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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

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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 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 Only in the USM, the ATP has been replaced by an artificial but theoretically authentic EWC, H^+ (given as HCl).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 *nanoprotoplasm*, is the ultimate physical basis of life. (See Post Script on page 111.)

IN 1834 Felix Dujardin introduced what was eventually known as protoplasm[1]. Five years later, Theodor Schwann (and Mathias Schleiden) announced the Cell Theory[2,3]. Less well known is the fact that Schwann also introduced the essence of the membrane pump theory as a part of his Cell Theory. That is, cells are membrane-enclosed cavities filled with clear liquid water[2a], and that the cell membrane has *metabolische Kraft* (metabolic power) that controls the chemical makeup of the fluids inside and outside the cell[2b].

In contrast, Max Schultze pronounced in 1861 that a living cell is "a membrane-less lump of protoplasm containing a nucleus"[4]. Continuing this line of thinking, Thomas Huxley came to the conclusion that protoplasm is the physical basis of life[5] and communicated this idea to a lay audience in an Edinburgh church. Huxley's speech was breathtaking from start to finish; notwithstanding, it had a disconcerting flaw. Since protoplasm was then characterized as a semi-fluid, sticky and transparent substance, it did not match the more rigid and darker nucleus. If the nucleus is not a part of the physical basis of life, what is it? Then again, not all living cells possess a nucleus.

The mature mammalian red blood cell, for example, has no nucleus; nor could the best microscope available then reveal the presence of an enclosing cell membrane. Therefore, the very existence of mature mammalian red blood cells suggested that, at their simplest, living cells are lumps of protoplasm without a nucleus or cell membrane.

In 1889, Emil Abderhalden discovered something crucial that challenged the view of membrane-less red blood cells[6]. Abderlalden found no sodium ion (Na^+) inside the red blood cells even though the blood plasma, in which the red blood cells spend their lives, contain a great deal of Na^+. The question arose, if the red blood cell has no enclosing membrane, what would prevent Na^+ from becoming equally distributed inside and outside the cell? In years to come, further investigations revealed that there is some Na^+ in red blood cells. Nevertheless, a strongly asymmetrical distribution of Na^+ exists not only in red blood cells but in virtually all types of living cells carefully examined as exemplified by the human red blood cell, the frog muscle and the squid axons (Table I.)

TABLE I. The water and Na^+ concentrations in three of the most extensively studied types of living cells and the Na^+ concentrations in their respective blood plasma.

	H_2O (%)	$[Na]_{in}$ (mM)	$[Na]_{ex}$ (mM)	q_{NaCl}	ref.
nerve axon (squid)	86.5	50	440	0.114	7
muscle cell (frog)	77.4	16.9	104	0.163	8, 9
mature red blood cells (human)	65	18	135	0.133	10

Doubt about the existence of a cell membrane was laid to rest not long after the invention in 1931 of the electron microscope by Knoll and Ruska[11]. Its much higher magnifying power enabled biologists to establish that the red blood cell[12] — like other cell types studied[13] — does indeed possess a (vanishingly thin) cell membrane. In the decades following, the membrane pump theory rose to the peak of its popularity. Meanwhile, interest in the protoplasm concept steadily declined.

In an article entitled *Meaninglessness of the Word Protoplasm*[14], microbiologist Hardin showed that between 1926 and 1951 citation in the *Biological Abstract* of the word, protoplasm, had plummeted 77% (p.118.) In recent years, Encyclopedia Britannica Online has repeated Hardin's message. "As the cell has become fractionated into its component parts, protoplasm, as a term no longer has meaning."[15] In the US at least, biology textbooks at both the high school and college level teach the membrane pump theory exclusively; they rarely if at all mention the word protoplasm[9, 9a, 16]. However, a scattering of cell physiologists worldwide believe that the rejection of protoplasm may be a painful but passing episode in history.

Ling, for example, suggested that the real cause of the dwindling popularity of the protoplasm concept could be that the relevant parts of physics, chemistry and biology needed to give a more cogent definition of protoplasm and move the concept forward were still things of the future[16]. With time the relevant branches of knowledge attained the needed maturity one by one.

Arriving on the scene at just the right time, Ling was able to put together in 1962 an elementary unifying theory of the living phenomena in a volume entitled "A Physical Theory of the Living State: the Association-Induction Hypothesis"[17–22].

The association-induction (AI) hypothesis grew up in the light of critical knowledge denied earlier investigators. That includes the knowledge on protein structure and the new branch of physics called statistical mechanics. From a historic perspective, however, the AI hypothesis is without question the heir to what Dujardin, von Mohl, Schultze, Huxley and others introduced in mid-19th century. Another 45 years were to elapse before a new name, nano-protoplasm, was coined[23] to replace what was once referred to as a *biological fixed charge system*[18a] or *an elementary living machine*[17a].

Protoplasm re-defined

The long-range goal of the AI Hypothesis is to interpret all macroscopic cell physiological manifestations in terms of the properties and activities of microscopic molecules, atoms, ions, and electrons. Thus, in the latest version of the AI Hypothesis, what Thomas Huxley called the physical basis of life is a specific but highly prominent example of macroscopic protoplasm[23]. Other varieties of macroscopic protoplasm make up respectively the cell nuclei, the cell membrane and other sub-cellular structures. Despite their great diversity in form and function, all macroscopic protoplasms have one thing in common. They all comprise a vast number of similar microscopic units called nano-protoplasm[23]. Accordingly, nano-protoplasm — rather than a specific kind of prominent macroscopic protoplasm — is the smallest unit of life and hence its ultimate physical basis[23].

In introducing a more detailed description of a typical nano-protoplasm, we start out with a single mature human (or mammalian) red blood cell — to be referred to henceforth as a rbc. The electron-microscopic cross-section of two such rbc's is shown in Figure 1. Note that they have no nucleus, nor sub-cellular organelles of any other kind. Consequently, the inside of a rbc is essentially homogeneous. Furthermore, cytologists of the past had demonstrated that a rbc could be cut into tiny pieces without spilling its intracellular protein, hemoglobin[24]. Armed with these relevant insights and an imaginary knife, we cut a rbc into smaller and smaller pieces until each piece contains just one single hemoglobin molecule — and more as indicated next.

Water makes up 64% of the rbc's total weight. Of the 36% solid, *97%* belongs to a *single* protein, hemoglobin[10]. Thus, water and hemoglobin together make up more than 98% of the total weight of mature mammalian red blood cells. Thirty percent (30%) of the remaining 1.1% solid is K^+. Even less belongs to organic substances including about 5 mM of {2,3-diphophoglycerate (2,3-DPG)[25] *plus* adenosine triphosphate (ATP.)[26]} All told, what is still unaccounted for is not more than 1% of the total weight of a rbc.

From all these facts and numbers, one can estimate what is in each of the smallest microscopic lumps of rbc cytoplasm that we carved out from a rbc. Thus, in addition to one hemoglobin molecule, there are also some 7000 water molecules, 20 K^+ and one (2,3 DPG + ATP.) Together, they make up a *nano-protoplasmic (NP) unit.* And it can be represented by a formula: $(Hb)_1\ (H_2O)_{7000}\ (K^+)_{20}\ (ATP\ or\ 2,3\text{-}DPG)_1$. (For a general formula for all nano-protoplasms, see ref. 23, p.124.) The molar concentration of these NP units in a rbc is equal to that of hemoglobin at approximately 5 mM. Assumed spherical in shape, each rbc NP unit measures 6.8 nanometers in diameter[23].

