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WATER AND THE LIVING CELL AS SEEN FROM THE VIEWPOINT OF  
A NEW PARADIGM(U) PENNSYLVANIA HOSPITAL PHILADELPHIA  
DEPT OF MOLECULAR BIOLOGY G N LING 1983

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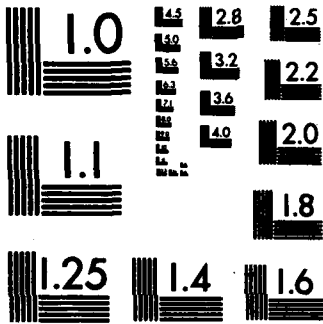
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by

Gilbert N. Ling

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Department of Molecular Biology  
 Pennsylvania Hospital  
 8th and Spruce Streets  
 Philadelphia, Pennsylvania 19107  
 USA  
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## 1. Introduction

It is common knowledge that without water there is no life. This dependency of life on water is unique: water not only furnishes an essential environment; water is also by far the major component of the substance of life, making up 75 to 85% of all living cells (Ling, 1962, p. xxiii).

In view of these self-evident reasons for anticipating prime roles of water in cell function, it is curious that history has evolved along such a tortuous path. Thus according to the widely held membrane theory, there is nothing unusual about water in life. It is simply a solvent, inside or outside the cell. The present report is to tell how this view is now changing, drastically, and in its place a new paradigm of the living cell has emerged (Kell, 1979).

To begin with, though the membrane theory has achieved supreme dominance in the last thirty years or so, and has been widely taught as truth rather than a hypothesis, it is not the only view expressed on the matter. Indeed strong objections to the membrane theory and its basic tenets have dogged its entire 100-year history, (Fischer, 1909; Moore et al, 1912; Devaux, 1916; Gortner, 1930; Lepeschkin, 1939; Troschin, 1951; Nasonov, 1959; Ernst, 1960; Ling, 1952, 1962). Opponents to the membrane theory often argued that there is a living substance or protoplasm which serves as "the physical basis of life" and that protoplasm is colloidal in nature.

The membrane theory reached the peak of its development in 1941 when Boyle and Conway published their masterful synthesis in the 100th volume of the Journal of Physiology. A sieve-like plasma membrane offered explanations for each of the 4 major subjects of cell physiology: (1) semipermeability, (2) selective solute accumulation and exclusion, (3) cellular electric potential, (4) osmotic swelling and shrinkage. At the same time, the colloidal school sank to the lowest point in its history; with the cessation of the publication of the Journal of Colloidal Chemistry, it seemed that colloidal chemistry itself had come to an end.

However, such was the unpredictable twist and turn of history, that immediately

following the publication of Boyle and Conway's paper, radioactive tracer techniques became available, enabling biologists for the first time to study directly and accurately the main parameter of the membrane theory, i.e., permeability. As new and often totally unexpected knowledge accumulated (Brooks & Brooks, 1941, preface), proponents of the membrane theory were forced to resort to more and more ad hoc remedial postulations, illustrated by the Na pump and the ever-lengthening list of other membrane pumps that followed (Ling et al, 1973). The warning that one pump alone would consume much more energy than the cell commands (Ling, 1952, 1962) was largely ignored, marking the beginning of rapidly waning confidence by at least some of the membrane theory's key supporters.

Energy insufficiency is, however, not the only difficulty of the membrane theory, or more accurately described from then on, the membrane-pump theory. In the following, I shall first briefly review the mounting evidence pointing to the fact that this theory is fundamentally wrong. I shall then briefly present an alternative view, the association-induction hypothesis (AI Hypothesis) with special emphasis on the physiological role of cell water. I shall end with a demonstration that this new hypothesis can explain the very same four major areas of physiological phenomena that the membrane-pump theory no longer can.

## 2. Evidence Against the Membrane-pump Theory

### 2.1 Lipid Bilayer Membrane as Semipermeable Barrier

The membrane-pump theory is built on Overton's lipoidal membrane theory. In its current version, lipid layer not only furnishes the semipermeable barrier of the cell; it also provides the enclosing water-immisible fluid phase to harbour the postulated "carriers" and "pumps". Obviously to serve these roles the lipid layer must cover the whole cell surface. Indeed the electron microscopic demonstration of the presence of a tri-layered "unit membrane" around many types of living cells led at one time to the belief that this is indeed the case and that the lipid membrane in general and the "paucimolecular" model of cell membranes in particular was confirmed. Subsequent studies made it difficult, if not impossible, to sustain this belief. Thus

it was found that extraction of 95% of the lipids did not alter the thickness nor the spacing of the laminar structure of the unit membrane (Fleischer et al, 1967; Morowitz and Terry, 1969). On the other hand, trilaminar structure was also demonstrated at the surface of "microspheres" prepared from lipid-free, pure proteinaceous materials (Fox et al, 1969).

