the assumed value of pK_a for $(glycyl)_1$-L-tryptophan of 8.20, and the degree of fluorescence 50%, $(glycyl)_2$-L-tryptophan, 8.00, 21%, $(glycyl)_3$-L-tryptophan, 8.00, 10%. The data suggest that inductive effect can be effectively transmitted over ***three*** peptide linkages to reach the functional group on a short saturated carbon side chain. (Data from Edelhoch *et al* 1967; Ling 1992 pp. 124–125)

4.4.3.1 RESONANCE AND SHORT CN BOND OF THE PEPTIDE LINKAGE

N-methylacetamide, shown below in Equation 5, is the smallest molecule that contains a single *peptide linkage* (CONH.) As such, it is a useful model of one kind of building blocks of all polypeptide and proteins. In 1950 Mizushima and his coworkers demonstrated that the bond linking the N and C atom in this molecule is much shorter than a normal N-C single bond (Mizushima *et al* 1950; Mizushima *et al* 1955.) This bond-length shortening indicates ***resonance*** between two states as shown below:

$$\begin{array}{l} CH_2C\text{-}NHCH_3 \\ \quad\| \\ \quad O \end{array} \leftrightarrow \begin{array}{l} CH_3C{=}N^{+}HCH_3\,. \\ \quad\quad| \\ \quad\quad O^{-} \end{array} \qquad (5)$$

In consequence of this resonance, the CN bond in the peptide linkage is 40% double bond and 60% single bond. There is extremely rapid switching between the two structures of this most simple model.

The ease of transformation between two alternative structures makes the polypeptide chain of proteins highly polarizable electronically. It is this high polarizability that enables the polypeptide chain to serve as the "highway" of information and energy transfer over large distance like the frictionless seesaw chains (Ling 1962, p. 93.)

In addition, this resonance also makes the CO and NH groups function as *dipolar anion* and *dipolar cation* respectively. Dipolar ions are special because they are essentially neutral when seen at a far distance. However, at close range, they become either cationic or anionic, depending on which direction one is approaching the dipole. In the AI Hypothesis, these dipolar ions play critical roles in protein-protein interaction and in protein-water interaction.

4.4.3.2 INFRA-RED SPECTRA OF SYNTHETIC POLYPEPTIDES

In the early 1950's E.J. Ambrose and A. Elliott studied the infrared absorption spectra of synthetic polypeptides. And soon they made a very important discovery (Bamford *et al* 1956 p.130; Ambrose and Elliott 1951; Elliott 1953.) That is, in water, the polypeptide does not assume a large variety of conformations, as the popular term "random coils" would lead one to expect. Instead, each polypeptide assumes only one or the other of two alternative conformations. In one conformation, the NH and CO groups are oriented in the same direction as the polypeptide axis; this is exactly what one would expect if the conformation assumed is that of ***α-helical conformation***. In the alternative conformation, the NH and CO groups are oriented perpendicular to the polypeptide axis; this is exactly what one would expect if the polypeptide is in the ***fully-extended conformation***.

The results were so consistent and convincing that all the authors, but especially Elliott, went out of their (or his) way to make their conviction known. Notwithstanding, they could not see a greater significance beyond the observations until the association-induction hypothesis came along with what was first called the *biological fixed charge system* (Ling 1962 p. 53), then as the *minimal unit of life* (Ling 1992 p. 425)*, then* as the *elementary living machine* (Ling 2001 p. 152*)* and finally as *nano-protoplasm,*—which in theory also exists in two alternative conformations at equilibrium. In one, it is essentially an ***α-helical conformation*** and in the other it is essentially a ***fully extended conformation*** (Ling 2007.)

4.4.3.3 STRICT OBEDIENCE TO THE (TWO-STATE) YANG-LING COOPERATIVE ADSORPTION ISOTHERM

Boltzmann was a scientist of vital importance to the understanding of life and its physical basis because he almost single-handedly invented Statistical Mechanics. In addition, I also profited a great deal from another theoretical physicist of comparable rank, my dear friend, Professor C. N. Yang. He was my roommate at the Tsing Hua University Graduate School in Kun-ming, China between 1943 and 1945. (C.N. Yang and T.D. Lee were awarded the Nobel Prize for physics of 1957 for their conjoint work on parity. For a brief vignette of Yang's contribution to statistical mechanics, particle physics etc., see Yang 1995.)

Yang was among the first to read and endorse my 1962 book, "A Physical Theory of the Living State: the Association-Induction Hypothesis" long before it was published. Next thing you know, the Yang-Ling adsorption isotherm was born (Ling 1964a; Ling 1984 p. 208)—based on the one-dimensional Ising method. As such, it was a quantitative extension of my simple (two-state) model of a protein molecule seen as an infinitely long chain of equally spaced sites of similar nature, which could adsorb either an ith or a jth solute (where either the ith of the jth solute could be vacancy.) (For the details of the derivation of the Yang-Ling isotherm, see Karremann 1980.)

However, to fully understand how such a simple equation like the Yang-Ling adsorption isotherm can quantitatively describe the diverse aspects of living phenomena and their models requires space. Indeed, that was why the article "Nano-protoplasm, the Ultimate Unit of Life," cited as Ling 2007a is 123 pages long. To keep the present communication as short as possible, only one of those cases will be reviewed. For those interested in more, a click on Article #9 on the front page of my Website www.gilbertling.org and you have it in full.

Figure 11 demonstrates how the exceptionally accurate data of oxygen binding on hemoglobin by Dr. R.L.J. Lyster obtained in the laboratory of, and under the tutelage of Prof. F.J.W. Roughton of the Cambridge University of England can be precisely described by the Yang-Ling isotherm. All 16 data points fall on the theoretical curve dictated by just two numerical constants, an *intrinsic binding constant* of oxygen binding, $K^{oo}_{j\rightarrow I}$ equal to 5.88 x 10^{-6} M and a *nearest neighbor interaction energy* ($-\gamma/2$) equal to 0.67 kcal/mole (Ling 1969, but also reproduced in Ling 2007 Figure 17 on p. 150.) For a full discussion on the poignant significance of this precise prediction by two numbers, see Ling 2007a, pp. 147–152.

This high degree of quantitative agreement shown between theory and experimental data demonstrates that the protein can indeed exist in two stable states: in one, oxygen is bound in all the sites and in the other, the binding sites are all vacant.

The two-state model will be dealt with again in more detail below. But before that we will consider the chemicals that act like the little mouse shown in Figure 3, which can determine which of the two alternative conformations the living see-saw chain assumes. Drugs offer one category of such chemicals.

4.4.4 Cardinal adsorbents

In the association-induction hypothesis, drugs do not belong to a stand-alone group of chemicals. Rather, along with hormones, ATP, Ca^{++} etc., drugs are examples of ***cardinal adsorbents***, which exercise strong influence on living phenomena at low concentration. Cardinal adsorbents are electronic agents that play a central role in living phenomena.

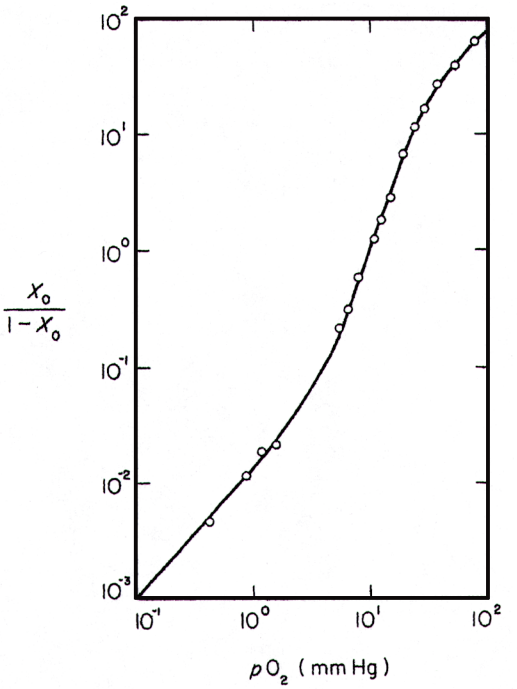

FIGURE 11. A log-log plot of the binding of oxygen by human hemoglobin in 0.6 M phosphate buffer at pH 9.1 and temperature of 19°C. Data from R. L. J. Lyster, as presented by Rossi-Fanelli *et al* (1964.) Points are experimental; line is theoretical according to the Yang-Ling adsorption isotherm presented as Equation 5 and 6 on page 142 in Ling (2007) with a $K^{oo}_{j\rightarrow I}$ equal to 5.88 x 10^{-6} M and (–γ/2) equal to 0.67 kcal/mole. (From Ling 1969)

Many drugs are made by Nature. Man makes others (Ling 1962 p.118 and p.420.) As a whole, cardinal adsorbents can be divided into three categories: *electron-withdrawing cardinal adsorbents* (**EWC**), *electron-donating cardinal adsorbents* (**EDC**) and *electron-indifferent cardinal adsorbents* (**EIC**).

A cardinal adsorbent as a rule has the *three-in-one power* to bring about (i) ***from here to there,*** (ii) ***one on many*** and (iii) ***making many respond as*** if they were one—when it binds onto a specific site on a cell protein called a ***cardinal site***. Cardinal sites include what we conventionally call *receptor sites* (for external molecular agents like drugs, hormones) but they also include other sites that interact with internal molecular agents like ATP (see below.) To demonstrate the three-in-one power of key cardinal adsorbents, I shall talk about three special cardinal adsorbents: Adenosine triphosphate or ATP is what I sometimes call the Queen of cardinal adsorbents for its overreaching power as an EWC. Ca^{++} is another important EWC. Ouabain is yet another powerful cardinal adsorbent but an EDC.

4.4.4.1 ATP

Discovered by K. Lohmann in 1929, ATP was for about 15 years widely believed to carry two high-energy phosphate bonds, each represented by the symbol ~P in a theory

proposed by Lipmann (Lipmann 1941.) This concept turned out to be mistaken as shown by Podolsky and Morales (1956.) They found no usable energy to do work in any one of the three phosphate bonds (Ling 1992 p. 179; Ling 2001 p. 234, 306.) ***This iconoclastic development left ATP without a function—***for six years only or ***altogether***, depending on one's familiarity with or lack of it with the association-induction hypothesis.

That apparently all the textbooks at high school and university level worldwide has ignored Podolsky and Morales's historical discovery shows that the inability to deal with major progress is not limited to my work but is an illness affecting the whole science. As such, it provided the legitimate causes for sentiments expressed in Chalmers's book, *What Is This Thing Called Science?* And other negative sentiments toward the future of science shared among some forty leading scientists in different fields, including science philosophers, Sir Karl Popper, Thomas Kuhn and Paul Feyerabend in John Horgan's book, *The End of Science: Facing the Limits of Knowledge in the Twilight of the Scientific Age* (Horgan 1996.) However, in the Preface of my 2001 book, *Life at the Cell and Below-Cell Level*, I pointed out that science has not come to an end, the mistaken notion was created in part by the failure to recognize the association-induction hypothesis, which gives ATP a new function as an important cardinal adsorbent. As such, it is distinguished by its strong binding energy with a standard free energy of binding, ΔF° equal to –14.3 Kcal/mole (Ling 1992 p. 180.) Thus the binding energy on myosin of ATP is ten times higher than the binding energy on myosin of its hydrolytic product, ADP (Ling 1992 p. 187.)

As the Queen of electron-withdrawing cardinal adsorbent (EWC), ATP has been demonstrated to show a stoichiometric relationship to the level of K^+ in a variety of living cells studied (Ling 1962 pp. 252–255) but most extensively in frog muscle cells. In this case, the same quantitative equilibrium relationship of 20 K^+ selectively adsorbed for each ATP occupying its specific cardinal sites persists regardless of which one of the eleven different poisons is employed to bring about the decline of ATP level very slowly. They include iodoacetate, 2,4-dinitrophenol, azide and chlorpromazine (Gulati, Ochsenfeld and Ling 1971; Ling 1992 p.189; Ling 2001 p. 72.)

ATP also shows stoichiometric relationship to intracellular Na^+ (and sucrose) concentration level. (For supporting experimental evidence, see Figure 8.18 and Figure 8.19 in Ling 1992; Figure 56 in Ling 2001.)

Since all sucrose in the muscle cell and (virtually) all intracellular Na^+ are dissolved in cell water, the control of ATP on their concentrations is achieved via its control of the dynamic structure of bulk-phase cell water. Now each kilogram of normal resting frog muscle contains 80% by weight or 44.4 moles of water and 5 nmoles of ATP. Resting frog muscle cell water has a q-value of 0.132 for sucrose and 0.100 for D-raffinose (Ling, Niu and Ochsenfeld 1993, p. 191.). A simple calculation shows that at least 40 moles of water in one kilogram of muscle cells are under the control of ATP. Put differently, each molecule of ATP adsorbed on its cardinal site controls at least eight thousand (8000) water molecules.

4.4.4.2 Ca^{++}

Like ATP, Ca^{++} is what I call a "*conservative cardinal adsorbent*" because its function is to maintain the resting physiological state of the cell rather than pushing the cell to a different active state (Ling 1992 pp. 171–172.) In agreement with this view, lowering external Ca^{++} concentration experimentally causes massive loss of cell K^+ in brain slices (Gardos 1960), liver slices and transplanted tumors (Gilbert 1972), carotid arteries (Jones

1973) and guinea pig *taenia coli*, a strip of smooth muscle along side of the intestine (Gulati 1973.) No further study of the role of Ca^{++} in terms of the AI Hypothesis has been reported since the early 1970s to the best of my knowledge despite the obvious success in work done and reported earlier.

Meanwhile, the textbooks of biology worldwide continue to teach that living cells are sacs of free watery solutions enclosed in cell membranes containing an unlimited number of inward and outward pumps. Among these membrane pumps studied extensively is the mighty Ca pump. It is allegedly able to maintain in human red blood cells an extracellular/intracellular concentration gradient as high as 45,000. (Bogdanova *et al* 2013.) Is this real?

Keeping in mind that none of these (membrane) pump concepts can be even called a scientific hypothesis—because by definition a scientific hypothesis provides a mechanism for the phenomenon observed. A much more extensively studied hypothetical membrane pump is the sodium pump. Glynn and Karlish (1975) who wrote the first of its kind of review under the title "The Sodium Pump" readily admitted that no mechanism for the pump has ever been proposed. Thus, even the so-called Na^+ pump hypothesis is only a rephrasing of an observation.

True, pumping of ions and molecules does occur in living systems but only in *bifacial* cells like kidney epithelium and frog skin (Ling 1981a.) For *unifacial* cells like frog muscle and human red blood cells, none survived careful testing. Indeed, what the proponents of the Ca pump have been doing more recently are almost exact duplicates of what have been done in the early days on the sodium pump.

As mentioned earlier on p. 6, I demonstrated in 1962 that the postulated sodium pump would require at least 15 to 30 times of the total energy the muscle cells command under the condition of the experiment. But that estimate was made before the demonstration of Morales and Podolsky that ATP does not carry extra usable energy in the so-called high energy phosphate bonds. When this fact is taken into account, the discrepancy between the maximum energy available and minimum energy needed would be far beyond a mere 15 to 30 times. Or put it in a simple way, the maintenance of a low Na^+ level in (muscle) cells simply does not require a continual supply of any amount of energy. The fact that we can preserve all kinds of living cells, including the human red blood cells and animal embryos containing all kinds of tissue cells, in liquid nitrogen or liquid helium indefinitely, leaves no room for arguments.

In 1980, Ling and Negendank also asked the question that investigators of the Ca pump have been asking years later: Do isolated vesicles pump sodium and the answer was no (Ling and Negendank 1980.) Does man-made phospholipid membrane-containing the alleged pump (Na, K-activated ATPase) pump sodium and potassium? The answer is again no (Ling 1992 pp. 22–24.) Does K^+-selective microelectrode faithfully tell us about the free K^+ concentration in living cells? The answer is again no (Ling 1984 pp. 252–257.)

But even these are not all. Other studies have shown that any intracellular ion-specific electrode can only see the concentration of a thin layer of water coming from the damaged protoplasm that the intruding electrode has produced. Thus in some way, the activity of ions and molecules inside living cells are like the life or death of the cat in Maxwell's black box. Opening the door to find out if the cat is alive will inevitably trigger the release of poison that instantly kills the cat and defeats the purpose of finding its state of health before opening the door. Inserting an ion-selective electrode or injecting a

dye, a photoprotein (e.g., aequorin), a metallochromic indictor (e.g., fura or quin 2) will as a rule produce unpredictable changes in the free Ca^{++} concentration and its state of binding from their respective natural state. Notwithstanding, there are indirect and proven and published ways of accurately determining the concentration of free and adsorbed ions or neutral molecules inside living cells but there is no evidence that investigators of the Ca^{++} pump are aware of their existence and take advantage of them.

4.4.4.3 OUABAIN

As a specific example of the far-reaching power of another cardinal adsorbent, this time an EDC, consider an experiment that Ling and Bohr conducted in 1971 (Ling and Bohr 1971.) We sterilely isolated frog sartorius muscles and incubated them at 25°C in a gently shaken known volume of sterile modified Ringer solution, which contained 2.5 mM K^+ and 100 mM Na^+ (Ling and Bohr 1969.) We then added to the solution in each one of half of the flasks a minute amount of the drug, ouabain to reach a final concentration of 3.26 x 10^{-7} M. After 72 hours of sterile incubation at 25°C, we took out the muscles and analyzed their ionic contents. Whereas the normal control muscles retained all their K^+, the muscles exposed to ouabain had quantitatively replaced one-on-one all their K^+ with Na^+. These findings provided us with the exact information on how many β-, and γ-carboxyl groups in the muscle protein(s) have changed the kind of its adsorbed cations. Assuming all the ouabain added to the incubation fluid to be adsorbed on its appropriate cardinal sites, we calculated that the binding of ***a single ouabain molecule*** has made ***one thousand and forty-two*** (1042) β-, and γ-carboxyl groups in the muscle protein(s) to shift from adsorbing K^+ to adsorbing Na^+ (Ling 2001 p. 262.) How such a far-reaching switch can be accomplished in molecular and electronic terms will be the conceptual cement joining the subject matters of the three subsections following.

4.4.5 c-value, c-value analogue etc.

As described in an earlier page, the pK_a is a convenient parameter representing the affinity of an acid for its H^+. Weak acetic acid has a pK_a of 4.76 while strong TCA has a pK_a of only 0.66. In presenting Ling's Fixed Charge Hypothesis, I have shown that the mechanism suggested for the selective accumulation of K^+ over Na^+ in living cells can be extended to explain selective uptake of K^+ in non-living systems as well. They include soil, glass as well as man-made *ion exchange resins*, which too selectively accumulate K^+ over Na^+ —although at selectivity ratios far below that seen in living cells (Ling 1952; see also Wiegner & Jenny 1927; Jenny 1932; Ling 1962 p. 56.) A later type of ion exchange resin, however, selectively accumulates Na^+ over K^+. In Bregman's review on the subject, he pointed out that the earlier form of ion exchange resin that prefers K^+ carries anionic sulfonate groups while the later type of resin that prefers Na^+ have anionic carboxyl groups (Bregman 1953.) Of course, the sulfonate group is a much stronger acidic group than the carboxyl group. Bregman, however, did not make anything out of this insight, preferring a difference in the *polarizability* of the different acidic groups as suggested in the theory of Teunissen and Bungenberg de Jong (1939.) However, I became very excited when I learned of this selectivity reversal in the new type of ion exchange resin.

There was a special reason for my interest in this selectivity reversal. I had been invited to join the basic research staff of the newly founded Eastern Pennsylvania Psychiatric

Institute in Philadelphia. And before my acceptance of the job, I had given a talk to my prospective colleagues, Donald Rudin, George Eisenman and James Casby. Included in the talk was my theory of selective accumulation of K^+ in living cells (Ling 1952) as well as the role of selective adsorption in generating the electric potential difference across the surface of nerve and muscle cells (Ling 1955.)

Some time afterward, Eisenman, Rudin and Casby suggested a new modification of my earlier theoretical model. They thought that if the field strength of the fixed negative charge does not stay put as in my original model but changes, then among the five alkali-metal ions, cesium (Cs^+), rubidium (Rb^+), potassium (K^+), sodium (Na^+) and lithium (Li^+), the most weakly hydrated Cs^+ will be the first to lose its hydration, followed by Rb^+ etc. so that 11 orders of the five alkali metal ions will be created (Eisenman *et al* 1957; Eisenman 1967; Ling 1984 p.153.) Extremely important as it was to me at the time, their theory was nonetheless entirely speculative. For that reason, I felt that the best way to proceed from there on was to do a detailed quantitative study on the subject. And to begin, I needed to invent a new independent parameter that underlies the pK_a value. The result is what I call the **c-value.**

Rigorously defined in my first book, *A Physical Theory of the Living State*, the c-value represents the electron density of the *negatively-charged oxygen atom* of an oxyacid like a β-, or γ-carboxyl group but given in the distance parameter of Angstrom units (Ling 1962 pp. 57–60; Ling 1984 pp. 155–156; Ling 1992 pp. 126–127.) A high c-value corresponds to a high electron density and a high pK_a ; a low c-value corresponds to a low electron density and a low pK_a. In contrast, the carbonyl oxygen atom of a peptide NHCO group is the *negative end of an electric dipol*e. A different parameter is introduced for its effective strength called the **c-value analogue** (Ling 1962, p. 57, 60.) Two other parameters were also introduced. The **c'-value** refers to the positive charge of a positively charged amino or guanidyl group and the **c'-value analogue** of the positive charge of dipolar peptide imino group, for example.

4.4.5.1 THEORETICAL PREDICTED REVERSAL OF K^+ / NA^+ PREFERENCE WITH C-VALUE CHANGE

With the c-value defined, I then decided to construct a **linear model**, in which a cylindrical cavity is carved out of the continuous dielectric of bulk-phase water. An array of interacting ions and water was then installed in the cavity. A singly charged oxygen atom is placed on one end of the cavity and one of the seven mono-valent cations: Li^+, Na^+, K^+, Rb^+, Cs^+, H^+ and NH_4^+ is placed on the other end of the cavity, separated from the oxygen atom by 0, 1, 2 or 3 water molecules. There are also two additional water molecules beyond the mono-valent cation. Instead of the simple Coulombic interaction considered in my 1952 model, seven other types of interactions were taken into account (for details see, Ling 1962 pp. 60–74.) The polarizability (α) of the oxyacid group was given three values (0.876×10^{-24}, 1.25×10^{-24} and 2.0×10^{-24} cm^3.) Only the results from the model with the highest oxygen atom polarizability (α) is shown in Figure 12 here.

Broadly speaking, the theoretical result confirms the idea of Eisenman, Rudin and Casby that with the increase of field strength, (given here as the c-value) there is a sequential change in the preference for any pair of two mono-valent cations considered. In contrast, by ignoring the polarizability or α value, Eisenman's simple model cannot account for the high H^+ over K^+ preference observed in virtually all kinds of living cells examined. Yet, this is accurately predicted with the model shown in Figure 12 with an

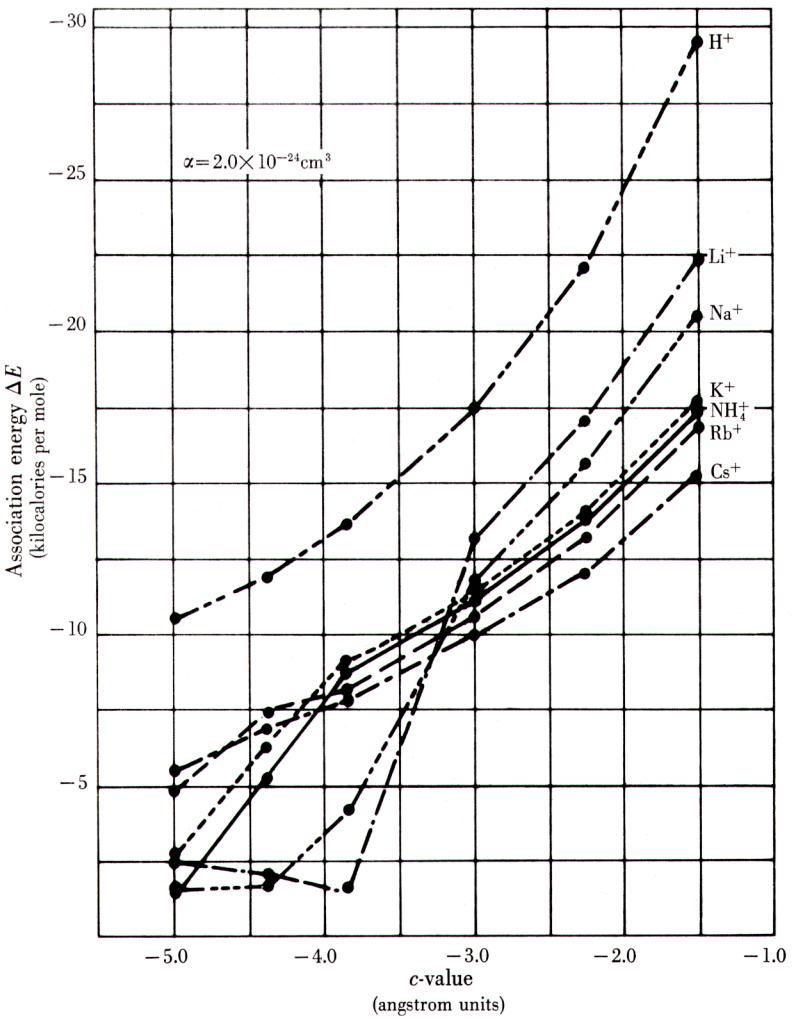

FIGURE 12. The theoretically computed association-energies in kilogram calories per mole are shown on the *ordinate* for six monovalent cations on a singly-charged oxyacid group with a polarizability of 2.0 x 10^{-24} cm^3 and c-value as indicated on the *abscissa*. An ion, say K^+, which shows a higher negative energy of association of –8.3 kcal/mole on a fixed oxyacid at the c-value of –4.0 Å is preferentially adsorbed over Na^+, which at the same c-value shows a lower negative association energy of –3.3 kcal/mole only. However, at a higher c-value of –2.5 Å, the preference is reversed since at this point the negative association energy of Na^+ at –16 kcal/mole is higher than that of K^+ at –13 kcal/mole. Preference reversal at different c-values is important in physiological activities according to the association-induction hypothesis as described in Figures 13 and 14 following. (From Ling 1962)

α value of 2.0 x 10^{-24} cm^3. However, the results from the two additional ions H^+ and NH_4^+—also not dealt with in Eisenman, Rudin and Casby's model at all—are of particular significance. Since all the fixed cations carried on proteins are variants of the NH_4^+ ion, the computed values for this ion as shown in Figure 12 offers more than its face value. That is, it also offers insight in the way c-value changes can alter the salt-linkage formation between fixed anions (mostly β-, and γ-carboxyl groups) and fixed cations (a-amino groups, ε-amino groups and guanidyl groups) in protoplasm.