The next section addresses the crucial question: How does a nano-protoplasm unit differ from a random mixture of the same chemical makeup and nano-metric dimensions?

Life and death at their most basic level

Unlike most objects in the dead world, nano-protoplasm can exist, in two alternative discrete states under the same atmospheric pressure and at the same temperature. An illustration of the "core structure" of a nano-protoplasm unit in its (lasting) *resting living state* is shown in the right-hand side picture of Figure 2. Another illustration of the core structure of either a nano-protoplasm unit in its (transient and reversible) *active living state* or one that is in its (irreversible) *dead state* is shown on the left of Figure 2[18a,20a,21d,22a]. In the resting living state, all the components of the core structure are directly or indirectly in contact with one another spatially (association) and electronically (induction.) In the active or dead state, the two major components, water and K^+ are set free mostly if not

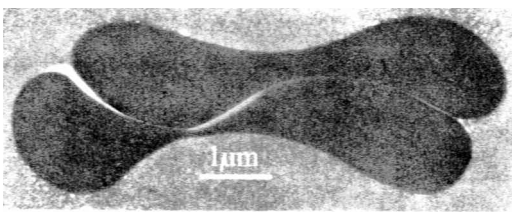

FIGURE 1. An electron micrograph of the cross section of two mature human red blood cells (in blood plasma). Cryofixed, freeze-dried and imbedded in Lowicryl. (Gift of Dr. Ludwig Edelmann)

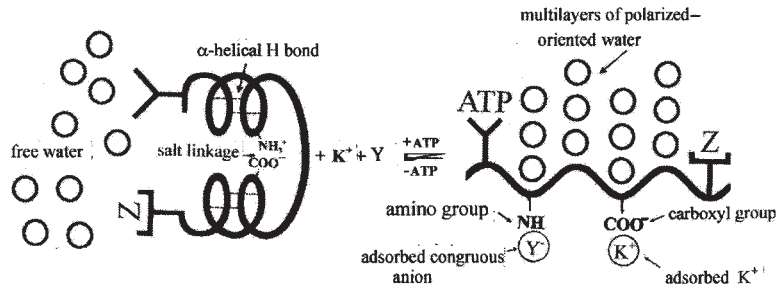

FIGURE 2. Diagrammatic illustration of the all-or-none transition between the folded and the fully-extended state of the core structure of a nano-protoplasmic unit.

totally[17c, 18e, 21g, 23c]. Thus, *it is the pervasive spatial and electronic connectedness that sets apart nano-protoplasm in its resting living state from a random mixture of similar chemical composition and miniscule dimension.*

While existence in the resting living state defines *living*, momentary reversible transitions between the resting and active living, state underlies *life activities.* As a rule, transitions in life activities are all-or-none; or, in the language of the AI Hypothesis, *auto-cooperative*[18b,21a,23e,27]. Center stage in these transitions is the auto-cooperative transition of the protein component of the nano-protoplasm between the folded *α-helical conformation* and the *fully extended conformation,* as diagrammatically illustrated in Figure 2.

Another player in the maintenance of two discrete living states and hence the auto-cooperative transition between them is the *salt-linkage*[28,29a]. Salt linkages are formed one on one between an equal number of fixed cations like the *ε-amino and guanidyl groups* and fixed anions like the *β-, and γ-carboxyl groups.* (See Figure 2 left.)

Neither the α-helical structure, nor the salt linkages — nor the *self-assembling* microscopic NP units making up macroscopic protoplasm — are joined together permanently. Rather, the structural connections in living protoplasm are *dynamic* — like that of a flock of wild geese in their migratory flight.

Nano-protoplasms are similar and different. Three major sources of their diversity are: (i) the amino acid composition-and-sequence of the protein(s) in the NP unit as defined by their respective genes; (ii) prosthetic groups, enzyme sites etc. that the protein may or may not carry and (iii) the location of the nano-protoplasmic (NP) cationic unit in the cell.

On the other hand, what make different nano-protoplasms (and hence different macroscopic protoplasm) similar are the shared components of the "core structures" of nano-protoplasm. They include: (1) a lengthy and highly polarizable polypeptide chain, serving as the highway of information and energy transmission; (2) close to the information highway, an abundance of fixed anionic β-, and γ-carboxyl groups carried respectively on short side chains of aspartic and glutamic residues; (3) the even more abundant peptide iminocarbonyl groups (CONH) — right on the information highway, the polypeptide chain; (4) the fixed cationic groups, including e-amino, guanidyl and (cationic) imidazole groups, which roughly match in total number the total number of fixed anions; (5) alternative partners for adsorption on these ubiquitous functional groups including K^+ (or another cation) for the carboxyl groups[29] and water molecules for the backbone carbonyl

groups; (6) adenosine triphosphate (ATP), the controlling *principal cardinal adsorbent* and specific sites on the protein that adsorb ATP, as well as *auxiliary cardinal site(s)* that adsorb other controlling agent(s) denoted by the symbol, Z (See Figure 2 right.)

ATP as the principal electron-withdrawing cardinal adsorbent

As a generic name for drugs, hormones, ATP , Ca^{++} and other agents that exercise powerful influence on the nano-protoplasm at extremely low concentration, are what we call *cardinal adsorbents* [17b,21e,23a]. As a rule, they act as the on-and-off switch of the all-or-none transitions of the nano-protoplasm between two discrete states. To serve that function, they adsorb onto (and desorb from) *specific cardinal sites* on the nano-protoplasmic protein. Depending on the consequence of their respective electronic impact on the protein, each cardinal adsorbent belongs to one of three categories: *the electron-indifferent cardinal adsorbent* or EIC, the *electron-donating cardinal adsorbent* or EDC, and the *electron withdrawing cardinal adsorbent* or EWC.

The most pervasive and powerful EWC is ATP. In conjunction with other auxiliary adsorbents or agents, ATP plays a critical role in maintaining (reversibly) the nano-protoplasm in *its resting living state* through its adsorption on a specific cardinal site. As such, its action can reach over long distances. ATP is unusual in yet another way. It can be rapidly removed through the action of a specific enzyme called ATPase. It can also be rapidly replenished through the activities of other enzymes of one kind or another.

In the original AI Hypothesis, two types of long-distance operations of ATP and other agents were presented. They are respectively called the (static) *direct F-effect*[17d, 21j] — a combination of direct or D-effect, transmitted through space and inductive or I-effect transmitted through intervening atoms, — and the i*ndirect F-effect*[18c]. The direct F-effect is local and static in action; its name remains unchanged though often replaced by the predominant inductive or I-effect alone. In contrast, the dynamic and far-reaching indirect F-effect has been renamed *AI cascade mechanism* {where the letter A and I used here stand for the (close-contact) *association* and the electronic *induction* respectively}[23b,17e].