Other major developments in the field of membrane research included the perfection of the technique of preparing phospholipid bilayers by Mueller and Rudin (1962, 1969). A phospholipid bilayer by itself has extremely low ionic conductance when it separates two bodies of isotonic KCl solutions. Introduction of the  $K^+$  specific ionophore, valinomycin ( $10^{-7}$  M) or monactin ( $10^{-7}$  M) increased the  $K^+$  permeability of the lipid bilayer by several orders of magnitude (for review, see Jain, 1972). Thus willy-nilly these investigators had provided a powerful tool to test one of the most basic assumptions of the membrane-pump theory, the assumption that phospholipid layer provides the permeability barrier of plasma membranes and membranes of subcellular particles. The result of investigations using this tool was dramatic.

Valinomycin, which increased  $K^+$  permeability of man-made phospholipid layer drastically, had no effect whatsoever on the conductance of mouse mitochondrial inner membrane (Maloff et al, 1978). Nor does monactin have any detectable effect on  $K^+$  permeability of giant squid axons (Stillman et al, 1970). Neither valinomycin nor monactin has any effect on the  $K^+$  permeability of the plasma membranes of frog muscle or ovarian eggs (Ling and Ochsenfeld, in preparation).

These findings demonstrated conclusively that in most living cells and subcellular particles, the surface semipermeable barrier is not that of a continuous lipid layer - a fundamental disproof of Overton's lipoidal membrane theory, on which, alas, the entire membrane-pump theory was built.

## 2.2. Accumulation and Exclusion of Ions and Other Solutes Due to Membrane Pumps

According to the membrane-pump theory, if an electrically neutral molecule does not distribute equally between the cell water and the external medium or if a charged ion does not follow the distribution pattern dictated by the theory of Donnan,

an outward or inward pump for this particular molecule or ion must be present to make up for the difference. Although no reason has been yet offered, it is often assumed that the  $K^+$  distribution across the living cell membrane follows the Donnan equilibrium and is used as the yardstick for detecting departures. I have already pointed out that there is not enough energy to cope with one pump alone.

An opportunity for another test of the membrane-pump concept came with the perfection of the technique for the removal of the cytoplasm from a giant squid axon and with the demonstration of perfectly normal electric behaviors of the axoplasm-free membrane sheath thus prepared (Baker et al, 1961; Tasaki, 1968). However, over a period of some 20 years, repeated attempts to demonstrate net transport of  $Na^+$  against a concentration gradient by the axoplasm-free squid axon membrane sacs have failed (Ling, 1977a). In contrast, employing an effectively membrane-pumpless open-ended cell (EMOC) preparation, I demonstrated that a functionally active membrane pump is not required for the selective accumulation of  $K^+$  or the exclusion of  $Na^+$  (Ling, 1978a).

So-called isolated "membranes" or vesicles, widely cited as exhibiting pumping activities turned out to be not hollow at all but to contain large quantities of cytoplasmic proteins, which in concentration equalled or even exceeded that in intact cells. These vesicles do not qualify as models of hollow membranes (Ling and Negendank, 1980). Similarly a widely publicized claim of  $Na^+$  and  $K^+$  pumping against concentration gradients by synthetic membrane vesicles could be more consistently explained as largely experimental artifacts (Ling and Negendank, 1980).

### 2.3. Cellular Electrical Potentials as Membrane Potentials

In Bernstein's membrane theory of cellular electrical potential (1902), the key role of external  $K^+$  in determining the size of the resting potential,  $\psi$ , was attributed to the permeability of the cell membrane to  $K^+$ , while the membrane is impermeable to  $Na^+$ . Later, Hodgkin and Katz (1949) made the important discovery that the transient variation of  $\psi$  during an action potential involved a momentary susceptibility of  $\psi$  to external  $Na^+$ . They attributed this increased  $Na^+$  sensitivity to an increase in the relative membrane permeability to this ion. This view formed part of their ionic

theory (HKI theory), in which the effectiveness of an external ion in depolarizing  $\psi$  depends on its relative membrane permeability. The more permeable an ion is, the greater should be its effect on depolarizing  $\psi$ . However, soon after the publication of the HKI theory, contradictory evidence began to appear. Thus in frog muscle, as in many other types of living cells, the permeability to  $\text{Cl}^-$  is very high (Ling, 1978b). Yet Hodgkin and Horowitz (1959) have found that variation of external  $\text{Cl}^-$  concentration had no effect on the steady level  $\psi$  in frog muscle as demanded by the HKI theory.