4.4.5.2 THEORETICAL PREDICTION OF SWITCHING BETWEEN α-HELICAL ↔ FULLY-EXTENDED CONFORMATION WITH CHANGE OF C-VALUE ANALOGUE OF PEPTIDE CARBONYL OXYGEN ATOM

C.B. Anfinson shared the 1972 Nobel Prize for Chemistry for the discovery that the protein *ribonuclease* denatured in a concentrated urea solution, can be completely returned to the original so-called "native state" by washing away all the urea taken up by the protein (Anfinson 1967.) (For evidence that this assignment of "native" state is inverted and thus erroneous, see Ling 2001, Sect. 16.6 (1.3) on pp. 243–246 and p. 314; Ling 2006, Sect. 2.5 on pp. 10–15.) From this observation, Anfinson concluded that the folding patterns, or secondary and higher structures are determined by the protein's unique amino acid sequence also called the *primary structure*. But he made no suggestion how the primary structure determines the secondary and tertiary structure. The AI Hypothesis as described in pages following fills this conceptual gap and in the process has corrected a major error in protein chemistry on what is truly native and what is denatured. But as far as I can tell, rank-file protein chemists have not paid any attention to the suggested changes yet.

In the preceding section, we have shown how the effective electron density as expressed by the c-value determines the strength of a specific mono-valent cation's adsorption. As also mentioned above, all the fixed cations of a protein—be it an α-amino group at the N-terminal of a protein chain, or an ε-amino group carried on the side chain of a lysine residue or a guanidyl group at the end of an arginine residue—are all modified ammonium ion (NH_4^+.) Since what are known as salt linkages are formed between pairs of fixed cations and fixed anions, and in the AI Hypothesis salt linkages constitute the dominant component of the tertiary structure of a protein, clearly the c-value of the fixed anions determines the tertiary structure in the AI Hypothesis. By analogy, I suggested in the AI Hypothesis that the effective electron density of the peptide carbonyl oxygen atom determines the secondary structure as a mix of α-helical structure and fully extended structure (Ling 1986.) Table 1 shows how satisfactorily confirmed this idea turns out to be in the end. It has been retroactively confirmed by the quantitative data from three independent groups of prominent investigators: Chou and Fasman (1978); Tanaka and Scheraga (1976); Garnier, Osgathorpe and Robson (1978.)

Now the propensity or potential of the NHCO linkage of a specific amino acid residue to form an α-helical structure is called *α-helical potential.* The data shown in Table 1 yield respectively the positive linear correlation coefficients of +0.77 (Chou and Fasman 1978), +0.75 (Tanaka and Scheraga 1976) and +0.72 (Garnier *et al* 1978), averaging +0.75 between the α-helical potentials of 19 amino acid residues and the *electron donating strength* of the amino-acid-residues's side chains—as revealed by the pK_a of the corresponding carboxylic acids (e.g., formic acid for glycine; acetic acid for alanine.) The

TABLE 1. The α-helical potentials from Chou and Fasman (1978), Tanaka and Scheraga (1976) and Garnier, Osguthorpe and Robson (1978) of 19 α-amino acids and the pK_a of the corresponding 19 carboxylic acids. The linear correlation coefficients of Chou and Fasman's series of α-helical potentials and the pK_a's is +0.77. That derived from the Tanaka and Scheraga series and the Garnier *et al* series are +0.75 and +0.72 respectively. The average of all three sets of linear correlation coefficients is +0.75. (From Ling 1986)

	Chou and Fasman (P_α)	Tanaka and Sheraga ($\omega_{h,j*}$)	Garnier et al. (j)	Corrected pKa of Analogous Carboxylic Acids
Glu (-)	1.51	1.188	164	5.19
Ala	1.42	1.549	151	4.75
Leu	1.21	1.343	118	4.77
His (+)	1.00	0.535	98	3.63
Met	1.45	1.000	139	4.50
Gln	1.11	0.795	96	4.60
Trp	1.08	1.105	98	4.75
Val	1.06	1.028	100	4.82
Phe	1.13	0.727	102	4.25
Lys (+)	1.16	0.726	109	4.70
Ile	1.08	0.891	92	4.84
Asp (-)	1.01	0.481	91	4.56
Thr	0.83	0.488	60	3.86
Ser	0.77	0.336	47	3.80
Arg (+)	0.98	0.468	77	4.58
Cys	0.70	0.444	73	3.67
Asn	0.67	0.304	35	3.64
Tyr	0.69	0.262	41	4.28
Gly	0.57	0.226	0	3.75

rule is that the higher the pK_a of the corresponding carboxylic acid, the greater is the propensity of the CONH group of that amino acid residue to engage in α-helical structure. Or in the lingo of the AI Hypothesis: *the higher the c-value analogue of the peptide carbonyl oxygen atom, the greater is the probability of that amino acid residue's peptide group forming α-helical structure.*

Our next question is an equally fascinating one. What is the alternative of a peptide linkage if it does not form an α-helical structure? Once more, the AI Hypothesis offers an answer decisively different from the conventional one.

The conventional answer is that it will become a part of a random coil. I believe that this is highly questionable. For example, the infrared absorption studies of Ambrose and Elliott (1951), especially of Elliot, mentioned above show that in an aqueous medium, the polypeptide exists only in one or the other of just two alternative conformations: the *α-helical conformation* and the *fully-extended conformation,* a conclusion I cited from these authors in Section 4.4.3.2.

Since we have already established that a protein in the fully extended conformation adsorbs and polarizes multilayers of water molecules, clearly a protein's two alternative choices are: (1) existing in the fully extended conformation and adsorb deep layers of water molecules or (2) assuming the *α-helical conformation* mixed with liberated free water molecules. And the data given in Table 1 demonstrate *that high c-value analogue favors the α-helical conformation whereas low c-value analogue favors the fully extended conformation—with multilayer adsorption of water molecules through self-propagating (polarization and) orientation.*

4.4.6 The AI cascade mechanism (until the year 2007, known as Indirect F-effect)

As pointed out earlier, a pervasive trait of living matter is its connectedness. We know that the transmissivity through a saturated carbon atom is 0.33 or as high as 0.48 and that the static Direct F-effect can transmit through three peptide linkages directly or in addition to a short saturated carbon chain. However, to achieve what underlies its triple (i) ***one on many,*** (ii) ***from here to there*** and (iii) ***making many respond as one*** capabilities, the AI Hypothesis offers what was once called the *Indirect F-effect* until the year 2007. From 2007 on, it has been given the new name, *AI Cascade mechanism* (Ling 2007a.) As in the title of the AI hypothesis, A and I stand for association and induction respectively. And you will soon find out why.

Once more I repeat an earlier declaration to present here an essentially unmodified narrative on the model of long-range information and energy transfer that I presented first in my 2001 book (Ling 2001 pp. 147–149; Ling 2007.)

The single inset in Figure 13 illustrates the type of relationships between c-value analogue and c'-value analogue and the alternative adsorbents. At higher c-, or c'-value analogue, a^+ and a^- are respectively preferred. At lower c- or c'-value analogue, b^+ and b^- are respectively preferred. Now, let us suppose that as shown in Figure 13-A, initially all the backbone CO groups shown as O and all the backbone NH groups shown as H are respectively at the high c-value analogue and c'-value analogue of 2. In Figure 13-B and 13-C, the adsorption of the EWC, **W** at the cardinal sites withdraws electrons from the nearest neighboring O site, decreasing its c-value analogue from 2 to 1. This decrease of the c-value analogue reverses the preference for a^+ over b^+ to b^+ over a^+. As a result, the a^+ originally occupying the O site is replaced by a b^+.

Now b^+ is a weaker electron-withdrawing agent than a^+. In consequence, the displacement of a^+ by b^+ releases electrons. Some of the released electrons go back upstream toward the cardinal site-**W** couple, enhancing its electron-withdrawing effect. Other electrons released go downstream to the nearest H site, causing a decrease of its positive charge and hence a fall of its c'-value analogue from 2 to 1. A reversal of its preference for a^- over b^- to b^- over a^- follows, leading to the displacement of a^- by b^-. Now b^- is a weaker electron donator than a^-. In consequence, the displacement of b^- for a^- withdraws electrons. Some of the electrons withdrawn come from the O site upstream, further decreasing the c-value analogue of the O site upstream. Some of the electrons come from the next O site downstream, lowering its c-value analogue from 2 to 1. A b^+ for a^+ exchange follows. And the cycle repeats itself until all the a^+ and a^- are replaced by b^+ and b^- respectively as shown in Figure 13 C.

In Figure 13 the side chains are not represented. In Figure 14 we take into account functional groups on short side chains also.

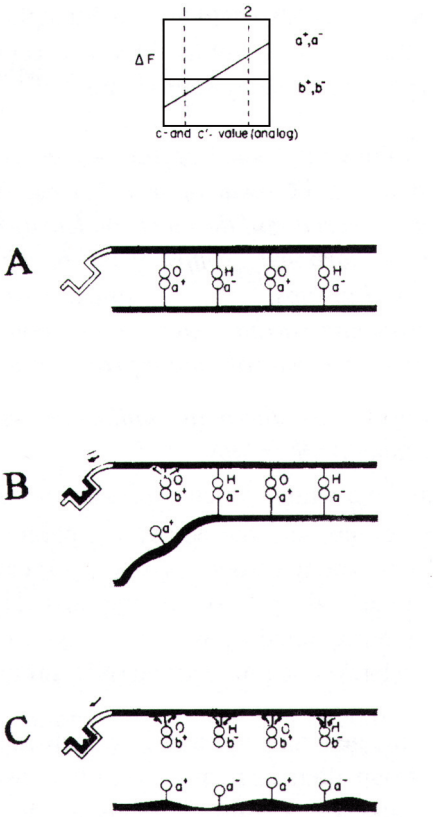

FIGURE 13. A theoretical model showing how adsorption of a cardinal adsorbent **W** on a controlling cardinal site of a protein brings about an (controlled) auto-cooperative transition resulting in an across-the-board uniform change in the electron density of the backbone NH and CO groups.
A sterically and electronically specialized region at the left end of the upper protein chain shown as a dark horizontal line makes up a *cardinal site*. Empty circles attached to the protein chains and labeled H and O represent respectively backbone NH and CO groups. Inset diagrammatically illustrates how changes in the *c-value analogue* of backbone CO groups alters the relative affinities for adsorbent a^+ and b^+ and how changes in the *c'-value analogue* of backbone NH groups alter the affinities for adsorbents a^- and b^-. See text for a description of how adsorption of the electron-withdrawing cardinal adsorbent **W**, on the cardinal site creates an inductively propagated across-the-board and uniform change in all the backbone NH and CO groups. (From Ling 2001)

In Figure 13 we treated adsorption on the backbone O and H individually. In Figure 14, however, each pair of adsorbent a^+ and a^- in Figure 13, (adsorbed respectively on the CO and NH groups of peptide groups belonging to a single amino acid residue) is treated as a single entity and represented by a rectangular box a. Figure 14A then represents a protein segment before an EWC is taken up. Here the electron-withdrawing power of the backbone cationic component (a^+) is represented as a downward arrow in the rectangular box, while the electron-donating power of the anionic component (a^-) is represented by an upward arrow.

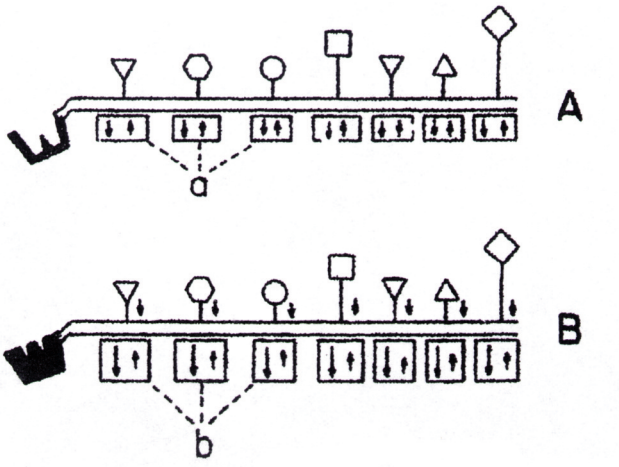

FIGURE 14. An alternative view of seeing the model of controlled auto-cooperative transition presented in Figure 13 above with focus on the impact of the electron-withdrawing cardinal adsorbent (EWC) shown as **W** on the various functional groups on side chains (shown as triangles, hexagons, circles and squares.) Displacement of the weaker (net-) electron-withdrawing **a** (representing Figure 13's a^+ and a^- together as a single net-electron-donating or a single net-electron withdrawing unit) on each peptide NHCO group shown in **A** by the stronger net electron-withdrawing **b** (representing Figure 13's b^+ and b^- together here as a single electron-donating or electron-withdrawing unit) shown in **B** is initiated by the adsorption on the cardinal site of the electron-withdrawing cardinal adsorbent (**W**.) This adsorption of **W** leads eventually to the across-the-board decrease of electron density (shown by downward arrows) of all side chain functional groups. Length of the upward arrow in the rectangular boxes indicates strength of the *electron-donating* effect; length of the downward arrow in the rectangular boxes (and along the side chains) indicates the strength of *electron-withdrawing* effect. For more details, see text.

Figure 14B shows the same protein segment after an EWC, **W,** occupies the cardinal site. As a result, the sequence of events described under Figure 13A to C takes place. And box b (which stands for b^+ and b^- together) now displaces box a (which stands for a^+ and a^- together). Since the O site, shown in Figure 13, to represent a carbonyl group (CO) is highly polarizable but the H site shown in Figure 13 to stand for an imino group (NH) is much less polarizable (Cannon 1955; Mizushima *et al* 1955), the *electron withdrawing effect* at the O site is strong and represented by a long downward arrow in the b box. In contrast, the *electron-donating effect* at the H site is weak and is represented as a short upward arrow. The *net effect* of displacing box a by box b is therefore an electron-withdrawing effect. Since this electron-withdrawing effect is repeated at each CONH peptide group, every functional group experiences a similar electron withdrawing influence as indicated by the downward arrows of the same length and in the same direction along each side chain as shown in Figure 14B on top of the polypeptide chain shown as a double narrowly-separated straight line. Of course, the functional groups at the end of the side chains differ and are diagrammatically represented variously as triangles, squares and circles. The functional groups that are on short side chains undergo the greatest change.

Now most abundant functional groups on short side chains are β-, and γ-carboxyl groups. A lowering of their electron density means a fall of their c-value (from an initial

high value.) As a result, a monovalent cation A^+ may be replaced by a B^+. This B^+ for A^+ exchange in turn reinforces the b for a exchange at the backbone as well as the adsorption of the EWC at the cardinal site. *Taken together, the backbone sites and the functional groups on short side chains and their respective adsorption partners (a/A^+/etc. versus b/B^+/etc.) constitute the alternative basic components of the auto-cooperative assembly diagrammatically illustrated in Figure 14B.*

In consequence of the interaction with the EWC, **W**, the backbone CO groups as well as the β-, and γ-carboxyl groups (and other functional groups on short side chains) undergo an across-the-board ***electron-density decrease*** from their initial higher values. And an exchange of adsorption partners occurs at both sets of sites. Conversely, with an EDC adsorbed onto the cardinal site of a protein segment, the backbone CO groups as well as the β-, and γ-carboxyl groups (and other functional groups on short side chains) may undergo an across-the-board uniform ***electron density increase*** from their initial lower values. And exchange of adsorption partners follow at both sets of sites in the reverse direction.

On account of their coherence, one may say that the whole "gang" of cooperatively linked functional groups on short side chains and the backbone carbonyl groups behave as if it were a single site. By the same token*, three-in-one impacts* of (i) *one-on-many*, (ii*) from here to there* and (iii) *making many sites behave as one site* capabilities produced by the binding or release of a cardinal adsorbent have all been achieved.

5 What is life

Charles Dickens wrote *Great Expectation* to tell about life in Victorian England. Cao Xue Qin wrote *Red Chamber Dreams* to tell about life of an aristocratic family in 18th century China. Leo Tolstoy wrote *War and Peace* to tell about life in Russia during the Napoleon invasion. In all these stories what I call life refers to social life. To portray social life, the writers tell about the lives of individuals, be it Pip in one, Jia BaoYu in another and Pierre in still another. The guiding rule is to explain life of a larger living entity in terms of the life of an entity one level smaller.

Accordingly, to explain an individual swimming across the English Channel, or an ordinary individual recovering from a stroke, we cite efficient muscle, powerful lungs and vulnerable brains. Each of these are part of the human body we call organs. But to understand organ physiology, we must descend another level smaller and that is the level of cells. In conventional bio-medical textbooks, cells are the last and thus the ultimate units of life. As a membrane enclosed sac of aqueous solutions, cells are like little soap bubbles, which can be broken but not resolved into still smaller repeating units. So what happened in conventional cell physiology to parts of the living cells like the nucleus, the mitochondria and the Golgi apparatus? They were given away to the department of biochemistry, department of genetics etc. and never came back to conventional cell physiology.

For we know by now that this conventional membrane (pump) view is completely wrong. And I am far from being the first to say this. Max Schultze in his *Protoplasmic Doctrine* said so already in 1861, when he called the cell a lump of protoplasm without a membrane. But as mentioned already on preceding pages, he too was only partly correct.

There are two shortcomings that Schultze and other early proponents of protoplasmic doctrine could not resolve. Again, the time was too early. First, they considered only one kind of protoplasm. Secondly, they could offer only gross *macroscopic* terms and concepts to define that protoplasm. Coming late in time and thus enjoying benefits denied earlier investigators, the association induction-hypothesis has in time corrected both shortcomings as will be shown next.

5.1 Definition of LIFE in terms of microscopic entitles

In the association-induction (AI) hypothesis, the smallest unit of life and hence life's ultimate physical basis, is ***microscopic protoplasm*** or ***nano-protoplasm***. In contrast, the living jelly oozing out of a broken cell like that shown in Figure 1 and once called sarcode or protoplasm is a specific type of ***macroscopic protoplasm***. As a rule, macroscopic protoplasm from one part or region of a living cell, say the cytoplasm, differs from macroscopic protoplasm from another part of the cell like the nucleus or cell membrane. But **each type of macroscopic protoplasm is, without exception, an aggregate of a vast number of the corresponding kind of *nano-protoplasmic units*** (NPU) (Ling 2007a.)

In the AI Hypothesis, life has two facets: being alive and engaging in reversible life activities. At the most fundamental level, being alive signifies the existence of the nano-protoplasm in a low-entropy but stable state called the *resting living state*. Life activities, on the other hand, involve reversible all-or-none shifts between the low-entropy resting living state and the alternative stable high-entropy *active living state*. Figure 15 provides a diagrammatic illustration of the salient features of these fundamental attributes and behavior of a nano-protoplasmic unit.

The low entropy of the nano-protoplasm in the resting living state—illustrated in the right-hand side picture of Figure 15—originates from the near total ***association*** among all the components of the nano-protoplasm: (1) virtually all the water molecules are directly or indirectly adsorbed on the peptide NH and CO groups of the fully-extended protein chains; (2) virtually all the K^+ in the nano-protoplasm are adsorbed on the β-, and γ-carboxyl groups of the nano-protoplasmic proteins and (3) the cardinal adsorbent, ATP

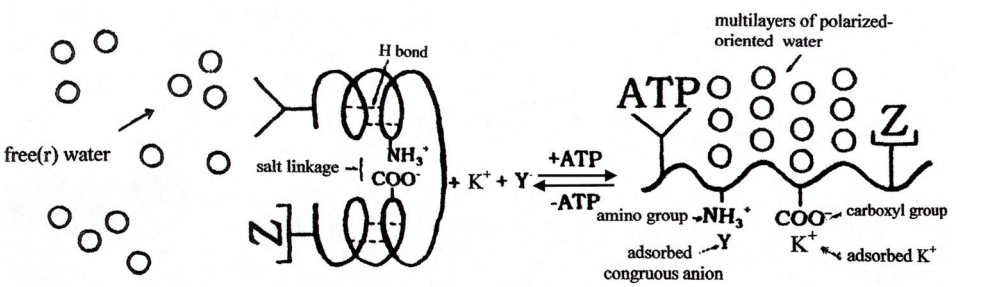

FIGURE 15. Diagrammatic illustration of all-or-none transition of a nano-protoplasmic unit between the fully-extended (resting living) state and the doubly folded (active living) state brought on by the binding onto, or removal of ATP from its specific cardinal site and/or of one or more *auxiliary cardinal adsorbent(s)* including (possibly) Ca^{++} and protein X etc. which are represented together here for simplicity by the single symbol, **Z** rather than by an undefined number of Z's (see Ling 1992 p. 184 including its Figure 8-14.)

is adsorbed on its specific *cardinal site* (Ling 1962 p. iiiv and p. 110.) Auxiliary cardinal adsorbents like that shown as Z in Figure 15 may remain adsorbed on their respective *auxiliary cardinal sites* during the resting living state and the active living state; they also may play a key role in controlling or modifying the shift between the resting and active living state as Ca^{++} and ouabain may do respectively.

For an illustration of the quantitative aspects of a typical nano-protoplasm in terms of molecules and ions, I choose for its exceptional simplicity the cytoplasmic nano-protoplasm of mature human red blood cells (rbc.) A typical rbc assumes the shape of biconcave disk as shown in Figure 16. It has neither nucleus nor other subcellular organelles. 65% of the rbc weight is water. 97% of the remaining 35% of the rbc's weight comprises vast number of copies of a single protein, ferri-hemoglobin (Hb) (Ponder 1948.) It is also known that the content of a rbc does not spill out when the rbc is cut apart (Best and Taylor 1946.) And that at 0° C, the rbc keeps its ATP content better than at higher temperature (Ling and Bohr 1969.) Accordingly, one can use a magic scalpel to cut a single rbc at 0° C into two equal halves and repeat the procedure again and again until at last each of the halves contains just one single Hb molecule. At this time, the content of each half is essentially that of a single rbc cytoplasm nano-protoplasmic unit (NPU.)

Based upon these and the other relevant facts, one can then tentatively represent the nano-protoplasmic unit of the rbc cytoplasm by the formula: $(Hb)_1(H_2O)_{7000}(K^+)_{20}(ATP)_1$. As our knowledge about each nano-protoplasmic unit increases, its formula will change. A general formula for all nano-protoplasm is given in the form of Equation 1, on page 124 of the article, *Nano-protoplasm, the Ultimate Unit of Life* (Ling 2007a.) Assumed to be spherical in shape, the diameter of each NPU of rbc cytoplasm is 8.6 nanometers (nm.) All these pertain to the rbc cytoplasmic nano-protoplasm in its ***resting living state***—as diagrammatically illustrated in the right hand side illustration of Figure 15.

The resting living state is under the pervasive control of the principal electron-withdrawing cardinal adsorbent, (EWC) ATP (in addition possibly to other as yet unidentified auxiliary cardinal adsorbents.) Through the operation of the AI cascade mechanism, the adsorption of ATP on its cardinal site sets in motion the chain reaction so that all the β-, and γ-carboxyl groups on the aspartic and glutamic side chains of the hemoglobin molecule are kept uniformly at a low c-value with preference for K^+ over competing Na^+. And

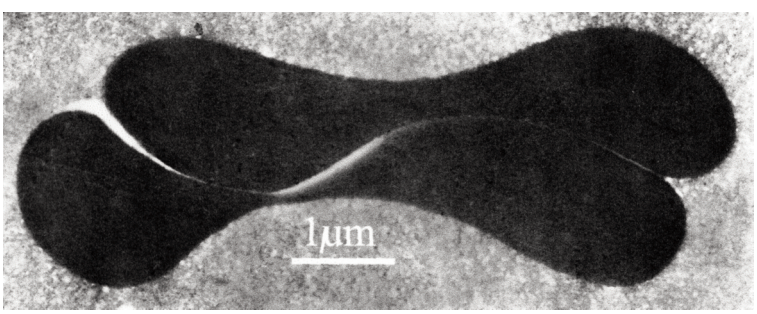

FIGURE 16. Electron micrograph of the cross sections of two mature human red blood cells (in blood plasma.) Cryofixed, freeze-dried and embedded in Lowicryl. (Gift of Dr. Ludwig Edelmann, from Ling 2007a)

all the backbone peptide carbonyl groups are kept uniformly at a low c-value analogue value with preference for multilayer water polarization-orientation.

In contrast, the left-hand side picture of Figure 15 depicts a nano-protoplasmic unit in the ***active living*** *(or* ***dead)*** ***state***, brought on by the removal of the EWC, ATP transiently (or permanently.) As such, the assembly as a whole has high entropy because all the water molecules and K^+ are set free. This follows from the fact that the removal of EWC, ATP sets in motion the AI cascade mechanism leading to a uniform rise of the c-value of all the β-, and γ-carboxyl groups on aspartic and glutamic side chains. And at the high c-value (and with the availability of fixed cations) the β-, and γ-carboxyl groups prefer to engage in the formation of salt-linkages, setting free the adsorbed K^+. Simultaneously, the ATP removal also leads to a uniform decrease of the c-value analogue of the backbone carbonyl groups. At the low c-value analogue, the backbone carbonyl groups prefer to engage in α-helical formation with NH groups of the fourth amino acid residues in both directions along the polypeptide chains. Release of all the multilayers of adsorbed water molecules follows.