It is the AI cascade mechanism that provides the means for the (ideally) non-attenuating, one-on-many, from-here-to-there influence of the cardinal adsorbent. In its mode of operation, the AI cascade mechanism resembles a falling domino chain — in that the energy that topples the first domino is the same as the one that topples the last domino, regardless how far apart they are. (See also ref. 17k, 21i.) (Regrettably, space limitation does not permit even a brief sketch of a more detailed account of the AI cascade mechanism. The interested reader may want to consult earlier presentations[17e] or a recent downloadable pdf version[23b].)

By the same token, it is the AI cascade mechanism that makes a *gang* of cooperatively linked sites behave as if it were a single site[17e]. It is this AI cascade mechanism that produces the across-the-board *uniform* rise or fall of the *c-values* of the β-, and γ-carboxyl groups and of the *c-value analogue* of backbone carbonyl groups along the lengths of the polypeptide chain. What is a c-value? What is a c-value analogue? What roles do their perturbations play in the phenomena of living and life activities? The answers follow next.

The effective electron density of a singly-charged carboxyl oxygen atom of, say a β-, and γ-carboxyl group, is expressed in Ångstrom units and called *the c-value*[18d, 21b,17i]. The-

oretical computations made in the late 1950's showed that variation of the c-value could decide adsorption preferences on these negatively charged functional groups[17g,18f]. Thus, according to what we now call the **c-scheme**, at low c-value, K^+ is preferred over Na^+; at high c-value, the reverse is the case. As a pervasive EWC, ATP adsorption on its special cardinal site — in conjunction with auxiliary agents of various kinds — keeps all the nano-protoplasmic β-, and γ-carboxyl groups at a low c-value. That is why K^+, and not Na^+, is adsorbed on these anionic sites and accumulates in the nano-protoplasm maintained at its resting living state as shown in Figure 2 right[29].

However, at both low and high c-value, H^+ is preferred over most other mono-valent cations often by a high margin[17g,18f]. Keep this in mind, as it will appear again in new experimental studies to be presented on later pages.

Similarly, the *c-value analogue* of the backbone peptide carbonyl group represents the effective electron density of the dipolar carbonyl group (CO)[18d, 21c], and as such it has a parallel capacity like that of the c-value. Based on the comprehensive and mutually consistent work of three groups of scientists on the "α-helical potential" of amino acid residues in proteins (Chou & Fasman, Tanaka & Scheraga and Garniere *et al*), Ling introduced in 1980 the theoretical concept later named the **c-analogue scheme**[30,17j,21k]. According to this scheme, at high c-value analogue, the peptide carbonyl group prefers to engage in α-helical H-bonds. On the other hand, at low c-value analogue, the peptide group prefers to assume the fully extended conformation. As such, they adsorb, polarize and orient multilayers of water molecules according to the *polarized-oriented multilayer or POM theory* of cell water[31,32].

The POM theory, short for the "polarized-oriented multilayer theory" of cell water and model systems — for some time also called the PM theory — was added to the *AI Hypothesis proper* in 1965 to explain the exclusion from the living cells[31](Table I) of (hydrated) Na^+ — i.e., Na^+ plus its more or less permanently attached coat of water molecules in an aqueous environment. As illustrated in the right hand side picture of Figure 2, all the water molecules in the nano-protoplasm in its resting living state adopt the dynamic structure of polarized-oriented multilayers. In this polarized-oriented water, the average water-to-water interaction energy is stronger than in normal liquid water—with important consequences[33,34,35]. Thus, it would take more energy to excavate a hole in the polarized-oriented water to accommodate a large hydrated Na^+ than the energy recovered in filling up the hole left behind in the normal liquid water from where this Na^+ came.

Accordingly, to move such a Na^+ (and its companion Cl^-) from the normal water outside a living cell into the polarized-oriented water inside a living cell entails extra energy expenditure. The Boltzmann distribution law then dictates that, at equilibrium, less Na^+ (and its companion anion, Cl^-) would be found inside the cell water than in the external-bathing medium[36]. This then constitutes the important *volume component* of the solute exclusion mechanism against large solutes like hydrated Na^+ (and Cl^-) but with less or no expulsive impact on smaller solutes like urea, for example, according to what has become known as the *size rule*[33,34,37,17f].

As mentioned above, ATP adsorption on the nano-protoplasmic protein exerts a pervasive and far-reaching electron-withdrawing effect. At the lowered c-value thus achieved, the β-, and γ-carboxyl groups favor K^+ over Na^+ adsorption *via* what is known as the ***c-scheme***. Similarly, the lowered c-value analogue of backbone carbonyl groups keeps the polypeptide chain in the fully extended state *via* the **c-analogue scheme**. Take away the

ATP and the system reverts in an all-or-none manner to the doubly folded state as shown in Figure 2 on the left, and in the process, liberates all or virtually all its adsorbed water and K^+ [17c, 21g, 23c, 38]. With that, the nano-protoplasm's ability to exclude Na^+ also disappears.

An *ultra-simple model* of the core structure of nano-protoplasm

The utter simplicity of the chemical makeup of the rbc's cytoplasmic NP unit offers an unusual opportunity. It makes it feasible to construct an *ultra-simple model* (USM) of the core structure of a NP unit from *pure chemicals exclusively.* On the vast collection of these models in say 0.4-ml of the mix in a dialysis sac, we can test the theory how its real-life counterpart works. If the ultra-simple model (USM) can indeed reproduce what the theory has predicted, we know that it is the pure chemicals we put together and nothing beyond them that did the job.

Philosophically, this new USM approach could offer the cell physiologist a way out of the inherent trouble of there being too many inseparable participants within the living cell being studied. Remember Occam's Razor, *One should not increase, beyond what is necessary, the number of entities required to explain anything.* For only when dealing with *all* the essential entities — and when they are only a few of them — can we keep our eyes on the *total* picture all the time and avoid being sidetracked into pursuing more and more (on less and less — ultimately to nowhere.)

However, given the advantage of reducing the number of entities involved, the full potential of a test with an ultra-simple model could be reached only when the physiological trait chosen for study is *critically important* and *it is shared by all or virtually all living cells***.**

To reach that goal, we believe that no other physiological manifestation could come close to the (reversible) *maintenance of Na^+ (as chloride) in the cell water at a level much lower than that in the surrounding medium* (Table I.)

The entire history of cell physiology[39] testifies to the veracity of this belief. To the best of our knowledge, all the major theories of the living cell were built around the critical phenomenon of Na^+ exclusion. They include Boyle and Conway's sieve version of the membrane theory[40], the pump version of the membrane theory once wrongly attributed to R.Dean[41], but truly due to Theodor Schwann as pointed out earlier, Troshin's sorption theory[42] and Ling's association-induction hypothesis[18].

With our strategy outlined, we proceed to test our theoretical concept of pervasively connected nano-protoplasm as the ultimate physical basis of life. First, we outline how we carried out the studies under Materials and Methods and attach it at the end of the Results and Discussion sections, which are presented in two parts labeled respectively R and D part 1 and R and D part 2. However, make sure to read the Materials and Methods section first.

Results and Discussion

R and D part 1

As described under Material and Methods, we incubated dialysis sacs (filled with initially a 40% hemoglobin solution) in solutions containing the same low concentration of NaCl

but different amounts of HCl. After equilibrium was reached, we determined the equilibrium distribution ratio of Na^+ or q_{NaCl} of the water inside each sac and plotted the result in Figure 3 as six bar graphs.

