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Introduction

The membrane (pump) theory of the living cell taught worldwide today and used as the foundation for biomedical research is in essence a copy of the one Theodore Schwann introduced in 1849 in his Cell Theory[1,2]. Namely, a living cell is a miniscule puddle of watery solution enclosed in a membrane carrying pumps. Ideally every educated person should know that this theory is wrong; in reality few do know.

As a simple starter, I may mention that a typical living cell is solid and not a water-filled hollow box. Cytologists found this out fast and made correction promptly [3p4]. But so far the great majority of cell physiologists and biology teachers have not done so. For a clue to this anomaly, I return to the time when I was a graduate student in the Department of Physiology of the University of Chicago.

On a spring afternoon in 1947, I was standing on the podium in the main lecture room of the Department, waiting for my late-coming audience to settle down. The title of my talk was “The Sodium Pump.”

I began with an apology. I told my audience that even though I had thoroughly searched the libraries, I had no worthwhile information on the sodium pump to share. It was just a name. I then went on to other related issues. When I finally stepped down from the podium, I was startled by what happened next. Two of my highly respected professors each individually took me aside and used almost the same words to tell me “ Gilbert, you don’t want to make yourself a martyr. Leave the sodium pump alone. It is a sacred cow.” I thanked both warmly for their concern about my personal welfare. At the same time, I also felt that they overly worried. Next thing you know, I was doing some simple bread-and-butter experiments to test the validity of the membrane (pump) theory.

In retrospect, that Schwann should have presented a wrong view of a typical living cell is not hard to understand. With the very limited resolving power of the early primitive microscope he used and intent on establishing that cells are basic units of life, he was naturally looking for the largest cell that he could find. What he did find was the large mature plant cell. As illustrated in Figure 1, each of these mature plant cells contains a huge central vacuole filled with clear liquid water. Regarding what he saw as a larger version of all living cells big and small, Schwann then elaborated on the structures of the typical living cell. In specific, he referred to the entire layer of substance surrounding the body of water as the cell membrane. He also recognized that the chemical makeup of the fluid in the cell water and the fluid outside the cell was different. To sustain this asymmetrical solute distribution, he then postulated the existence in the cell membrane of microscopic devices that regulate the chemical contents of the fluid inside and outside the cell. Though he did not explicitly use the word pump, clearly his microscopic devices are what one would call pumps today. But how and why did the membrane pump become a sacred cow?

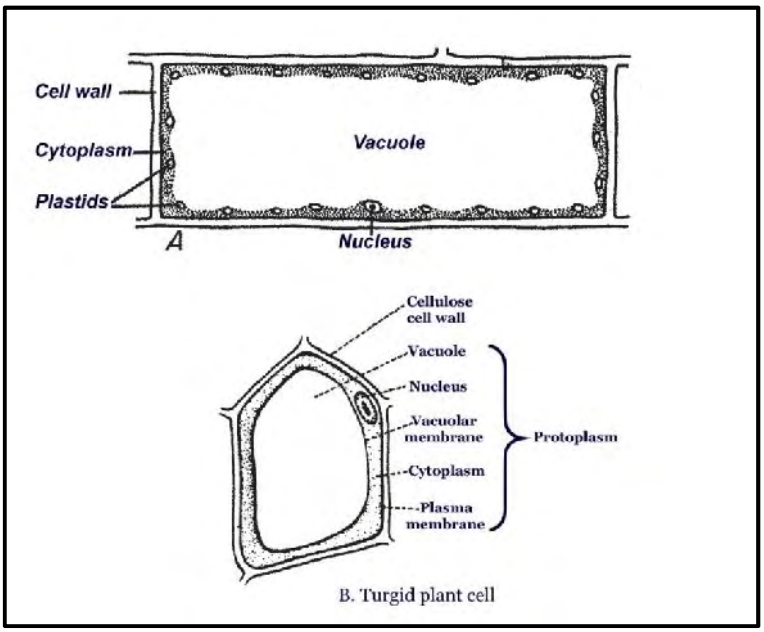


Figure 1 – Mature Plant Cell

The key to understanding this specific strange event could be in the "unquestioning acquiescence of the German textbooks" toward what were introduced in Schwann's Magnus Opus [4p106]. But I have no idea whether or not that was what my two professors had in mind when they advised me to leave the sodium pump alone. A safer guess is that they were thinking in general terms not to do publicly what I did at the departmental seminar.

I did not leave the sodium pump alone because above all, I thought it was a very serious question that needed to be resolved. I also doubted that anyone would care whether a lowly graduate student thinks or not thinks. Furthermore, I had all the tools and frogs to do a test. So I went ahead. I had no idea at the beginning that my associates and I would one day be able to present the results of not one, two or even three but six sets of independent experimental studies.

Six independent disproof of the sodium pump and the membrane theory:

(1) *Energy insufficiency*

My early attempt to do some simple study on the validity of the sodium pump grew into more and more precise inquiries over a period of ten years. The final and most accurate studies were conducted in September of 1956. Under rigorously controlled condition, the minimal energy needed to operate the sodium pump is from 15 to 30 times the maximum available energy if the cell uses all its available energy for pumping sodium only [5p211;6]

(2) *Membrane and postulated pump dispensable*

Frog sartorius muscle cells with intact cytoplasm but without functional membrane pumps was given the name, "Effectively Membrane-pump-less Open-ended Cell" or EMOC preparation. Frog sartorius muscle cells in EMOC preparations continue to keep intracellular K^+ at levels many times *higher* than that in the external bathing medium and to keep intracellular Na^+ level *far below* that in the external medium--- both as found in normal living cells [7p105].

(3) *Cytoplasm-free, membrane sac does not work*

With both ends tied, exoplasm-free squid axon membrane sacs were filled with seawater and nutrients and incubated in seawater. This membrane sac preparation neither pumped K^+ into the sacs nor pumped Na^+ out of the sacs against concentration gradients [8p215].

(4)*Mobility of intracellular* K^+ in healthy cells severely reduced

From a total of seventy-two (72) sets of independent studies, the average mobility of K^+ in healthy frog muscle cytoplasm is one eighth (1/8) of the K^+ mobility in a dilute salt solution. The K^+ mobility rises to 1/2 of that in dilute salt solution in killed muscles that had kept their non-contracted normal dimensions. The mobility of K^+ in injured cytoplasm falls to between 1/8 and 1/2 of that in dilute solution [9]. The validity of an earlier claim that K^+ mobility in squid axons with cut ends is close to that in plain seawater is in doubt on account of the likelihood of death or severe injury of the axoplasm studied [10p21].

(5) *Postulated pump does not have energy to pump* Na^+ *or* K^+

According to the theory of Nobel Laureate, Skou, an enzyme in the cell membrane called Na,K- activated ATPase is the sodium pump. The enzyme

was supposed to provide the needed energy for the pumping by splitting and liberating the energy stored in the high-energy phosphate bond of ATP. However, Podolsky and Morales have demonstrated clearly that there is no extra energy to perform work in the mistaken theory of high-energy phosphate bond [11].

(6) *Cell membrane and postulated pump(s) unnecessary to sustain low Na^+; cytoplasm alone does it faultlessly*

A mature human red blood cell does not have a nucleus or any other intracellular organelles. 65% of its weight is water; 34% of its weight is that of a single pure protein, ferri-hemoglobin. Ling and Ochsenfeld filled sacs made from narrow dialysis tubing with both ends tied, a 40% solution of pure ferri-hemoglobin in water and incubated the filled sacs in solutions containing the same concentration of 10 mM NaCl but different concentrations of HCl until equilibrium was reached. Analysis of the equilibrium sac contents revealed a lowering of the Na^+ concentration in the sac water to between 15% to 30% of the Na^+ concentration in the water bathing the sac if its final pH is in the range 2 to 3.. This ultra-simple model (USM) does not have a cell membranes as such nor the essential constituents of cell membrane and yet it maintains a low "intracellular" Na^+ level precisely matching that found in normal mature human red blood cells and other living cells. The finding demonstrates that to keep the low level of Na^+ in red blood cells and other living cells, the postulated sodium pump is at once wrong and superfluous. The cytoplasm alone can do job perfectly [12]. By the way, I will return to this set of study below under the heading of selective solute accumulation on the significance of the low pH used.

The mutual agreement among the respective conclusions from these six independent disproof extends to other evidence not presented here. Since anyone of these six sets of evidence alone is enough to topple the hypothesis, together they leave absolutely no doubt that I have not exaggerated the case by saying that the currently widely taught and used theory of the living cell is wrong.

The once popular but now abandoned concept of protoplasm

In 1945 French proto-zoologist, Felix Dujardin described a viscous substance oozing out of crushed protozoa that is glutinous, transparent, insoluble in water and drawn out like mucus. He gave this living jelly the name sarcode, meaning fleshy[13]. Years later, the German plant physiologist Hugo von Mohl also discovered a similar substance from plant cells and gave it the name protoplasm

[14]. It was Robert Remak who suggested using the same name protoplasm for both sarcode and protoplasm [15] and his suggestion is adopted. After that landmark episode, two other major historical events occurred in regard to protoplasm. In 1861,Max Schultze announced his "Protoplasmic Doctrine" according to which, living cells are membrane-less lump of protoplasm with a nucleus." [16]. In 1868, Thomas Huxley gave his famous lecture in an Edinburgh church, in which he referred to protoplasm as the physical basis of life [17]. Generally speaking, the concept of protoplasm remained popular untill at least the end of the 19th century.

Then the two World Wars came. Among the wreckage was the once optimistic outlook for the future of life science. More and more began to believe that the concept of protoplasm is wrong. As an example, the *Encyclopedia Britanica online* announced: " As the cell has become fractionated into its component parts, protoplasm as a term no longer has meaning." My futile search for the word, protoplasm through the indices of the ten most popular US high school biology textbooks confirmed my worst fear. It is no longer there.