The  $\text{Cl}^-$  contradiction is not the only contradiction. Indeed a large amount of research has been carried out in the last 30 years to test the HKI theory as well as other versions of the membrane-pump theory. The results showed that while  $\psi$  varies logarithmically with extracellular  $\text{K}^+$  concentration most investigators could find no logarithmic relation between  $\psi$  and intracellular  $\text{K}^+$  (and  $\text{Na}^+$ ) concentration as predicted by the membrane-pump theory. (For review, see Ling, 1978b).

Serious discrepancies like these led to the increasing reliance on another ad hoc remedial postulation, the so-called "electrogenic pump" which was defined as follows (Kernan, 1970): "The electrogenic pumping of ions may be recognized by a change of membrane potential which cannot be accounted for in terms of passive ion movement and which has some characteristics of a metabolic process..." This is probably not the best way to put forth a serious scientific hypothesis; it has a slight vitalistic accent and is without much predictive value. It is true that Mullins and Noda (1963) put the electrogenic pump hypothesis in more quantitative terms. Nevertheless, their equation for  $\psi$  offers no better explanations than the HKI theory for the independence of  $\psi$  to external  $\text{Cl}^-$  concentration or to internal  $\text{K}^+$  (or  $\text{Na}^+$ ) concentration mentioned above.

The growing number of new findings incompatible with the HKI theory and their variations is further illustrated by the recent observations of Maloff et al (1978). In their study of the mitochondrial resting potential, they found that without valinomycin,  $\psi$  was more or less indifferent to external  $\text{K}^+$ ; in the presence of valinomycin,

† showed a pronounced sensitivity to external  $K^+$ . Yet, as pointed out earlier valinomycin had no effect whatsoever on the  $K^+$  permeability of the same liver mitochondrial inner membranes. The expected relation between † and  $K^+$  permeability demanded by the membrane-pump theory was not observed.

#### 2.4. Cells as Osmometers in Swelling and Shrinkage

Swelling and shrinkage of living cells were subjects of great interest to early cell physiologists. Traube's discovery of the semi-permeable copper-ferrocyanide gel membrane (Traube, 1867) led to the quantitative study of osmotic pressure and eventually the formation of the membrane theory. Cellular swelling and shrinkage according to the membrane theory are primarily the consequences of an enclosing semi-permeable membrane and varying concentrations of impermeant solutes in the external medium. That is, living cells behave like osmometers.

Two types of experimental evidence have made untenable the osmometer explanation of swelling and shrinkage behaviors of living cells.

a. Frog muscle cell segments without an intact membrane swell in hypotonic or isotonic KCl solution in a way indistinguishable from intact muscles (Ling and Walton, 1976).

b. The major cation in most living cells is  $K^+$ . According to the membrane theory, cell  $K^+$  must be all completely free to account for the osmotic activity of the living cells. Using 4 different techniques, 3 laboratories across the world have recently established unanimously that the bulk of the  $K^+$  in frog muscle cells is not free but is in an absorbed state (Edelmann, 1977, 1978; Ling 1977b; Trombitas and Tigy-Sebes, 1980). (This subject is reviewed by Edelmann elsewhere in this volume.)

### 3. The Association-induction Hypothesis (AI Hypothesis)

#### 3.1. The living state and cooperative adsorption

In spite of its long history, the membrane theory has offered no clearly enunciated definition of the meaning of "being alive" at the cell level. The AI hypothesis, on the other hand, provides the following definition: the presence of all the necessary chemical ingredients which are associated with one another and interacting electronically in such a manner that the

whole assembly is maintained at a specific high energy cooperative state, the living state.

Protein, water and  $K^+$  are the most abundant ingredients of living cells. The association among them as postulated by the AI Hypothesis include: (1) adsorption of  $K^+$  singly on  $\beta$ - and  $\gamma$ -carboxyl groups (and under certain conditions, backbone carbonyl groups) and (2) multilayer adsorption of water on the backbone NHCO sites of certain proteins existing in an extended conformation. The confirmation of the first type of adsorption was mentioned above. The even more recent confirmation of the second type of adsorption will be discussed next.