The first bar graph on the right came from ultra-simple models in which the final equilibrium pH of the bathing medium averaged 7.1. After correcting for a small amount of adsorbed Na^+ in the sacs, we obtained an *equilibrium distribution coefficient* of NaCl or q_{NaCl} of 1.0 within error of ± 0.06. This unity q_{NaCl} shows that at neutral pH, the water in this ultra-simple model does not exclude NaCl. As such, this finding agrees with the unity q-value demonstrated earlier for sucrose and other small and large non-electrolytes in similar neutral USM of hemoglobin systems[37]. The finding also agrees with the theoretical prediction mentioned earlier that in the absence of ATP or an alternative effective EWC, hemoglobin exists in the introverted doubly folded state (Figure 2 left.) And in that state, the hemoglobin exercises virtually no influence on the physical state of the bulk-phase water. (However, see ref. 43 and 44 for more on the subject.)

The five short bar graphs in Figure 3 tell an entirely different story. Here, the right amount of H^+ had been added and it has caused the q_{NaCl} to fall consistently to between 0.1 and 0.3. As such, it ***quantitatively*** matches the q_{NaCl} found in most if not all healthy resting living cells (Table I.)

Two conclusions can be drawn from this quantitatively exact matching. *First*, it contradicts the membrane pump theory for the maintenance of low Na^+ level in living cells. *Second*, it affirms the AI hypothesis that nano-protoplasm is the seat of basic physiological functions exemplified by Na^+ exclusion.

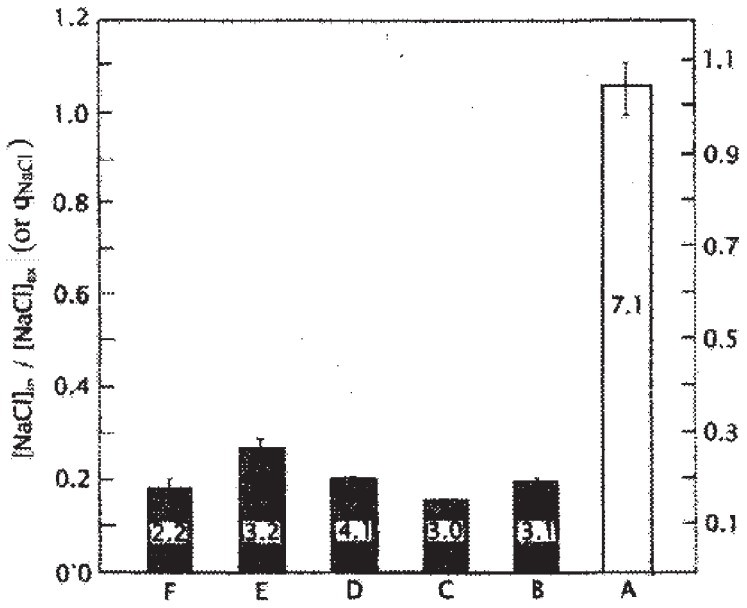

FIGURE 3. The *equilibrium distribution ratio* of NaCl, or q_{NaCl} of ultra-simple models of red blood cell cytoplasmic nano-protoplasm (A) not treated with HCl; (B, C, D, E, F) treated with appropriate amount of HCl. Numbers in bar graphs indicate equilibrium pH of the media bathing that particular set of dialysis sacs.

Thus, the data summarized in Figure 3 provides yet a fourth set of evidence against the membrane pump theory of Na^+ exclusion, — in addition to three other sets published earlier, which are: i) energy insufficiency[18e, 45, 16]; ii) intact membrane without cytoplasm does not work[46,47,48]; iii) cytoplasm without functional membrane (and pump) does work[49,17m].

The new data presented here suggests once more that the low Na^+ level in living cells does not come from an impermeable cell membrane barrier, nor from the ceaseless activity of (postulated) sodium pumps in the cell membrane. Indeed, the new evidence is so plain, that one doubts that any one with an open mind could miss what the data tell. *Not a trace of the cell membrane nor postulated pumps exists in the USM preparation that has quantitatively reproduced the 0.1–0.3 q_{NaCl} seen in most living cells*.

Of far greater significance, this concordance between the experimental and real life q_{NaCl} fulfills what we set out to do. Namely, proving that a mix of four pure chemicals — hemoglobin, water, NaCl and HCl — can indeed reproduce not only qualitatively but *quantitatively* one of the most fundamental physiological attributes shared by living cells. What is more, it can be turned on or off by the introduction or removal of the agent, HCl.

Having completed part 1 of our Result and Discussion, we begin R and D part 2 with a brief introduction.

R and D part 2

The addition of HCl to a mix of three pure chemicals and the conversion of normal liquid water in that mix into one that exhibits a 0.1 to 0.3 q_{NaCl} are by themselves totally unconnected events. The only logical connection between them comes from the AI Hypothesis. From a broad perspective, the quantitative confirmation of a predicted result implies that all the interweaving steps giving rise to the predicted results have already been confirmed. However, in dealing with a science as complex as cell physiology, a deeper look into the validity of each of the intervening (three) step sequence is a must. Indeed, to do that is the purpose of the second part of our study that has yielded R and D part 2.

In the **first step** of the three-step sequence, the right amount of the *premiere* EWC, H^+ given in the form of HCl binds onto appropriate sites on the hemoglobin in the ultra-simple model.

In the **second** step, this binding of the EWC, H^+, lowers the *c-value analogue* of local backbone carbonyl groups of the hemoglobin molecules — *via* the direct F-effect. The local c-analogue fall then sets in motion the complex third step**.**

In the **third step,** an auto-cooperative transition takes place producing in an all-or-none manner the following trio of interlocking changes: (a) converting contiguous NHCO groups from their original high c-analogue to low c-analogue — *via* the *AI cascade mechanism*; (b) converting sections of the hemoglobin chains from their original a-helical folded conformation to the fully extended conformation with the backbone NHCO groups exposed — *via* the *c-analogue scheme*; (c) converting a large number of free water molecules to the state of multi-layer polarization and orientation — on the exposed NHCO groups of the fully-extended hemoglobin chains — to assume the dynamic structure distinguished by a 0.1-0.3 q_{NaCl}.

Before going into our next section, we point out that there are already repeatedly confirmed evidence in support of step 2 and step 3c. Thus in regard to step 2, there are mutually supportive evidence that the step 2 inductive effect can be launched by the formation of new ionic or H-bonds as in the more familiar formation of new covalent

bonds[21m]. In addition, four sets of independent evidence exist demonstrating that the direct F-effect can effectively transmit through a length of the polypeptide chain plus segments of saturated hydrocarbon chains[17d, 21j]. In reference to step 3c, one may mention the following: Proteins which may for structural reasons as in gelatin (i.e., high contents of non-helical forming proline and hydroxyproline residues)[50] or in response to denaturants (e.g., NaOH, guanidine HCl, urea) exist in the fully-extended conformation[51]. In that case, they have been shown to adsorb at the physiological relative vapor pressure (0.9969) enough water molecules that match the living cells in amount[52] and in the extents of exclusion of sucrose, free amino acids and Na_2SO_4 — with the exception of NaCl[53].