The Association-Induction Hypothesis

Within the half century since I gave the departmental talk on the Sodium pump, I have also introduced a unifying theory of living phenomena called the *association-induction (*AI*) hypothesis* [5], put it to extensive experimental testing and verified it in essence---without any major setback.

Historically speaking, the association-induction model is an heir to the old protoplasmic concept, which as pointed out above, has been abandoned for its inability to account for the diversity in color and texture of different intracellular structures. The resolution of this specific difficulty is one of the many rewards that the momentous revolutionary change brought to cell physiology by the AI Hypothesis. In substance, this change echoed what had happened earlier to physics between mid-18th and early 20th century with the introduction of the Kinetic Theory of Gases [18]. This theory offered the new concept that gases are not homogeneous matter but are collections of vast number of small particles or molecules moving randomly in all directions. As such, the theory explains why gases exert pressure on the container wall in all directions---a fact totally unexplained until then. It also led eventually to the long-delayed acceptance by physicists of the reality of atoms (and other microscopic particles.)

From the standpoint of the four independent revolutionary physicists involved, it was mostly a lonely and heart-breaking experience. Of the four

who participated, at least one (possibly two) took his own life. And he also happened to be the great Austrian mathematician-physicist, Ludwig Boltzmann, who had almost single-handedly given us the major branch of modern physics called *statistical mechanics*. One hundred and seventy (170) years elapsed between the first introduction of the Kinetic Theory of Gases by Daniel Bernoullli in 1838 and its eventual acceptance in 1908.

For the change brought on by the AI Hypothesis involves the parallel introduction of *microscopic* physiology in which molecules, atoms, ions and electrons replace and give new meanings to *macroscopic* concepts of membranes, pumps, rigid pores, semi-permeability etc. Central to this revolutionary leap under the banner of the AI Hypothesis is the introduction of the concept of the (unit of) microscopic protoplasm or *nano-protoplasm* as the smallest unit of life [19].

Collections of vast number of similar nano-protoplasmic units, in turn, make up what was once called protoplasm and then discarded. It is now revived and given the new name, *macroscopic protoplasm*. Different macroscopic protoplasm in turn makes up the diverse sub-cellular structures including the cytoplasm, the cell membrane or the cell nucleus. But macroscopic protoplasm is not the ultimate physical basis of life just as cells are not the smallest units of life. *Microscopic protoplasm or nano-protoplasm (unit) is the smallest unit of life as well as life's ultimate physical basis*.

To demonstrate its minute size and simplicity, consider a nano-protoplasmic unit from the cytoplasm of a mature human red blood cell. It can be represented by the formula: $(Hb)_1(H_2O)_{7000}(K^+)_{20}(ATP)_1$. Here $(Hb)_1$ indicates the presence in the nano-protoplasmic unit of one molecule of the protein specific to red blood cells, ferri-hemoglobin or Hb. The other three symbols in the formula indicate respectively the number of water (7000), K^+(20) and ATP (1) molecule(s) or ions associated with each ferri-hemoglobin molecule. Assumed spherical in shape, each nano-protoplasm unit measures 8.6 nanometer or 86 angstrom units in diameter.

As a rule, the physiology of the next larger living structure can always be understood in terms of the then existing knowledge on the physiology of the living structure one-level or more levels smaller. Accordingly, the vision of the step-by-step ladder: nano-protoplasm → macroscopic protoplasm → organelle → cell → organ → individual suggests that the AI Hypothesis is indeed a comprehensive unifying theory of life that has the potential of explaining all living phenomena [20].

But for this short summary of the association-induction hypothesis, my task is first to tell how nano-protoplasm and its component proteins work. The article will end on the four classic physiological phenomena at the cell and

below cell level: **solute distribution**, **water and solute permeability**, **cellular electrical potentials** and **cell swelling and shrinkage**. For AI interpretation of physiological activities above the cell level, there is a brief summary in reference 20 p54.

Nano-Protoplasm

The three most abundant components of living cells and their constituents are water, protein and potassium ion (K^+). The individual units of each of these components in the form of single molecules and single ions are all directly or indirectly in contact or *associated* with one another. Through electronic polarization or induction, each entire nano-protoplasmic unit or their bigger aggregate can function coherently.

Before presenting the essence of the association-induction hypothesis proper, I shall briefly describe two subsidiary theories of the AIH. In what is known as *Ling's fixed charge hypothesis* (FCH) first presented in 1952 [21], I pointed out why fixation in space of an ion enhances its association with its oppositely-charged counter-ion. The intensity of mutual attraction between the counter-ion and the fixed ion varies directly with the product of their charges and inversely proportionally to the square of the distance between them. Although a naked Na^+ is smaller than a naked K^+, a hydrated K^+ is smaller than a hydrated Na^+. Accordingly, a hydrated K^+ can stay closer to the singly charged oxygen atom of an oxyacid group. As a result, preferential electrostatic adsorption of K^+ over Na^+ follows. It was pointed out parenthetically that in muscle cells, the contractile protein, myosin alone carries enough fixed anionic sites to adsorb all the cell's K^+.

The second subsidiary theory is called the *polarized (oriented) multilayer* (PM or POM) *theory* of cell water first published in 1956 [22;23;24]. This subsidiary theory demonstrates why all or nearly all the water molecules in a living cell are adsorbed as polarized-oriented multilayers on the fully extended protein chains. When both subsidiary theories of the AIH are considered together, the full association of all the individual units of all three major components of the nano-protoplasmic unit is the result.

A nano-protoplasmic unit is an electronic machine. As such, it can exist in one of two alternative states: the *resting living state* and the *active living state* (Figure 2).

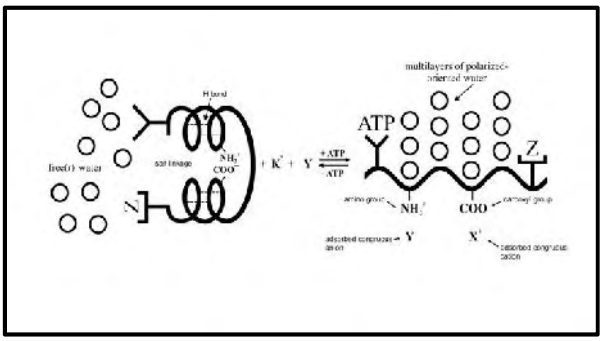


Figure 2 - Resting Living State and Active Living State

Now, the broader subject of life in general also has two components: *being alive* and *engaging in life activities*. Staying in the resting living state signifies being alive. Engaging in reversible shift between the resting and active living state represents life activity. Irreversible shift into the active state ends in the *death state*.

In the resting living state, nano-protoplasm (or its larger aggregate) is at equilibrium **and therefore does not require continual energy expenditure for its maintenance**. Instead, for a nano-protoplasm unit to assume and sustain its resting living state, a special region (or site) of the protein component must be occupied and acted upon by a specific molecular agent called ATP. A product of the cell's energy metabolism, ATP serves its functions by its steady adsorption as such and exerts a far-reaching and powerful electronic impact on the protein (and its associated molecular and ionic partners.)

In the AIH, both internally produced agents like ATP and external applied drugs and hormones belong to what are collectively called *cardinal adsorbents* [5p118]. Cardinal adsorbents fall into two categories: electron-withdrawing cardinal adsorbents or EWC and electron-donating cardinal adsorbents or EDC. (A third kind, called electron-indifferent cardinal adsorbents or EIC is of rare theoretical significance and can be ignored.) ATP and calcium ion (Ca^{++}), for example, are highly important EWC's. To explain why and how the binding of a cardinal adsorbent like ATP achieves its far-reaching physiological impacts, we need some additional knowledge on the structure and function of proteins and other chemicals..

Among the three major components of living cells, protein alone is made only by living cells of one kind or another and thus unique to life. As a rule, the proteins in different kinds of nano-protoplasm are different. As a long-chain polymer, the building blocks of a protein are some 20 different small molecules called α-amino acids or simply amino acids. Each amino acid can be represented by the general formula, NH_2CHR_iCOOH, where R_i differs among

different amino acids but the remainders are almost always the same. The symbol R_I for the amino acid, glycine, is a simple neutral H atom, that for alanine is a neutral methyl group, that for aspartic acid is $COOHCH_2$ with a negatively charged, anionic β-carboxyl group hanging on its end. For the amino acid, glutamic acid, it is $COOH(CH_2)_2$ with an anionic γ-carboxyl group at its end. For the amino acid lysine, it is a $NH_2C(H)_2(CH_2)_3$ with a terminal positively-charged cationic ε-amino group. For arginine, it is $(NH_2)_2H(CH_2)_3$ with a terminal cationic guanidyl group.

Equation 1 below shows how a miniature protein or short polypeptide or tripeptide containing two peptide linkages (CONH) is formed by joining three free amino acids with the loss of four hydrogen atoms and two oxygen atoms in the form of two water molecules.

$$NH_2CHR_1COOH + NH_2CHR_2COOH + NH_2CHR_3COOH \leftrightarrow$$

$$NH_2CHR_1CONHCHR_2CONHCHR_3COOH + 2\,H_2O \qquad (1)$$

As a part of a protein molecule, what remains in the polypeptide or protein of each amino acid is called an *amino acid residue*. The symbol R_I that each amino acid carries is called a *side chain*. In a neutral medium, the β- and γ-carboxyl groups are fully ionized and thus each endows the protein with a single negative electric charge of a fixed mono-valent anion. Each ε-amno group and guanidyl group, on the other hand, endows the protein with a positive charge in the form of a fixed mono-valent cation. β- and γ-carboxyl group may function as adsorption sites for K^+, Na^+ or fixed cations. Pairing of a fixed cation and a fixed anion forms a *salt linkage* [25]. The peptide linkage, CONH, may bind onto similar peptide group on the same protein chain fourth removed in either direction, forming an *α-helix* structure or alternatively bind multilayers of water molecules. The choice between the two is determined by the electron density of the sites involved, a subject which the section farther below will discuss.