Since ATP is the end product of all energy metabolism (Ling 1981) and in cells like the voluntary muscle, there is also a large store of creatine phosphate, which through the action of the enzyme *creatine kinase* maintains a constant level of ATP. Accordingly, the replacement of lost ATP is usually rapid and complete. When for one reason or another, the replacement of ATP fails, the nano-protoplasm will enter the irreversible ***dead state***.

In summary, ***life*** comprises ***being alive*** and ***engaging in life activities***. At the most basic level, *being alive* signifies the existence of nano-protoplasm units in the low entropy *resting living state*, in which all the major components of nano-protoplasm are in direct or indirect association with one another—, an act made possible by the adsorption of the controlling ATP on its specific cardinal site (and possibly adsorption of as yet unverified (auxiliary) cardinal adsorbents also.) The adsorption of the powerful EWC, ATP brings about a uniform decrease of both the c-value of the β-, and γ-carboxyl groups (and K^+ adsorption) and the c-value analogue of the peptide carbonyl oxygen atoms (and multilayer H_2O adsorption) via the AI cascade mechanism. ***Engaging in life activities***, on the other hand, signifies reversible all-or-none (auto-cooperative) shifts between the *resting living state* and the *active living state*. In contrast to the resting living state, the active living state is distinguished by its high entropy in consequence of the liberation of all its K^+ ions and water molecules and the assumption of the nano-protoplasmic protein of the double-folded states through the formation of α-helical intra-polypeptide H-bonds on one hand and the formation of salt linkages between fixed cations and fixed anions on the other.

5.2 Verification of theory on an ultra-simple model

If a theory of life is correct, it would provide the foundation of the basic mechanisms for understanding all living phenomena. Or more correctly, understanding of life at the ultimate lowest level would explain all living manifestations at one level higher and those perceptions would in turn provide the basis for understanding life phenomena at another level still higher—until all living phenomena will be explained—in a way in full harmony with our understanding of the entire Universe comprising both the living and the dead.

To begin with our knowledge at the nano-protoplasmic level, our task is to understand phenomena of cell physiology. The four classical topics of cell physiology are (i) solute (and water) distribution; (ii) solute (and water) permeability; (iii) cellular electrical

potentials; (iv) cell swelling and shrinkage. Progressively more up-to-date explanations of the manifestations of cell physiological activities under each of these four categories have been presented in two books and a more recent lengthy review. They are respectively *A Revolution in the Physiology of the Living Cells* (Ling 1992), *Life at the Cell and Below-Cell Level* (Ling 2001) and *Nano-protoplasm, the Ultimate Unit of Life* (Ling 2007a.)

Other physiological activities of the cell beyond the four categories listed above and their explanations based on prior versions of the AI Hypothesis are found in two earlier books: *A Physical Theory of the Living State: the Association-Induction Hypothesis* (Ling 1962) and *In Search of the Physical Basis of Life* (Ling 1984). In addition, there are also individual review articles on specific subjects including the following: Ling 1977 (energization of biological work performance); Ling 1981 (oxidative phosphorylation & mitochondrial physiology); Ling 1981a, 1990 (active transport across frog skin and other bifacial systems); Ling, Reid and Murphy 1986 (cancer); Ling and Ochsenfeld 1991 (muscle contraction.)

While the interested reader may choose to consult these publications directly, I am going to present below the Abstract of a very special paper by Ling and Ochsenfeld (2008) described immediately below as an illustration of the simplicity and effectiveness of explaining life at one level above nano-protoplasm. The title of the paper reads:

A Historically Significant Study that at Once Disproves the Membrane (Pump) Theory and Confirms that Nano-protoplasm is the Ultimate Physical Basis of Life—Yet so Simple and Low-cost that it Could Easily Be Repeated in Many High School Biology Classrooms Worldwide

Abstract.
In 1889 Abderhalden reported his discovery that there is no (or as shown later, little) sodium ion (Na^+) in human red blood cells even though these cells live in a medium rich in Na^+. History shows that all major theories of the living cell are built around this basic phenomenon seen in all the living cells that have been carefully examined. One of these theories has been steadily evolving but is yet-to-be widely known. Named the association-induction hypothesis (AIH), it has been presented thus far in four books dated 1962, 1984, 1992 and 2001 respectively. In this theory, the low Na^+ in living cells originates from (i) an above-normal molecule-to-molecule interaction (energy) among the bulk-phase cell water molecules, in consequence of (ii) their (self-propagating) polarization-orientation by the backbone NHCO groups of (fully-extended) cell protein(s), when (iii) the protein(s) involved is under the control of the *electron-withdrawing cardinal adsorbe*nt (EWC), ATP. A mature human red blood cell (rbc) has no nucleus, nor other organelle. 64% of the rbc is water; 35% belongs to a single protein, hemoglobin (Hb). This twofold simplicity allows the concoction of an ultra-simple model (USM) of the red blood cell's cytoplasmic protoplasm, which comprises almost entirely of hemoglobin, water, K^+ and ATP. Only in the USM, the ATP has been replaced by an artificial but theoretically authentic EWC, H^+ (given as HCl). To test the theory with the aid of the USM, we filled dialysis sacs with a 40% solution of pure (ferri-) hemoglobin followed by incubating the sacs till equilibrium in solutions containing different amounts of HCl (including zero) but a constant (low) concentration of NaCl. We then determined the

equilibrium ratio of the Na^+ concentration inside the sac over that in the solution outside and refer to this ratio as q_{NaCl}. When no H^+ was added, the q_{NaCl} stayed at unity as predicted by the theory. More important (and also predicted by the theory,) when the right amount of H^+ had been added, q_{NaCl} fell to the 0.1–0.3 range found in living red blood (and other) cells. These and other findings presented confirm the AIH's theory of life at the most basic level: in the *resting living state*, microscopic, or *nano-protoplasm*, is the ultimate physical basis of life. (End of Abstract.) (Figure 17 presented in the main text of the article is transplanted here for emphasis and clarification.)

The following is a list of the highlights presented in the full-length article and their ***new global significance***.

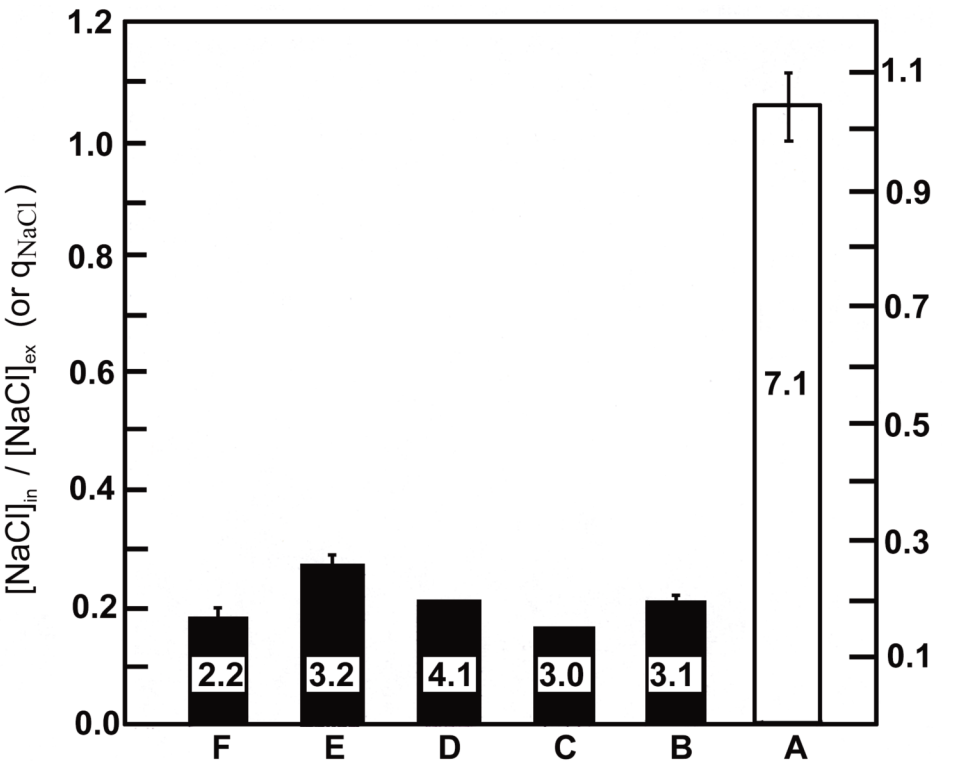

FIGURE 17. The equilibrium distribution ratio of NaCl or q_{NaCl} of ultra-simple models of red blood cell cytoplasmic nano-protoplasm (A) not treated with HCl; (B,C,D,E,F) treated with appropriate amount of HCl. Numbers in bar graphs indicate equilibrium pH of the media bathing that particular set of dialysis sacs. Data were obtained with the aid of two different experimental techniques: equilibrium dialysis studies carried out by M. M. Ochsenfeld and radioactive tracer studies carried out by Dr. Hu Weixiao. (From Ling and Ochsenfeld 2008)

5.2.1 q_{NaCl}

This article presents the first fully successful demonstration in history in a model system of a q value between 0.1 and 0.3 for NaCl at the low (10 mM) concentration range. The bulk of prior work on q_{Na} from my own laboratory and from some other laboratories were based on the use as probes of sodium sulfate or sodium citrate and as a rule at very high concentration. Other studies showed that in those model systems like linear oxygen-containing polymers and NaOH-denatured hemoglobin, the demonstrated q_{NaCl} were as a rule, not far from unity. The low q_{NaCl} profile demonstrated here is shared by most living protoplasm as the theory predicted. The **diversity** of dynamic water structure (all under the heading of polarized-oriented multilayer theory) and their experimental evidence will be introduced by us hopefully not too long from now. Included is the evidence that the dynamic structure of the bulk-phase water in maximally deviated **cancer** cell is different from that in normal cells like muscle for example.

5.2.2 AI cascade mechanism

As many as 500 water molecules have been demonstrated to become "non-solvent" for Na^+ by the attachment of a single H^+ on an H^+-binding site of the hemoglobin molecule. This demonstration confirms the even farther reach of the AI cascade mechanism operating in intact living muscle cells discussed in Section 4.4.4.1 on page 40, where each ATP molecule controls the dynamic structure of an average of 8000 water molecules.

5.2.3 ATP as EWC

The physiological role of ATP as an electron-withdrawing cardinal adsorbent (EWC) has received further confirmation because H^+, its substitute in the present study, is nothing more than a positive charge and hence by definition an EWC.

5.2.4 Chloride binding confirms role as congruous anion adsorbed one-on-one in close-contact in rbc

The extensive adsorption of Cl^- ion on hemoglobin described in detail in the text but not mentioned in the abstract, confirms yet another important theoretical postulation of the AIH: Cl^- is the principle congruous anion of red blood cells (Ling 1992 p. 183; Ling 2001 p. 153.) That Cl^- binding has produced an across-the-board *increase* of the c-value analogue hence the percentage of backbone carbonyl oxygen atoms existing in the α-helical fold conformation—further confirms that the binding of the Cl^- is adsorbed one-on-one in close contact as the theory has predicted.

5.2.5 Explaining Edelmann's LiCl promotion of Cs^+ adsorption

Using laser-microprobe mass-spectrometer analysis (LAMMA), Edelmann discovered that the presence of 100 mM LiCl produced what appears to be a fivefold increase of Cs^+ adsorption on the β-, and γ-carboxyl groups of myosin at the two edges of the A bands of frog muscle (Edelmann 1980.) Like H^+, Li^+ too is a simple and small cation and as such its binding on myosin could in theory produce a qualitatively similar effect on myosin as

that produced by the binding of H^+. Now 100 mM Li^+ is equivalent to a pLi of 1—certainly high enough in concentration to match the H^+ concentration at pH of 2, which produced the minimum physiological q_{NaCl} of 0.1 to 0.3 of the bulk-phase water as shown in Figure 17. In theory, this was accomplished by decreasing wholesale the ***c-value analogue*** of the backbone carbonyl groups. Of course, according to the AIH, a wholesale decrease of the ***c-value analogue*** of the backbone carbonyl groups via the AI cascade mechanism would inevitably be accompanied by a wholesale decrease of the ***c-value*** of the β-, and γ-carboxyl groups simultaneously— but that subject was not pursued in the 2008 Ling & Ochsenfeld study described. Yet what that work did reveal to us led me to the belief that the influence of a high concentration of Li^+ in promoting Cs^+ adsorption is by way of decreasing the c-value of the β-, and γ-carboxyl groups by the adsorption of Li^+. And at the lowered c-value, the adsorption energy of Cs^+ is increased (and the relative binding energy of competing Li^+ decreased) as shown in Figure 12. And a consequence would be what Edelmann had discovered and reported in 1980: increased binding of Cs^+ on the edges of the A bands of frog sartorius muscles. (It bears mentioning that the Cl^- ion that came with Li^+ and Cs^+ will play a similar role as that described for the Cl^- ion that came as HCl in the full-length article of the Ling-Ochsenfeld study.)

5.2.6 Polar NP surface or array of polar NP-NP-NP chains

The NP-NP-NP system of oriented fully extended protein chains with its backbones directly exposed to bulk-phase water have been shown in the (full-length) article to have far-reaching impact on the dynamic structure of the bulk phase water—functionally almost as effective as an NP (or NP-NP system) of a checkerboard of negatively charged N and positively charged P sites uniformly distributed on a two-dimensional surface—which was shown to have the potential of polarizing-orienting water molecules *ad infinitum* under idealized conditions. (For other independent evidence for long-range water polarization-orientation of other NP-NP-NP models, see Ling 2001's Figure 30 and Ling 2006's Figure 14.)

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A 2004 Unanswered Letter to the Economist Magazine Requesting a Retraction (And Apology)

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Abstract: This is a copy of (the bulk of) a letter I mailed on May 13, 2004 to Sir Robert P. Wilson, President, and three editors of the magazine, the Economist. With the letter, I also sent each recipient a copy of my latest book, "Life at the Cell and Below-Cell Level" as a gesture of good will.

THE MAIN objective of the letter, however, was to ask the Economist to publish a retraction in a forthcoming issue of the magazine, of the unfounded attack on my reputation and my life's scientific work in a 2003 issue of the journal. Nearly ten years have passed. My letter (and attached gifts of books and articles) were never answered or even acknowledged. The request for retraction was ignored.

I have now decided to publish my letter in order to keep on record of the unprovoked and unfounded attack and my full rebuttal of the attack. It is disappointing to witness that a company so skillful in selling worldwide more and more copies of its magazine, makes no effort to stand behind what it sells in the magazine. Obviously, at the time when I mailed my letter I had a more hopeful view on the prospect. All that, however, does not rule out the possibility that one day a younger leadership may take over the company and change for the better. That said, I now return to my letter.

After pointing out the fallacy of the claim that the denigration of my scientific reputation came from "most scientists", I offered the author of this slanderous article the chance to defend her position in a written debate. That is, with the full understanding that a "no response" constitutes an admission of total defeat and a mandate for printing the retraction.

I had spent time and effort to write this long letter, at least in part out of my belief in fair play. For I realized that the person who wrote the slanderous article and the person(s) in a position to undo the harm done and retract the article are not the same. For the

person(s) to undo the harm meaningfully, he or she must know more than what is on the surface. Indeed, he or she must know the essence of the *whole truth*.

However, there is an additional even more serious reason for suggesting the debate and for sharing the essence of the whole truth (see below.) For I strongly suspect that the unprovoked attack on me is only the tip of a massive submerged iceberg of accepted destructive selfishness that is threatening the long-range wellbeing of the future Humanity as a whole.

To illustrate the subjects of my concern, I quoted five recently published books at the time: Sir Alan Rees's "*Our Final Hours*" (2003); David Goodstein's "*Out of Gas; the End of the Age of Oils*"(2004); Laurie Garrett's "*The Coming Plague*" (1995); Merrill Goozner's "*The $800 million Pill*" (2004) and John Horgan's "*The End of Science: Facing the Limits of Knowledge in the Twilight of the Scientific Age* (1996.)

The first four books portray the grave dangers humanity is facing or soon to face—even though they are frequently rejected as untrue or shrugged off as unimportant but mostly unknown to the vast majority of the inhabitants of this planet and their (too many) poorly educated, myopic leaders. The last book tells us that *basic science*, the mind-opening enterprise that had in the past produced admirable defenses against serious problems again and again, may itself be ending. Is the future of Mankind doomed? The answer is a qualified no. It depends (see below.)

The remaining pages of the letter—under the heading of "The Rest of the Whole Truth" represent a detailed analysis of the backgrounds of the situation that Mankind finds itself in, at once dazzlingly advanced and abysmally backward.

At the outset I remind the readers of my letter that science is far more than a systematized collection of knowledge. It is, above all, a cooperative effort to *search for the whole truth* by scientists, living, dead and yet to come. That science could get to where it is today was no accident. It depended on a *code of behavior* adopted and subscribed to by the scientific leadership of the past, notably in the late 19th century and the early 20th.

This code of behavior was described in the (1924) Textbook of General Physiology written by the English *general physiologist*, Sir William M. Bayliss. It says that what makes a scientist great is not his never making mistakes but his readiness to admit a mistake when it is made. (And then turn around and pursue with full vigor and enthusiasm, the once-opposing view, now proven correct.) If the subject matter is deep and far-reaching enough, this turn around could constitute the core event of what is known as a *scientific revolution*.

I then cited three major scientific revolutions of the past, each respectively in chemistry (Lavoisier), biology (Darwin) and physics (Planck). In every case, despite relentless last-ditch resistance from the defenders of the old (erroneous) faith, the search for truth continued on in the hands of a younger generation of scientists. These youthful scientists enjoyed the freedom of new adventure on roads that the subscribed *code of behavior* of the enlightened had paved.

But, sadly, things have drastically declined during mostly the mid- and late 20th century. To begin with, there were too many scientists competing for the limited support and positions available to them. Worse, two major causes arose that stood in the way of normal scientific progress. To explain how they misguide the enterprise of science, I invoked the similarity between research in *cell physiology,*—which is the only largely unexplored major field of basic science and of particular importance to Mankind's future welfare—and the solution of a *cross-word puzzle:* each uniquely has one and only one solution.

One major cause that blocks normal scientific progress with its (occasional) revolution(s) was totally unexpected. It is the (three) rich, autonomous and long-standing Institutions. Each was erected to promote and facilitate the progress of science. All metamorphosed at times into fortresses sheltering the entrenched obsolete *status quo*.

Leading the trio is the Nobel Prize Institutes for the as-yet-immature sciences like physiology and medicine. It is followed by the research funding agencies with their established selective procedure called *peer review* and lastly the giant textbook-printing corporations. How each of these three institutions does the damage is in turn explained with the help of a bright 9-year old's solution of a New York Time crossword puzzle. However, a deeper insight into the cause of their harmful modes of operation can be found in one tragic fact. None of the key players of these institutions has apparently been taught in their critical early formative years the vital *code of behavior* mentioned above

The second major cause halting normal scientific progress is continuing fragmentation. In the beginning, fragmentation like that of dividing natural science into physics chemistry and biology was helpful but continued fragmentation into still smaller and smaller specialties becomes counterproductive. Like trying to solve a crossword puzzle by first tearing the puzzle into small pieces and then enlisting different people to solve the torn pieces separately, continuing fragmentation by itself is not to lead to the unique solution.

However, once more, the situation is not entirely hopeless. Indeed, in theory at least, one can remedy the harmful consequence of fragmentation by inventing ***an all-encompassing unifying theory*** and testing the theory again, again and again until it has been proven completely and unequivocally correct. That fully confirmed unifying theory, when widely accepted, would guide future human progress in time to come.

However, generally speaking, such an all-encompassing unifying theory is not something you see displayed in show-windows everywhere. It is rare and sometimes downright unattainable—especially if the maturation of the relevant underlying fundamental sciences of physics and chemistry needed for constructing the unifying theory are still a thing of the future.

A short account of the real-world history of cell physiology then follows. As I pointed out earlier, cell physiology is the only remaining major field of basic science as yet mostly unexplored. Its maturation could produce vital new knowledge that would help solve many of the pressing problems.

The science of the small began with the invention of the microscope. And the invention was twofold: the discovery of *cells* and the discovery of *protoplasm*. For various understandable reasons, the cell was (incorrectly) described as a membrane-enclosed water-filled cavity. Notwithstanding, it is currently being taught *as unqualified truth* in all high school and college biology textbooks worldwide—half a century after it has been unequivocally disproved.

It is hardly surprising that to my best knowledge, none of these biology textbooks for 9^{th}–12^{th} graders and for colleges in the US and elsewhere mention the *code of behavior* that the Textbook of General Physiology of Sir William Maddox Bayliss once did at the turn of the 19^{th}–20^{th} century.

In contrast, the protoplasmic approach was widely adopted for some time and then abandoned. That is, until a distant heir to the protoplasm theory came into being. It is called the association-induction (AI) hypothesis.

The central theme of the AI Hypothesis was first published in 1962 in a 680-page monograph entitled: A Physical Theory of the Living State: the Association-Induction

Hypothesis. Three more full-length monographs followed in the years following, 1984, 1992 and 2001. Each new monograph records continuing theoretical advance and steady experimental confirmation of all aspects of the AI Hypothesis without any major reversal in the years following till now. At this moment in 2013, to say that ***the association-induction hypothesis has been fully confirmed*** is no exaggeration.

Above all, the association-induction hypothesis is nothing less than a full-fledged *unifying theory of cell physiology.* As pointed out earlier, the only way to heal the damage that fragmentation has done to fundamental cell physiology is a verified unifying theory. That it was possible to construct such a unifying theory is to no small extent because the necessary foundation of chemistry (proteins) and of physics (statistical mechanics) have finally matured—before my generation of cell physiologists arrived on the scene.

With all these in mind, it would sound facetious to say that all in all, curing fragmentation with a verified unifying theory is the easy part, when one compares this with the task of getting all the biology textbooks to teach the association-induction hypothesis rather than dead-wrong membrane pump theory. As mentioned above, tragically but most likely that none of the key players in the three "fortresses" guarding the (erroneous) *status quo* has been taught early in their education the *code of behavior* described earlier. Still, it is never too late to correct this mistake by teaching the *code of behavior* to all the coming generations of young people that would one day inhabit this planet. And my writing this letter in 2004 and my printing it in this year 2013 has all that purpose as its ultimate goal. That said, I now return to the association-induction hypothesis.

The central theme of the association-induction hypothesis tells how *association* and electronic polarization (or *induction*) provide the basic molecular mechanisms that put the three major components of living matter, *protein, water,* K^+ and a small number of controlling agents, *ATP* etc. into a coherent assembly. This assembly can exist in an all-or-none manner in either of two alternative states. In one state, called the *resting living state*, ATP and other essential auxiliary cardinal adsorbents stay adsorbed on the protein on their respective binding sites called cardinal sites. In the resting living state, the protein exists in the fully extended state and all the four major components are connected throughout spatially and electronically.

More specifically, all the K^+ are adsorbed *one-on-one*, *in close contact* on the exposed β-, and γ-carboxyl groups carried respectively on the side chains of aspartic and glutamic residues of the fully extended protein(s) involved. In contrast, all the water molecules are adsorbed directly or indirectly as polarized-oriented multilayers on the exposed CO and NH groups of the same fully extended protein chains. In the alternative state called the *active living state (*when reversible) or *dead state* (when irreversible) all the K^+ and water molecules are set free.

The letter went on to explain how the cytoplasmic protoplasm made of vast number of these more basic microscopic protoplasmic units (to be named *nano-protoplasm* four years later in 2008) can selectively accumulate K^+ at a level many times *higher* than K^+ in the external bathing medium, while at the same time, keeping its chemically almost identical Na^+ at a level many times *lower* than found in the bathing medium. Accordingly, there is absolutely no need for the postulation of membrane pumps in most of what is known in unifacial cells like muscle, nerve and red blood cells. (See below.)

The letter then went back in time to tell how British scientists, Alan Hodgkin, Andrew Huxley and Bernard Katz made their Nobel Prize-winning discovery of the key role of

Na^+ in the creation of the muscle and nerve impulse or action potential. My early graduate thesis work produced evidence apparently in harmony with the membrane pump theory, which is the foundation of the research of Hodgkin, Huxley and Katz. In those early days, we became friends.

Then I discovered that the membrane pump hypothesis is in violation of the Law of Conservation of Energy. More experimental studies paved the way to the eventual introduction of the Association-Induction (AI) Hypothesis. I sent copies of my earlier writing on what I found to many friends including Hodgkin and other cell physiologists in Cambridge, England. Many responded favorably.

Then suddenly, like a thunderbolt out of a blue sky, I found myself no longer treated as a friend by my Cambridge colleagues. Worse, Prof. Richard Keynes, a student of Prof. Hodgkin announced publicly that I had committed a major heresy. It was not intended as a joke. Indeed, before long, he and his helpers went on to "excommunicate" me and in other ways made it very hard for me to continue my life as a cell physiologist. As an example, one of their former American graduate students used the position he held as the head of the Physiology Study Section of the National Institute of Health (NIH) of the United States—by that time, my wife Shirley and I had become US citizens—to stop funding my research permanently. That wish became a reality in 1988.

The following section tells how the Nobel institutes also acted as if my decisive disproof of the membrane pump theory had never existed and awarded Nobel Prizes for work on the thoroughly disproved theory again and again.

And lastly, the Nobel Prize for Physiology or Medicine was awarded to chemist, Paul Lauterbur and physicist, Peter Mansfield for inventing Magnetic Resonance Imaging or MRI but not to physiologist-physician Raymond Damadian, who in my opinion was MRI's true inventor.