Accordingly, the subject matter that most urgently calls for confirmation include: (i) identifying H^+ (of the HCl added) as the true causal agent that brings about the q_{NaCl} fall from unity to 0.1-0.3; (ii) determining the nature and number of the binding sites for H^+; (iii) establishing the role of the AI cascade mechanism in creating the low q_{NaCl} observed; and (iv) verifying the way an effective EWC actually transforms a protein like hemoglobin from its α-helical folded conformation to the fully extended conformation (that produces the low q_{NaCl} in the bulk-phase water the extended protein chain adsorbs, polarizes and orients.)

We end this introduction with a question, How did we find "just the right amount of HCl" — as pointed out earlier — to produce the five short bar graphs of Figure 3?

Just right amount of HCl

Once more, we repeat**.** We incubated dialysis sacs (containing initially a 40% hemoglobin solution) in solutions containing the same concentration of NaCl but different amounts of HCl. After equilibrium was reached, we determined the q_{NaCl} of the water inside each sac and plotted their averages against the final pH's of their respective bathing solutions in Figure 4.

The data demonstrate that as the amount of HCl added increased step by step, both the pH and the q_{NaCl} fell steadily to lower and lower values — until the pH reached a value of 2.2. From this point on, further addition of HCl continued to lower the pH but not the q_{NaCl}. Instead, the q_{NaCl} started to rise with further HCl addition. Seen as a whole, the curve has the shape of an asymmetrical U. As such, it comprises three distinct segments designated 1, 2 and 3 respectively, counting from the high pH end. A minimum of q_{NaCl} occurs at pH 2.2. The amount(s) of HCl needed to create this minimum in this, and in other companion sets of studies, are referred to as the "right amount of HCl" used to produce the five short bar graphs in Figure 3.

The specific set of data shown in Figure 4 was obtained with the Na-electrode after incubation of the sacs at around 9° C. Two additional full sets of data were obtained after incubation of sacs at 25° C with the radioactive tracer method and they produced similar asymmetrical U-shaped curves. However, the minimums occurred at pH closer to 3 than 2. Notwithstanding, the values of the q_{NaCl} measured at or near these pH's also fall between 0.1 and 0.3 and are among those shown as short bar graphs in Figure 3.

In what follows, we will pursue further what underlies the data presented in Figure 4 in two sections. We begin with a section on the "*falling limb"* (Segment 1 and 2) on the right of the asymmetrical U-shaped curve. After that, we will continue with a section on the *minimum* and the *rising limb* of the curve to the left of the minimum at pH 2.2 (Segment 3.)

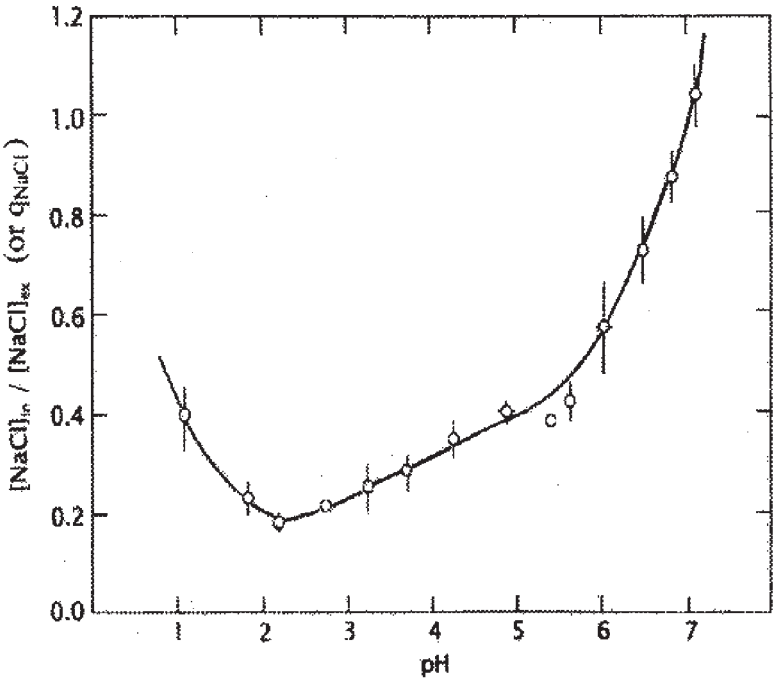

FIGURE 4. The *equilibrium distribution ratio* of NaCl, or q_{NaCl} of ultra-simple models of red blood cell cytoplasmic nano-protoplasm in dialysis sacs treated with different amounts of HCl. Data plotted against the final pH of the bathing solution.

What underlies the falling limb?

From the formula of rbc cytoplasmic NP unit given earlier, one can deduce that each *principal cardinal adsorbent* (ATP or 2,3-DPG) — and its supporting auxiliary agents — keeps 7000 water molecules in its dynamic structure that produces a q_{NaCl} of 0.13 (Table I.) Frog muscle contains 5 mM ATP[17c] but little or no 2,3-DPG. 78% of its total weight is water[8]. Accordingly, each ATP molecule (and its auxiliary helpers including creatine phosphate and K^+)[21h] controls more than 8000 water molecules. (For other evidence of the far reach of another cardinal adsorbent, see ref. 17n.)

Of course, our present study did not use ATP as the principal cardinal adsorbent. Instead, we chose an artificial but theoretically authentic EWC, H^+. As shown above, when enough H^+ had been added, it can also produce a q_{NaCl} to match those seen in living red blood cells or frog muscle under the domination of their natural principle EWC, ATP.

Thus, unlike ATP, which has just one (principal) cardinal site on each hemoglobin molecule, H^+ can bind onto a multitude of sites on a nano-protoplasmic protein molecule. Bovine hemoglobin, for example, carries three kinds of acidic functional groups that bind H^+ between pH 7.0 and 1.0. They comprise 34 imidazole groups (pK_a 6.0), 64 β-, and γ-carboxyl groups (pK_a 4.0) and 4 α-carboxyl groups (pK_a 2.0)[54]. Given their different pK_a values, one expects that as more and more HCl is added, H^+ would bind onto the imidazole groups before binding onto the β-, and γ-carboxyl groups. From the more or less

steady fall of q_{NaCl} between pH 7 and 2.2, one draws the tentative conclusion that the q_{NaCl} of the bulk phase water falls in a more or less unchanging direction each time another H^+ binds onto one of these three types of acidic groups. Put simply, the impacts produced on the q_{NaCl} by different (bound) H^+ add up *via* what is known as the *additivity principle* to be described below.

Experimentally, one can also roughly estimate how many water molecules each bound H^+ controls with the help of another parameter, $(1 - q_{NaCl})$. For a q_{NaCl} equal to, say 0.2, $(1 - q_{NaCl})$ would be 0.8. As a *working hypothesis* — but not so literally in the latest version of the definitive theory, so far published only in parts [33, 34, 35, 43] — a $(1- q_{NaCl})$ equal to 0.8 could be seen as indicating that 80% of the bulk-phase water has completely lost its natural solvency for Na(Cl), leaving completely unchanged the remaining 20% in its normal solvency for Na(Cl).