Above all, alternative structure of the peptide linkage undergoes extremely rapid transition or *resonance* [26], which makes the polypeptide chain highly polarizable and thus suitable for long-distance information and energy transfer. In addition, resonance also endows short-range negative electric charge to the carbonyl oxygen atom and positive charge to the imino NH group. That fact too is of great importance.

The c-value and the c-value analogue and their respective role in determining selective K^+ and water adsorption.

As mentioned already, in a neutral medium like that provided by the tissue fluid, blood plasma or Ringer's solution, the β-, and γ-carboxyl groups are fully ionized because their respective *acid dissociation constants* K_a's are high enough. Another modified quantitative parameter is the pK_a; it is the negative logarithm of K_a to the base 10. As an illustration, the pK_a of vinegar or acetic acid is 4.76 while the trichloroacetic acid (TCA) is so much stronger acid that its pK_a is below unity. The cause for this difference is the classic *induction* mechanism. Now, each chlorine atoms has 17 positively charged protons in its nucleus whereas a hydrogen atom has only one proton in its nucleus. Each proton carries a net positive electric charge. Thus replacing the three H atoms in the acetic acid CH_3COOH with three chlorine atoms to produce a TCA, also generates a far-reaching *inductive effect* so that the electron density of distant singly-charged oxygen atom of the carboxyl group COO^- becomes greatly reduced. As a result, a sharp fall of its pK_a occurs as just pointed out above.

However, pK_a is not an independent parameter, it is limited to interaction with a specific partner, the H^+ ion. For our broader inquiry, I introduced as part of the association-induction hypothesis, the independent parameter called the c-value. Rigorously defined elsewhere [5p58;10p126], it is in units of angstroms and it can be regarded as a measure of the electron density of the singly charged oxygen atom of an oxy-acid group. A high c-value is equivalent to a high pK_a. A low c-value is equivalent to a low pK_a.

With the c-value defined, I was able to compute the theoretical binding energies of K^+, Na^+, Rb^+, Cs^+. Li^+ as well as those of H^+ and NH_4 on fixed anions with different c-values [5pp62to78]. Figure 3 represents one set of the theoretically computed results, which shows that for any pair of ions like K^+ and Na^+, there is a cross-over point at a certain c-value. In other words, if the c-value of a group of β-, or γ-carboxyl groups is kept low, they would have a selective preference for adsorbing K^+ over Na^+. On the other hand, if the c-value of the same β-, or γ-carboxyl groups is made to rise to a higher c-value, they may change to preferring Na^+ over K^+. In addition, I also introduced a c'-value, which represents the positive charge density of fixed cations like an ε-amino group of a lysine residue.

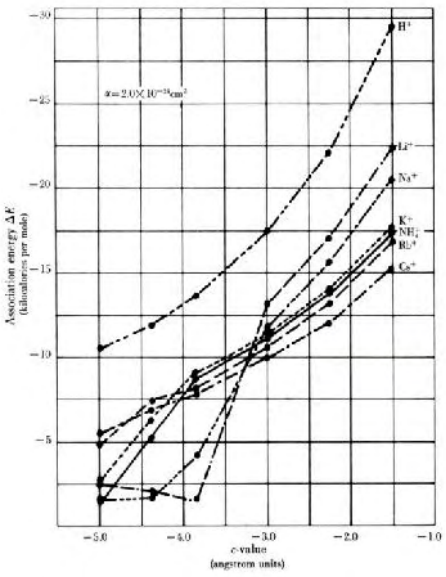


Figure 3

The binding or removal of cardinal adsorbent

like ATP is the main controlling events. However, under what is called "Principle of Additivity" [5p95], the AI Hypothesis also suggests that anything that binds onto the protein component of a nano-protoplasm exercises an electronic impact, though the polarity and magnitude vary from one adsorbent to another.

An in-depth examination of the amino acid composition of many proteins led to the conclusion that physiologically active proteins as a rule contain a high percentage of aspartic and glutamic acid residues. As a result these proteins contain a high percentage of β-, or γ-carboxyl groups. For reasons that would be made clear in sections to come, one may say that these carboxyl groups on relatively short side chains function as the eyes and hands of the smallest unit of life, *nano-protoplasm*. Another type of important reactive functional groups of cell proteins is the carbonyl groups (CO) of the peptide linkages (CONH). The peptide linkage is bipolar. Seen from a distance, the group as a whole is electrically neutral. However, as one approaches the group closer and closer from different angles, the individual carbonyl oxygen atom becomes a fixed anion and the imino NH becomes a fixed cation. For the counterparts of the c-value and c'-value, I also introduced for the carbonyl oxygen and for the imino group the independent quantitative parameter *c-value analogue* and *c'-value analogue* respectively [5p57].

As a part of the AI Hypothesis, I have shown that a list of each amino acid's empirically-determined relative tendency to form α-helical structure called the *α-helical potential* is strongly correlated with the electron donating strength of the side chain of each amino acid (Table 1) [27;28;29].

	Chou and Fasman (P_α)	Tanaka and Sheraga (ω_{h_j*})	Garnier et al. (f_j)	Corrected pK_a 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

Table 1

In consequence, the carbonyl oxygen with high c-value analogue has a high tendency to form α-helix, driving the protein to form a folded secondary structure. As its alternative, carbonyl oxygen atoms with a low c-value analogue prefer to stay open, polarizing and orienting multilayers of adsorbed water molecules.

Physiological reversible degradation of ATP drives the cell into the active living state of a life activity. On the other hand, with the key controlling agent ATP in place, individual molecules or ions of the bulk of all three major

components are directly or indirectly in contact with one another and function coherently together. This "connectedness" is achieved through a combination of both short-range inductive and (falling-domino-like) long-range information and energy transmission mechanisms. Basically they are versions of electronic polarization of one kind or another. Substitution of one covalently linked chemical group by another covalently linked group produces in theory two kinds of impacts. An effect mediated through the intervening space is traditionally called a Direct effect or D-effect; the impact on a target group mediated through intervening linked atoms is called an Inductive or I effect [30]. Combined D and I effect is called an F-effect [31].

The classic Induction theory introduced by G. N. Lewis[30] is limited to changes in covalently linked atoms or radicals. In developing the AI Hypothesis, I demonstrated that both the *initiator groups* and *target groups* of an inductive effect can be linked to the protein involved by ionic or H-bonds [10pp113to115]. In addition, distance action was sorted out into two kinds: Short-range static effect is called Direct F-effect and long-range dynamic effect is referred to as Indirect F-effect. In the year 2008 I gave the Indirect F-effect a new name, the *AI cascade mechanism*, where the letter A and I stand for association and induction respectively [19p137].

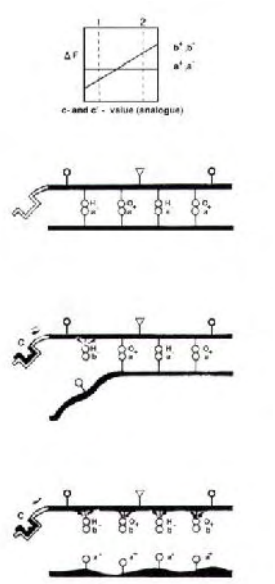


Figure 4

We now know that the *Direct F-effect (mostly inductive) can be transmitted through three peptide linkages with or without an additional small stretch of saturated carbon chain* [32pp47to50]. Like a chain of frictionless tethered seesaws, the *AI cascade mechanism in theory can go on as far as it takes to reach the end of the protein molecule*. The Direct F-effect is simple and readily understood. In contrast, the AI cascade mechanism as illustrated in Figure 4 is more complex as described in ref. 10 pp 147-149 and in ref. 20 pp 90-95. In broad terms, the AI cascade allows a cardinal adsorbent to achieve three mutually complimentary goals: (1) from here to there; (2) from one to many; (3) make many behave like one. Indeed, in Figure 5, the quantitative correspondence between the theoretical predicted patterns relationships as shown in A and experimental observations shown in B, C, D and E demonstrate the power of the combined actions of the short-range Direct F-effect and long range AI cascade mechanism [33p176].

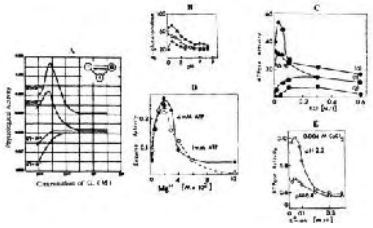


Figure 5

Now that we have completed the presentation on how the ultimate unit of life, nano-protoplasm works, we are ready to present the AI interpretation of the four classic cell physiological subjects namely**solute distribution**, **water and solute permeability**, **cellular electrical potentials** and **cell swelling and shrinkage**

Classic cell physiology:

Solute distribution

The main subject in Figure 11.21 of ref. 79 shows the equilibrium K^+ contents of frog muscle in Ringer solution with different K^+ concentrations but a fixed Na^+ concentration of 100 mM. In outline, the curve bears strong resemblance to that of oxygen uptake in intact human red blood cells shown in the inset. Both curves are S-shaped [34p346]. In the case of oxygen uptake, the conventional view is that the oxygen is taken up by binding onto the cell protein hemoglobin. As such, it indicates that as the external oxygen concentration rises steadily, the heme site with weak binding energy for oxygen takes up oxygen first, followed by uptake by heme sites with stronger binding energy. To explain this anomaly, the "Crevice theory" was suggested. In this concept, the heme sites with strong binding energy are buried inside "crevices" of the hemoglobin molecule while the heme sites with weak binding energy are outside the crevice and thus can bind oxygen first. Perutz's precise work on the structure of the hemoglobin molecule revealed that all 4 oxygen-binding heme sites are on the protein molecule and none buried, leaving the prominent S-shaped curve un-explained. This shortcoming was resolved in 1964 with the introduction of what has become known as the (auto-cooperative) "Yang-Ling adsorption isotherm" [35;10pp137 to 140].