Parenthetically I pointed out why without the AI Hypothesis, or more specifically its subsidiary Polarized-Oriented Multilayer (PM or POM) theory of cell water (and model systems,) there would be no or very little chance that MRI would be a reality today—for the following reason.

In the polarized-oreinted multilayer (POM, or PM) theory, I suggested for the first time in history that the bulk of cell water in healthy resting living state is polarized and oriented and thus dynamically ***structured***. So if a machine can detect the motional freedom of the water molecules (H_2O) and expressed it in quantitative parameters called T_1 and T_2, that machine would record a shorter T_1 and T_2 of the water in living cells than those of normal liquid water. That is, if the PM theory is correct. In fact, a machine, called nuclear magnetic resonance (NMR) spectrometer, can do just that.

In time, four individual (or group of) investigators took up the challenge. They belonged to a younger generation of scientists and I knew none of them before. They are Freeman Cope, Carlton Hazelwood, Hollis *et al*, and lastly Raymond Damadian. Each acknowledged in their publication that they knew beforehand the PM theory and how it predicts dynamically structured cell water. Before long, they reported unanimously that the T_1 and T_2 of water protons in the living cells they examined are much shorter than those of normal liquid water in a dilute salt solution. All four also acknowledged publicly and privately that the results are in harmony with the (subsidiary) Polarized Multilayer theory of the AI Hypothesis. The story of Raymond Damadian is particularly telling.

To begin with, most educated people worldwide are brought up on the belief that water in healthy living cells is plain unstructured free water. Therefore, the brief remark, which

Albert Szent-Györgyi made in a footnote of a book that cancer cells have less water structure makes no sense at all except to those few who happened to know beforehand that the bulk of water in healthy resting cells is in fact (dynamically) structured. And that perception prompted one of the few informed investigators, Raymond Damadian, to study the T_1 and T_2 of water protons in three varieties of malignant cancers—side by side with those of a variety of normal rat tissues. The much longer T_1 and T_2 seen uniformly in the cancer tissues when compared to their normal counterparts set the stage for his next move.

The opening sentence of Damadian's report in the Science magazine describes his intention of using the differences in T_1 and T_2 as the factual basis to construct a machine that would detect cancer. In time, he and his two graduate students did just that. And that machine they put together was given the name, "**Indomitable**." Can anyone in his right mind deny that this is a landmark event in the history of the invention of MRI?

Or deny the significance of what Damadian wrote on November 9, 1977 in a letter to me? "The achievement—of the world's first MRI image of the live human body—originated in the modern concept of salt, water biophysics, on which you are the grand pioneer with your classic treatise, the association-induction hypothesis."
The following further corroborates my belief that the Polarized Multilayer Theory of cell water has played a vital role in the invention of MRI.

Now a few additional words on why I think that the members of the Nobel Prize committees committed another serious mistake by giving the award to Lauterbur and Mansfield but not to Raymond Damadian.

To be sure, compared to the current-day model of MRI designed with the technological inputs from Lauterbur, Mansfield and others, the "Indomitable" is far more primitive. But could that be held as fair reason to deny that the Wright brothers are the inventors of airplane and give the credit, instead, to the inventor of modern jet planes? After all, who could deny that the Wright brothers's flying machine is also much less sophisticated than the modern jet planes?

After I have displayed the logic and evidence that my scientific work played a key role in the invention of MRI, I raised the question, who stands to profit by denigrating my scientific work. With that and my answer, my letter came to its end.

In conclusion, I emphasize that I am not interested in punishing the wrong doer who has slandered and "fettered" me in my effort to help mankind. I do hope, though, this letter may get others to start thinking more about humanity's future. In particular, I hope to get them to seriously think of teaching all young children in their critical age, the code of behavior as a part of the first biology course. For without doubt, that it would enhance the chance of mankind's continuing survival and prosperity and turn it into a certainty.

A correction: I have in this letter and elsewhere repeatedly mentioned that science is an invention of the West. This was a mistake and I hereby make correction. One does not deny that the West has played a dominant role in the later development of modern science. However, it is also widely acknowledged that modern science began with the invention of what is known as *the Scientific Method*. In the Western literature, the invention of the scientific method has been almost always attributed to scientists of the 16^{th} to 18^{th} century like Galileo, Roger Bacon, and Francis Bacon. The startling truth unearthed in my belated discovery is that the scientific method was actually invented many centuries earlier during the European Dark Age. More specifically, the Arab scholar, Ibn al-Haythem alias Alhacen, who was born in Basra in 965 and died in Cairo in the year 1019, invented the

scientific method. The reader can find what could be an entirely unknown world of the Arab golden age and many incredibly brilliant Arab achievements by legions of polymaths (universal scholars) in Wikipedia, the free encyclopedia online.

Sir Robert P. Wilson, President
The Economist
25 St. James's St
London, SWIA 1HG, UK May 13, 2004

Dear President Wilson:

The following comments on my scientific work appeared in the quarterly Technology section of your journal, The Economist (12-5, 2003), in an article entitled "MRI's Inside Story":

> "Following an obscure theory devised by Gilbert Ling, a physiologist … Most scientists consider Dr. Ling' ideas wacky at best…" (Wacky is slang for irrational, crazy, Webster Dictionary)

Honestly, you could not have interviewed all the world's scientists (and found that most of them consider my ideas wacky at best.) It is equally unlikely that you have invented this all by yourself. That leaves only one alternative. You have interviewed a miniscule fraction of the world's scientists and passed its defamatory attack as a fair evaluation of my life-long work by most scientists.

I ask, what motivated your journal to risk its reputation of honesty and intelligence in harming someone who had never done you harm? After all, if you should succeed in denigrating my credibility as a scientist, what could your journal or your readers gain? The answer is less than nothing. That too leaves only one alternative. You have done somebody else's hatchet job—unknowingly I trust.

The people who had succeeded in getting your journal to do its hatchet job did not act alone. It was a part of a sick but powerful clique, which in various ways resembles the 17th century Catholic Church under Pope Urban VIII. Both were willing to go to extremes in order to preserve their image of infallibility and the goodies that come with it.

To warn off others, who might also doubt that the Earth is the center of the Universe, Pope Urban VIII burnt Bruno and imprisoned Galileo for life. To warn off others (like myself), who might also doubt that pumping of sodium ion from living cells spells the difference between the living and the dead, the modern "Popes" use obfuscation through "creative truth telling" and manipulation of life-giving money and jobs—ostensibly for unfettered search for scientific truth.

Now, I ask, Do you know why your journal singled me out by name as the target of the defaming attack? My guess is that in the dense fog of engineered darkness, you could not perceive the real answer. Yet knowing the right answer is the unavoidable first step in undoing the harm you have unwittingly done to me and to yourself (as a trustworthy reporter.)

The remedy to undo the harm produced by telling untruth is telling the truth. But the truth told must be "the whole truth", because only by knowing the whole truth could one distinguish truth from falsehood paraded as truth. Of course, to learn the "whole truth" takes time and effort. But taking that time and effort may mark the beginning of the greatest investment of all the investments that your journal has been making in the future of humanity—since the 19th century.

For such a small investment might raise your head above the manmade darkness and evaluate judiciously my claim that (genuine) science as the last resort in Mankind's struggle for survival is very ill. And that the (seemingly trivial) attack on my reputation is in fact the tip of an immense iceberg—lying squarely in the path of our swiftly moving planet toward its destiny. The titles and subtitles of a list of recent books and articles offer a glimpse of this iceberg from different angles.

Book I. "***Our Final Hours***: A Scientist's Warning: How Terror, Error, and Environmental Disaster Threaten Humankind's Future in the Century—on Earth and Beyond" (2003) by Sir Alan Rees, England's Astronomer Royal. Is Sir Reese overly worried in forecasting a fifty-fifty chance that we might not be able to make it to the end of this century? I surely hope so. Nonetheless, when it comes to questions of life and death of all humanity, being overcautious is the only sensible way. For as Intel CEO Andrew Grove warned us: "Only the Paranoid Survive." Only the paranoid survive because they stay awake when Captain Smith of the unsinkable Titanic went to sleep.

Book II. "***Out of Gas: The End of the Age of Oil"*** (2004). David Goodstein, a physicist, added something else for us to worry about. That is, we would also have used up most of our fossil fuels (including uranium) by the end of the century. If the estimate proves accurate and we do not take prompt and effective measure to forestall its consequences now, this sudden withdrawal of the major energy source would further tip the 50/50 chance forecasted to a ratio closer to certainty.

Goodstein pointed out that the most promising way to deal with the energy problems lies in a prompt and concerted global effort to develop fusion energy and other alternative (lasting) energy sources and in drastically raising the efficiency in the consumption of the remaining fossil fuels. This prescription in turn calls for a can-do and upbeat (basic and applied) science and a wide-awake voting public that wholeheartedly supports it.

An upbeat and vigorous science with broad and vigorous public support is equally indispensable in coping with another unnerving subject calling for immediate action—AIDS.

Book III. "***The Coming Plague***: Newly Emerging Diseases in the World Out of Balance." Here, author Laurie Garrett reminded us that AIDS was only ONE of a list of (the then-) recently discovered diseases and by sharing genes, new drug resistance has left vancomycin the only antibiotic still effective in combating what used to be harmless Staphylococcus. Nonetheless, her 750-page book made no mention of the Mad Cow Disease, SARS and Bird Flue as killer diseases. These all came after 1994.

Skyrocketing increase in physical contacts between humans and humans, between humans and killer microbes and between killer microbes and other killer microbes have a predictable consequence. They would make gene sharing among killer microbes increasingly commonplace. As a result, harmless microbes could turn into deadly ones and deadly ones turn even more deadly.

And it would be foolhardy not to expect that AIDS virus would one day develop resistance to desiccation and become airborne like flu and SARS .

As microbial invasiveness and deadliness continues its relentless upward flight, immunization and quarantining would become more and more difficult to administer and less and less effective. That would leave the science of drug design and manufacturing our last ditch defense to keep at bay our irreconcilable microbial enemies.

But what is the status of our current science of drug design and manufacturing? In fact, we already know the answer from the records of our wars on cancer and AIDS. And they are not encouraging.

Clifton Leaf, in a recent Fortune magazine article, asked in its title "Why We're Losing the War on Cancer? " He showed that since President Nixon launched the War on Cancer over thirty years ago, a huge amount of money (ca.$200 billions) has been spent in attempts to conquer cancer. But despite that and the parade of one new "cancer curing " wonder drug after another like Avastin, Erbitux, Gleevec …, death from cancer continues unabated. Thus, the average number of (innocent) Americans killed by cancer per day now stands at half of the (innocent) Americans killed on the day 9-11. Or as Dr. Dan-Farber put it: "It is as if one World Trade Center Tower was collapsing on our society every single day." But Americans make up only 5% of the total world population. Worldwide, cancer deaths would be equivalent to 20 World Trade Center Towers collapsing each passing day.

AIDS, described as "the greatest weapon of mass destruction on the earth today" (Colin Powell) is even more terrifying. Unlike the more or less steady cancer death rate, AIDS death rate has been steadily and rapidly climbing. Take the case of India. There were only a few thousands of HIV/AIDS patients in early 1990's. In just ten years, it has risen to between 3.8 million and 4.6 million (in 2002). In 2010, just six years from now, it is predicted to rise to 25 million. What would that number be in 2020, in 2030, in 2040, in 2050, in 2060……?

However, India is not the worst hit; Africa is. In 2003, there were already 12 million HIV/AIDS orphans in Africa. Meanwhile, men and women in the most productive age-bracket are dying like flies. The dismal overall picture provides those living in luxury and comfort today a peep into what could happen to all humanity in time to come if we would merely make the small mistake of waiting in indecision a bit too long. As I pointed out above, the key issue is how soon can we design drugs that can cure cancer, AIDS or any other new diseases yet to come.

As it is well known, despite massive efforts and money spent, no drug has been discovered that cures (cancer or) AIDS. The best drugs available only ameliorate further progress of the disease. Then, there is the question of who can afford to buy these drugs. Keep also in mind that it is in the sick bodies of untreated patients that new killer viruses and other lethal microbes have the best opportunity to swap lethal genes and become even more out of control.

Book IV. "***The $800 Million Pill"*** by economics journalist, Merrill Goozner. Goozner reported that (so inefficient is the production of useful drugs) that on the average, it would cost the drug company $800 millions to produce just one drug. And to recover the cost, the drug has become so expensive that that payment for prescription drugs is threatening bankruptcy of the Medicare program in the United States, the wealthiest in the world.

Then there is the other side of the problem. When creating new drugs has become so inefficient and so costly, drug companies—whose main objective is to make money—can no longer afford continue making them even if they exist. Vancomycin was already the last resort antibiotic against Staphylococcus killer in 1993; it remains so ten years later today—only two cases of vancomycin-resistant Staphylococcus have already been reported (60 Minutes, 5-2, 2004.)

But why are we so inefficient in producing new drugs when compared to producing, say, new and better automobiles or new and still better computers?

To introduce my answer, I cite Prof. Alfred Burger from his monumental treatise "Medicinal Chemistry" (2nd edition) on thousands of drugs.

> "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." (p. 19.)

This statement was made in 1960 but things have not changed much since that time. The bottom line is that all the drugs in existence were obtained not through understanding and rational design, but ***by chance*** or ***random trial and error***. To give you a perspective in evaluating this seemingly acceptable fact, let us ask ourselves this question: How many of our modern weapons used against human enemies were obtained by chance and random trial and error?

I would say, none. But then, How and why were our modern weapons against our human enemies developed differently? In answer, I offer you a thought experiment.

Suppose with a time machine, we send to Queen Victoria a transistor radio. Let us also suppose that she was immensely pleased with the gift and enjoyed the heavenly music that little box delivered from nowhere. One day, the radio stopped singing. Terribly upset, she vowed to have it repaired regardless of cost.

Yet, you and I know with certainty that even if she emptied the treasury of the entire British Empire, she would not be able to repair that radio. And if she insisted, a lot of money would be wasted with no tangible return. The reason for the predictable failure is simple. At her time, even electrons were not yet discovered and the transistor radio is an electronic machine. However, once the basic science of electricity and magnetism was understood, the transmission of electromagnetic waves over distance comprehended, this basic knowledge would be harnessed to produce all kinds of practical devises including the transistor radio. It would then cost next to nothing to make her silent radio singing again.

Indeed, it was by following the same sequence of steps that most of the sophisticated modern weapons against humans were developed. Now, physics (and chemistry) is the basic science underlying the science of modern weaponry. (Since living cells are the basic units of all life forms,) the science describing how living cells work or ***cell physiology*** underlies drug action and design.

From what Professor Burger said and quoted above, it is obvious that the theory of cell physiology that he—and just about everyone else—was taught as truth and depended on (known as the membrane pump theory) is so primitive and so unrealistic that it has nothing to offer on how any drugs work—let alone designing cheaper and better ones.

In summary, humanity is facing an unprecedented crisis. Fossil fuels, on which virtually all our busy world depends from cooking meals to flying supersonic jets, may be gone by the end of this century. Cancer kills 10 times more innocents every single day what terrorists killed on 9-11. AIDS is out of control in Africa and threatening to do so in India, New microbial diseases unknown in all human history appear and old ones once thought conquered come back more deadly than ever. Even the willingness to pay $800,000 for each new drug has no future because drug discovery by chance and random trial and error cannot go on forever. Random chance is by definition rare; random trial and error too expensive and increasingly unproductive.

There are not that many options left Mankind to overcoming our manifold crises. Those that remain all point to a single direction: a vigorous science on track, the recruiting of the best and brightest in its cause and vigorous and unwavering public support. But is this what we see in the real world today? Not from the title and subtitle of a fifth book.

Book V: "***The End of Science***: Facing the Limits of Knowledge in the Twilight of the Scientific Age" (1996) by John Horgan.

The author of this volume is a science reporter for the magazine, Scientific American. He wrote this book after interviewing some forty prominent scientists in different fields. Among the physicists interviewed were Hans Bethe, Richard Feynman, Freeman Dyson and Murray Gel-Mann. Among chemists interviewed were Ilya Prigogine, J.D. Bernal and Francis Crick. Among biologists interviewed were Stephen Jay Gould, Bentley Glass and John Eccles. Among science historians interviewed were Thomas Kuhn and Paul Feyerabend. It might be mentioned that Sir John Eccles who received the 1966 Nobel Prize for Physiology or Medicine, is a cell physiologist specializing in the study of nerve function.

Thus, what Horgan told us that the end of science is here or close at hand is not just his personal opinion but the shared opinions of the forty-some leading scientists he interviewed for the book. As physicist Richard Feynman pointed out, each discovery can only be made once. Sooner or later all the discoveries that can be made have all been made. The publication of "The End of Science" in 1996 showed that at least a 40 some leading scientists and science-philosophers believed that the end of science was already here or near. Cell physiology was no exception.

Yet cell physiology is the foundation for rationally designing drugs that would protect humanity against the "Coming Plague." But is it good enough that drug companies could depend on it to make effective drugs cheap enough that all patients in need ot them could afford to buy? Thus, in fact if not intention, Horgan and the leading scientists he interviewed already answered our questions. They told us that what we could understand have already been understood. Other subjects not yet understood including cell physiology and drug action—are beyond the limit of the reach of the human mind.

Perhaps, it was a similar hopelessness that New York Time's science correspondent, Dennis Overbye encuntered. In his review of Sir Alan Reese's "Our Final Hours", he began with the title "It Was Fun While It Lasted" and ended on a plea, "I would be grateful for any good news."

But is there any good news of comparable weight?

There is but not without irony. The good news or at least the seed of potentially good news of comparable weight now lies hidden in what your journal described as "obscure—ideas that are wacky at best?"

That said, let us take a look at the real face (in two parts) of what your usurper(s) tried to get your journal (and its readers) to spit upon.

Part 1: Forty-two yeas ago, I published a book. In Chapter 8 of this book, I presented evidence that the sodium pump hypothesis—the theory of cell physiology taught as truth worldwide to this day and the only "guiding light" for drug manufacturing to this day—violates one of the most fundamental laws of physics. This law violated is called the First Law of Thermodynamics or the Law of the Conservation of Energy.

Part 2: The remaining 17 chapters of the book were devoted to the presentation of a unifying theory of life at the cell and below cell level, called the **association-induction (AI) hypothesi**s, which has been by now extensively verified in essence from half a century of worldwide testing. *From its beginning, the AI Hypothesis has provided in broad outline of a molecular-electronic mechanism for how drugs bring about physiological and pharmacological responses of the living cells*—a giant forward step in the direction toward the future high tech of drug design and production of effective good drugs free of undesirable side effects and cheap enough for all patients in need.

Three years later, the subsidiary theory of (dynamically) structured cell water was added. NMR testing of its predicted motional restriction of water protons led to the invention by Dr. Raymond Damadian (with assistance from Drs. Paul Lauterbur and Michael Mansfield, who provided outstanding technical improvements) of what is now called magnetic resonance imaging, or MRI.

These exciting (though largely unknown) developments show clearly that it was wrong to claim at this time that understanding cell physiology is too difficult for the human mind. It was too difficult so far—because the majority was barking up the wrong tree. Find the right tree with the help of the right theory, we will be well on our way in defeating cancer and other killer diseases caused by our steadily gaining microbial enemies. This is, of course, easier said than done. But as Tao Te Ching says. A thousand-mile journey begins with one step. And taking that first step is what this letter is all about.

On the following four pages, you will find what I believe to be a simple and effective first step to undo the damage your earlier publication has done. More importantly, in the process of undoing the damage, you will also initiate and put in motion a far-reaching movement toward producing medicinal weapons developed from fundamental understanding of basic cell physiology.

My real hope is that you will take the time to read all of the remaining pages of the letter, which I have spent a good part of three months to put together on the essence of the documented "whole truths"—largely for your convenience.

A simple way to undo the damage done and to alert in time the sleeping Captain of what may happen to our space ship Earth

As a beginning step in undoing the damage done, I would like to suggest that you request your informer-usurpers to present published documentary evidence item by item to back up their claim that "most scientists consider Dr. Ling's ideas wacky at best." In case they fail to respond (as I fully expect) that failure would not be a nonevent to be dismissed and soon forgotten—as it has happened again and again in the past. Rather, that failure to respond would constitute a part of the historic record of the true nature of the attack: unfounded false allegations. As such, this failure to respond must be made known to your worldwide readership—thus far remaining misinformed since December 5, 2003.

Of course, your informer-usurpers might give their names, affiliations and documented evidence in support of the view that my scientific ideas are wacky at best and their contention that most scientists of the world shared this low opinion of my science and me. In that case, I would examine their statements carefully and present a written point-by-point rebuttal. The debate can then go through a number of rounds until one side or both sides admits that it had nothing more to add to the debate—would represent Part 1 of the formal written debate—in which your journal would have the honor of officiating as referee as well. And as such, your primary responsibility includes making certain that both sides follow the rules of debate and that no evasion or any other below-belt maneuvering be allowed to pass.

Part I of this written debate was in fact initiated by the published December 5, 2003 attack on my science and me. It is now my turn to initiate Part 2 of this debate—after a brief explanation of why I believe that this debate offers the best, perhaps even the only way to resolve the grave problem we have on hand.

Socrates chose death to underscore a historical message. To avoid disastrous decisions made on the basis of fads or superstitions, he argued that to survive, a democratic society needs the leadership of an intelligent, wise and courageous philosopher-king.

Out of the many democracies on-going and emerging, one struck me as a close approximation of the Socratic ideal. This is Singapore. A tiny former British colony the size of ancient Athens, rose in three decades from the third world to the first.

I can count at least two reasons for this spectacular success. An intelligent, well-educated, courageous and honest Prime Minister, Lee Kuan Yew was a modern "philosopher king." The second reason was the endless person-to-person parliamentary debates that enabled Lee to win the trust of the voters and election after electron, for upward of thirty continuous years to achieve what the world have come to admire. Through these debates he also succeeded in introducing a succession of new (revolutionary) measures opposed by powerful forces in favor of the status quo. Parliamentary debate is, of course, a major contribution from England.

Knowing all this, it seems almost beyond belief how the culture of fundamental science, on which everything of a modern society depends, does not in the least resemble a parliamentary democracy. Indeed, in its mindless suppression of new ideas and persecution of their authors and would-be subscribers match history's most detested tyrants. Indeed, the trademark of the modern arm of this new tyranny is the studied refusal of those in power to engage in debates (For documented record, see www.gilbertling.org/lp21a.htm.)

Now, I think that you and your journal may have a chance in exploding this ancient relic of enslavement by sponsoring the proposed open and refereed debate. Indeed, the debate I visualize if successfully carried out, would be the first and only formal and earnest debate with cross-examinations in the basic biomedical science of cell physiology—between proponents of the membrane pump theory and those of the association-induction hypothesis.

(I wonder if some future historian with a tender heart might not shed a tear for the many innocent children, women and men who could have been saved (but were not) if such a debate had taken place decades earlier?)

As such, I will open Part 2 of the debate by presenting fourteen sets of key evidence that in my view have disproved the membrane pump theory and confirmed in essence the association induction hypothesis years and years ago.

But in the search for scientific truth, nothing could be taken for granted on faith or say-so of anyone. All evidence of weight must be re-examined again and again with the passage of time and in the light of new findings, which might make what once seemed certain, uncertain and the once uncertain certain. Then, there is no better way to find out than through a full-fledged formal written parliamentary debate with cross-examinations under the watchful eyes of judicious referees—like the one I am proposing.

Thus to launch part 2 of the debate, I would request that you hand over these 14 sets of evidence to the defenders of the membrane pump theory and ask them to respond to each one of them and present item-by-item documented and published evidence. Again there should be the chance of back and forth exchanges thereby serving the equivalent role of legal cross-examinations in court trials to reveal the underlying truths. Once more I would hope that these records in its unaltered form will be published long with the documents of part 1 of the Debate in a future issue of your technology quarterly in which the "wacky at best" story first appeared.

The next question is who should defend the sodium pump hypothesis in this written debate? I would recommend that in this task you should do everything possible and leave no stone unturned to make sure that the most competent and the most capable and as many of them as needed be included—not excluding the informer-usurpers if they would identify themselves and accept the invitation.

The following organizations and individuals might be profitably approached for suggestions of the names of possible participants. The Nobel Committee for Chemistry and the Nobel Committee for Physiology or Medicine; (Both have been awarding Nobel Prizes to subscribers of the membrane-pump hypothesis, see pp. 104–105.) Other possible candidates for debate include Prof. Richard Keynes of Institute of Animal Physiology, Baabraham, Cambridge, England, and Prof. I. M. Glynn, Physiological Laboratory, Cambridge University, Cambridge, England. Also known for its support of work based on the membrane pump hypothesis is the Howard Hughes Medical Inst., Chevy Chase, Md. Its President, Dr. Tom Cech might be able to provide names.

However, if past experience (as described in the Website mentioned above) is any guideline, there would be a good chance that you could find no one willing to engage in such a proposed debate. In that case, as pointed out above already, that failure to respond would be part of the record, in the same way that no show is not a non-event but the seal of defeat as in any game of competitive sport.

When published in all its details in a future issue of the Technology section of your journal, the ball would be in the hands of the people of the world and their leaders who have the responsibility for the future security of not just the citizens of their respective nation but of the entire world to decide what to do next. That would be where the buck stops.

In my view, the most powerful nations that have dominated this world for a long time, have botched the job of keeping alive this crown jewel of the West's contribution—too often seen as a stepping stone toward some other "higher" goals such as a Nobel Prize, some high official positions, a reelection etc—rather than what it really is or could be: the final defense of the human race's continued existence on this paradise of a planet. Perhaps, it is the turn for the leaders of a small emerging nations like Singapore, Qatar and their likes—with effective and far-seeing leadership and your journal's (proverbial) ability to reach them.

What is needed, in my opinion, is nothing less than an entirely different kind of science culture, in which defense against microbial enemies via basic and applied scientists is seen as serious a national concern as the defense against human enemies and given equal or at least comparable support.