### 3.2. The Multilayer Polarization of Water by Extended Protein Chains

3.2.1. Theory. That gaseous molecules condense on solid surfaces more than a single layer thick led de Boer and Zwikker (1929) to formulate a theory of polarized multilayer adsorption. Brunauer, Emmett and Teller (1938) severely criticized this theory as applied to noble gas adsorption and advanced their own theory, later known as the BET theory. However, Brunauer et al pointed out that their criticism did not apply to gaseous molecules (like water) that have a permanent dipole movement. In that situation "it is feasible that many layers may be successfully polarized by the mechanism of de Boer and Zwikker. This case has been treated by Bradley (1936)".

In 1965, I further elaborated the AI Hypothesis to include the polarized multilayer theory of cell water (Ling, 1965a, 1972), which can now be described in 7 postulates.

Postulate 1. Water can exist in the state of polarized multilayers when found in the vicinity of fixed polar sites of certain geometry. This could be either surfaces of a checkerboard of fixed positive polar sites (P) alternating with negative polar sites (N), separated from the nearest neighbors by distances approximately that of the diameter of a water molecule, or a matrix of linear chains bearing N and P sites at similar distances apart.

Postulate 2. The intensity and extent of water polarization is enhanced if two polarizing surfaces are brought close together, forming what is called an NP-NP system or

if many parallel polar chains are brought close together forming a matrix, called an NP-NP-NP system. Similar but weaker long-range polarization of water may be achieved if either the P or the N sites are replaced by nonpolar, vacant sites (O), forming NO-NO, OP-OP, NO-NO-NO or OP-OP-OP systems. The distances between the nearest-neighbor N and P sites must then roughly equal twice the diameter of a water molecule.

Postulate 3. Water in the state of polarized multilayers excludes solute molecules roughly in direct proportion to the complexity and sizes of solute molecules. Small spherically symmetrical molecules may not be excluded at all; large hydrated ions and molecules may be substantially excluded. The physical basis for the exclusion are entropic and/or enthalpic (Ling, 1965b; Ling and Sobel, 1975).

Postulate 4. The polarized multilayer state of water represents a dynamic rather than a static, crystalline structure. Water in the state of polarized multilayers has lowered freezing temperature because it is separated by a larger activation energy from ice than normal liquid water.

Postulate 5. The bulk of water in a typical resting cell exists in the state of polarized multilayers.

Postulate 6. This state of polarized multilayers is achieved by interaction of cell water with certain proteins present in all living cells, forming a more or less continuous matrix throughout the entire resting cell.

Postulate 7. These proteins exist in an extended state with their positively charged NH (P) and negatively charged CO (N) groups directly exposed, constituting an NP-NP-NP system and as such polarize cell water in multilayers. Proteins whose backbone NHCO groups that are locked in  $\alpha$ -helical, pleated sheet or other intra- or inter(macro) molecular H-bonds do not have the long-range polarization effect. This dependency of water polarization on the conformation of the key proteins permits control of the state of water by agents which interact with the protein as cardinal adsorbents. Among these one of the most important is ATP.

### 3.3.3. Experimental Evidence

That bulk-phase water anywhere can exist in a physical state different from either ice or liquid water represents a major new concept. It must be tested with methods that can yield unequivocal quantitative answers. The method chosen satisfies these criteria and was based on probe-solute distribution or  $\rho$ -value studies. The  $\rho$ -value, or apparent equilibrium distribution of, say, sucrose, measures the distribution coefficient of sucrose between water under the influence of proteins or other polymers (or otherwise specified) and normal liquid water. The  $\rho$ -value obtained yields a firm estimate of the minimal amount of water affected by the protein or polymer. Thus a  $\rho$ -value of say, 0.4 for sucrose would mean that at least 60% of the water must be made different from normal liquid water. The same data also yields a quantitative operational parameter, called minimal "non-solvent" water (MINOW) expressed as grams of ("non-solvent") water per gram of dry polymer, even though in reality there is no categorically non-solvent water.

Table 1 and Figure 1, taken from Ling, Ochsenfeld, Walton and Bersinger (1980), show that all native globular proteins gave a  $\rho$ -value close to unity for  $\text{Na}_2\text{SO}_4$ , while gelatin yielded a  $\rho$ -value of only 0.537. To explain this difference, we pointed out that gelatin with its well-known amino acid sequence of glycine-proline-hydroxyproline does not form  $\alpha$ -helix. Therefore a substantial portion of the backbone amide groups of gelatin is directly exposed to bulk phase water, causing multilayer polarization and solute exclusion effects. In support, polyvinylpyrrolidone (PVP) and poly(ethylene oxide) (PEO), which also cannot form  $\alpha$ -helical conformation but are not in the form of gels or coacervates, also exclude  $\text{Na}_2\text{SO}_4$ .