To show how useful this adsorption isotherm could be, I have plotted in Figure 6 the theoretical curve to match the unusually accurate experimental data of Lyster carried out in the laboratory of Prof. F.J. W. Roughton of Cambridge University [36]. All 16 data points fall on or very close to the theoretical curve. And there are just two variables in the theoretical curve: the *intrinsic equilibrium binding constant*, $K^{oo}_{j\rightarrow i}$ and the *nearest neighbor interaction energy*, $-\gamma/2$, as the energy gain each time a new ij pair is formed on two adjacent sites. Similarly, it was shown that the K^+ and Na^+ equilibrium distribution also follows the dictate of the Yang-Ling auto-cooperative

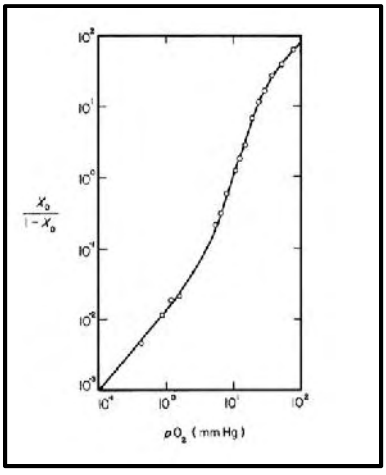


Figure 6

isotherm and as such also described by just two constants of similar magnitudes. Oxygen binds onto the 4 heme sites on hemoglobin; K^+ and Na^+ bind onto many more β-, and γ-carboxyl groups. Yet, the heme sites we refer to as closest neighbors are in fact far apart-- separated by different number and kind of amino acid residues. Similarly, the nearest- neighboring β-, and γ-carboxyl groups are also far apart and separated by different number of amno acid residues. The AI hypothesis can readily explain these because the AI-cascade mechanism makes a long chain of peptide-to-peptide linkages as if they were one. That is, like the energy of one falling domino, it is the same as the next one. In the AI Hypothesis, cooperative changes like those just described belongs to the category of *spontaneous cooperative transition* (Figure 7A) in contrast to the category of *controlled cooperative transition,* (Figure 7B) a subject we will deal with next.

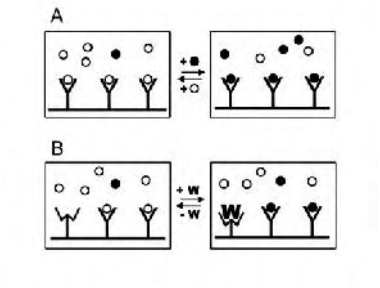


Figure 7

Since we do not have the technology to study a single nano-protoplasmic unit, mature human red blood cells are a special gift to investigators like myself because they contain virtually just a single kind of protein, ferri-hemoglobin and mature human red blood cells are also easy to obtain anywhere. We have already used mature human red blood cells to illustrate the small size and simplicity of a nano-protoplasm unit and Lyster had unwittingly demonstrated the accuracy of the Yang-Ling cooperative adsorption isotherm.

Another gift to the like of myself is the frog muscle. A frog muscle cell is a more complex cell than a human red blood cell. Indeed, what we often study is usually not a single cell but a collection of similar (and some dissimilar) cells belonging to a single whole muscle organ. And yet as a rule, it too behaves like mature human red blood cells, thereby demonstrating that physiologically active cells share proteins that are not unlike hemoglobin.

Figure 8 shows what happened to a collection of frog muscles when exposed to a low concentration of the metabolic poison, sodium iodoacetate and radioactively labeled sucrose. Notice that as the ATP concentration fell, its time course of decline runs parallel to that of the falling concentration of K^+ but anti-paralleled to those of the rising curves of both Na^+ and sucrose. For solutes like Na^+ and sucrose, which exist inside the cell at concentration below their respective concentration in the bathing solution, the extra intracellular concentration gained could be either adsorbed or free.

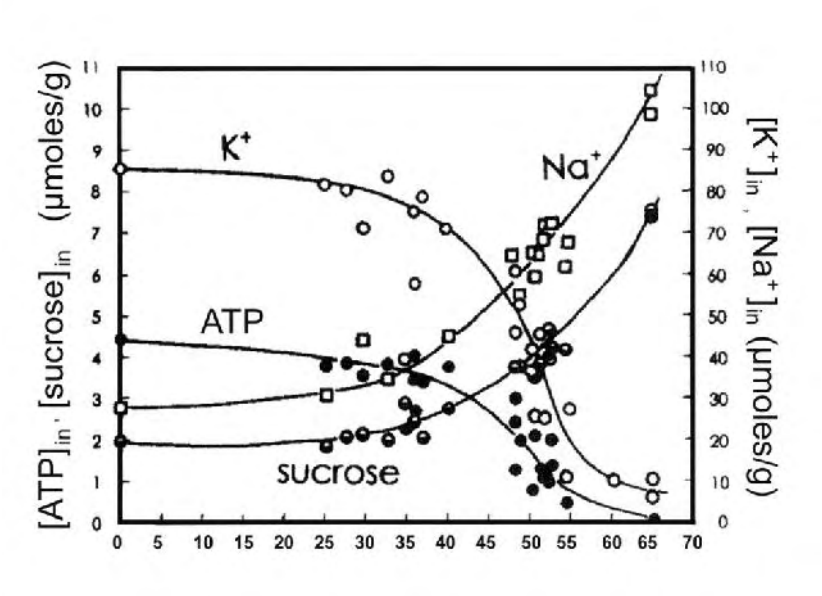


Figure 8

We have previously demonstrated that sucrose shows strictly rectilinear distribution in frog muscle [34], indicating a lack of significant binding in muscle cells. With this in mind, we reached the conclusion that the parallel rising time courses of labeled sucrose and Na^+ reflect a steady desorption of the bulk phase cell water and steady rise of their respective q-values. This, in turn, affirms that the ATP concentration determines the state of polarization-orientation of the cytoplasmic nano-protoplasmic water--- like that illustrated in Figure 2. Since the backbone carbonyl groups adsorb water in multilayers at low c-value analogue, the data adds yet another set of data affirming that ATP is an electron-withdrawing cardinal adsorbent or EWC. In the next section, I present results of a parallel study on K^+ and Na^+ distribution in similar frog muscles under the influence of an EDC.

To avoid inadvertently perturbing the ATP level of the frog muscle at the same time, we added an extremely low concentration of an electron-donating cardinal adsorbent (EDC) a cardiac drug called ouabain [37] to the bathing solution containing an increasing ratios of the concentration of K^+ over Na^+. All the data points in Figure 9 are experimental and the pairs of X-shaped curves are once more theoretical according to the Yang-Ling isotherm. What the drug

did was to reduce the value of $K^{oo}_{j\rightarrow I}$ from 100 to 21.7. The result shows that at the physiological concentration ratio of 2.5 x 10^{-2}, the entire population of β-, and γ-carboxyl groups changed its adsorption partners from all K^+ to all Na^+.

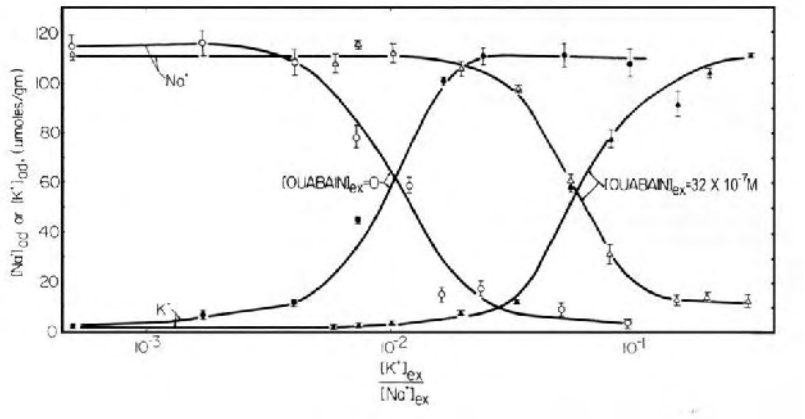


Figure 9

In yet another similar low-ouabain level experiment [38], we assumed that the muscles's cardinal sites took up all the added ouabain added to the bathing solution. We then showed that each one of the adsorbed ouabain molecule would have caused one thousands and forty two (1042) β-, and γ-carboxyl groups to switch their adsorption partners from all K^+ to all Na^+. In still another parallel analysis of the far reaching impact of ATP, we showed that at least 8000 water molecules are under the influence of one adsorbed ATP molecule[20p40].. Both sets of number illustrate and confirm the far-reaching influence mediated by the AI cascade mechanism.

Lastly, I return to the study already described as the 6th disproving the memebrane pump hypothesis [12]. In that study I pointed out how according to the AI Hypothesis of Na^+.partial exclusion from cell water began with a higher water-to-water interaction of cell water. It follows that more energy required to install a hydrated Na^+ in the cell water than that recovered in filling up the hole left behind in normal liquid water. And the water-to-water interaction energy is higher because all the cell water is adsorbed as polarized-oriented multilayers on the exposed polypeptide chains of cell proteins. The existence of cell proteins in the fully extended state in turn is in consequence of the adsorption on key cardinal sites of the protein of ATP, which is an electron withdrawing cardinal adsorbent or EWC. And in the super-simple model or (SSM) studied we did not include ATP as such, which is only assumed to be an EWC. Instead, we replaced it with an artificial EWC, the H^+, *which being nothing but a positive electric charge, is thus by definition an EWC* . The fact that our assemblies of artificial human red blood cell cytoplasmic nano-protoplasm units did reproduce precisely low Na^+ level as seen in normal red blood and other

cells affirms not only the AI hypothesis of solute distribution, it also added another set of convincing evidence that ATP is an EWC.