Choose to establish a small number of (a highly coveted) positions that a Nation can support and award them through a system of fair competitive examinations open to any qualified contestants from anywhere—a practice perfected in two thousand years of the Chinese history in choosing their "philosopher-kings" to run the country. And award the winners not a short-term grant but with life-time support for both living expenses and business (research) cost—as we routinely award life-time support to bureaucrats and to conventional type of soldiers.

Properly designed examination questions would be one of the most effective and economic ways of bringing about badly needed changes in the teaching and direction of research. These changes may include ways to reach deeper understanding by "making whole" history's artificially fragmented science (e.g., division of natural science into physics, chemistry and biology/ cell physiology.) and in updating (obsolete) ideas in the training of future scientists. Thus, if examination writers demand competence at once in mathematics, physics, chemistry as well as cell physiology, it would create quickly all-around competence—from nothing more than description of observations to the cutting edge of fully unified natural science covering all its subjects.

With the best of the future generation liberated from the shackles of the power of the status quo and given the freedom and support to pursue what evidence point to and what ingenuity and imagination take further beyond, we may be able not only to swerve out of the way of the immense unseen iceberg in time but continue to make our earthly paradise better and more secure than ever before and lastingly so for all time to come.

Sincerely yours,

Gilbert Ling

The Rest of the Whole Truth

In what follows, I shall tell you in more detail the "whole story" on how we got to where we are today—in grave danger of losing our survival battle altogether amidst exhilarating unprecedented prosperity and affluence.

Taken together, the experimental disproof of the prevailing sodium pump hypothesis and the extensive worldwide affirmation of the essence of the unifying theory called the association-induction hypothesis constituted what is known as a **scientific revolutio**n. The concerted effort of the sodium pump alliance to discredit and to make invisible all these new developments was in principle not different from what Pope Urban VIII attempted to do in the seventeenth century to ***the* scientific revolution** of all scientific revolutions introduced by the Polish astronomer, Nicolas Copernicus.

History shows how the burning alive of Bruno at the stake and the imprisonment of Galileo for life had achieved their desired goal. No one dared to continue what these scientists did and the once flourishing Mediterranean science came to an end—only to revive in Western Europe years later. But the reign of the sodium pump alliance is global. There is nothing like the Western Europe of the 17^{th}-18^{th} century for legitimate science to be relocated and growing again.

However, we also have something that the 17^{th} and 18^{th} century did not have. They include the means of instant communication and global news reporters like your journal, the Economist. It is my hope that you take seriously your avowed dedication to the guardianship of Capitalism, which rests upon the integrity of the democratic institutions including that of science.

I now return to what followed the execution of Bruno and imprisonment of Galileo and the revival of science of England, Holland and France. That revival of reason in Western Europe has a great deal to do with the arrival of the Age of Enlightenment and the birth of ***modern science***.

To me, the invention of modern science in the 17^{th} and 18^{th} century Western Europe was not merely the introduction of a new scientific method,—which of course gave Mankind a way of testing and thus verifying or disproving a scientific hypothesis. Just as important, it also introduced a new kind of ***all-inclusive, cooperative enterprise to search for truth by all scientists—living, dead and yet to come***.

The reason that this great forward leap happened then-and-there and not anywhere else or at any other time, has many causes (A fuller account of them will be discussed in a book that I am in the early stage of writing.) But one key component was the adoption at

that time of a code of behavior for all participants. It was the strict adherence to this code that has made it possible for a concerted global changes to be made when an old belief proved wrong and a new and better theory emerged in what I have already mentioned by name, a ***scientific revolution***.

Now, each scientific revolution has two phases. The first phase is what I call a scientist's scientific revolution. This was what Nicolas Copernicus had done and divulged in his treatise, *Opus de Revolutionibus Coelestibus.* The second phase is what I call the historian's scientific revolution, in which the scientific community as a whole broadly accepts the new idea. Phase II is much more difficult to achieve because it involves the conversion of many others who have vested interests in the preservation of the old (but now disproved) hypothesis. It is in facilitating this difficult transition that the code of behavior was developed, taught and religiously followed as its guiding light.

For an example, Sir William Bayliss described this code of behavior in 1927 in his magnificent "Principles of General Physiology" (4th edition, p.xviii) thusly:

> "Shake your counter as boldly every whit,
> Venture as warily, use the same skill,
> Do your best, whether winning or losing it" (Browning)
>
> "But at the same time, there must never be the least hesitation in giving up a position the moment it is shown to be untenable. It is not going too far to say that the greatness of a scientific investigator does not rest on the fact of his having never made a mistake, but rather on his readiness to admit that he has done so, whenever the contrary evidence is cogent enough."

(Perhaps one may say that this is a more detailed rendition of what *fair play* or even s*portsmanship* says in a broader and more plebian context. It is also in full harmony with the twin Confucian teachings: ***Chung*** or do your best and ***Shu***, or don't do to others what you don't like done to yourself.)

When experimental tests and other means of determining which hypothesis is closer to truth were carried out and the one you have been following turns out to be wrong, one must graciously relinquish the old and familiar gestalt and adopt as one's own and foster the new (and closer-to-truth) one in its place.

Note also that though it is usually not explicitly spelled out in defining fair play or sportsmanship or in Sir Bayliss's admonition, each rests upon accurate score keeping. That is, each contending party knows and makes it promptly known to all others, not only how many goals its own side has scored but just as accurately and as promptly, how many goals the opponent side has scored. Indeed, without fair score keeping, fair play is just two words with no meaning.

Then there is the inviolable right of the ownership of the original authorship of theories and key experimental findings. Stealing either is condemned as plagiarism. But what is plagiarism? Is it simply stealing? It is worse than that. Rather, it is more like a Supreme Court Justice picking someone's pocket.

In fact, long before Sir Bayliss's poetic instruction, this code of behavior including fair score keeping and respect for the ownership of original authorship was well understood and practiced. Thus, Joseph Priestly (1733–1804)—an English Unitarian minister, linguist, scientist of incredible width and depth, a member of the Birmingham Lunar Society, the discover of oxygen and of the inverse-square law governing electrostatic interaction, but later named after Coulomb—was also an opponent of the French chemist, Anton Lavoisier. That is, until new findings showed that Lavoisier was right after all.

Priestly then turned a full 180-degree around and became one of the staunchest advocates of Lavoisier's idea. With overwhelming admiration and enthusiasm, he wrote: "There have been few, if any, revolutions in science so great, so sudden and so general …of what is now named the new system of chemistry." Rapid and widespread acceptance of Lavoisier's new system soon followed.

Only five years after writing the Preface for Sir Bayliss's book cited above, Professor A.V. Hill was to show how true he too was to the code of behavior Sir Bayliss had outlined. Hill's having been awarded the Nobel Prize did not deter him from admitting a mistake he had once made and vigorously defended, when the contrary evidence becomes cogent enough. In an article he wrote for the Physiological Review under the title: "The Revolution in Muscle Physiology', he wrote: "He laughs best, who laughs last" only it was Gustav Embden, Hill's long-time and equally strong-minded opponent, that did the last laughing. (Physiol. Rev.12: 56, 1932.)

Universal practice of what Sir Bayliss put down as a guiding principle ensures the ideas of both contending sides and their respective supporting evidence to be all put on the table and thus made visible to all. As a result, the younger generation could choose according to their respective judgment based on all the facts and the search for truth of the entire scientific community could then continue but now in a new and productive direction.

To see the critical importance of the true freedom of the younger generation to make their own choices, I quote from three of history's great revolutionary scientists each respectively in the field of chemistry, biology and physics in that order.

> " I do not expect my ideas to be adopted all at once..... Meanwhile I observe with great satisfaction that the young people are beginning to study science without prejudice…" (Anton Lavoisier, in "Reflections on Phlogiston.")
>
> "Although I am fully convinced of the truth of the view given in this volume under the form of an abstract, I by no means expect to convince experienced naturalists …but I look with confidence to the future—to young and rising naturalists, who will be able to view both sides of the question with impartiality." (Charles Darwin in his "Origin of Species.")
>
> "A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die and a new generation grows up that is familiar with it…" (Max Planck in his "Scientific Autobiography.")

In each case, the success of a major revolution was not due to a lack of stiff and open resistance. That resistance was entirely healthy and to be expected. Rather, it was the honest score keeping, full protection of original authorship and freedom of the new and coming generation of scientists to make their own deliberation and choice that had made possible rapid progress along a new and fruitful direction. Note that any tampering of score keeping and of authorship would pose a deadly threat to the survival of the whole enterprise. That is why in any competitive sport, professional umpires and referees are indispensable and in court trials, any tampering of evidence is itself punishable by law.

That said, I must return to the question, why we need scientific revolutions.

The answer is simple. If a just revolution were effectively blocked, that branch of basic science would wither and die on the vine. Since all truths are part of a whole truth, death of its part spells the death of the whole. In contrast, a successful revolution enables that specific branch of basic science to move rapidly forward in a new and promising direction. A successful revolution would also pave the way for the invention of new practical devices—based on what up to that was a hidden part of Nature. That practical device in turn would father new industries, spreading life-enhancing benefits,

financial profits and rewarding employment in an ever-widening circle of prosperity and happiness.

Thus, 50 years after Michael Faraday made the revolutionary discovery of magneto-electric induction, electric power industry came into being in England. Thirty years after James Clerk Maxwell introduced his revolutionary unified theory of electromagnetic waves, Marconi obtained a British patent for the future radio industry. One hundred and thirty years after Mendel published the revolutionary law of inheritance he discovered in the Journal of Brno Natural History Society, detailed knowledge on DNA, the physical basis of Mendel's Law of Inheritance, began to save innocent people from execution for crimes they never committed. Thus the benefits what could follow a successful basic scientific revolution as a rule went far beyond what could be anticipated by even the most visionary.

Looking back on history, one sees that in the course of the two and half (or ten-eleven) centuries since the birth of modern science almost every branch of basic science has successfully gone through at least one major revolution. These successes testified to the broad acceptance of the basic code of behavior for all scientists and of honest keeping and publishing of scores for and against each side of the contending parties and of guarding of original authorship and of the freedom enjoyed by the coming generation of scientists to switch from an old (but wrong) constellation of ideas to the new viable one. All these really happened even though there was no official umpires or whistle-blowing referees, police, court-trials, jails and sentences for wrong doers.

So if on a later day, a new brand of scientific movers and shakers would argue openly or take for granted that scientists are a new breed of humans that can be depended upon to police themselves and to stay honest without the threat of punishments (which they imply are necessary for all their nonscientist brothers and sisters,) they are not without past evidence in support of these contentions. But the bottom line is that others believed these preposterous contentions and had made major decisions of great importance based on the truthfulness of that preposterous contention.

At this juncture perhaps it is worth remembering an analogous situation. Not that long ago, many believed the self-appointed guardianship of the integrity of industry by Arthur Anderson Inc., the gold standard of accounting profession—that is, before money from consulting came into the picture. Then everything changed. In that case, Law 1001 put a stop to the hemorrhage. Can 1001 serve a similar role in putting a stop to what is happening in basic cell physiological science? It might.

As mentioned above, while each major field of science has seen at least one major scientific revolution completed, there is one notable exception. That exception is the branch of basic science closest to the wellbeing of the human species, the science of life at its most basic cell and subcellular level, ***cell physiology***. One asks, Why?

There were two major reasons for this long delay, one was dictated by logic and thus unavoidable, the other one was man-made and thus theoretically at least reversible. I shall concentrate on the first one here and return to the second one later.

The unavoidable cause for the delay is unique to cell physiology. One can understand this cause more readily with the help of a simpler model, the crossword puzzle.

First, if one compares cell physiology to a crossword puzzle, one sees that each one has ***a unique solutio***n—on that there is not the kind of lubricating give-and-take practiced in almost all other human undertakings. Second, in place of the proper collection of right words to fill the empty squares in an ordinary crossword puzzle, it is the placements of

the right combination of correct physico-chemical concepts that lead to the solution of the living crossword puzzle. Thus, in theory, attempt to solve the cell physiological crossword puzzle should not begin until the relevant parts of the sciences of chemistry and physics have reached maturity.

Unfortunately, that was not what happened. Long before the maturation of the relevant parts of chemistry and physics, research in cell physiology had already begun. Almost all the trouble this letter tells you about the science of cell physiology today can be traced directly or indirectly traced to this premature beginning.

The results of this premature activity are like that of a curious 9-year old trying to do a New York Times crossword puzzle. The child's small vocabulary limited the choices of words used to fill the empty squares. And no matter how bright he is and how hard he tries, sooner or later, he would be stalled on an unfinished wrong "solution."

Meanwhile, two new developments evolved. One was what you may call spontaneous. Men with the best of intentions to benefit humanity created the other.

The spontaneous movement is that of fragmentation. In the word of philosopher Will Durant: "We suffocate with uncoordinated facts, our minds are overwhelmed with science breeding and multiplying into speculative chaos for want of synthesis and a unifying philosophy." The situation has gotten worse since Will Durant wrote this passage in his "Story of Philosophy" in 1933.

Returning to our cross-word puzzle analogy, this continuing fragmentation is the counterpart of tearing the cross word puzzle into smaller and still smaller pieces and assigning different people to do each torn piece independently. It is obvious that continuing work on the smaller and smaller pieces would not add up to the unique correct solution of the whole and intact puzzle. Instead, each would just dry up and die on the vine. Seeing a whole bunch of them dying on the vine might well give the impression that that branch of science has reached the limits of knowledge as John Horgan and the scientists he interviewed have suggested in Horgan's book, "The End of Science."

While the fragmentation went on and on, three new institutions designed to promote the pursuit of science came into being. In decreasing pecking order, they are the Nobel Prize, the government and private research funding agencies and the publishing of mass-distributed biology textbooks at all levels of learning. Each one of these three institutions is—like the 17th century Roman Catholic Church—of lasting duration and in possession of inexhaustible funds. And each enjoys the kind of autonomy that it can decide to do whatever it chooses to do and sticks to it—year in and year out with no end in sight. So if they made a mistake, the impact would be far and wide beyond imagination.

And in time, these three institutions did make a grave mistake. In fact, it was the same mistake—as if in a perfectly choreographed *pas de trois*.

What is that mistake? That shared mistake is forgetting the fact that different branches of science were in widely different stages of development and must be treated entirely differently.

As an example, mathematics is mature. Cell physiology and medicine, on the other hand, are still in their infancy. By ignoring their differences, immature cell physiology was falsely raised to the status and level of the "trustworthiness" of mathematics. Nor does it take deep thinking to see how it happened that way.

Consider the difficulty of persuading a panel of top cell physiologists that their brand of science is lower than that of their fellow professors, you will see that if some had tried

this, he or she must have failed. So everyone was, so to speak, raised to the status of a four-star general and happy. The unhappiness will come later and to other people facing airborne AIDS virus as it might be.

Ignoring that cell physiology and medicine are not at the advanced stage of development as mathematics and theoretical physics had far-reaching consequences.

For one, the Nobel Committee of Physiology would be required routinely to reward the equivalents of the 9-year-old's "solution" as if it were like the Pythagorean Theorem, Einstein's Theory of Relativity and Planck's quantum mechanics. Based on that (false) belief, the 9-year old would be asked to play a key role in the selection of the following year's Nobel Prize winner years in and years out, each adding to and elaborating on the original 9-year-old's bright but totally wrong "solution." Thus, by forgetting the widely different stage of development of cell physiology from the truly mature sciences, a slow-releasing poison is being drop by drop instilled into the vital organs of the most respected institution supporting human intellectual achievements, the Nobel Prize.

A second adverse consequence is even more deadly. Since all branches of sciences are uniformly raised to the four-star status by the most prestigious king-making institution of the Nobel Prize, the concept that profound changes identified as scientific revolution would be against the culture. Accordingly, following the saying if the shoe does not fit, operate on the foot, the word and concept of scientific revolution was sidelined. This deduction would offer an explanation for the very unreasonable change of heart of science historian, Thomas Kuhn, from its enthusiastic champion to its skeptic detractor. And it would also explain partially at least why the sodium pump hypothesis can survive decisive experimental disproof after disproof, while the association induction hypothesis though experimentally verified again and again is described as wacky at best.

Taking their clue from the Nobel Prize Committees as the supreme arbiter of scientific truth, research grant awarding agencies, both public and private, would be hard put not to favor those planning to pursue the same direction of research founded by the original 9-year-old genius. But that is only one of the one-two punch leveled at legitimate science.

The other one is, again in harmony with the foot and shoe analogy, a politically savvy adaptation of the fund-distributing agencies to the sickness of fragmentation. It is, so to speak, the research funding agencies' way of rewarding more and more money to all those manning the smaller and smaller pieces of the torn crossword puzzle. And to that end, the funding mechanism is divided into many little medieval "monarchies", each with its independence and power to give (taxpayer's) money to anyone they choose while they are "on the throne" but also thereafter when their successors, whom they recommend, take over the reign. The overall impact on progress is more and more fragmented factual details and less and less understanding in the true sense of the word. (For more factual details of the National Institute of Health and the National Science Foundation and their peer review system, see my website, <www.gilbertling,org/lp11.htm>

Endowed with ample research grant money, these followers of the creative 9-year old Nobel Laureate would fill all the vacant academic appointments and teach what made their lives so successful consciously or by example to their students.

And further down the line, you have the (mass-distributed) textbook writers. How could they not follow the trend? They do. And next thing you known each and every one of the ***coming*** generations year in and year out with no end in sight will be indoctrinated on the original 9-year old's creative mistake as proven scientific truth. Since the latest sci-

entific products are all fragmented, the textbooks reproduce the same. More and more names and colored illustrations and less and less that connect them into even halfway decent coherent stories.

Now, suppose at this juncture, the needed parts of physics and chemistry have reached maturity. Translated into the language of our hypothetical model, this means that finally our 9-year old has grown up and graduated from college. He would easily point out where as a 9-year old he was mistaken. A few substitutions and several new words added to the right places, Lo and Behold we have what is on the way to becoming the correct (unique) solution. It is that simple. But what do you think would happen to him then?

Yes, you are right. It would be a replay of the Urban VIII story all over again as I tried to tell you early in this letter—unbelievable as it might well have been to you then.

The confusion and backsliding thus generated have produced the second major (man-made) reason for the delay of Phase II of a major scientific revolution in this, the most relevant of science to Mankind's future welfare and security.

That said, my next task is to return to the reality of cell physiology, which began a little over one century and a half ago in France and Germany.

Within a span of five years, two major discoveries marked the beginning of what is now cell and subcellular physiology. Theodor Schwann's discovery that living cells are the basic unit of life (1840) and Felix Dujardin's discovery of what became eventually known as protoplasm, an even more fundamental unit of life (1835.) Each of these seminal discoveries initiated one of the two alternative directions of research of the early days—not to be united until the arrival of the association-induction hypothesis more than a century later. In retrospect, each discoverer, brilliant as they were, made the same mistake of over-generalization.

Protoplasm is what Dujardin saw oozing out of a broken protozoan cell and was described by him as living jelly (For a photograph of a similar specimen of protoplasm from a plant cell, see Figure 3 on page 18 of Book 4. For the source of this book, see p. 88 below.) Later, the brilliant and eloquent British naturalist, Thomas Huxley pronounced protoplasm as the "physical basis of life" in his famous Sunday evening lecture on November 8, 1868. With such an auspicious beginning, where do you think the concept of protoplasm stands today? Would it not shake your basic trust in science, when I tell you that the concept has been eliminated from the minds of most biologists. From my recent search through ten of the most popular US high-school biology textbooks, the word, protoplasm, has not been found even once.

Is this wholesale abandonment of such a once highly cherished scientific idea based on irrefutable evidence that proved it wrong? The answer is a decided No. It only ***seemed*** in trouble at one time in the past. In part, the early investigators made the mistake of regarding ***all*** protoplasm as existing in the form Dujardin saw flowing out of a broken cell. But even more important, it was—as I have said again and again—because the necessary physical and chemical knowledge to define protoplasm correctly was not yet available. A wrong definition of protoplasm was offered and that caused its eclipse—until the AI Hypothesis arrived. But the AI Hypothesis itself was made invisible by creative truth tellers as you will find out below.

Meanwhile, the cell theory took center stage. As mentioned above, it too suffered from an incorrect overgeneralization. That overgeneralization, however, did not cause the theory to be abandoned as in the case of protoplasm. Instead, it had led to an even graver misadventure worse than premature abandonment. It became widely adopted like my hy-

pothetical 9-year old's "solution" of the advanced cross word puzzle, thus initiating a cascade of mishaps threatening everything it touched.

More specifically, Theodor Schwann and other early workers thought that the huge mature plant cells, which are truly sacs of water solution enclosed by some kind of a membranous covering (See Figure 1 on page 7 of Book 4. See p. 88 below for availability of Book 4), are typical examples of all living cells. The truth is that most living cells (like those making up the meaty part of a beefsteak) are not hollow but are solid. But this discovery came too late. By that time, the basic notion of cells as membrane-enclosed body of watery solution is already widely taught and believed under the name, the **membrane theory**. For some time, this simple theory **seemed** to have received a wide range of supportive evidence (See pp. 10–25 of Book 4,) only to be proven wrong one by one in later times.

In the version widely taught, all cells are tiny sacs of watery solution, covered by an extremely thin membrane. As routes for the traffic of chemical substances, the cell membrane was postulated to contain tiny but rigid pores. Through these pores only molecules, and (electrically-charged entities called) ions smaller than the width of the pores can enter or leave the cells. This theoretical postulation offered an explanation why only the smaller hydrated potassium ion (hydrated, meaning covered with a more or less permanent layer of water molecules) accumulate within the cells. The larger (hydrated) sodium ion stays out permanently or so it was thought.

This whole constellation of ideas under the canopy of the membrane theory collapsed in the late 1930's and early 1940's, when better methods of determining what can enter or leave the cell and what cannot, became available (e.g., radioactive tracer technology.) It was then revealed one by one that all substances examined small or big, enter and leave the cells with relative ease, including the large (hydrated) sodium ion.

In retrospect, this would be the time to re-evaluate all alternative ideas and make judicious decisions according to Sir Bayliss's instruction. Instead, those at the helm for (undisclosed) reason(s), chose the easy way out (thus casually planting "landmines" in the paths of many if not all biomedical scientists and teachers to come.) As a result, what is now known as the **membrane pump theory** was born and soon too became widely taught as truth. In this new version of the membrane theory, ceaseless activity of numerous hypothetical devises in the cell membrane called ***sodium pumps*** keep the concentration of this ion low in the cell water in spite of its constant inward diffusion.

It was at this point that my Ph. D. thesis study of cellular electric potentials brought me into contact with the sodium pump hypothesis. As time went by, I became more and more uncomfortable with this hypothesis. It all seemed so arbitrary. Why do we discard a wrong theory only to replace it with a makeshift alternative destined to fail? Destined to fail, because to keep the cell afloat, postulating one or any limited numbers of pump(s) would not be enough. Indeed, the number of pumps that needs to be postulated increases endlessly as chemists continue to synthesize more and more new chemicals that can traverse the cell membrane but are found at steady levels different from those in the outside medium.

In 1951 I began to study the energy balance of the postulated sodium pump hypothesis. My immediate purpose was to find out if frog muscle cells would have enough energy (under a rigorously controlled experimental condition) to operate the postulated sodium pump. In the course of the next five years I had been steadily improving the methods for study. In the end I carried out some seventy-eight (78) sets of complete and incomplete experiments, all pointing exactly in the same direction: There is not enough energy.

The last three sets of my studies completed in 1956 were the most accurate. They show that even if the muscle cell used all its available energy for just one purpose, namely to pump sodium ion, the ***minimum energy need*** of the postulated pump would still be from 15 to 30 times or 1500% to 3000/% of the ***maximum energy available***. The details of this study was published as Chapter 8 of my first book, "A Physical Theory of the Living State", which appeared in print in 1962.

Within the next ten years after the publication of my first book, the essence of my finding was twice confirmed and none publicly challenged my method or my conclusion (see p. 110 of Book 4.) However, that original book has been out of print for some years now. To make the findings more easily available, I have reprinted the entire Chapter 8 in 1997 as Appendix 1 in an article entitled: "Debunking the Alleged Resurrection of the Sodium Pump Hypothesis." The main part of the article was devoted to clean up some "garbage" masquerading as science and to reaffirm, update and further sharpen the correctness of the conclusion made 35 years earlier. (To download a copy of "Debuking…", click Article No. 1 listed by titles on the front page of my Website, www.gilbertling.org.)

The remaining 17 chapters of the 1962 book was devoted to presenting an altogether new and ***unifying theory*** of the living cell, called the association-induction (AI) hypothesis. Why should I be able to write such a revolutionary and unifying theory whereas some of history's great physiologists like Carl Ludwig, Emil DuBois Raymond, Ernst von Brücke and Ludwig von Helmholtz had failed to do so? There are two reasons. They were working on what is known as organ physiology. The living cell was only discovered recently and the methods for its study had not been evolved yet. The second reason can be easily understood again with the aid of the crossword puzzle. The necessary physicochemical concepts were yet to come in the future.

At about the time when my generation of young scientists arrived on the scene, the relevant parts of physics and chemistry had finally reached maturity and the methods of studying isolated living cells have become readily available. Thus, I was able to do what my predecessors could not do, because I happened to be at the right place at the right time.

In many ways, the association-induction hypothesis is a long-delayed resumption of the concept that living cells are made of protoplasm,— a concept that was, as mentioned above, abandoned partly because of a misleading overgeneralization that all protoplasms are a viscous liquid, but even more importantly because the necessary physical and chemical knowledge needed to explain the properties of living protoplasm were not yet in existence. But again I repeat that the relevant parts of physics and chemistry did mature and it was my privilege to continue this correct but abandoned approach nearly a full century later.