By taking also into account the equilibrium water content (not shown) and the $(1 - q_{NaCl})$ calculated, one can estimate the minimum number of water molecules made "non-NaCl-solvent" at different points along the q_{NaCl} vs. pH curve of Figure 4, counting the right-hand most point as point 1. Thus, at point 5, the number of water molecules made "non-NaCl-solvent" by each H^+ bound is 500. In contrast, the additional number of water molecules made "non-NaCl-solvent" by each additional H^+ bound between point 5 and point 11 averages only 66.

The large difference between these two numbers (500 and 66) suggests that each of these two averages cover a wide spread of numbers. Thus at pH above 6.5, the number of water molecules made "non-NaCl-solvent" by each H^+ bound could exceed substantially 500. And, at pH below 4.0, the number could fall substantially below 66. It is the lowest below 66-number that would set the limit of water molecules made non-NaCl solvent by each H^+ adsorption *via* the direct F-effect alone. Anything above that lowest number could only have come about *via* the AI cascade mechanism. All told, it is clear that most of the water polarized and oriented got there by way of the AI cascade mechanism.

In summary, one may say that the falling limb of the q_{NaCl} vs. pH plot in Figure 4 is a profile of the all-or-none transition from the active living (or dead) state back to the resting living state in "slow motion." Only in this slow motion version with the binding of a large number of H^+ to the same protein are details of the progress of the transition of the protein revealed that would be hard to detect when there is only a single cardinal site adsorbing a single cardinal adsorbent (ATP) — as is the case in the real-life nano-protoplasm. This is just another example of the bonuses from studying an ultra-simple model.

What lies behind the q_{NaCl} minimum and its secondary rise?

If H^+ alone determines c-value analogue of all the backbone carbonyl groups and the dynamic water structure, one would expect the q_{NaCl} to continue its decline with further HCl addition until a constant q_{NaCl} value is reached and then stays unchanged from there on with still more HCl addition. That the q_{NaCl} in fact exhibits a *minimum* followed by a rise suggests that some other player(s) might have entered the arena. What could that player be?

A moment of reflection provides an answer. That new player could only be the chloride ion (Cl^-) that was added systematically with H^+ in the form of HCl since nothing else was added.

As mentioned earlier, H^+ is very strongly bound to fixed anions[17g, 18f, 23f]. Accordingly, H^+ would have no trouble displacing and thus liberating the fixed (and some free) cation

originally bound to a fixed anion in the salt linkages they formed together (as illustrated in Figure 2 left.) In contrast, the Cl^- is by itself too weakly bound[55] to compete against and displace a fixed anion in a salt linkage. However, given the opening provided by the spearhead effect of H^+, it will now seize the opportunity to bind itself onto the liberated fixed cation. Put differently, Cl^- introduced as NaCl, for example, will not be able to displace the fixed anion of a salt linkage and becomes adsorbed on the liberated fixed cation as the study of Carr has shown[55]. Only the Cl^- introduced as HCl can bind onto the fixed cations.

(The experimentally established Cl^- binding further affirms the theoretical conclusion that for statistical mechanical reasons given, fixation of ionic sites on protein chains propels counter-ions to engage in close-contact association with fixed ions[56, 29]. This point known as the *principle of enhanced counter-ion association with site fixation* is made here in anticipation of the traditional aversion to the concept of ionic association among some influential protein chemists.)

Furthermore, there is another principle introduced in the AI Hypothesis that comes into play here. It is the *additivity principle*[18c] mentioned a few paragraphs above. As an example, we can say that the effective electron density of each backbone peptide carbonyl group expressed as its c-value analogue is determined not only by the intrinsic value of that group alone. Rather, it is that, *plus* the algebraic sum of the inductive effects of all other near and far linked groups and their respective adsorbents — according to the distance of separation between the inducing group and the target group and the target group's polarizability. Armed with this *additivity principle*, we examine next how adsorbed Cl^- could affect the q_{NaCl}.

Like its counterpart H^+, a Cl^- is also an uncomplicated single atom. However, unlike H^+, which carries a *positive* electric charge and acts as a *bona fide* EWC, a Cl^- carries a *negative* electric charge and acts as a *bona fide electron-donating* agent, or EDC. The adsorption of a Cl^- would therefore in theory produce a direct F-effect on the hemoglobin molecule opposite to that produced by H^+.

Now is the time to put this train of theoretical speculations and deductions to a test. To that end, we studied the (equilibrium) binding of both H^+ and Cl^- (added together as HCl) on a bovine hemoglobin solution, at a concentration of 19.2%. The results are shown in Figure 5. Note that before the H^+ binding curve (labeled A) reaches its maximum at pH 2.0, it too comprises two distinct segments — like the q_{NaCl} curve shown in Figure 4. However, there is also a difference. In Figure 4, Segment 1 has a higher slope than Segment 2; the opposite is the case of curve A in Figure 5. We will return to this subject shortly.

Since the midpoint of Segment 1 in Figure 5 is close to the pK_a of the imidazole groups (6.0), it confirms our earlier speculation that Segment 1 of Figure 4 originates from the binding of H^+ (alone) on the imidazole groups of the hemoglobin molecule. From the existing points in Figure 5, one can draw a curve. And from that curve, we then roughly extrapolate to a maximum of between 10 and 20 (averaging 15) moles of H^+-binding imidazole groups per mole of hemoglobin (or rbc NP unit) as the pH approaches zero. Subtracting this estimated 15 moles/ mole from the maximum of 92 moles/mole of the H^+-binding curve at a pH of about 2, we obtain a figure of 77 which is not too far from the total number of β-, and γ-carboxyl groups at 69 moles/ mole of hemoglobin[54]. This and a midpoint close to the pK_a of the β-, and γ-carboxyl groups (4.0) confirms our second

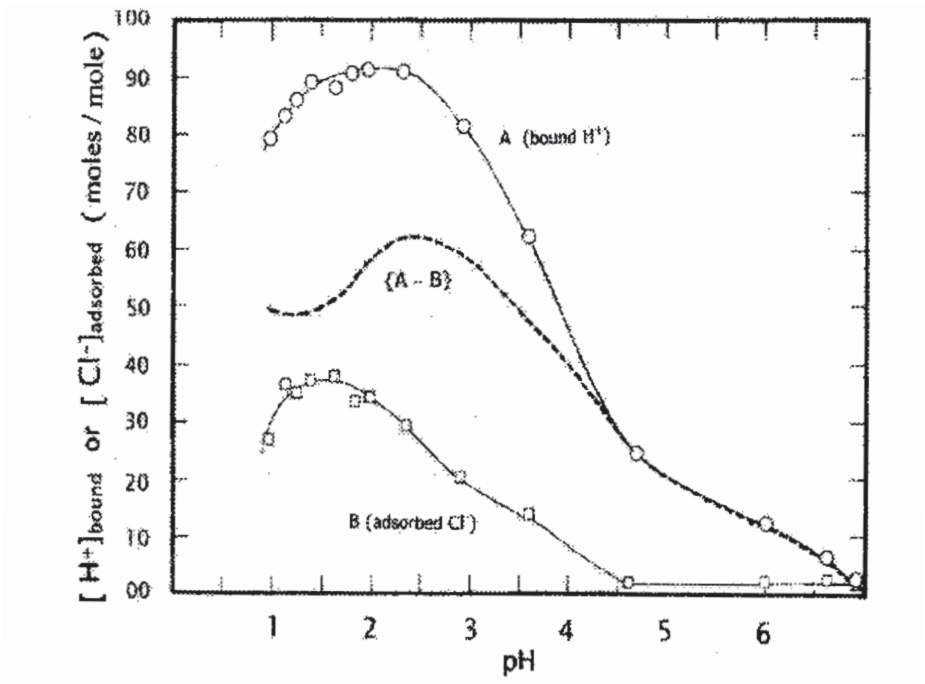

FIGURE 5. The binding of hydrogen ions (curve A) and adsorption of chloride ions (curve B) by 19.2% aqueous solution of bovine hemoglobin when different amounts of HCl were added to the bathing solutions. Curve labeled {A – B} is obtained by subtracting point by point Curve B from Curve A.

tentative assignment of Segment 2 in Figure 4 to originate mostly from the binding of H^+ on the β-, and γ-carboxyl groups.