Solute and water permeability

Radioactive elements used as tracers in biological studies became available in the wake of WWII. *For the first time in history, biologists could measure the genuine permeability of cell membranes to solutes like Na^+ for example*. Soon the historic macrosopic concept that the normal cell membrane is absolutely and permanently impermeable to Na^+ was unequivocally disproved. It was in the crisis thus created for the suvival of the membrane theory, that the membrane pump concept introduced by Theodor Schwann was brought center stage again. Ironically, it was with the deployment of similar radioactive Na^+ tracer that the sodium pump idea was put away for good. But that was only the beginning of adventures brought on by the radioactive tracer technology in cell physiology.

The most abundant component of nano-protoplasm is water. According to the AI Hypothesis, each water molecule is linked to the protein directly or indirectly as a part of polarized-oriented multilayers of water molecules. Furthermore, as a general principle, the nano-protoplasm making up organelles like the cell membrane should not be profoundly different from nano-protoplasm making up the bulk-phase cytoplasm. Two iconoclastic predictions would follow as a result. First, the traditional belief that the cell membrane is primarily a continuous (phospho) lipid bimolecular layer is wrong. Second, the rate of diffusion of labeled water molecules could be similar in their passage through the cell membrane as in their passage through the bulk-phase cytoplasm. In the terminology of diffusion lingo, this type of diffusion is a *bulk-phase limited diffusion* in contrast to a *surface limited diffusion.* With the help of advanced mathematics of diffusion on hand, it was not difficult for me to introduce what is called an *influx profile analysis* to detect and find out which is which [39]. An additional requirement for making accurate studies of bulk-phase- or surface limited diffusion is very large cells. Those we chose are the frog ovarain eggs and the giant muscle cells of giant barnacles.A third critical requirement is radioactively labeled water. This was available in the shape of tritiated water that can be measured in a β-counter.

Thus equipped, my associates and I carried out two main projects: one on frog ovarian eggs by Ling, Ochsenfeld and Karreman [39] and the other on giant barnacle muscle fibers by Reisin and Ling [40]. The results were unequivocal; the *diffusion of labeled water in both of these giant cells was bulk-phase limited.*

A by-product of this study is the diffusion coefficient of labeled water throughout the inside of the two types of giant cells. It is equal to 30% to 60% of that of normal liquid water in the frog ovarian eggs and 55% of that of normal liquid water in the giant barnacle muscle fibers. Furthermore, these figures agree perfectly well with figures from four groups of other independent investigators of the giant barnacle muscle fibers, using different experimental methods.of study. Thus, in units of 10^{-5}cm/sec, their results are 1,34 (Caillé and Hinke), 1.56 (Abetasedarskaya *et al*), 1.20 (Finch *et al)* 1.35 (Walter and Hope), averaging 1.36 ±0.13 (s.d.). From eight sets of studies, our result are 1.35 ± 0.125 (s.d.) [19p190]. This pair of investigations produced yet another set of major confirmation of the AI Hypothesis and refutation of the membrane (pump) theory.

This finding also provides an explanation why (the failed) antibiotic, valinomycin increases a thousand-fold the permeability of K^+ through pure phospholipid membranes, have no influence at all on the K^+ permeability of frog ovarian eggs, frog muscle, human lymphocytes, inner membrane of rat liver mitochondria (Figure 10) [41]. Valinomycin has no detectable impact on the their K^+ permeability because the dominant continuous phase of the cell membrane of these cells are polarized-oriented water molecules, not phospholipid bilayers.

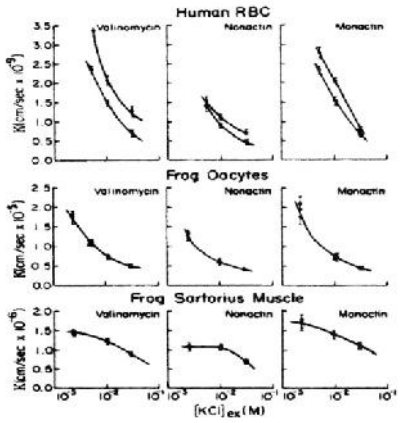


Figure 10

Next I shall deal with the membrane permeability toward vital nutrients like D-glucose that is carried in the blood stream. The problem raises the question of extreme diversity, which is lipid membrane theory does not provide.Thus to reach the cell interior of the functional cells in need of that specific nutrient, D-glucose must be able to pass through speedily the membrane of the blood capillary wall in addition to the membrane of the recipient cells. For that, the requirement is quick permeation and hence high membrane permeability [42]. In contrast, for animals like the frog which spend its life in large body of pond water, the outer skin must have low permeability toward D-glucose or else they will all be lost to the pond water [43]. Again the AI Hypothesis provides a reasonable answer of this striking divergence: in theory the degree of polarization and orientation of the rate-liming water multilayers in the cell membrane are not fixed but flexible.

Thus, the excess water-to-water interaction energy in water assuming the state of polarized-oriented multilayer is a very small figure (126 cal for bulk phase frog muscle cytoplasm), which is a minute number when compared to the total water-to-water interaction energy or vaporization energy of water (10,000 cal.) Thus in theory, a doubling the tiny flexible number 126 cal could greatly slow down the permeability toward large solutes like D-glucose while halving it would greatly increase its permeability. Yet doubling or halving a minute number produces no more than two more minute numbers and as such readily achievable. This theoretical flexibility is well affirmed by experimentally-determined extremely high permeability toward large solute like D-glucose in normal frog muscle cell membrane and orders of magnitude lower permeability toward nutrients like D-glucose in membranes of outer frog skin.

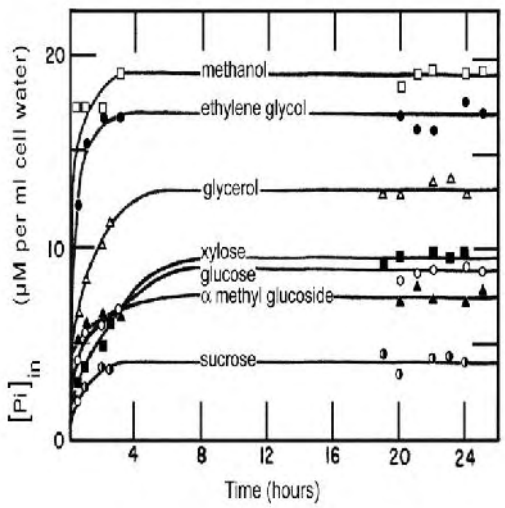


Figure 11

High permeability to glucose, even sucrose is shown in Figure 11 and exceedingly low permeability of the inverted frog skin toward L-glucose and sucrose as shown in Figure 12.

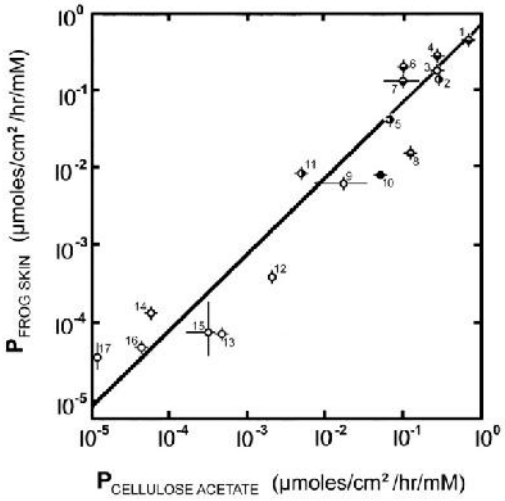


Figure 12

In contrast, without the fundament flexible provided by continuous phase of polarized water, lipid membrane models like that studied by Collander would make all cell membranes even more impermeable to glucose than the inverted frog skin[10p207].No cells with that kind of membrane could survive or indeed exist.

Cellular electric potential

In mid-19th century, Emile DuBois-Reymond, a student of Johannes Müller at the Berlin University and a member of the "Reductionist Four", introduced the name, *demarcation* or *injury potential* for the standing electric potential between the injured end of a muscle or nerve and its intact surface. He also gave the name, *action potential* to the *negative Schwankung* (negative variation) that propagates along the length of a nerve or muscle fiber. A student of another member of the "Reductionist Four", Hermann von Helmholtz was Julius Berstein. Bernstein was in his sixties when he introduced in 1902 his *membrane*

theory of cellular electrical potentials. In this theory, a semi-permeable cell membrane bars the passage of Na^+ and all anions, thereby converting what would be a transient diffusion potential difference into a lasting one. Bernstein also suggested the theory that during the passage of an action potential, the cell membrane becomes momentarily permeable to all ions. Subsequent study of giant squid axons, however, revealed that the action potential does not represent a mere transient annulment of the inside-negative membrane potential but involves a transient polarity reversal creating the an inside positive "overshoot" [44].

When I began my carrier as a graduate student under Prof. Ralph W. Gerard of the Department of Physiology of the University of Chicago, my immediate task was to improve the capillary glass electrode used to measure the membrane potential of individual impaled frog muscle cells or fibers. Success in my early efforts of perfecting the production and use of the microelectrode permitted us to make accurate measurements of the membrane potential of frog muscle cells. (And in time to come, also measuring a great variety of big and small living cells and intracellular organelles by other investigators.) My early work confirmed the proportionality of the resting potential to absolute temperature and to the logarithm of the external K^+ concentration. Notwithstanding, the unequivocal demonstration of membrane permeability to Na^+ threw a monkey wrench in the Bernstein's membrane theory, making the theory as such no longer tenable.