Here are the names and qualifications of three reviewers who had read the 680 pages of the book "A Physical Theory of the Living State: the Association-Induction Hypothesis" and made the following comments:

> "Thus there must be some very comprehensive and basic principles at the molecular level that underlie and illuminate all the special manifestations of living systems. Ling offers no less than such a general molecular theory of life phenomena."
>
> (Professor Ralph W. Gerard, Department of Physiology, University of Chicago, Chicago, author of "Unresting Cells" 1940, Harper, New York)
>
> " At a time when we look forward to the merging of the physical and biological sciences, this is a most stimulating book, distinguished by a bold and inquisitive attitude on the one hand and careful experimental methods on the other."

(Professor C. N. Yang, Nobel Laureate in Physics, Institute of Advanced Studies, Princeton. Author of the later Yang-Mills non-Abelian gauge theory.)

"Your book…strikes me as being one of the most important and advanced contributions to the understanding of the structure of the living system which I have seen in the last 10 or 20 years."

(Professor Lancelot Law Whyte, Cambridge University, Cambridge, England and Standford University, Berkeley, CA, USA, Author of "The Unitary Principle in Physics and Biology" (London, reset, New York, Holt, 1949.)

Three years after the publication of this volume, I introduced the subsidiary Polarized Multilayer Theory of Cell Water, thus making the unifying AI Hypothesis complete.

Next, I shall present as briefly as I can what this association-induction (or AI) hypothesis is about. But before I begin, I want to call your attention to another envelope I have also mailed to you (beside the one enclosing this letter.) In this separate envelope I am enclosing as a gift to you a copy of my latest book published in 2001, "Life at the Cell and Below-Cell Level." (ISBN 0-970-7322-0-1) In the following (and above) I shall refer to this book as Book 4, as it is the fourth one of the books I have so far published. (In addition, I also enclosed in the envelope several key reprints, which I have referred to above or will refer to below.)

I am sending you this book for several reasons. First, it is a gesture of good will. Second and most important, it might help you find information that have been made invisible and beyond reach by the sodium pump alliance in one way or another.

Thus, this book presents the ***first and only full history of cell physiology*** ever written—covering the more than one century and half from its very inception to 2001.

(The volume also contains a bibliography of over 500 single and multiple references, thus in fact acting as a "road map" to the origins of all or most of the key relevant publications in the development of his branch of science. It also contains a **Superglossary** with more than 900 terms and concepts that you may find in the book but not in standard texts or dictionaries. For my immediate object in mind, this volume could help you to understand what I will describe in the pages immediately following about the association-induction hypothesis and its by-now extensive supporting evidence.)

With the contents of the second envelope described, I now return to describe some key features of the association-induction hypothesis.

The first word, association of the title, association-induction hypothesis, indicates that—in diametric contrast to the membrane pump theory—, all the major components of the living cells are associated with one another directly and indirectly, mechanically and energetically— in the same sense that boroughs and precincts of a modern metropolis are linked directly and indirectly, mechanically and energetically.

Now, the largest component of all living cells in volume is water, the next is proteins. In number, the largest component is again water; the next is potassium ion. Though closely resembling sodium ion in most physico-chemical properties, potassium ion is found in living cells at levels as high as 40 times its concentration in the surrounding tissue fluid, in which the cells spend their entire lives. Sodium ion, in contrast, is found at a concentration only about one fifteenth that in the surrounding medium. (In units of millimolarity, the concentration of potassium ion in the cell is about 100 millimoles per kilogram of fresh cells, that of sodium ion in the cell is only about 15. The concentration of

potassium ion in the outside bathing medium is about 2.5 millimolar and that of sodium ion in the outside solution is 100 millimolar.)

(A millimolar solution of sodium ion means that in one liter of that solution one finds $1/1000^{th}$ of 1 mole of that ion. One mole of sodium ion or any other chemical represents the same (Avogadro's) number of sodium ion or any other chemical and that (Avogadro's) number is 6.02×10^{23} or 0.602 trillion trillion.)

Reduced to the simplest terms of the AI Hypothesis, protoplasm is the collective name of the closely associated and electronically interacting system of proteins, water, potassium ions and other critically important but small concentrations of potent agents called ***cardinal adsorbents.*** One of the most important cardinal adsorbent is the end product of energy metabolism called adenosine triphosphate or ***ATP*** for short (See Figure 44 on page 153 of Book 4.)

The basic composition of protoplasm is qualitatively similar but quantitatively widely varying. In physical form, it varied from that of a viscous liquid (Fig 3. in Book 4) to that of a hard gel. As such, protoplasm is the seat of all physiological activities, depending primarily on its location in the cell—where it is maintained with the aid of adsorbed ATP and other cardinal adsorbents at a low entropy state, called the ***resting living state***. (See right-hand side picture of Figure 44 on page 153 of Book 4.)

The protoplasm can undergo reversible changes between **the** ***resting living state*** and the ***active living state***, thus performing physiological activities (***living activity***.) (Figure 44 of Book 4.)

{In contrast, no definition of either living or living activities has been proposed on the basis of either the original membrane theory or its later version, the membrane pump theory, beyond rephrasing an observation. In his otherwise excellent 1981 book, "Life Itself, Its Origin and Nature" (ISBN 0-671-25562-0), Nobel Laureate, Professor Francis Crick of the double-helix fame, wrote, " It is not easy to give a compact definition of either 'life' or 'living' " (p. 49.)}

As mentioned above, the sodium ion is found at around one fifteenth of the concentration in the outside bathing solution. In contrast, the potassium ion is found in the cell at a concentration some forty times higher than in the bathing solution. As you know by now, this asymmetry in distribution is not the consequence of ceaseless pumping as postulated in the membrane pump theory. In fact, alternative concepts have been introduced long ago but I did not know of their existence until long after I had received my Ph. D. degree in (cell) physiology. So complete was this opaqueness to alternative theories that even my mentor and teacher, Professor Ralph W. Gerard, who was the personification of intelligence, integrity and open-mindedness, rarely if ever mentioned the ideas of the like of Moore and Roaf.

Yet clearly in 1913 Professors Benjamin Moore and Herbert Roaf of the University of Liverpool pointed out some highly relevant facts. That is, the similarity of the asymmetric distribution of potassium and sodium ion in living cells and in soils, which too selectively accumulate potassium ion over sodium ion. However, they did not offer a molecular mechanism for either phenomenon (See p. 35 of Book 4.)

It was some 39 years later in 1952 that I first proposed such a (quantitative) molecular mechanism for the selective accumulation of potassium ion over sodium ion in living cells as well as in non-living model systems like (old) soils and (new) man-made ion-exchange resins (p. 48 of Book 4.)

This new theory can be divided into two parts. The first part is called the ***Principle of Enhanced Association by Site Fixation***. It is a physical theory why molecules and ions stick to, or adsorb on spatially immobilized or fixed objects. The second part (to be elaborated in the next paragraph) explains how **close-contact association** with fixed negative charges makes possible for the selective uptake or adsorption of the smaller hydrated potassium ion over the larger hydrated sodium ion.

As mentioned above, (hydrated) potassium ion is smaller in size than (hydrated) sodium ion. Each potassium and sodium ion carries a unit positive electric charge. As such, they are attracted to and stay associated with a (fixed) site carrying a single negative electric charge.

According to the Coulomb Law, (which in my opinion should be called Priestley-Coulomb Law), the strength of electrostatic attraction between a positive electric charge and a negative electric charge is inversely proportional to the square of the distance separating the centers of the opposite charges. Hence the smaller the distance of separation, the stronger the attraction. Since the (hydrated) potassium ion is smaller, it can reach closer to the center of the fixed negative charge and thus experiences a stronger electrostatic attraction than the larger (hydrated) sodium can. The *statistical mechanical law* called the ***Boltzmann distribution law*** would then predict that among the trillions and trillions of negatively-charged sites in the living cell, many times more of the smaller (hydrated) potassium ion would be the preferred partner of the fixed negative sites over the much smaller percentage of sites found associated with the larger (hydrated) sodium ion.

With the basic molecular mechanism for selective potassium over sodium selection explained, our next task was to find what in the protoplasm of the living cell can provide enough negatively-charged fixed sites required from the basic knowledge on what a protein is. Keep in mind that water and potassium ions can be found almost anywhere on this planet. Proteins, on the other hand, can only be found in living beings and in their products.

Now, each protein molecule is a chain of linearly arranged basic units and in that it resembles a printed English word but much longer. The uniqueness of each protein lies in the specific sequential order and kinds of the basic units called *amino acid residues* in the long protein chain. There are in most proteins 20 kinds of amino acid residues, each derived from a corresponding free α-*amino acid* or simply amino acid. While the 26 alphabet letters in different assortment and order of arrangement spell Shakespeare, the 20 amino acid residues in different assortment and order of arrangement spells life.

We recognize that e-a-t is different from a-t-e, because we say so. That difference between the comparable sequence of the three amino acid residues, glutamic acid-glutamic acid-glycine or glu-glu-gly and another sequence, glu-gly-glu is because the laws of Nature dictate so.

Now, each amino acid residue is a part of a protein chain when it is joined to two other immediately neighboring amino acid residues in a protein molecule, which may contain thousands of amino acid residues. What is called a polypeptide or polypeptide chain contains much fewer amino acid residues but otherwise quite similar to most (giant) protein molecules.

Each free amino acid has two ends. One end of each free amino acid is always the same, consisting of, for simplicity, what one may call a left limb and a right limb. When one free amino acid reacts with another, the left limb of one amino acid is joined to the right limb of the neighboring amino acid and forms what is called a *peptide bond*. Each protein contains a long chain of such peptide bonds, which together form the "backbone"

of a protein. As mentioned above, one end of each amino acid or amino acid residue is always the same. However, the other hand differs from one kind of amino acid to another. As part of a protein chain, this part of each amino acid residue is called a side chain. As a rule, it is the kind and sequential order of the different side chains that uniquely defines a specific protein.

Earlier, I mentioned that we needed to find out what in the cell can provide a large number of fixed negatively charged sites that would adsorb and thus selectively take up the smaller (hydrated) potassium ion over the larger (hydrated) sodium ion. Now, we are in a position to describe what they are. One kind belongs to the glutamic acid residue in a protein. (Parenthetically, the sodium salt of this (free) amino acid is known as monosodium glutamate (MSG), which has been used as a food additive for ages in China and Japan before arriving at the West and marketed in a more or less pure form under the brand name, "Accent".)

As mentioned above, it is the different ends of different amino acid residues that provide a protein with its unique assembly of side chains. The specific side chain that a glutamic acid residue carries at its end is an acidic group called a **carboxyl group** or more precisely, a γ-carboxl group. Vinegar or acetic acid carries a similar carboxyl group, so does the near relative of glutamic acid residue known as the aspartic acid residue carrying at its end a β-carboxyl group.

Now we return to the lengthy "backbone" of a polypeptide chain or protein. The electrons in a polypeptide chain do not have a single pattern of distribution. Instead, they may assume either one of two alternative configurations, which energetically speaking are not too far apart. In that way, they are like a chain of well-balanced seesaws tethered end to end with flexible strings. In both, a small disturbance at one end of the chain may set up a wave of perturbation travelling all the way to the other end. Put differently, the polypeptide chains are highly **polarizable** and thus able to conduct information by a falling-domino like mechanism.

As mentioned earlier, each polypeptide chain consists of a long sequence of peptide linkages, each of which is composed of carbon (C), oxygen (O) and hydrogen (H) atoms in the structure, NHCO, where the NH group is positively charged and the CO group is negatively charged. But different from the side-chain carboxyl groups, these polypeptide-chain groups are **dipolar** in nature, whereas the side-chain carboxyl groups are **monopola**r. A monopolar charged group carries a single negative or positive electric charge and no other residing electric charge of the opposite kind nearby. Each dipolar charge, in contrast, is inescapably accompanied by an opposite electric charge in close vicinity. A monopolar site like a side-chain carboxyl group tends to offer strong electric field near and far according to the inverse square law mentioned earlier. A dipolar electric charge may offer fairly strong electric field at location very close but the strength of the electric field falls off much more rapidly with distance.

As a rule, each protein molecule can exist in two alternative folding patterns (See Figure 44 , Book 4.) They are respectively the folded *α-helical conformation* and the *fully extended conformation*. The textbook teaching is that in what is called native conformation (one that occurs in Nature), the protein exists in the α-helical conformation and in the damaged or denatured state the protein exists in the fully extended conformation. But this assignment is by and large mistaken. According to the association-induction hypothesis and its abundant experimental supports, the major protein making up the bulk of each healthy resting living cell is as a rule in the fully extended conformation.

In retrospect, we mentioned that side-chain carboxyl groups offer potential adsorption sites for intracellular potassium ion. And since the concentration of intracellular side-chain carboxyl groups are, as a rule, quite high and their affinity for monovalent cations like potassium strong, we now can understand why in living cells there is such a high concentration of potassium ion even though its concentration outside the cell is meager.

It is now over one half of a century since we began to test this theory of selective potassium accumulation in living cells. The evidence is by now overwhelmingly confirmative. For details, I suggest that you consult Book 4 from page 48 to page 73. Note in particular the beautiful contributions from the German scientist, Dr. Ludwig Edelmann cited again and again.

Now we are ready to tackle the question how come exactly the opposite holds for the sodium ion. Its concentration is much higher outside than inside the living cell. Again I reiterate that this is not due to the ceaseless activity of a hypothetical sodium pump in the cell membrane. Indeed, alternative ideas have also been suggested as early as 1909 by the brilliant and courageous American physiologist-physician, Dr. Martin Fischer, the son of two German immigrants.

Buried deep in a 657 page-long article in the Transcript of the College of Physicians of Philadelphia, was what Fischer wrote: for substances occurring at a concentration higher than in the surrounding medium, **adsorption** may offer the mechanism. For substances that occur at a concentration lower than in the surrounding medium, the **Law of Partition** may provide the mechanism (see p. 36 of Book 4.)

With all the extensive studies we have made in the forty years since the completed association-induction hypotheses was introduced, I can say with no hesitation that the nearly completely forgotten Fischer was right on both accounts.

But that was also as far as Martin Fischer went. For unexplained reason, he did not further pursue this subject of how the partition law could function in living cells. He did, however, suggest that the inside of living cells is colloidal. Again neither he nor any other colloid chemist offered a molecular mechanism as to what makes colloid different from non-colloids until the PM theory of colloids was offered (Compare old definition given to colloid quoted on page 30 to new one given on page 84 of Book 4.).

The Polarized Multilayer (or PM) theory of cell water and model systems offered for the first time, a molecular mechanism for the reduced level of sodium in cell water and in solutions containing inanimate colloids (without the need of continual energy expenditure.) The theory was first presented at the Symposium on Forms of Water in Biological Systems held in New York in 1965 (See Chapter 9 of Book 4 for A. S. Troshin's important contributions.)

According to the PM theory of cell water, **all or virtually all** the water in a typical living cell assumes the ***dynamic structure of polarized-oriented multilayers*** (See Figure 20 on p. 76 of Book 4.). In that basic postulation, the PM theory is unique and first of its kind. Note also that strictly speaking, it is not correct to refer to the PM theory's concept of cell water simply as "structured water" because the structure involved is not static as found for example in ice but constantly changing like the **dynamic structure** of a flock of migrating geese. The next question is what makes the bulk of cell water take on this dynamic structure?

In the PM theory, in each living cells there is a parallel-arranged matrix of fully extended protein chains with their negatively charged CO groups and the positively charged

NH groups of their backbones directly exposed to, and polarizing and orienting (directly and indirectly) multilayers of water molecules of the cell. In cells like the frog muscle, the average number of water layers between adjacent proteins chains is no more than ten and that is all it takes to polarize and orient all the cell water. (For the more up-to-date information on this subject, go to my Website, www.gilbertling.org and click Articles No. 2 and No. 5 listed by titles on the front page of the Website. In Section 2.5 of Article No. 5 is a detailed exposition why protein conventionally called native is not native in the sense that it is in this form it occurs in Nature. For this reason, in all subsequent reference to this form of protein, we will put quotation marks on it like "native".)

Nor is the theory merely to explain the existence of dynamic structure of water in living cell *per se*. Just as important or even more important is how the dynamic water structure can offer explanations for a whole gamut of cell and subcellular physiological properties that in the past have often been wrongly attributed to different causes like the sodium pump (For a list of these properties, see p.78 of Book 4.)

One of these physiological phenomena is the ability of living cells to exclude to varying degree from its cell water ions like sodium, molecules like cane sugar (or sucrose) and a whole variety of other substances that occur in Nature or were created for the first time by Man.

As a result of the multilayer polarization and orientation, the average water-to-water interaction energy in cell water is higher than that in normal liquid water. Accordingly, if you move a large dissolved substance or solute molecule from its normal liquid water in the outside bathing solution, a large hole of the right size must be dug in the cell water (with stronger water-to-water interaction energy) to accommodate the solute. This would entail the expenditure of more energy than the energy recovered in filling up the hole left behind by the solute in the normal liquid water outside the cell where it came from (with weaker water-to-water interaction energy.) Again the Boltzmann distribution law dictates that more sodium ion would stay outside the cell because fewer sodium ions would have enough energy to run up the hill, so to speak.

This is the main energy or enthalpy component for the low level of large (hydrated) sodium ion in living cells.

There is also an unfavorable entropy component due to the more restricted motional (especially rotational motional) freedom in the "stickier" cell water than in the external normal liquid water. In language of statistical mechanics, being stickier means that there are less quantum-mechanically allowed energy levels in the cell water than outside in the normal liquid water, this disparity of allowed energy levels also "drives" the larger hydrated sodium ions to the outside and stays outside.

Both the energy and the entropy component become more and more unfavorable as the molecular size increases to higher and higher values. Hence what is called the "**size rule"**—seen in the equilibrium distributions of solutes in living cells and in the right kind of models. One example of the right model is a solution of gelatin. Gelatin molecules exist at least 50% in the fully extended conformation (See right hand side picture of Figure 44 in Book 4.) In contrast, the size rule is not obeyed for solutes found in water in solutions of the so-called "native" proteins like isolated "native" hemoglobin, which you can buy from a biochemical supply house that comes in a bottle in crystalline form. These "native" proteins exist mostly in the folded α-helical conformation (See left-hand side picture in Figure 44) because being folded, the charged NH and CO groups of the back-

bone are already neutralized and thus no longer free to interact with water molecules. However, denature the hemoglobin and cause it to assume the fully extended conformation, it too now behaves just like gelatin, able to cause change in the dynamic structure of surrounding water (See Inset A of Figure 28 on page 97 of Book 4.)

Furthermore, the quantitative formulation of the PM theory of solute distribution has made it possible to determine the excess water-to-water interaction energy due to the multilayer polarization-orientation in living cells or model systems. The theoretical equation introduced (Equation A3 in Appendix 1 on page 282 of Book 4.) could account for the divergent q-values (or *true equilibrium distribution coefficients*) of 23 solutes ranging in molecular volume from 18 to 1055 cc. In addition, it also offers quantitative explanation why seven of the solutes studied are known cryoprotectants, whose use allowed the preservation of living cells at liquid nitrogen temperature (See Figure 29 on page 97 of Book 4.) These molecules apparently have surface structures that fit better the polarized-oriented multilayers of normal cell water, thereby stabilizing it and make it able to withstand the intensely low temperature in liquid nitrogen or even in liquid helium during cryopreservation.

However, this letter is already far too long to continue on in order to convey to you other exciting experimental confirmation after confirmation with singular dependability years after years. Fortunately, I can refer to Chapter 11 of Book 4, which you could if you so choose, read at your leisure.

The confirmation of the theoretical predictions of the theory of cell water in the living cell and in the right kind of inanimate models (i.e., satisfying the theoretical requirements) but not in models missing the key features required by the theory is collectively called **triple confirmation.** On page 78 of Book 4, you will find records of triple confirmation of all eight attributes of cell water investigated worldwide since the publication of the PM theory in 1965.

Next, I will try to tell you how disproving the sodium pump hypothesis (in specific and the membrane pump theory in general) and introducing the association-induction hypothesis including its subsidiary polarized multilayer theory of cell water were received by my fellow-cell physiologists. But before plunging into that story, I want to add that in the last fifty-some years, I and my associates have further strengthened the disproof of the membrane pump theory and the affirmation of the essence of the association-induction hypothesis. Additionally, I have recorded these findings as well as new theoretical development in three other books. The 4th and last one is already in your hands. The first one, "A Physical Theory of the Living State" is, as mentioned earlier already out of print. Two other volumes are still in print. They are:

Ling, G.N. 1984 "In Search of the Physical Basis of Life". Plenum Publishing, ISBN 0-306-41409-0, 791 pages.

Ling, G.N., 1992 " A Revolution in the Physiology of the Living Cell". Krieger Publishing Co., Malabar, Florida, ISBN 0-89464-309-3, 378 pages.

(The respective *Table of Contents* of all 4 books can be found in the website: <www.gilbertling.org/lp7a.htm>.)

I now give a brief account of several additional sets of critical findings in disproving the membrane pump theory and in affirming the association-induction hypothesis:

- A sausage-like sac was made from a segment of a giant nerve axon with its internal protoplasmic content or axoplasm removed and replaced with seawater containing energy sources. After tying both of its open ends, the preparation was incubated in seawater. If the membrane pump theory is correct, potassium ion would gradually move into the sac and sodium ion move out of the sac, both against concentration gradients. If the association-induction hypothesis is by and large correct, no significant transport of either ion should occur. Experiments attempted by some of the most skilled workers failed to demonstrate outward movement of sodium ion or inward movement of potassium ion against concentration gradients (See section (4) on page 112 in Book 4.)
- In contrast, an effectively membrane (pump) less open-ended (EMOC) muscle cell preparation continues to accumulate potassium ions to concentration many times higher than in the source solution and to maintain an intracellular sodium ion concentration many times lower than in the source solution—again contradicting the membrane-pump theory and supporting the association-induction hypothesis (See pp. 52–54 including Figure 7 and 8 of Book 4.)
- By varying the salt (sodium chloride) content of their bathing medium, human red blood cells can be made to lose all, some or little of its hemoglobin, which makes up 97% of normal red blood cell's total protein content. When the swollen "ghosts" thus prepared are "resealed" in solutions containing the normal isotonic concentration of sucrose, salts and ATP, potassium ion re-accumulated in the resealed ghosts and sodium extruded from them. The levels finally attained for both ions are quantitatively dependent on the amount of hemoglobin remaining in the resealed ghosts but in opposite directions. In "resealed ghosts" with intact cell membrane but no or virtually no intracellular proteins (mostly hemoglobin) both potassium and sodium ion concentration remained unchanging.

  This set of studies at once refutes the membrane pump theory and confirms the association-induction hypothesis. (See p. 111 including Figure 33 in Book 4.)

Now I shall begin to tell you how my disproof of the membrane pump hypothesis and how my introduction and the steady verifications of the association-induction hypothesis were received. But a few words on my personal history before that.

I came to the US from China after winning in a nationwide competitive examination, the (single) biology slot in what was known as the Boxer Scholarship Program for further study in the US. I sought and was given permission from Professor Ralph W. Gerard to study under him for a Ph.D. degree in the Department of Physiology in the University of Chicago. Now Professor Gerard himself once studied under Professor A. V. Hill in England. This was a great beginning for me because in more than one way, I have learnt from Professor Gerard's example the critical importance to seek a broader perspective than the experimental subject being pursued at any one time—as for example was well represented by Sir William Bayliss's incomparable textbook of General Physiology I cited earlier.

My early work with what was once known as the Ling-Gerard microelectrode (which I think should be referred to as Gerard-Graham-Ling microelectrode) supported (or so it seemed) the membrane theory. Sir Alan Hodgkin came to Chicago to visit our laboratory where I had the honor of teaching him how to pull microelectrodes etc. He also on his own induced the prestigious Physiological Review to invite me to write a review on my

work, and they complied—all before I even got my Ph.D. degree. But all that feeling of belonging was soon to disappear with brutal suddenness.

Two responses came in the year 1966 four years after the publication of the AI Hypothesis proper and the disproof of the sodium pump. In retrospect, each response (or lack of response) was foreboding in its own way.

Richard Keynes, Professor of Physiology of the Cambridge University and a pupil of Sir Alan Hodgkin (Nobel Laureate of Physiology, 1963) of the Physiological Laboratory of the Cambridge University announced publicly in a lecture and in print that "Ling is responsible for a major heresy in this field." Now in history, the word heresy has been used again and again to justify putting someone to death. Its use in describing a purely scientific matter was to my naïve mind hard to understand. For a while, I asked myself if this was intended to be some kind of a joke. After all, up to that point in time, I thought that Sir Hodgkin and I were friends. We certainly wrote letters back and forth on scientific topics. I also asked myself, would it not make things easier if Professor Keynes should discuss with me in private what was bothering him? But he never made such an attempt.

In the same year, Sir Bernard Katz (Nobel Laureate of Physiology, 1970) published a small book, entitled "Nerve, Muscle and Synapse" in which he wrote: "These authors (Ernst, Troshin and Ling) take the view that the potassium ions …possess selective affinity and are chemically bound to the proteinate. (This sentence has misrepresented my view, in which potassium ions are adsorbed electrostatically and not chemically bound, added by GL.) It seems, however, very difficult to support this view in the face of the following pertinent observations by Hodgkin and Keynes (1953.) These results are discussed in detail because they are of crucial importance in the still persistent argument about the validity of the membrane concepts. …It was clear therefore that the labeled (potassium) ions that had entered the exoplasm continued inside cells, to behave as free ions with approximately normal mobility…"

What struck me hard was not what was in the book. It was what was not in the book, even though he must have been quite aware of the existence of the book since he cited "A Physical Theory of the Living State" by name in the reference list. Thus, he made no mention of the evidence against the sodium pump hypothesis (Chapter 8). Nor did he say a single word about the new unifying theory of the living cell, the association-induction hypothesis, nor the fact that the association-induction hypothesis has been receiving steady confirmation again and again.