The lower-most curve in Figure 5 is labeled B; it represents the Cl^- binding curve. Note that there is little binding of Cl^- from the HCl added until the pH has reached about 4.5. This shows that under the condition of the experiment, the Cl^- ion does not bind onto cationic imidazole groups at all. Instead, for the reason given above, they readily bind onto the (liberated) fixed cations when the pH falls below 4.5.

The absence of Cl^- binding at pH between pH 7 and 4 is important on two accounts. (1) It shows that we were right in attributing (without proof up to this moment) that the impact of HCl on the q_{NaCl} (as shown in Figure 3) to be caused by its H^+ component alone as an EWC. Now that we do have proof, it would be fitting to count this as an additional piece of supportive evidence that ATP, which acts like H^+, is also an EWC. (2) It explains why Segment 1 in Figure 4 has a higher slope than Segment 2. In Segment 1, H^+ acts alone in reducing q_{NaCl}, while in Segment 2, the *electron-withdrawing* influence of bound H^+ is counteracted and thus reduced by the simultaneous adsorption of the *electron-donating* Cl^-.

We turn next to what happens after the pH has fallen below 4.5. From there on down, Cl^- binding is on the rise. To better visualize its overall impact on q_{NaCl}, we began by making a simple assumption. That is, the electron withdrawing impact on q_{NaCl} created by the

binding of each H^+ is equal to, but opposite in direction to that produced by the binding of each Cl^-. (Space limitation forbids telling more about the detailed reasoning that led us to make this assumption.)

Based on this assumption, we subtracted point by point curve B from curve A and plotted the difference as the dashed line labeled {A – B.} Since we know in theory at least that H^+ binding lowers the q_{NaCl}, while Cl^- binding raises it, the theoretical prediction of the overall effect of H^+ and Cl^- binding together is revealed as the curve {A – B} *turned upside down*. And this upside down {A – B} curve also has the shape of an asymmetrical U with a minimum at pH between 2 and 3. In other words, the upside-down {A – B} curve in form replicates the observed q_{NaCl} vs. pH curve in Figure 4. A positive causal relation between the binding of both H^+ and Cl^- and q_{NaCl} is thus substantiated experimentally.

Additionally, we also begin to understand a little more about the fine structure of the asymmetric U-shaped curve of Figure 4. Thus, a minimum occurs at pH 2.2 because just before the pH has fallen to this value, the H^+ binding has already attained its full maximum while the Cl^- binding curve still has a way to go before reaching *its* maximum. Since H^+ binding lowers q_{NaCl} while Cl^- binding raises it, it is at this pH that q_{NaCl} sinks to its minimum.

As the pH falls still further below 2.2, q_{NaCl} begins to rise again (Segment 3), because the Cl^- binding continues to increase while the H^+ binding had ceased to increase and is beginning to decrease.

The parallelism between the inverted {A – B} curve in Figure 5 and the q_{NaCl} curve in Figure 4 affirms the 3 step scheme presented at the outset of R and D part 2. Regrettably, however, it does not tell us explicitly about the intermediate step 3b, i.e., the transformation of the protein from the folded α-helical conformation to the fully extended conformation. Indeed, step 3b tells us that in theory the percentage of the protein that stays in the α-helical conformation at each final pH value should also follow the inverted {A – B} curve and it too should show a minimum near pH 2 — just like that of the q_{NaCl} vs. pH curve shown in Figure 4. It was thus with great joy that we came upon belatedly a 19-year old paper telling us something about just that.

Predicted unfolding of α-helical fold and reversal retroactively affirmed

In 1990 Goto, Takahashi and Fink reported their study on the folding and unfolding of β-lactamase, cytochrome c and apomyoglobin in response to HCl[57]. They showed that increasing HCl caused all three proteins to undergo progressive loss of their α-helical content, reaching a maximum of unfolding at pH 2, or put differently, reaching a *minimum* of folding at pH 2. From there on, further pH decrease brought on by the addition of still more HCl caused the proteins to refold again. They then showed that the rise of α-helical content below pH 2 was caused by the binding of Cl^- to (each of) the protein under study.

In short, Goto and his coworkers had retroactively confirmed what the *c- analogue scheme* described as step 3b has predicted. And, we repeat that *pari passu* with the change of q_{NaCl} with the addition of HCl, the α-helical content follows a similar asymmetrical U pattern with a minimum at the pH close to 2. Thus, just as the data shown in Figure 4 provides a direct link between H^+ and Cl^- binding and the lowering of q_{NaCl}, Goto *et al*'s work establishes a direct link between H^+ and Cl^- binding and the unfolding of the α-helical conformation of the proteins that precedes and is instrumental in the lowering of q_{NaCl},

Materials and Methods

In our ultra-simple model (USM), we chose the hydrogen ion or H^+ as the principal cardinal adsorbent. It is the smallest atom minus its electron or a proton carrying a single positive electric charge. As such, H^+ is, in theory, our premiere authentic EWC (and will be used from now on as a standard EWC.) We chose H^+ as the principal cardinal adsorbent in our ultra-simple model over say, ATP, because our evidence that ATP is an EWC are entirely empirical — strong and consistently so as they are [17h, 21f, 58]. Since K^+ is not included in the makeup of the USM, H^+ would also substitute for K^+ in its role as the partners of fixed anions as shown in Figure 2 right (See below.)

Experimentally, we began by preparing a 40% solution of (exhaustively dialyzed) *pure* bovine methemoglobin (ferri-hemoglobin) (Sigma Chemical Co, St Louis, Mo., Catalogue No. H-2625, H-2500.) 0.4 gram portions of the 40% hemoglobin solution were steadily but forcefully injected through a catheter into bubble-free dialysis sacs made from quarter-inch Spectra Por 2 dialysis tubing (cut off point: 12,000-14,000 Daltons.) The bathing solutions of a fixed volume in which the filled sacs were incubated contained a constant concentration of 20 mM (or 10 mM) NaCl but varying initial concentration of HCl, ranging from 0 to 200 mM.