In 1949 Hodgkin and Katz made a historically great discovery [45]. Namely, the increase of membrane permeability during the passage of an action potential does not involve an increase in the permeability toward all ions but is restricted to that of one ion, Na^+. Furthermore, the height of the overshoot is directly proportional to the logarithm of the external Na^+ concentration. In face of the by-then widely recognized permeability of the cell membrane to Na^+, Hodgekin and Katz then introduced the so-called Hodgkin-Katz-Goldman equation for the cellular electrical potential, ψ:

$$\psi = RT/F\{ \ln (P_K[K^+]_{in} + P_{Na} [Na^+]_{in} + P_{Cl}[Cl^-_{in}) / (P_K[K^+]_{ex} + P_{Na} [Na^+]_{ex} + P_{Cl}[Cl^-]_{ex}) \}, \quad (2)$$

where R and T are the gas constant and absolute temperature respectively. F is the Faraday constant. P_K , P_{Na} , P_{Cl} are the permeability constant for the three ions respectively and $[K^+]_{in}$ and $[K^+]_{ex}$ are the intracellular and extracellular K^+ concentrations, etc.[45]. Then, just as the demonstrated permeability to Na^+ wrecked Berstein's theory of membrane potentials, demonstrated indifference

of the cellular resting potential toward external concentration of the highly permeant Cl^- [46] made untenable the Hodgkin-Katz-Goldman equation.

In this tumultuous time, I received my Ph.D. degree in physiology. The year was 1948. My Ph.D. thesis [47] as well as my first four major papers published conjointly with Prof. Gerard and Walter Woodbury all bore the name, membrane potential in their respective titles [48]. Since all these are part of the membrane (pump) theory, which I began to suspect to be wrong. I began to think of rejecting Bernstein's membrane potential theory and constructing an alternative theory of the cellular resting and action potential. But at that time, all I knew was what I had been using as a theoretical guideline is wrong. But I had no idea whatsoever what to replace it with until three years later.

Then on a fine day in 1951, a new idea suddenly dawned on me that would provide a new way of selectively accumulating K^+ over Na^+ without continual energy expenditure. Announced briefly in 1951, it was published in a full-length article in 1952 and would be referred to henceforth as Ling's Fixed Charge Hypothesis[21].

Shortly after the publication of Ling's Fixed Charge Hypothesis, I announced in 1955 and again later, at the Federation Meeting at Atlantic City my new belief that the resting potential of living cells share a fundamental mechanism with that of the glass electrode [49;50;51]. The name eventually given many years later to this new model is *Closest contact surface adsorption potential* (CCSA) [33p.206;p226].Two simple equations were first introduced for what I will henceforth refer to as the non-committal resting potential:

$$\psi = RT/F\{\ln (K_K[K^+]_{in} + K_{Na} [Na^+]_{in})\}, \quad (3)$$

and

$$\psi = RT/F\{\ln (1 + K_K[K^+]_{in} + K_{Na} [Na^+]_{in})\}, \quad (4)$$

where K_K and K_{Na} are the adsorption constants for K^+ and Na^+ on the surface of the living cells.

The change from the membrane potential model to the close-contact surface adsorption model in fact represented a shift from a macroscopic model of membranes and semi-permeability to a microscopic model of ion adsorption on and desorption from β-, and γ-carboxyl groups at the cell surface. Historically speaking, this change has a rich and interesting background.

In 1881, Ludwig Helmholtz measured the electric potential difference across two electrolyte solutions separated by a thin glass membrane [52]. At that time, it was believed that the glass membrane is permeable to H^+ and

impermeable to other ions. Max Cremer, professor of physiology at the Berlin University, suggested for the first time in history that the glass membrane is a suitable model for the living cell membrane [53]. Then something extraordinary happened. A scientist by the name, Horovitz alias Lark-Horovitz showed that a regular glass membrane electrode with no permeability toward the silver ion (Ag^+) acquired a sensitivity to this ion after soaking in a silver nitrate solution overnight [54]. Horovitz attributed the electrical potential difference to originate from the presence of negative electric charges on the surface of the glass electrode and accordingly called this potential a surface adsorption potential. But this concept was not new even then.

In 1892, physicist W.H. Nernst first suggested that the Law of Macroscopic Electro-neutrality forbids the movement of significant electric charges within each phase. It was only at the phase boundary, that ions accumulate and generate electric potential differences [55]. Baur and Kronmann demonstrated the addition of strychnine to one electrolyte solution separated from another by an oil layer, generates what they call surface adsorption potential [56;57]. However, glass membrane and oil layer are not the only materials used to construct inanimate models of cell electric potentials. A third kind is made of collodion.

Nitrocellulose also known as gun cotton is made by exposing cellulose to concentrated nitric acid. In the commercially available form called collodion, nitrocellulose is dissolved in a mixture of alcohol and ether. Dipping a glass tube in collodion, followed by drying in a humidified atmosphere, a “thimble” of membrane electrode could be slipped off for studying membrane potentials. In response to Horovitz’s startling conclusion on the key role of fixed electric charges, Leoner Michaelis and W. Perlzweig pointed out that their colloidion thimble electrode does not carry fixed charges on its surface and therefore continues to affirm Bernstein’s theory of membrane potentials[58].

Then World War II broke out. The German chemical company could no longer provide Michaelis’s laboratory with more collodion to make their collodion membrane models. In response, Michaelis’s students began to make their own colloidion. To their disbelief, the purer the collodion they made, the worse they became as a model for the membrane potential. The models made from the perfectly pure collodion, showed no electric potentials at all.

Eventually, the cause of this mystery was discovered. It turned out to be an impurity in the Schering collodion that generated the electric potential. What is that impurity? It turned out to be our familiar carboxyl groups [59]. This strange turn of events again demonstrated that it is the microscopic surface carboxyl groups that give rise to the potential. It is not a macroscopic potential across a semi-permeable membrane.

But oil membrande continues to yield interesting knowledge. Thus Collacico tried to measure the electrical potential difference between two potassium chloride (KCl) solutions of different strength separated by a layer of oil. But he found none. However, when he added some anionic detergent like sodium dodecyl sulfate to one KCl solution, then the oil surface facing that solution becomes a cation-electrode and as such sensitive to the K^+ concentration in that compartment but totally indifferent to the K^+ concentration in the other compartment. On the other hand, if instead of the anionic detergent, sodium dodecyl suface, he added a cationic detergent like cetyltrimethyammonium bromide then the surface of oil layer facing this KCl solution becomes an anion electrode sensitive to the Cl^- concentration. Now it is no longer sensitive to the K^+ concentration in that compartment [60]. This study was further confirmed and extended by Tamagawa and Nogata [61]. All in all, the Colacicco study dramatically affirmed the microscopic surface adsorption model like that in the CCSA model to come later.

Next, I shall describe how my associate and I combined the two failed model (glass membrane model and collodion membrane model) into a spectacularly successful model called (oxidized) colloidon-coated glass (CCG) electrode [62]. The procedure of preparation is to coat a Corning O15 glass electrode with a layer of collodion, allowed it to dry in a humidity chamber. A brief exposure to a NaOH solution followed, causing oxidation of the nitrocellulose to form more carboxyl groups on the electrode's surface.

It is almost unbelievable how closely this model behaves electrically like a living frog muscle cell: (1) both show a relative sensitivity toward the alkali-metal ions in the order $Rb^+ > K^+$, $Cs^+ > Na^+$ (2) both are totally insensitive toward Mg^{++}, (3) both are two orders of magnitude more sensitivity toward H^+ than to K^+.

In explanation, I point out that cellulose like protein can also exist in a folded and extended conformation. The carboxyl groups appear to resemble living cells in having a low c-value. This too is understandable because the nitro group added onto the cellulose is a well-known electron-withdrawing radical[5p162fig47]. As such its incorporation took the place of the binding of the EWC ATP in the living frog muscle.

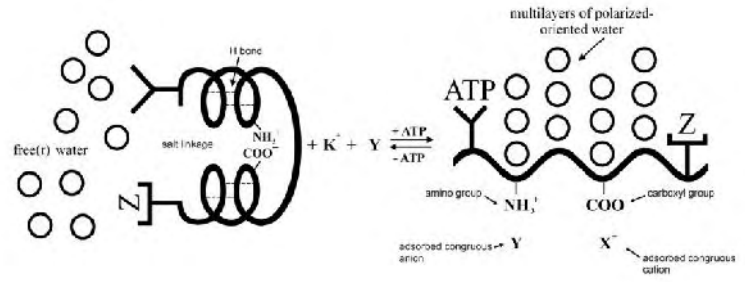
The action potential

According to the theory widely taught in textbooks, the action potential involves the sequential opening of specific sodium gates and potassium gates. Driven by an alleged sodium potential, Na^+ from the external medium rush into

the cell, creating the overshoot. Subsequent opening of the potassium gates allows K^+ to leave the cell creating the *after potentials*.

Like the long-disproved membrane potential, this widely taught action potential model is also untenable. First, the assumption of the existence of standing "Na^+ potential" is unrealistic because the resting cell membrane is highly permeable to Na^+. And if such a "Na^+ potential" existed, it would promptly be discharged [33p225]. Similarly, it has been established that the alleged sodium gates also permit the passage of K^+ [10p303p310;33p226]. That means when you open the sodium gate, not only would external sodium ion rushing into the cell interior, intracellular K^+ would at the same time rushing outward also, therefore annulling the potential change due to Na^+. But above all, the disproof of the membrane (pump) hypothesis makes it superfluous to consider these details. They are presented to demonstrate that local issues also disagree with the widely taught models.

The AI Hypothesis of the action potential stands on the solid foundation of the model of the living cell in general and the resting potential in specific as described earlier. There is another outstanding feature of the muscle, nerve and other excitable cell surface involved: Unlike the cell interior, these fixed anions are not accompanied by an equal number of fixed cations as in the nano-protoplasmic unit shown in Figure 2. Following the Faraday Cage Effect, their surface layer is primarily anionic. Another distinguishing feature of the excitable cell surface is the controlling influence of the (auxillary) EWC, Ca^{++} [64]. Local electrical perturbation created by the advancing front end of a coming action potential or the depolarization of end-plate potential [5pp358to380] causes the detachment of the Ca^{++} (Figure 13).