After all, he pointed out that it was of crucial importance to examine the mobility of potassium ion in living cells to substantiate his belief that the membrane (pump) theory is right. How could he then ignore the (energy) evidence showing that the membrane (pump) theory is not right while a new alternative does fit most of, if not all well-known facts examined in the 680-page long monograph?

Indeed, it was precisely this issue of contradictory evidence against one's favorite theory that the code of behavior enunciated by Sir Bayliss, or the simpler concept of fair play and sportsmanship, was all about.

This violation of the basic code of behavior for a scientist by such a prominent cell physiologist, a knighted Nobel Prize Winner is not a light matter that can be easily shrugged off. After all, receiving such high honors is not a one-way trip to self-glorification. It implies the acceptance of the leadership and its implicit responsibility. Top of all that responsibility is the responsibility of upholding the integrity of the relevant domain of knowledge.

Katz has set a very bad example. For what his pointed omission has done was to announce to the world of cell physiologists that the reign of honor and integrity that had made the modern world so wonderful is over. From here on, it would be acceptable for scientists to get rid of unwelcome scientific facts against one's favorite theory by ignoring them.

As you will find out in more details, this omission, intentional or otherwise was one of the earlier developments of the equivalent of what the industrial world has known too well under the name, ***creative accounting.***

Because Katz set such a store on Hodgkin and Keynes' 1953 paper, I decided to send (along with Book 4) a copy of that paper and labeled it Paper 1. What follows is a simple summary of what this paper tells us along with a few explanatory notes of my own.

The basic units of our nervous system are the nerve cells or neurons. Each neuron contains a cell body and a nucleus and other cytological structures much like other living cells. Unlike most other living cells, however, each neuron also contains a long process called the axon. Most axons are very thin threadlike structures but in squids and cuttlefish, some of the axons are as wide as one millimeter in diameter or wider.

This extraordinary width and its length in centimeters have made the giant axons a remarkable experimental material for investigations of the electrical activities of the nerve fibers. And it is the experimental material that Hodgkin and Keynes used in their potassium mobility study referred to by Prof. Katz above.

Hodgkin and Keynes set out to determine if the movement of radioactively labeled potassium ion measured in the axoplasm is similar to or different from that measured in seawater. As pointed out by Prof. Katz, they reached the conclusion that potassium ion travels inside the axon at a rate not substantiality different from that in normal seawater. Hence their conclusion that inside of the axons the water is like that in normal sea water in agreement with the membrane pump theory but against the association-induction hypothesis and other similar views that the potassium ion inside cells are adsorbed or bound and thus expected to move slower.

However, in hindsight, I would like to mention that there might be a flaw in the way Hodgkin and Keynes determined the state of health of the isolated axons they studied, i.e., by monitoring the electric activities of the axon membrane. This is not to deny that it could be a good way to determine the health of the axon but then only if one has already accepted the validity of the membrane (pump) theory. For in this theory, only the cell membrane is really alive in the axon preparation and in other cell preparations. Thus, if the cell membrane continues to function normally, the axon could be considered normal.

On the other hand, if one also considers the alternative protoplasmic models of the living cell like the association-induction hypothesis, the adequacy of assessing the health of the axoplasm by monitoring the (membrane) electric activity would be unwarranted. This follows from the fact that in the protoplasmic model like the AI Hypothesis, both the cell membrane and the axoplasm are alive. Accordingly, the health of one does not prove the health of the other. This non sequitur is especially significant here because the axon preparation used by Hodgkin and Keynes was not a part of an intact and healthy cell. Rather, it was a "limb" surgically removed from a once intact nerve cell. Thus even the chance that some coherence might normally exist between one part of the cell and another is annulled by the axon preparation's separation from the cell body, which contained the nucleus and other vital organelles. Nonetheless, for a decisive conclusion on the subject, we

needed more incisive experimental studies. They came twenty years later after the publication of Hodgkin and Keynes's 1953 paper on cuttlefish axons.

In 1973, my associate, Margaret Ochsenfeld and I published the results of a parallel study on another elongated but much more easily accessible type of living cells, i.e., the sartorius muscle cells from North American leopard frogs. The great advantage in using this material over the cuttlefish axons is that with frog muscle one can be routinely obtained in intact form as witnessed by the fact that they can be maintained healthy in an artificial medium two weeks or longer. For this reason, it is as easy to study the diffusion of radioactively labeled potassium ion in perfectly normal intact cells or on cells deliberately injured or killed.

In seventy-two (72) sets of completed studies, Ling and Ochsenfeld were able almost quantitatively to reproduce what Hodgkin and Keynes observed, i.e., potassium ion mobility close to that in a dilute potassium ion solution (in cuttlefish axons), if the muscle cells were deliberately killed with metabolic poisons before the study began. In perfectly healthy muscle cells, the mobility of potassium ion is only one eighth (1/8) of that in normal water solution. In the injured region of the muscle cells, the potassium mobility was somewhere between the normal value and that from the killed cells.

The results from our work threw doubts on the validity of the conclusion reached by Hodgkin and Keynes in 1953 and the opinion of Katz expressed in his book in 1966, namely cell potassium ion is free. On the contrary, we concluded that they fully supported the prediction of the AI Hypothesis and other similar views. Other relevant events occurring after the publication of our paper can be found summarized on pp. 56–60 of Book 4. Our feeling is that no matter how you feel about our results, this work deserves to be read by the leading cell physiologists.

Indeed, if Prof. Bernard Katz had followed the guideline of behavior expressed by Sir Bayliss, he would feel honor-bound to respond to our new findings. The sad truth was that neither he, nor Sir Alan Hodgkin, nor Professor Richard Keynes made any comment on our findings then or later. Yet it was exactly on a subject that was once considered to be of such crucial importance, in the words borrowed from Prof. Katz.

Coming from scientists of such eminent stature, this about face on a piece of key scientific information has dealt a deadly blow to the (self-policed) integrity of cell physiological science in particular and fundamental science in general. In hindsight, I may say with infinite sadness, that the construction of a canopy of darkness had thus begun—from of all places, what has become broadly and increasingly accepted as the Mecca of cell physiological science. But before I could fully understand what all these portend, something else equally bad or even worse followed.

Dr. Paul Horovitz was another former student of Sir Alan Hodgkin. Once, for purely scientific reasons, I had criticized the opinions expressed by them conjointly in an earlier paper. I lost sight of Dr. Horovitz until in 1973, when he became the (powerful) chairman of the Physiology Study Section of the National Institute of Health of the US. It was at about this time that my NIH research grant was up for renewal, and in my progress report attached, I had elaborated on the potassium mobility study described as part of the progress achieved in the preceding grant period. I thought that the reviewers would recognize the relevance of the new truth we had unveiled and make some appropriate and kindly remarks. But this naïve expectation on the assumption of a shared goal of finding truths and through the truth discovered bettering the human condition was almost comically misplaced. But what actually followed exceeded my worst nightmares.

To wit, the Physiology Study Section not only recommended rejection of my renewal proposal but also suggested that my research support from the National Institute of Health of the United States should be terminated permanently henceforth. Apparently, the Study Section members saw nothing wrong in their violating the law practiced in any civilized country—against black-listing a (qualified) citizen's right to apply for the award of taxpayers' money.

However, my work did not end then and there but only through the intervention of two honest and dedicated scientist-administrators, the deputy NIH director, Dr. Thomas Malone and the Associate Director of NIH's Division of Research Grant, Dr. Steven Schiaffino. They read my point-by-point rebuttal of the detailed recommendations from the Physiology Study Section and eventually decided to initiate what was called **special study section,** comprising scientist-advisors who had no direct conflict of interest with my work to review my future proposals.

Similar policy adopted by another courageous and dedicated scientist-administrator, Dr, Arthur Callahan of the Office of Naval Research (ONR) made it possible for our proposal to ONR to be also reviewed by neutral reviewers. With our work periodically reviewed by these special study sections our work continued for another 15 years.

Throughout it all, the regular Physiology study section continued to support generously studies based exclusively on the membrane pump theory and to deny support any work sympathetically linked to the association-induction hypothesis (For more details, see <www.gilbertling.org/lp 11.htm>.)

Eventually, Dr. Schiaffino and Dr. Callahan both retired, in my view before their times. The suggestion to deny permanently support for our work by the Physiology Study Section headed by Dr; Paul Horovitz soon became a reality. After review by a panel still called "special study section" but manned by some of our most determined and ruthless scientific opponents, my laboratory was closed at the height of its productivity in August of 1988. But that would be many years ahead in the future.

While fighting desperately for the survival of my laboratory, I was too preoccupied to put myself in the shoes of my gathering of young graduate and postgraduate students. If after so many years of struggle, I was not sure of being able to continue, what is the chance for them to do better? Nonetheless, I did not think about that at the time and was completely devastated when suddenly virtually all my graduate and postdoctoral students left my laboratory *en masse*.

It was heart breaking to see how each of these truly bright, motivated and promising young scientists had to go through just to continue making a living—in this the otherwise wonderful land of America. In order to be accepted into the fold of the membrane pump camp, each had to perform a two step ritual: renouncing their former association with me and my laboratory and inventing some (lame) scientific reasons to indicate that their turn around was not motivated by fear (for being unable to get jobs) but out of legitimate scientific reasons.

I was ready to cry, when I learned that Jeffrey Freedman came up with what his past scientific opponents must have loved to hear. In due time, it turned out to be still more evidence for, rather than against the AI Hypothesis. Indeed, the red blood cell ghosts experiments reiterated on page 95 above began as an alleged verification of the membrane pump theory. At the time, Freedman thought that he had prepared some perfectly hollow cytoplasm-free red cell membrane vesicles and showed that they could re-accumulate potassium and extrude sodium ions. These vesicles eventually were shown to be not hollow at all but solid to different extents, depending on who was the donor of the blood used.

I have since then lost touch with virtually all of them. (How they must have suffered when they woke up at nights and remembered their carefree and better days long gone.) But occasionally I heard about the later lives of one or two of them. Some were so well rewarded that a student of one of my former graduate students Chris Miller, has just been given the Nobel Prize for Chemistry—on something that sounds familiar (For details, see p. 104 below.)

It did not take deep thinking for me to realize that the key element for continued scientific progress—***the freedom of the younger generation of scientists to choose whatever they believed to be true is no longer***. The date of the demise of what took so long and so many to achieve could be pinpointed with some accuracy. It was 1973 or thereabout. But that was when it started. More hair-raising stories were yet to follow.

As the number of scientific journals and research reports kept steadily increasing, it has gradually become very difficult if not impossible even for the most conscientious to keep abreast of current developments. As a result, more and more scientists obtain their up-to-date knowledge second hand from scientific reviews. One of these reviews that many cell physiologists relied on was the Annual Review of Physiology.

In 1975, the Annual Review of Physiology published the first-of-its kind of review on the subject of the sodium pump. The two young scientists Drs. I. M. Glynn and S.J. D. Karlich were from the Physiological Laboratory of the Cambridge University, the home ground of Sir Alan Hodgkin and Prof. Richard Keynes.

The review began with the following comment: "The present startling growth of the literature on the sodium pump makes a review timely, but it does not make the task of writing easier. If the great mass of work had led to…a hypothesis accounting for the working of the pump, we could have described that hypothesis and then considered the evidence for it. Unfortunately, no such hypothesis exists…" (Ann. Rev. Physiol. 37: 13, 1975, p. 13.)

This passage has frankly told one aspect of the story. The sodium pump hypothesis is not really a theory in the true sense of the word. It is just a bunch of words rephrasing the observation. This coming from the same Institution which Sir Alan Hodgkin and Professor Richard Keynes have made famous, for a second I was jolted with the sudden thought that maybe my one time friend, Alan and his student Richard Keynes might have finally come around closer to my position? The moment of fantasy soon ended.

But this frank admission was not what had made this review a historical watershed. What made this review different from all reviews I encountered before was forebodingly pointed out by Prof. J. Catchpole of the University of Illinois in these words:

> "The first comprehensive review, which mentioned the sodium pump in its title, was that of Glynn and Karlish. Glynn and Karlish listed 245 references in support of the sodium pump and none opposed. Yet Ling's idea had been around for 25 years, so had ours, so had Troshin's…" (Persp. Biol. Med. 24: 164–165, 1981)

Pope Urban VIII captured Bruno and burned him alive for committing heresy. He also showed Galileo the torture chamber twice and extracted a retraction and then imprisoned him for life. All together, the ongoing scientific revolution in astronomy around the Mediterranean science came to an abrupt end in southern Europe. That was centuries ago.

Now we see how a professor from the Physiological Laboratory of Cambridge University could also pronounce someone holding a different scientific view as committing a major heresy. And followed it by what amounted to an excommunication of not just one or two heretics but all those who did not join the alliance paying homage to the "non-existent" sodium pump hypothesis. The reviewers, Glynn and Karlish did not tell lies.

They just did not tell the whole truth. This is the historical landmark of the coming era of ***creative truth telling***.

To find out how the rest of the cell physiology community as a whole responded to this mind-boggling new development, I made a literature search in 1986 and found no less than six other reviews published on subjects directly on the sodium pump or related topics. Each one followed rigorously the style set out by Glynn and Karlish, citing all papers supporting the sodium pump hypothesis and leaving out all evidence contradicting it. (For more details, see www.gilbertling.org/lp15.htm.)

The latest of the review on the sodium pump was again in the Annual Review of Physiology. It was by Dr. I. M. Glynn alone and the title of this review is "A Hundred Years of Sodium Pumping".

Again the review listed only references in support of the sodium pump hypothesis and ignoring all evidence against the sodium pump hypothesis. By now, the art and science of creative truth telling has become apparently an accepted behavior. Is it not the irony of irony that the extremely honorable behaviors of the early scientists have allowed institutes to be set up with no overseeing facilities? That betrayed trust is now devouring what is left of the science of cell physiology and, if I am not entirely mistaken, also the chance of future humanity to continue enjoying the good life of modern living—in comfort, health, freedom and happiness.

Next, I shall present a bird's eye view of what is happening to other related enterprises that eventually could be seriously affected by the breakdown of the honesty and trustworthiness of our basic science in its potentially most promising field, cell physiology.

I shall start with a subject that one of your latest issue of Economist has addressed—in response to pressure to abandon global free trade for a return to protectionism. And I think that you correctly pointed out that the solution is not high tariff but continuing education. By that it must mean that many workers must have acquired and maintained a strongly positive attitude toward scientific and technological education—as it was introduced and nurtured from their early days of schooling. But in the US at least, all kinds of indications are pointing to just the opposite.

For decades now in America, there is a widespread public perception that something is seriously remiss in our educational systems, especially in our science education. As years went by, the concern began to focus more and more on biology teaching. Thus in 1990, *Fulfilling the Promise: Biology Education in the Nation's Schools* was published conjointly by the Board on Biology, Commission on Life Sciences and National Research Council. The authors pointed out that in the widely adopted high school curriculum in the U. S., biology holds a pivotal position. It is at the start of the series of science courses. At best, an inspiring biology course might invoke interest in not just biology but other sciences as well. In most cases, it did not turn out that way.

Of the 1200 students tested in 1988 for their knowledge on biology, 50% of those who never took a course in biology actually did better than 40% of those who did. As the (high school) students leave the biology course, their typical parting comment is "never to take another science course unless made to do so."

A major cause for the trouble, according to the authors of the "Fulfilling the Promise" is the poor quality of the biology textbooks. They de-emphasize the drama and excitement of discoveries and "portray biology as the worst kind of literature—all characters and no story."

Ten years later, the American Association for the Advancement of Science (AAAS) (Project 2061) arrived at more or less the same conclusion from its own independent

investigation. It showed that 9th through 12th grade biology textbooks uniformly fail to convey "big ideas." Of the ten most popular textbooks examined, none escaped the indictment.

On closer look, I found that each of these ten most popular high school biology textbooks (as well five of the most popular college biology textbooks) ,—teaches the sodium pump hypothesis unequivocally and exclusively as scientific truth forty years after it has been unequivocally proved to be wrong.

Did Project 2061 recommend a way to restore the missing "big ideas" in high school biology textbooks? It did—by pointing out what it had used as a benchmark for deciding why it concluded that big ideas are missing. As a matter of fact, there are three of these benchmark books, two of which AAAS itself produces and a third by the National Research Council. Did anyone of these three books mention the association-induction hypothesis? And the revival of the protoplasmic view of the living cell that had revolutionized the entire field of cell physiology in the second half of the 20th century? No. No. No. Instead, each of these benchmark books described the living cell is a way closely similar to the one given on p. 63 of "Science for All Americans" (AAAS);

Under the section title, "Cells", the text says and I quote:

> "All living cells have similar types of complex molecules that are involved in these basic activities of life. These molecules interact in a soup, about 2/3 water, surrounded by a membrane that controls what can enter and leave…"

If you have grasped what this review says, it would make you weep for the future of humanity. For what Project 2061 recommended as the missing "big ideas" is not the (in essence verified) association-induction hypothesis. Instead, it is the old membrane theory,—which was disproved 60 years ago, while its replacement, the membrane pump theory, which features in every single biology textbooks Project 2061 examined and condemned, was disproved only 40 years ago.

Embarrassing as this must have been to the many truly well intentioned and dedicated people involved, it is not all unexpected. When it is all darkness and creative truth telling, it has become well-nigh impossible to tell what is past and what is future or what is truth and what is falsehood. So if one argues that what creative truth telling does to science is a new "Dark Age", one could not find a better piece of evidence than the up-side–down story of Project 2061, a major attempt to improve but lost in its direction.

Since the five most popular college biology textbooks do the same, one wonders if all the college students are being indoctrinated in the same "backward to the future" direction. An answer of sort was provided by one of my former graduate students before he returned to the fold of the membrane pumps camp. And here are excerpts from these signed testimonials (See <ww.gilbertling.org/lp18.htm>.)

> " The following is a reproduction as well as I can recollect of a conversation I had with a Professor of Molecular Biology who had just delivered a lecture to my first-year class in Biophysical Chemistry:
>
> Prof. (with obvious irritation):
>
> " …..Besides, look, this is a business like any other, and you have to protect your security. You know, if I consider Ling, I'll hear repercussions, and my position is threatened. So, I won't consider Ling. I have a wife and children…"

So it would seem that those who know the truth—like the professor as well as my former student writing down this experience—are intimidated to such an extent that they

would rather not pass it on to the students, because their more immediate concern is to care for their families. Can you really condemn them for telling lies? Was it not essentially the same story that Victor Hugo immortalized in "Les Miserable", where Jean Valjean stole bread to feed his sister's hungry children?

But if all of us do the same and let science itself be scuttled, with what are we to fight new threats to the survival of our species—including countless innocent children—in another hundred year's time? Do you think that you too might lose your job if you do what the professor could not afford to do—tell the whole truth? Not if your journal is what others believe it to be—a synthesis of intelligence and integrity and the courage to support new ideas.

Meanwhile, I would like to conclude this series of inquiries on the long term impact of spreading darkness by evaluating what has happened to the institution that has long appointed itself as the final arbiter of what is the most admirable achievement in the search for truth, the Nobel Prize Committees. Are their dedicated members able to see through the half-natural and half-manmade darkness that has overtaken the research as well as teaching communities engaged in exploring the last great frontier of the most relevant of relevant knowledge, the science of life?

Sadly, since its beginning in 1901, the decision-making Nobel Committees have been singularly opaque to external inquiries. When asked, their typical response has been that their decision-making and other relevant data would not be disclosed until 50 years after the award has been made.

I will leave you to draw your own conclusion, after allowing me to tell you of something that pervasive darkness might have kept you from seeing in its totality. That something is in four parts, seemingly separate but in truth different aspects of the same phenomenon. The first two concern the awarding of two Nobel Prizes of Chemistry for research work on the hypothetical membrane pump (many years after its disproof.) The third concerns the award of another Nobel Prize for Chemistry for research that can be seen as being plagiarized from my earlier published work. The fourth and last concerns the Nobel Prize of: Physiology or Medicine for the invention of Magnetic Resonance Imaging or MRI, ending on a return to the quote from your journal with which I began this letter.

Professor Peter Mitchell received the Nobel Prize of Chemistry for the year 1978 for his Chemiosmotic Hypothesis for a mechanism of the membrane pump—twelve years after my categorical disproof the membrane pump concept. It is also astonishing because I have never known any prior award of this widely-regarded as highest honor for scientific achievement given for the introduction of a hypothesis—a hypothesis that has not been experimentally confirmed then or later. In a critical review I published in 1981 entitled "Oxidative Phosphorylation and Mitochondrial Physiology: A Critical Review of the Chemiosmotic Theory and Reinterpretations by the Association-Induction Hypothesis" I showed that this Chemiosmotic Hypothesis is full of holes and offered an alternative interpretation, which is in far better accord with all the relevant facts.

Thus, according to the Chemiosmotic Hypothesis, the energy needed to synthesize ATP in mitochondria comes from dissipating what he calls a "Protomotive Force", a composite of a hydrogen-ion gradient and an electric potential gradient across the inner membrane of mitochondria. However, it was soon discovered that the hydrogen-ion gradient is negligible in magnitude if in existence at all. And the electric potential gradient actually measured, instead of being maintained at the theoretically required inside negative voltage of 200-300 mV., turns out to be only 10-20 mV and in the wrong direction (Physiol.

Chem. Phys. 13:29.) Nineteen years later, another Nobel Prize for Chemistry was given to another sodium pump hypothesis worker. His name is Professor Jens C. Skou.

Prof. Skou from the University of Aarhus of Denmark won the Nobel Prize for Chemistry (1997) specifically for his work on the hypothetical sodium pump—thirty-five years after the disproof of this hypothesis. To seek deeper understanding of his work, I read most if not all of his published work. What he published in one paper is most telling for our present discussion,

In 1990 Skou gave the Fourth Datta Lecture. Its printed version carries the title: "The Energy Coupled Exchange of Na^+ (sodium ion) and K^+ (potassium ion) across the Cell Membrane, the Na^+-K^+ Pump" (FEBS 268:314.) In the opening section of this paper, he wrote, "that the energy from metabolism of the muscle was not high enough to account for the sodium flux…. The answer to the problem was given by (Hans) Ussing (of the University of Copenhagen) namely, that beside the active transport (or pumping) of sodium, there is a sodium-for-sodium exchange, an exchange diffusion, which energetically is neutral." (p. 314)

This statement is what my extensive search could reveal, the first, and also the last, Skou wrote on the problem of energy shortage. What is puzzling is that he made no mention whether or not the exchange diffusion hypothesis had been experimentally verified; yet, an unverified hypothesis is not much more than an idea, which could be true or untrue. In fact it was worse. Not only is there no experimental verification of this hypothesis, there are four sets of published *refutations* of the hypothesis.

Thus, between 1955 and 1970, four independent laboratories across the world tested this hypothesis on four kinds of living cells. They unanimously reached the conclusion that Ussing's exchange diffusion hypothesis has no validity (Hodgkin and Keynes, J. Physiol. 128: 61, 1955; Hoffman and Kregenow, Ann. NY Acad. Sci. 137: 566, 1966; Buck and Goodford, J. Physiol. 83:551, 1966; Ling and Ferguson, Physiol. Chem. Phys. 2: 516, 1970.)

Thus Skou (and the Nobel Prize Committee for Chemistry of 1997) continued to believe that the energy shortage problem had been successfully resolved by Ussing's exchange diffusion hypothesis— long after the exchange diffusion mechanism itself had been thoroughly disproved. Without the help of the hypothetical exchange diffusion mechanism, the energy shortage persists and as such invalidates the sodium pump hypothesis as well as the broader membrane pump hypothesis.

However, other than verifying my contention that Nobel Committees are not always infallible, the Skou tragedy was really no more than a minor footnote in history. To prove or disprove the sodium pump and the larger membrane pump hypothesis requires weightier evidence. Indeed, that was what I attempted to do some fifty years ago and summarized above.

Half of the 2003 Nobel Prize for Chemistry was awarded to Dr. Roderick MacKinnon, a student of my former graduate student, Chris Miller, for his work on the so-called potassium channel in the cell membrane. In a letter I wrote him on December 3, 2003 I told him why I thought that he might be a victim-unknowing perpetrator of the sodium pump alliance and as a result, he was at risk " of committing plagiarism (of my earlier published work.)" I ended the letter with a plea: "Shouldn't you and other intelligent and caring scientists like you, who have now the visibility and public trust that come with the Nobel Prize, join me in righting the wrongs in basic cell physiological sciences, wherever it may be?" With my letter to Dr. McKinnon I also enclosed a copy of my book,

"Life at the Cell and Below-Cell Level" both for the information it carries and as a gesture of good will.

Years went by and no answer came. Eventually, I published in 2007, in Volume 39, pp. 89–106 of Physiological Chemistry and Physics and Medical NMR an article, repeating my appeal in public. The interested reader can download it by going to my Website, www.gilbertling.org and click Article No. 7 listed by title on the Website's front page.

We now come to the 2003 Nobel Award of Physiology or Medicine for the invention of the new medical technology, Magnetic Resonance Imaging. The Prize was divided between Dr. Paul Lauterbur, a chemist, and Peter Mansfield, a physicist. This is unusual because neither one has done work on either Physiology or Medicine whereas the Prize is specifically for outstanding work in the field of Physiology or Medicine. It is doubly unusual because Dr. Raymond Damadian who is a physiologist and physician and who had spent most of his life making the seminal discoveries and in many other ways brought what is now known as MRI into this world.

To see just whether or not something wrong has happened in what led to the decision made by the Nobel Committee, we have to know the full history of how and when the trail that led to the development of MRI began.

It seemed safer for me to assume that you might not be thoroughly familiar with the nuclear magnetic resonance phenomena and how it has become a tool for the investigations of both inanimate and the animate world. For that reason, I have taken the liberty of sending you along with Book 4, a document labeled #4.