These findings, of major importance in the AI Hypothesis, also offered a new answer to an old question: What is a colloid? Thomas Graham (1861), who introduced this term ( $\kappa\omicron\lambda\lambda\omicron\sigma$ , glue), wrote "As gelatine appears to be its type, it is proposed to designate substance of the class as colloids." I suggest that the colloidal state is not just distinguished by the size of the macromolecules involved, as was once believed. Rather it is the long-range polarization of water that truly distinguishes

gelatin, living protoplasm as well as other colloids.

Solutions of native proteins show little exclusion of  $\text{Na}_2\text{SO}_4$  or sucrose (and glycine, data not shown) (Figure 1). Urea and guanidine HCl, known to unravel the secondary structures of protein thereby exposing the backbone NHCO groups, reduce the  $\beta$ -value for sucrose (and glycine, data not shown) and multiply MINOW more than five-fold. Denaturants sodium dodecyl sulfate (SDS), and n-propanol, known to unravel only tertiary structure but do not unravel  $\alpha$ -helical and other secondary structures, had no effect. The data presented also permits calculation which showed that more than one layer of water molecules were affected by each oxygen site.

As mentioned above, Brunauer, Emmett and Teller (1938) showed that electrical charged sites can polarize more than one layer of the gaseous molecules like water with a large permanent dipole moment. In other words, proteins and other polymers can influence distant water molecules only by a mechanism of propagated electrical polarization involving both induced and permanent dipole moments of the "target" as well as intervening water molecules. This is just another way of saying that under the long-range effects of polymers like gelatin, PEO, etc. water can only exist in the state of polarized multilayers.

### 3.3. Interpretation of the 4 Major Physiological Manifestations of Living Cells in Terms of the AI Hypothesis.

In Section 2 I have shown how the 4 major areas of physiological activities which once seemingly offered strong support for the membrane theory, have in the light of new knowledge, turned around, offering strong evidence refuting it. In this section I shall demonstrate how these same physiological manifestations are generally speaking in harmony with the AI Hypothesis.

#### 3.3.1. Polarized Water as the Semipermeable Surface Barrier of Living Cells

Long-range polarization of water required a matrix of chains properly spaced polar sites. Like PVP and PEO, cellulose acetate apparently can provide the NP-NP-NP sites, also. Figure 2 taken from Ling (1973) presents a plot of the permeability of water and 11 hydroxylic compounds through a hydrated cellulose acetate membrane against the permeability of a living membrane (inverted frog skin). Note that this figure demon-

Figure 2  
here  
strates more than just a good correlation ( $r = +0.96$ ) but direct correspondence: the best-fitting curve has a slope of 0.99 and that both the ordinates and abscissa are in the same units.

I concluded that surface barriers of the living cells are primarily water in multilayers polarized by certain cell surface proteins. This model brings the cell surface properties under the physiological control of cardinal adsorbents (e.g.,  $\text{Ca}^{++}$ ) which occupy and electronically interact with cardinal (or receptor) sites of these surface proteins.

### 3.3.2. Selective Accumulation and Exclusion of Ions and Other Solutes.

In the same year, 1951, that I published the earliest version of the AI Hypothesis explaining selective  $\text{K}^+$  accumulation in living cells (Ling, 1951), Soviet scientist A. S. Troschin (1951, 1966) also presented his sorption theory for non-electrolyte distribution. Troschin argued that an intracellular sugar may be either free or complexed and the total concentration in the cell can be thus described by a two-term equation. He explained the low level of sugars in the cell water on the ground that the cell exists as a colloidal "coacervate", citing similar exclusion (and accumulation) in inanimate coacervate systems like gelatin, which was known to exclude  $\text{Na}_2\text{SO}_4$  (Holleman, Bungenberg de Jong and Modderman, 1924).

Ling, Ochsenfeld, Walton and Bersinger (1980) came to a somewhat different conclusion concerning solute exclusion in gelatin. In our view, it is the multilayer polarization of water rather than the assumption of the coacervate state that holds the key to solute exclusion. Thus, PEO and PVP do not form coacervates under the conditions of our studies but possess just as effective exclusion property for  $\text{Na}_2\text{SO}_4$  as gelatin.