Hermetically sealed in paraffin film (*Parafilm*), each screw-capped glass tube containing the bathing solution and one or more dialysis sacs was then shaken for 5 or 7 days in a constant temperature water bath maintained at 25°C or in a refrigerator kept at about 9°C. This is 15 to 21 times longer than that required for the NaCl to reach diffusion equilibrium (i.e., 8 hours, from time course study.) Inclusion of the antiseptic thymol in trial runs, showed that in its absence, the systems studied were not adversely affected by microbial or fungal contamination.

During the incubation, the sacs, especially those exposed to a higher concentration of HCl, gained varying amount of water — reaching equilibrium in 4 days. Meanwhile, the contents of these sacs exposed to the higher concentrations of HCl, turned into a stiff gel, which could keep their shape after the dialysis sac membrane has been peeled off.

We used two methods of assaying the Na^+ concentrations. In the experiments to be described in detail, we employed a Na^+-selective combination glass electrode purchased from Thomas Scientific Co., of Philadelphia (Cat. #4230819.) In supportive experiments using radioactive Na-22 labels, we followed the detailed procedure given by Ling and Hu in 1988[37]. For pH and chloride ion measurements, we used respectively a Fisher glass membrane electrode (Catalog. # 315-75) and an Orion chloride ion electrode, Model 94-17A.

In the H^+ and Cl^- binding studies, the hemoglobin solution was not kept inside dialysis sacs, its hemoglobin concentration therefore stayed unchanged at 19.2%. This particular concentration of hemoglobin was chosen to approximate the hemoglobin concentration in the swollen dialysis sacs with final pH substantially below neutrality for reason that would become self-evident later. In this H^+ and Cl^- binding studies, we deliberated upon but decided against adding NaCl to the 19.2% hemoglobin solution. See ref.59.

Our main findings were expressed quantitatively in terms of the ratio of the equilibrium NaCl concentration in the bulk-phase water in the sac over that in the bathing medium. This ratio is, of course, the (true) *equilibrium distribution coefficient* or q-value of Na^+ (as chloride) and will be represented by the symbol $q_{NaCl.}$ This symbol rather than the

original designation will be cited frequently in Results and Discussion without further explanation.

As mentioned above, we will present results of our experimental studies in two parts. The first part shows the result of our attempt to use an ultra-simple model to test the theory that nano-protoplasm is the physical basis of life. The second part describes what we found out in our efforts to understand how and why we got what we got in the first part. And then there is also something else we did not count upon at first but is just as exciting.

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59. We decided not to add NaCl to the 19.2% hemoglobin solution for the H^+ and Cl^- binding studies for several reasons. Two will be cited here. First, the contents of the dialysis sacs, which produced the data of Figure 4, are highly variable and often much lower than the initial concentrations due to the low q_{NaCl} — known only after the experimental data are in. This has made it hard to decide before the experiment what and which concentration of NaCl to duplicate. Second, as shown by Carr[55], in the form of NaCl, Cl^- does not bind onto hemoglobin significantly Thus, whatever concentration of NaCl we may choose to add, it could not conceivably give us any tangible benefit.

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Post Script

In order to reach a larger numbers of scientists, teachers and others directly or indirectly involved with biomedical science, we submitted this manuscript on August 6, 2009 to the Nature magazine of Great Britain — though it bore at that time a different title: *A Test of the Theory of Microscopic- or Nano-protoplasm as the Physical Basis of Life.*

Our article was promptly rejected without an explanation. Since we believe that this paper is of major historic importance, its offhand rejection represents an event that many would have believed impossible in the 21st century. To help them to understand the true situation, we decided that it would be worthwhile to reproduce below both our introductory letter to Nature's Editor-in-Chief, Dr. Philip Campbell and Nature's unsigned summary response.

We also decided to keep the manuscript in the format Nature requires. We made this decision to add another reminder of the major efforts long past due to improve our science education worldwide — of not only the practicing scientists themselves but all the others entrusted with the awesome responsibility of making critical decisions in science. Over time their decisions may have profound influence on the future of not only pure science itself but the future of humanity which needs trustworthy basic science more than ever (See also references 9 and 48.)

August 4, 2009

Dr. Philip Campbell,
Editor-in-Chief
Nature Magazine
Macmillan Building
4 Crinan Street
London N1 9XW

Dear Dr. Campbell:

I (and co-author) submit the enclosed article for consideration of publication in Nature. I made this choice for three reasons.

First, the results of the critical test conducted clearly and unequivocally show that Thomas Huxley was correct in advocating that life has a physical basis — despite the fact that most bio-medical scientists and teachers have rejected the concept in recent times for what I believe to be wrong reasons. In addition, the publication of our article in Nature would be a triumphant sequel to what the journal's founder, Sir Joseph Lockyer printed in the very first issue of your journal, his article, *Protoplasm at the Antipodes.* Since Sir Lockyer was also the first Editor-in-Chief and apparently wrote the overall objective of the journal still being printed on current issues, I can cite his words in persuading you to accept our article for publication in Nature.

Second, the work described is highly unusual in combining unprecedented *incisiveness* and utter *simplicity.* In one stroke, it has once again disproved the membrane pump theory (of the living cell) and establishes the alternative theory called the association-induction hypothesis — in a manner so plain that an average high school student would have no problem understanding its essence (if not all the details) in one reading.

Third, given the wide circulation your journal enjoys, the publication of our article in Nature could be, from a historical point of view, the first step in giving *all the young people of the world a uniting, shared purpose*. That is, to seek the one and only *truth* by way of the one and only *scientific method* and relying on the one and only *honesty* to make certain that the theory thoroughly refuted by objective evidence be promptly abandoned by all involved. In its place, the alternative theory that has been confirmed by overwhelming evidence be promptly and universally adopted and taught — until new evidence points to a still better theory. Failing that, we should continue to develop this established theory to serve as part of the established knowledge on which to build an ever better place for all humanity and other living creatures that share our planet to live and prosper in.

For your convenience, I am enclosing a copy of my fourth book, "Life at the Cell and Below-Cell Level." Skimming through the Preface of the book alone will give you an overall perspective far beyond what I can write here. I may also mention that very recently two Russian scientists at the Pavlov Institute of Physiology and the Leningrad Institute of Cytology have combined their resources in translating this book into Russian. The initial response has been very encouraging. Next I return to our submission and your standard procedure for deciding which paper to publish and which to reject: the peer review system.

In my opinion, the conventional peer review system is fundamentally not a good thing but perhaps tolerable for a science like astronomy, which has seen its revolutionary struggles centuries ago. For a science like cell physiology whose major progress still lies in the future, peer review is as tolerable as cyanide pills. However, if you are not allowed, or do not want to review our paper personally and if you have trouble finding *truly* qualified peers — for cause, see p.8 of www.gilbertling.org, on the front page of which are titles of (downloadable) relevant recent publications — I enclose the names and addresses of three world-class scientists who are men of high integrity and qualified to review our article.
Finally, I want to explain why I submit the article directly to you. It is too serious an event to be trusted to anyone else I know. True, you are an astronomer and not a cell physiologist. That is a distinct advantage I count upon. You have no peers to fear. The scientific issues involved are simple enough for any intelligent and fair-minded person to handle. After all, Sir Joseph Lockyer was also an astronomer. That did not prevent him from judiciously evaluating and supporting Thomas Huxley' work on protoplasm.

Sincerely yours,
Gilbert Ling, Ph.D.