This document has three parts. Part A and B are taken from my book, "In Search of the Physical Basis of Life" (1984). Part A described succinctly the relevant parts of the basic physics of NMR. Part B summarizes the biological investigations made with NMR methods up to about 1984. Part C is a summary I put together for your convenience. It tells about the so-called nuclear electric quadrupole moments of elements like sodium (Na^{23} is the sodium isotope making up virtually all existing sodium on this earth.) And how NMR study of sodium (ion) can provide a unique way of determining whether the sodium ion in living cells is adsorbed electrostatically as proposed in the association-induction hypothesis or freely dissolved in (normal) liquid cell water as according to the membrane pump theory.

With these three sets of documents on hand, I am at liberty to move ahead without the need of frequent interruptions to explain names and details. Thus prepared, I can share with you what has been so far largely unseen part of the history of the invention of MRI.

The great advantage offered by nuclear magnetic resonance methods is that it can tell about the amount and properties of elements (like sodium ion) and molecules (like water) within fragile and unstable structures like the living cell without destroying or even perturbing the cell. Of course, that is also why the invention MRI is so valuable to detecting cancer and other life-threatening diseases without surgery or even exposure to X-ray. The basic instrument used is called an NMR spectrometer.

There are two types of NMR spectrometers: the high-resolution NMR spectrometers and the low-resolution spectrometers. Then there is a third variety called pulsed NMR, which can be both high resolution and low resolution. High resolution, continuous wave (CW) NMR instruments are the most widely used.

First, all these instruments have the potential of determining the amount of water (or sodium ion) in a given sample. Second, they can also determine the twin parameters T_1 and T_2 (respectively called the spin-lattice and spin-spin relaxation times) of the two pro-

tons of the water molecules or the sodium ion. These parameters measure the rate of dissipation of electromagnetic energy of the (proton or sodium) nuclei involved.

This dissipation of electromagnetic energy is similar to the dissipation of heat energy from a pot of hot water in that the rate of energy dissipation strongly depends on the environment. A pot of hot water sitting in cool air would take a much longer time to cool off, than if it is sitting in cool water. The phenomenon of energy dissipation is called ***relaxation*** and the time for the energy dissipation is called the ***relaxation time***.

For the relaxation of water (protons) in an NMR machine, the most important environment that determines the relaxation rates is other nearby water molecules. If the bulk phase water molecules are adsorbed directly or indirectly on some immobilized sites, the T_1 and T_2 of its protons are expected to be shorter than water molecules in free liquid water. While T_1 and T_2 values can be accurately determined by using the Pulsed NMR methods, a rough estimate of the value T_2 can also be obtained from a regular continuous wave NMR spectrometer by measuring the width of the NMR (water) proton peak at half height since that width is equal to $2/T_2$.

But having shorter relaxation times does not prove that the bulk phase water studied in living cells or elsewhere is adsorbed in the form of polarized and oriented multilayers. The measured T_1 and T_2 could also be shortened if a small amount of paramagnetic ions like manganese, iron or nickel is present in the water or if these is a small fraction of tightly-bound water (on some proteins) in rapid exchange with a large body of normal liquid water (or with a large body of polarized and oriented water.)

This is to say, that if the T_1 and T_2 of the bulk-phase water of some living cells are found to have the same high values seen in normal liquid water, it would be a piece of strong evidence in favor of the membrane pump theory. On the other hand, if the T_1 and T_2 of water protons in living cells or model systems are much shorter than those of normal liquid water, it would be in harmony with the polarized multilayer theory of cell water but it does not prove that theory. For more definitive evidence that the cell water really assumes the dynamic structure of polarized-oriented multilayers, one must look for other types of evidence which is incisive (e.g., solute distribution patterns.)

In contrast, NMR studies can provide definitive evidence for the electrostatically adsorbed (potassium or) sodium ions in living cells if one can demonstrate quadrupolar (40-60) splitting of the NMR signals of the sodium ions. The reason is this. The critical condition to generate the 40-60 signal splitting is the presence of an asymmetrical electrical gradient on the sodium ion. And such an asymmetrical electric gradient is precisely what the association-induction hypothesis has provided for the mechanism of selective ionic adsorption and accumulation.

However, it must be made clear that this is the latest and I believe the definitive explanation for the first order quadrupolar splitting. At the time when Cope (alone) and later Ling and Cope made their studies, the interpretation they offered was not completely correct though in the right direction. For full details of the long and round-about trail leading to the latest interpretation—perhaps occurring more often in the history of science than on the record—see pp. 188–190 of Book 4.

Soon after Block, Purcell and their respective coworker completed their pioneering studies of the NMR of hydrogen protons and given the Noble Prizes, the one-time tool of physicists was rapidly made into a powerful tool for the study of chemistry. With the ever-improving techniques, one soon was able to “see” on the strip chart the chemical structure of a hitherto unknown organic chemical from a tiny sample. The temptation must be

great for someone who had access to such a marvelous machine to put samples of living cells in the NMR tube and to see what does it tell about the most abundant component of the living cell—water.

So that was how it began. Eric Odeblad, who had his training as a physicist and a physician, put all sorts of samples from live human patients and rats, ranging from cervical mucus during the menstrual cycle to human milk, to human saliva and reported his findings in no less than 40 papers. T. M. Show, on the other hand, used NMR methods to determine the water contents of various animal and plant foodstuffs. J. R. Singer correlated the line-width of NMR signal of water protons in flowing blood to the speed of blood flow—a pioneer work with fruitful results in the future.

In 1965, Bratton, Hopkins and Weinberg demonstrated that during tetanic contraction of frog muscle the line width of water protons shows a 20% narrowing. The authors suggested that this line width narrowing or increase of T_2 of water proton was due to the release from binding of a small fraction of tightly bound water molecules (in rapid exchange with the bulk phase water molecules.) This averaging due to rapid exchange causes the overall water relaxation time of the cell water to fall to a lower value.

All living cells contain some 20% of their weight in the form of various proteins. Many so-called "native" proteins studied contain 0.2 to 0.3 gram of *hydration water* per gram of dry protein. With these facts in mind, it was not surprising that the most common explanation for the wider line width (or shorter T_2) of water protons in living cells or protein-containing solutions is by a fast exchange mechanism. Since the membrane pump theory offers no theoretical function of this minor fraction of hydration water found indiscriminately in all the so-called "native" proteins examined, all this type of study has been exploratory in nature and once reported rarely followed through further.

However, the year Bratten and coworkers published their work described above was also the year that I published my Polarized Multilayer Theory of Cell Water. As pointed out earlier, in this theory all the cell water is not normal liquid water but assumes the dynamic structure of polarized and oriented multilayers. And as such, it could account for the reduced level of sodium ions and sucrose found in most living cells— as an example of the gamut of cell physiological phenomena that can be given a new interpretation than in the historic past.

This subsidiary theory and the parent association-induction hypothesis soon caught the attention of two young scientists unknown to me before. Freeman Cope who had a physics degree from Harvard and an MD degree from Johns Hopkins Medical School. Carlton Hazlewood had his Ph.D. degree of physiology from Johns Hopkins University. Before plowing into the details of their NMR work, I would like to quote Cope explaining why he made his NMR studies of living systems.

Thus in a review article Cope wrote in 1976 entitled " A Primer of Water Structuring and Cation Association in Cells: II. Historical notes, present status and Future Directions", he said:

> "Unlike the work of the Brattan group, the NMR measurements of Na^+ (sodium ion, added by GL) by Cope were intended specifically to test the concept of Ling." (Physiological Chemistry and Physics 8: 569, 1976.)

Actually, like Bratten and coworkers, Cope also studied water (proton) NMR in living cells. Cope's NMR study of cell sodium was published in 1967 and his water study published in 1969, the year in which Carlton Hazlewood and his coworkers also published

their NMR study on cell water. But before going into that central subject, I want to discuss a little more of Cope's study of cell sodium.

The phenomenon Cope was dealing with is what is known as First Order Quadrupolar signal broadening. This branch of the physics of NMR was an outgrowth of the study of NMR of solid crystals. However, since in solid crystals the electric field experienced by the sodium (and other) nuclei are usually balanced due to crystalline symmetry, the signal splitting seen occurs only in imperfections due to contaminants or other aberrant causes. However, in living cells the negatively charged carboxyl groups are as a rule far apart (see Book 4, pp. 248–249.) And according to the association-induction hypothesis, adsorption of sodium ion would expose the quadrupolar nuclei to a truly asymmetrical electric field and cause the 40-60 signal splitting or broadening. This is truly remarkable: The concentrated efforts of physicists aimed at better understanding of dead crystals would find its virtually perfect application in one of the key problems in cell physiology.

Soon afterward, I became acquainted with Dr. Cope. He, my associate Grace Bohr and I then cooperated in work that was to be published in two papers each under the respective authorship of Ling & Cope and Ling & Bohr. Together, they provided by themselves another set of totally independent refutations of the membrane pump hypothesis and further verification of the association-induction hypothesis. I shall discuss just one set of these experiments: the ouabain experiment.

One of the experimental findings cited again and again by supporters of the membrane pump theory is that the cardiac glycoside called ouabain (a highly water soluble digitalis that was used by Africans as an arrow poison) causes living cells to lose potassium ion in exchange for sodium ion. Skou and many others have suggested that the sodium pump is in fact an enzyme called *sodium-potassium-activated ATPase.* When this enzyme is isolated from fractions of cell debris considered to contain cell membranes and studied in test tubes, its activity appears to be also slowed down by ouabain at the same concentration as that causing the potassium for sodium exchange in intact living cells studied. This was thought of as strong evidence for the sodium pump hypothesis. In this hypothesis, both the potassium ion displaced and the sodium gained are free ions as they are found in dilute solutions. Therefore, the prediction is that the sodium ion signal would be bigger but remain perfectly normal width as found in a normal salt solution.

In the association-induction hypothesis, however, ouabain acts as a ***cardinal adsorbent***, its function being to increase the relative affinity of the side-chain β-, and γ-carboxyl groups for sodium ion in comparison to potassium ion. Therefore, the sodium ion gained in response to ouabain is adsorbed.

We now know that quadrupolar splitting of the sodium NMR signal can only be produced by adsorption of the sodium ion onto a fixed negatively charged site like the (widely-spaced) side-chain β-, and γ-carboxyl groups. Therefore, if the sodium gained by living cells on exposure to ouabain shows the 40-60 splitting, that would add yet another set of evidence contradicting the membrane pump model and affirming the association-induction hypothesis. The work published by Ling and Bohr was based partly on our cooperation with Dr. Cope. It demonstrates that the sodium ion gained on exposure to ouabain indeed shows 40-60 splitting.

Having made this important point clear, we now return to the subject of water molecule polarization-orientation according to the polarized multilayer theory of cell water, a subsidiary of the association-induction hypothesis.

As mentioned above, in the association-induction hypothesis, not only are potassium and sodium ions associated or adsorbed, so is the bulk phase cell water. However, there is

a difference. The potassium and sodium ions are adsorbed singly, one to a site, on side chain β-, and γ-carboxyl groups. In contrast, the bulk phase cell water is adsorbed as polarized-oriented multiulayers directly or indirectly on the exposed NH and CO sites of the backbones of a matrix of parallel-arranged, fully extended protein chains.

This postulation of the multilayer polarization-orientation theory of all or virtually all the bulk phase cell water of the association-induction hypothesis was also put to a test with the help of NMR methods by three scientists of the younger generation. Beside Freeman Cope, they were Carlton Hazlewood and a still younger new comer, Raymond Damadain. Note that each came on their own and none was too concerned that by associating with me and my work they would come to grief one day—not at the time at least (see below for later events.)

Each of this trio of scientists (and their coworkers) independently concluded that their studies confirmed the predicted dynamic polarized-oriented multiplayer theory of cell water (Cope, Biophys. J. 9: 303, 1969; Hazlewood *et al*, Nature 222: 747, 1969; Damdaian, Science 171: 1151, 1971.)

However, Damadian took the study one step further. To understand what he did, we need to visit the earlier work of another prominent and colorful Hungarian biochemist and Nobel Laureate, Albert Szent Györgyi.

In 1957 and thus ten years before the publication of my Polarized Multilayer Theory of Cell Water, Szent Györgyi published a small book called "Bioenergetics."(Academic Press.) In a footnote on page 136 close to the end of the book, he suggested that cancer cells may have less water structure than in their normal counterparts, apparently based on his idea expressed earlier in this booklet that "…water within the cell may not be random water but 'liquid ice' ."

However, the concept of liquid ice is hard to understand, because by definition, when ice turns into liquid, it becomes liquid water. Water cannot be liquid water and ice at the same time, for the same reason that a pregnant woman cannot be not pregnant at the same time. Nor is there any experimental evidence demonstrating the existence of such "liquid ice" but there is evidence that no ice exists in the living cell (See p. 74 in Book 4.).

Furthermore, in a later book Szent Györgyi published in 1972 entitled "The Living State", he wrote: "What is important to the biologist is not so much the structure found in the bulk of water but the structure formed around solids" (Szent Györgyi "The Living State" 1972, p. 12.) Now the solids that are ubiquitously present in all living cells are proteins. In textbooks, structured water around proteins is, of course, the familiar hydration water mentioned above. As mentioned, it occurs at the rate of about 0.2 to 0.3 grams of (hydration) water per gram of dry protein. Since some 20% of cell weight is proteins, this would add up to about 4 to 6 grams of water in a total of about 80 grams of water in 100 cc of living cells.

Since Szent Györgyi's original idea that a cancer cell has less water structure was referring to the bulk phase water (and not to a small fraction of hydration water,) his apparent abandonment of the liquid-ice idea has left his 1947 idea that cancer cells have less water structure dangling with nowhere to go.

The publication of my polarized multilayer theory of the bulk phase cell water changed all that. When the PM theory of the bulk phase cell water is combined with Szent Györgyi's idea that a cancer cell has less water structure, a new hypothesis was born. In this new hypothesis, or combined hypothesis, water in cancer cells would be less intensely polarized and oriented than in their normal counterparts. As such, an NMR study like those already done by Cope and Hazlewood *et al* on the water protons in cancer cells, would

reveal longer T_1 and T_2 than in their corresponding normal cells from which the cancer strain evolved.

It was this exciting idea that was to have a powerful impact on the career of the third young scientist who came to test the PM theory, Raymond Damadian.

It was about this time that I must have introduced Cope to Damadian. This was important because Cope's NMR machine at the Naval Base in Johnsville, Pa. was not capable of making the needed study. Indeed, Cope had earlier made contact with a manufacturer of a more advanced pulsed NMR machine than the one Cope used earlier. The company, called NMR Specialties, was located near Pittsburgh, Pennsylvania. With their more powerful pulsed NMR machine it would be possible to study water itself rather than deuterium oxide (D_2O), which Cope studied earlier as a substitute. A comparative study of normal and caner cell water proton was within reach. Such was the energy and dedication of Damadian that the next thing you know he had completed such a beautiful and fruitful study.

In a paper he subsequently published in the Science magazine in 1971, Damadian showed that he had not only confirmed the PM theory of cell water with its prediction of shorter T_1 and T_2 of water protons in four kinds of normal rat cells and in three kinds of cancer cells—thus further confirming and expanding what Cope and Hazlewood *et al* had done earlier (Damadian, Science 171, p. 1151 column 2). In addition, Damadian also showed that Szent Györgyi's postulation, made meaningful by being cast in language of the new concepts of the PM theory of cell water, was right too. The T_1 and T_2 of water protons of three strains of cancerous tumors are substantially longer than the T_1 and T_2 of water protons in their normal counterparts (p. 1153.) The T_1 and T_2 of water protons of different normal tissues also varied among themselves.

The MRI images to be made in time to come are all built on this seminal diversity of the relaxation time differences of cell water protons. Therefore, Damadian's discovery was indispensable to whatever sophisticated MRI methodology one might find in the future.

And so was Szent Györgyi's idea that cancer cells have less water structure. And so was Ling's the PM theory of cell water assuming the dynamic structure of polarized-oriented multilayers. And so was Cope and Hazlewood *et al*'s seminal NMR study aimed at finding the answer to the key question if cell water suffers motional restriction as according to the association-induction hypothesis.

And here is then a brilliant demonstration that it takes a physician to find ways to treat patients. It is their preoccupation to do so. Thus the opening statement of this seminal paper of Raymond Damadian, M.D. began with the idea that NMR as "an exterior probe for the detection of internal cancer," which to this day remains perhaps a most appropriate description of one function of MRI.

And even more incredible was that in another six years time he and two graduate students, Larry Minkoff and Michael Goldsmith had not only made the first NMR scanning machine, called Indomitable, but also made the first successful NMR study of an intact human body. On November 9. 1977, Damadian wrote me a letter containing the following passage:

> "On the morning of July 3, at 4:45 A.M.…we achieved with great jubilation the world's first MRI image of the live human body. The achievement originated in the modern concepts of salt water biophysics, on which you are the grand pioneer with your classic treatise, the association-induction hypothesis."

However, I moved ahead before my story was fully told. To resume our earlier history, I point out that what Damadian (with the help of Freeman Cope) made the historic discovery, that event was not unnoticed. There were two groups of followers worth mentioning. One group was from the Johns Hopkins University, comprising Dr. Don Hollis and his students Leon A. Saryan and another scientist from Howard University, Harold P. Morris. They repeated and confirmed what (Cope and) Damadian did earlier.

In their final report, published in the Johns Hopkins Medical Journal (121: 441), Hollis, Saryan and Morris concluded that "Recent research by Cope (4), Hazlewood (5) and Bratten (6) using NMR relaxation measurement has added substantially to our understanding of the physical nature of cell water. ..short NMR relaxation times are generally associated with hindered molecular motion, particularly rotational motion, it was concluded that water does not move as rapidly as ..distilled water. ;…Such an interpretation is consistent with the hypothesis of Ling (Ann NY Acad, Sci. 125: 401) that cellular water is absorbed to cell proteins in a number of polarized layers." At the end of the article, they thanked NMR Specialties for help in obtaining the data— as Damadian did earlier at the end of his 1971 paper.

Only then did another scientist enter the picture. His name is Paul Lauterbur, a chemistry professor at the University of New York State at Stony Brook. NMR was a subject close to his interest. Thus he was involved in doing carbon 13 spectroscopy and carbon 13 labeling of proteins. And at the time he was expecting to purchase a piece of equipment from NMR Specialties. One thing led to another. Next thing you know, Lauterbur became the president of NMR Specialties. He wrote later "it was measurements that I observed Saryan carrying out in September of 1971 that caught my attention." Thus inspired, he began the idea of making a spin map different from the one Damadian used in his Indomitable and involved the application of a magnetic gradient and making a 2-dimensional scan.

In 1974, Peter Mansfield, an NMR researcher at the University of Nottingham in England, published his own idea of imaging crystals using NMR. Once he realized that he could achieve spatial imaging, he began to look for other more rewarding applications. And before long he came across what Damadian had discovered, medical imaging. The essence of what makes NMR imaging today was then more or less complete.

We are now at a position to wind up my narrative and go back to where we started: your defamatory attack on my credibility as a scientist now given in a fuller version as it appeared in your journal.

"Following an obscure theory devised by Gilbert Ling, a physiologist, Dr. Damadian believed he would be able to distinguish cancerous from healthy tissues on the basis of the cell's water structure. Most scientists consider Dr. Ling's ideas wacky at best. Undeterred, Dr. Damadian experimented by analyzing excised tumors of rats using a machine at NMR Specialties a now defunct company…"

What you say here is that Damadian made the seminal discoveries of T_1 and T_2 differences among normal tissues and between cancer tissues and normal tissues, not guided by a sound theory but despite being misguided by a wacky theory. In other words, Dr. Damadian's contribution was nothing more than a random piece of good luck—in that way no more worthy of a Nobel Prize than say, Dr. Odeblad.

Thus with one stroke of your pen, you have wiped out the entire real history behind the real and fully documented history of the true origin of what is known as MRI.

When in doubt, look for the party that has something to gain from the misdeed. As I mentioned early your journal had nothing to gain from this misrepresentation. It is not hard to see that your misrepresentation would make the award of the 2003 Nobel prize of Physiology or Medicine to Lauterbur and Mansfield while excluding Damadian seemingly more defensible and in giving the credit for the invention of MRI exclusively to Lauterbur and Mansfield fair and square.

It would also make the sodium pump alliance people happier. Now that they do not have to answer the question why the AI Hypothesis, which they took great effort to ignore and/or make invisible, has given rise to a Mankind-enhancing technology of great importance.

And further down the line, this denigration of the association-induction hypothesis might also make it justifiable to claim that the $800 million pill is the best we can do to protect humanity from cancer, AIDS and other diseases. Life phenomenon is just too difficult for the limited capability of the human mind.

All this is fine and dandy, except one thing. Are you, as a guardian of Capitalism and hence all humanity, willing to accept the fate of all future humanity as portrayed singly and together by the AIDS stricken Africans too poor to buy the $800 million pills? I do not think so.

If you agree with me, then we better get started on the journey toward the designing and manufacturing of reasonably priced and target specific drugs for cancer and other killer diseases in the same way we have been designing and mass producing the myriads of sophisticated weapons against our human enemies. It is a big order. But we don't have alternatives.

Short Note

Preface for the Updated Chinese Translation of Gilbert N. Ling's *Life at the Cell and Below-Cell Level*

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TO THOSE familiar with modern cell biology but unfamiliar with the work of Gilbert Ling, the message in this book will come as a surprise. Ling's view of cell biology will appear to have come from another planet. It is entirely different from the textbook view. On the other hand, we have come to know that the view from outer space can reveal insights that are not easily discernible from vantage points on the planet itself. And, that is what is brought by the monumental contribution of Gilbert Ling's extraordinary and unique insights.

I first met Gilbert Ling at a small meeting in Hungary in the mid 1980s, although I'd known of his alternative views for many years. For me, this meeting was a turning moment. The strength of his evidence, the logic of his arguments, and the sheer sense of resonance created by his paradigm convinced me that he was onto something of fundamental significance. And, others at the meeting shared my views.

New to this field, I not only read his books and papers avidly, but also dispersed them to the best of my students and fellows, who devoured them. Not one of them thought the message was any less than very close to ground truth, and I soon realized that my initial response may have been correct after all. Ling had apparently identified the most foundational features of the living cell, and our laboratory began turning its attention in that direction. Although my own book, *Cells, Gels and the Engines of Life* (Ebner and Sons, 2001) moves in a slightly different direction, it nevertheless builds on the central concepts identified by Gilbert Ling.

This book is Ling's attempt to summarize his views for the non-expert. Please don't expect an easy read. Because the book is built on orthodox physical chemistry, any attempt to circumvent the basics will have resulted in a piece that could easily come across as superficial. This book is anything but superficial. Hence, non-experts will need to spend some time dwelling on the many conceptual gems in this crown of a book. And, the reward will be great because Ling provides a fresh foundation on which to build.

To me, Ling's message contains two striking departures from convention. The first is that the cellular machinery considered by cell biologists to lie mainly in the cell membrane actually lies in the cytoplasm. Ling disputes, for example, the existence of cell-membrane pumps. One needs to take his arguments seriously because they are based on

evidence that has yet to be seriously challenged, though biologists continue to "discover" more and more membrane pumps. The story is fascinating—so much so that more than a few students to whom I've shown his arguments have been compelled enough to change their research directions.

A second departure from convention is long-range water ordering. Ling disputes the widely held view that most cellular water is ordinary bulk water, and argues instead for long-range ordering. If cellular water is ordered, then the milieu inside the cell is qualitatively different from convention, which holds that solutes readily diffuse through the cell. Ordered water excludes solutes, which would evidently have difficulty diffusing through such a milieu. I'm pleased to say that our own experiments have confirmed Ling's prediction even more powerfully than perhaps even he might expect: next to hydrophilic surfaces, ordering out to even millions of molecular layers can occur in some circumstances. Hence, Ling's assertion appears to be valid. It fits his construct very well, while modern cell biology has no easy way to deal with this feature, which in itself implies that the textbook view must be fundamentally erroneous.

So, please do read this book. It will open your eyes to fresh views of how biology may really work.

I cannot close without making reference to the emerging system of doing science. In this system, Gilbert Ling is an anomaly. While modern science has become incremental in nature, Ling fits more naturally with the older system of doing science in which kudos were given to approaches that tackled big questions. Gilbert's questions are indeed big. If he is right, then the way the cell really operates is grossly out of accord with the way textbooks would have it. For many, such an upending of the prevailing view borders on the impossible, for the entire—or almost entire—scientific world has come to a single foundational view, and looks upon those who are audacious enough to challenge that view with considerable skepticism. It has become virtually impossible to challenge a foundational construct without risking one's career.

In that sense Gilbert Ling is a scientific hero. He has bucked the establishment for more than a half century and has continued, year after year, to strengthen his basic position and to further open up new avenues of exploration. On this point, I believe that the substantially updated content of this volume will be a more eloquent persuader than I, regardless of how many words of praise I can add to this preface.

So, I urge the reader to immerse himself/herself with an open mind. Ling's book may appear to have been written by a scientist from another planet; but, after all, can one be certain that life on another distant planet might not be more advanced than life on earth?

Erratum

An article entitled "*Nano-protoplasm: the Ultimate Unit of Life"* was published in 2006 in this journal in Volume 39 from page 111 to page 234. In this article, an Equation 10 is presented on page 149 as follows:

$$n = \exp (- \gamma / 2). \qquad (10)$$

In this equation, n stands for the *Hill coefficient*. The symbol exp stands for natural exponential function to the base e. $- \gamma / 2$ stands for the *nearest neighbor interaction energy.* Unfortunately, as such this equation is incorrect because something has been left out. The corrected equation reads:

$$n = \exp (- \gamma / 2 RT). \qquad (10)$$

Here R represents the gas constant equal to 0.987×10^{-3} kcal/mole and T is the absolute temperature. At room temperature of 25°C, or absolute temperature of 298 °K, RT equals 0.593 kcal/mole. For a $- \gamma / 2$ equal to +0.67 kcal/mole, the corrected equation yields a Hill coefficient n of 3.1. This value agrees with other n-value equivalents of different $- \gamma / 2$ values given in Table 3, which appears on the same page where Equation 10 appears.