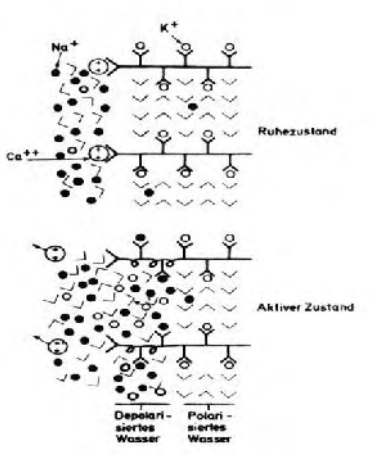


Figure 13

The ensuing action potential is an expression of the surface macroscopic protoplasm made of vast number of nano-protoplasmic units like that

shown in Figure 2 from right to light. The main event is the description of the adsorbed-oriented multilayer of cell surface water. This liberation of water molecules instantly creates a transient sodium potential and with it, Na^+ from the plasma or Ringer's solution rushes into the cell producing the "overshoot". The K^+ liberated during the auto-cooperative shift as well as the K^+ displaced by the inflowing Na^+ give rise to the action potential.

A critical support of the AI model of the action potential was provided by the work of Villegas *et al* [65] and shown in Table 2. The data obtained showed that repeated stimulation of giant squid axons increase the permeability of the squid axon surface to erythritol, mannitol and sucrose, which are all found only in the cell water, none adsorbed. Their increased entry during the passage of the action potentials is thus the likely consequence of the transient depolarization and liberation of the nano-protoplasmic water. In farther support of this belief, the increase of these non-electrolytes uptake will not take place in the absence of Na^+ in the bathing solution.

	Permeability in 10^{-5} cm/sec[a]			
Molecule	Resting axon (axon a)	Stimulated at 25/sec (axon b)	Net increase 25 stimulations/sec (paired data) (b - a)	Calculated permeability during activity[a]
Erythritol	3.6 ± 0.4	6.1 ± 1.0	2.5 ± 0.8	110
Mannitol	2.3 ± 0.4	4.0 ± 0.5	1.7 ± 0.3	75
Sucrose	0.9 ± 0.1	1.8 ± 0.3	0.9 ± 0.3	40

Table 2

Cell swelling and shrinkage

Abbé Nollet was a science teacher of King Louis XV of France. An opponent of Benjamin Franklin's "One fluid theory of electricity", Nollet was also the first recorded investigator of the *osmotic phenomena* generated by a *semi-permeable membrane* [66]. To begin, Nollet filled a bottle with alcohol. He then covered the mouth of the bottle with a flattened piece of pig's bladder and tied it down firmly with a piece of string before immersing the bottle in a tub of water. Hours later, he found that the bladder membrane bulged outward, indicating more fluid had gone into the bottle than out of the bottle. On the other hand, if he filled the bottle with water and immersed the bottle in alcohol, the membrane would bulge inward. Nollet concluded that the pig bladder membrane was more permeable to water than to alcohol. After this pioneering work, nothing much happened on this frontier for a long time.

Then in the middle of the 19th century, a Berlin tradesman, Moritz Traube enlivened this field by inventing the first man-made semi-permeable membrane

[67]. He did that by filling the open end of a glass tube with a solution of potassium ferrocyanide. He then dipped the filled end of the tube into a solution of copper sulfate. A reddish brown precipitate formed at the interface in the shape of a thin membrane of a finite thickness, because once formed the membrane is impermeable to both copper and ferrocyanide ions. To explain this phenomenon, Traube introduced what he called "Atomic Sieve Theory". That is, the membrane has pores but they are so small that only water molecules can pass through but not the larger copper and ferrocyande ions. Unfortunately this artificial membrane was too fragile to handle and to conduct experimental studies on. At this juncture, Wilhelm Pfeffer, a chemist with strong interest in botany, came to the rescue [68;69].

Pfeffer made the copper ferrocyanide barrier to form within the interstices of an unglazed porcelain pot by placing one of the two solutions in the pot and by immersing the pot in the other solution. Now, the artificial semipermeable membrane has strong mechanical support and can be handled safely.

Pfeffer introduced a concentrated sucrose solution inside the pot and connected the open upper end of the pot to a manometer. When he placed the filled pot in a bucket of pure water, that water began to seep through slowly into the pot. However, if a positive pressure of the right magnitude is applied to the inside of the pot, it could completely stop the water flow. That pressure was equal in magnitude to the *osmotic pressure* (π) of the concentrated sucrose solution within the pot. Pfeffer's highly accurate measurements demonstrated that the osmotic pressure, π, is directly related to the concentration of sucrose inside the pot and inversely related to the absolute temperature.

When the Dutch investigator, Hugo deVries [70], heard about these outstanding achievements, he was all excited and passed the information to his young Dutch fellow-scientist, J. H. van't Hoff [71;72;73]. It did not take too long for van't Hoff to reach the conclusion that the osmotic pressure can be quantitatively described by the following equation:

$$\pi V = R' T, \qquad (5)$$

where V represents the volume of the solution containing one mole of sucrose. R' is a constant. From the data provided by Pfeffer, van't Hoff showed that this constant is very close to the gas constant, 1.987 cal/degree.

The success of the Pfeffer's model stimulated intensive effort further to improve the instrument. Thus, in the hand of Morse [74], an osmotic pressure created by a sucrose solution he studied lasted 60 days [74p66]. In a parallel study by de Vries himself, the protoplast of the root hair of red beets stayed shrunken in a concentrated NaCl solution for as long as 7 days [75]. Understandably, these landmark records were once highly treasured and taken as convincing evidence that van't Hoff's semipermeability indeed demonstrates the existence of two kinds of substances. Some can go through the semipermeable membrane; others cannot go through the semipermeable membrane. When they cannot go through, they stay so not just for now, or for a few days, weeks, months or even years but forever.

In the new perspective provided by the association-induction hypothesis, these macroscopic notions ought to be replaced by their corresponding realistic microscopic models. For example, as far back as 1909, Bigelow and Bartell [76] had already shown that unglazed porcelain pots without copper ferrocyanide or other additives also produce an osmotic pressure and yet the pores could measure 0.37 micron (3700 Å) in diameter and thus two orders of magnitude wider than the diameter of a "impermeant" sucrose molecule (9 Å). In term of the microscopic model of the AI Hypothesis, this is a different kind of phenomena from the macroscopic membrane permeability problem discussed above. These gigantic pores were not empty cavities but space filled with polarized-oriented multilayers of water molecules. This dynamically structured water offers strong resistance to the passage of large molecules like sucrose---as we have clearly demonstrated in the case of inverted frog skin in the earlier section on Permeability. We could quantitatively measure the extremely low permeability because we had the powerful tool of radioactive tracer technology. But even molecules many times bigger than sucrose is not impermeable to the best of Pfeffer's modification of Traube's "semipermeable" membrane. In general principle, at above absolute zero temperature, the impermeable will all permeate through sooner or later.

In contrast, we have more than just speculation to explain de Vries's 7 day long maintained shrunken protoplast in concentrated NaCl solution of the red beet root cell. Nasonov & Aizenberg [77] and by Kamnev [78] showed how in the 1930's.

Curve A in Figure 14 shows the time course of shrinkage of isolated frog muscles in a Ringer solution

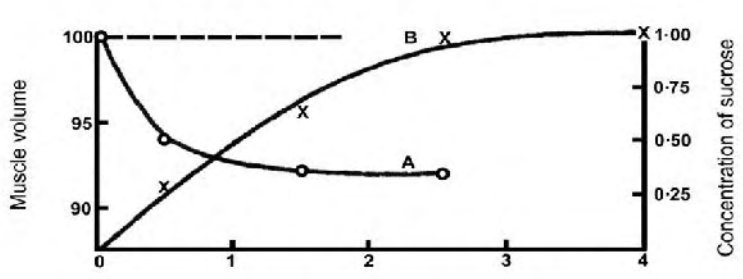


Figure 14

containing 4% sucrose. However, the cell membrane of frog muscle turned out to be , quite permeable to sucrose as indicated by its accumulation in the cells (curve B.)

In fact, to explain swelling as well as shrinkage in living cells in various permeant solutes like sucrose, the AI Hypothesis offered a new theory. Its testing and confirmation were conducted on many non-living "extrovert" models [10p107]like gelatin, oxygen-carrying linear polymers like polyethylene glycol (PEG) polyethylene oxide (PEO), acid or alkali denatured proteins---models that cause the polarization-orientation of water molecules but not in "introvert" models[10p107] including all so-called "native proteins" [79].

Figure 15 shows an example [79;10p256]. Enclosed in ¼ inch dialysis tubing with both ends tied, the model expanded or shrank in volume in a lasting manner when incubated in dilute and concentrated solutions of sodium citrate, to which the dialysis tubing is fully permeable.

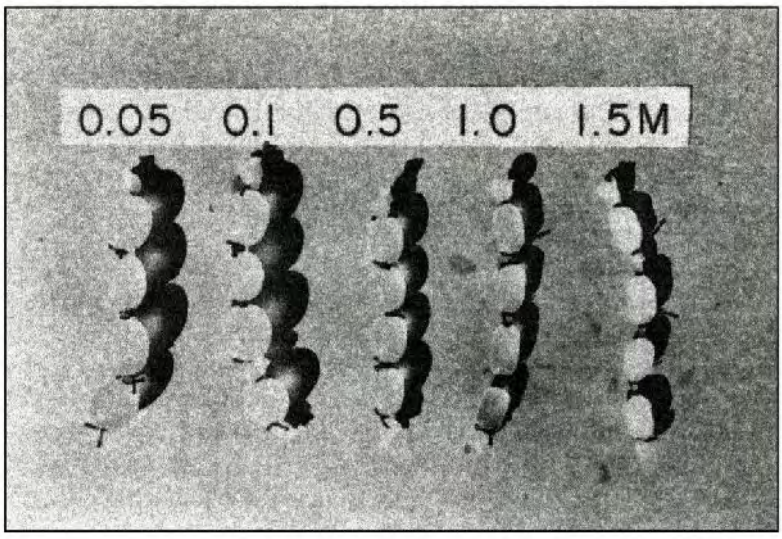


Figure 15

At about the same time the Russian scientists did their above-quoted study on muscle shrinkage, the English protein chemist, Dorothy Jordan-Lloyd of the Cambridge University measured the degree of swelling of living frog muscle as a function of pH [80;5p248]. Two peaks of extensive swelling were seen. The rising limbs of each peak have a midpoint equal to the pK's of major fixed cations and anions respectively. As a part of the AI hypothesis, I suggested a simple explanation: In normal frog muscle cells, salt linkages formed between fixed cations and anions help to keep the steady volume of the muscle cells. When the pH of the bathing medium approached the pK's of the fixed anions and fixed cations, the salt-linkages began to dissociate, thereby exposing the polypeptide chains, on which more water becomes adsorbed and swelling occurs as a result.

But that was how far I could go in 1962. With the introduction of the subsidiary polarized (oriented) multilayer theory of cell and cell model water in 1965, a new concept emerged: That is, if not restrained by salt linkages, the polarized oriented multi-layers of water molecules would grow much deeper and create more extensive swelling. But H^+ and OH^- are not the only external mono-valent ions that at high enough concentrations can cause swelling in frog muscle. Some familiar alkali-metal ions can also do the same.

It is well known that in an isotonic or 120 mM NaCl solution , frog muscle cells can maintain their normal volume indefinitely. Yet von Korosy [81] and Martin Fischer [82] had repeatedly demonstrated extensive swelling of isolated frog muscle in an isotonic KCl solution. In trying to explain this phenomenon, my associates and I pointed out that the osmotic or vapor pressures of these salt solutions at the same molarity are virtually identical. Nor had the frog muscle's unusual sensitivity to KCl anything to do with the intact cell membrane barrier. For muscle fibers cut into 2 mm and 4 mm open-ended segments that do not regenerate new cell membranes continue to respond to isotonic KCl solution by extensive swelling. It was then pointed out that in healthy animals, a normal amount of the EWC, ATP maintains the nano-protoplasm in its resting living state. In this state, the β-, and γ-carboxyl groups are maintained at their respective low c-value state, at which K^+ is the overwhelmingly preferred over Na^+. As a result, the muscle cells remain indifferent to the impact of isotonic NaCl in their blood and tissue fluids but swell promptly on exposure to isotonic KCl.

Isotonic KCl is not something you find in your immediate surroundings. Isotonic NaCl, on the other hand, is part of the milieu that keeps all your cells alive. It is the major constituent of your body fluids or blood plasma. But it is harmless under normal conditions.

However, every child can probably tell you how a fall or some other mishap could cause a part of your body to undergo painful swelling. Physicians and pathologists can also describe how a major auto accident, say, can cause a concussion and life-threatening brain swelling. Once more the AI Hypothesis sees a theoretical cause for the injury-induced tissue swelling of one sort or another.

The critical change is that injury destroys or interrupts the local energy metabolism to replace the ATP lost through injury. Being a most powerful EWC, the failure of its replacement means that the c-value of the
β-, and γ-carboxyl groups will become higher sharply in consequence. Figure 3 cited on page 9 shows that such a sharp rise of c-value, the normal strong preference of the β-, and γ-carboxyl groups for K^+ gave way to strong preference for Na^+. Suddenly, the normally harmless, indeed vitally-needed

near isotonic level of Na^+ becomes as deadly as isotonic KCl to normal body tissues. The corresponding breakup of shape and size-maintaining salt linkages by the Na^+ (and Cl^- ions) caused tissue swelling.

To put this theory to an experimental testing, Ling and Kwon kept isolated mouse kidneys, brain and other organs in a cold room maintained at 4°C in a oxygen-poor modified mammalian Ringer's solution [83]. Each solution contained a mixtures of salt and sucrose so that anyone mixture is isotonic (see abscissa of Figure 16.) After some time, the tissues were weighed and their water contents determined. The results show that the final weight over initial weight ratio ($W_{final}/W_{initial}$) of the kidney tissue rose sharply in NaCl and LiCl solutions of increasing concentration. To produce maximum swelling, both the specific cation Na^+ (or Li^+) and Cl anion are essential. In solutions containing only sucrose and $MgSO_4$, no swelling occurred at all.

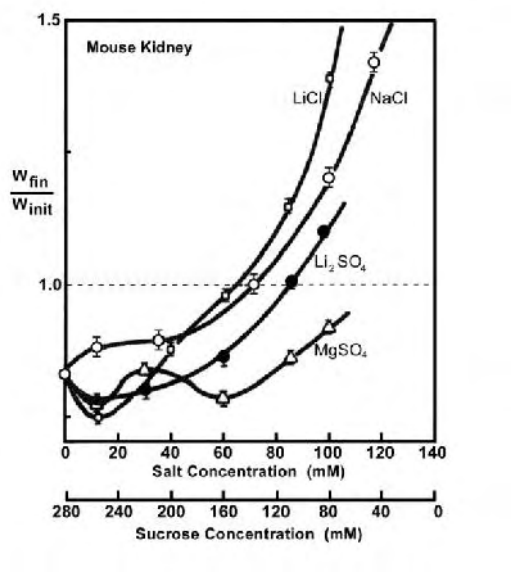


Figure 16

Figure 17 plots the concentration of ATP determined in isolated brain tissue after different length of time in the cold and anoxia (time indicated in number of hour near each data point in a similar cold and anoxic isotonic NaCl.) The data demonstrated increasing swelling with decreasing ATP contents. The maximum swelling and hence highest water content was found in brain tissue containing the least amount of ATP after the longest period of cold and anoxic exposure.

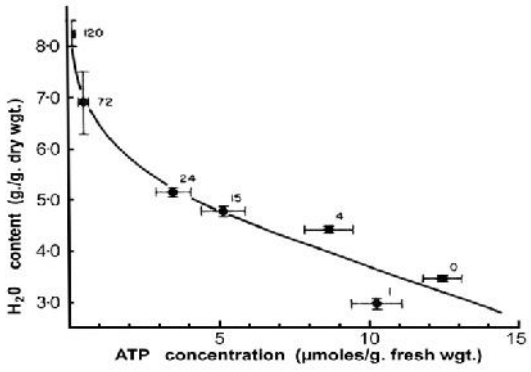


Figure 17

Together, the data given in the last two figures confirm the theoretical interpretation of the main mechanism of injury-induced tissue swelling: ATP depletion raised the c-value of the β-, and γ-carboxyl groups and as a result

Na^+ became a strongly preferred cation. Since the tissue fluid and blood plasma all contain a high concentration of NaCl, extensive tissue swelling is the consequence. On this note all my past presentations of this story ended. I did not notice then that there is a hidden question that needs to be answered. And now the hidden question will be revealed and answered.

To do so, requires the invocation of the "*Principle of Additivity*", which I introduced in my first book published in 1962 [5p95], including experimental evidence supporting the Principle then available [5pp176to179]. Best of all, the very recently published work of Ling and Ochsenfeld [12] provided additional clear-cut confirmation. In the shortest way of saying it, everything adsorbed on the protein of a nano-protoplasm unit exercises an influence on its electronic profile, though the polarity and magnitude varies from one adsorbent to another.

As the reader already knows, the electron density of each polypeptide carbonyl oxygen atom is expressed in terms of its c-value analogue. For partners, the carbonyl group has two alternative choices. On one hand, at high c-value analogue, it prefers other peptide imino groups to form α-helical H bonds. On the other hand, at low c-value analogue, the carbonyl groups prefer to polarize-orient multilayers of water molecules. With the fall of ATP concentration, the c-value of β-, and γ-carboxyl groups all rise higher, causing the displacement of the fixed cations engaged in form- and size maintaining salt linkages by Na^+. Uptake of more water and cell swelling follow. The hidden question now raised is: Would this electron-donating activity due to the fall of ATP level also cause an enhancement of the high c-value analogues of the carbonyl oxygen atoms and thus strengthen these form- and size-maintaining salt linkages? The answer is no--- due to a strategic exercise of the "Principle of Additivity".

This Principle tells us that every single positively charged Na^+ adsorbed on a β-, or γ-carboxyl group is itself an electron-withdrawing agent. Together, the electron-*withdrawing* influence of the multitude of adsorbed Na^+ combine to reverse the initial electron-*donating* action caused by the lost ATP to produce a net electron *withdrawing* impact on all the form- and size-maintaining carbonyl groups. At the low c-value analogue, all the backbone carbonyl oxygen atoms shift to binding multiple layers of water molecules and swelling follows in consequence

There are two mutually dependent advantages that make this transition inevitable: numerical superiority of the number of *effectors* and much closer proximity to the *target* groups.

From the work on cell swelling that Ling and Peterson published in 1966, we discovered that alkali-chloride at high concentration could liberate between 100 to 200 mmoles of β-, and γ-carboxyl groups per kilogram of fresh muscle

cells from the volume-and-shape maintaining salt linkages. In comparison, each kilogram of fresh muscle contains only 5 mmoles of ATP. This numerical superiority of the *effector* Na^+ offers the first overwhelming advantage for the electron withdrawing effect.

The second overwhelming advantage is proximity to the *target* groups. Adsorbed on a high c-value sites, the Na^+ is primarily in what is called Configuration 0 [5fig4p61]. As such, the Na^+ is fully dehydrated and thus in its small naked form. Furthermore, the β-, and γ-carboxyl groups are carried on their respective short side chains. Together, the positive charge at the center of each adsorbed sodium ion is very close physically to the local peptide carbonyl groups, In contrast, there is only 5 mM of cardinal sites for ATP. Their electron donating influence is on the average far away from the backbone carbonyl oxygen atoms under their influence. The overall result is that the adsorbed Na^+ strongly lower their c-value analogue, cause the dissociation of the form- and size-maintaining salt linkages and bring about extensive water polarization and orientation in multi-layers. And injury induced tissue-swelling follows as we know well from experience.

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