The general equation for solute distribution I presented first in 1965 (Ling, 1965b) takes into account site-to-site cooperative interaction as well as the control by cardinal adsorbents of the property of cell water and the adsorption and desorption of major ions such as  $\text{K}^+$  and  $\text{Na}^+$  (Ling and Bohr, 1971; Ling, 1970; Ling and Ochsenfeld, 1972).

Mostly through the brilliant efforts of Ludwig Edelmann (1977, 1978), whose article in this volume reviews the issue extensively, the fundamental assumption of

the AI Hypothesis that the bulk of cell  $K^+$  is in an adsorbed state has also been established.

### 3.3.3. Electrical Potentials as Surface Adsorption Potentials

According to the association-induction hypothesis,  $\psi$  is a surface adsorption potential (Ling, 1962) determined by the concentration of fixed ionic sites on the cell surface and the concentration of external ions and the affinity of these ions the surface adsorption sites. Furthermore,  $\psi$  can be controlled by cardinal adsorbents which vary the relative affinity of the surface sites for competing ions (e.g.,  $K^+$  and  $Na^+$ ).

Edelmann (1973) tested in guinea pig heart muscle the alternative predictions of the HKI theory and the surface adsorption theory according to the AI Hypothesis. He concluded that the relative effectiveness of an external ion in depolarizing the resting potential,  $\psi$ , does not depend on the relative permeability of the ion, as according to the HKI theory, but it does depend on the relative adsorption energy of the ion on surface anionic sites as according to the AI Hypothesis. In earlier studies Ling and Ochsenfeld (1965) provided evidence that the surface anionic sites in frog muscle cells have pK of 4.6, identical to that of the  $\beta$ - and  $\gamma$ -carboxyl groups, confirming the AI Hypothesis.

Recently I have further elaborated the equation of cell resting potential by taking into account the cooperative interaction among the surface anionic sites (Ling, 1979). The usefulness of this new equation and the theory behind it are illustrated in Figure 3. The data points are those of Maloff et al (1978) mentioned earlier, representing mitochondrial electrical potentials measured at varying external  $K^+$  concentration in the presence and absence of valinomycin. The solid lines which fit the data are theoretical, calculated on the basis of the new equation and the numerical value that valinomycin acting as a cardinal adsorbent increases the relative affinity of the surface anionic sites of the mitochondrial inner membrane for  $K^+$  by a factor of 3.

### 3.3.4. Osmotic Swelling and Shrinkage

An argument of A. V. Hill in the 1930's, played a pivotal role in the subsequent dominance of the membrane theory (see Ernst, 1960, p. 112).

Since living cells are in osmotic equilibrium with a 0.1 M of free and fully dissociated NaCl;  $K^+$  salts, which are found at roughly equal molar concentration in the cells, must also be fully dissociated and free (Hill, 1930; Hill et al, 1930). After nearly half a century, it is now firmly established that virtually all intracellular  $K^+$  is not free but adsorbed. What then produces the osmotic activity of the missing free  $K^+$  ions? I believe that within the confines of the membrane theory there is no reasonable answer. The disproof of one of the basic tenets of the membrane-pump theory (the tenet of free intracellular ions and solutes) uproots the entire system of interwoven and mutually dependent tenets which together constitutes the membrane-pump paradigm.

To present the interpretation on the basis of an alternative paradigm, I shall first review some basic knowledge. Osmotic pressure is the pressure needed to counter and check the flow of water from a dilute solution where the water activity is high, to a concentrated solution, where the water activity is low. The effect of a solute, such as sucrose, in reducing water activity is referred to as the osmotic activity; within limits, this osmotic activity of sucrose is proportional to the molar concentration. Like that of a dilute sucrose solution, a dilute solution of a globular protein also exhibits osmotic activity in direct proportion to the molar concentration of the protein. Now if proteins in cells also behave in a similar manner, there can be little osmotic activity from these proteins; their molecular weights are too large and total molar concentrations too low. Indeed, this is the reason why proponents of the membrane-pump theory have traditionally disregarded the role of water-protein interaction in the osmotic behavior of living cells.

The findings described in Figure 1 and Table 1 contradict these old ideas, and clearly show that proteins existing in an extended conformation react with water differently and profoundly. However, the data presented describe directly the solvent properties of the affected water. In work yet to be published, I and my colleagues have further demonstrated that water under the influence of an NP-NP-NP or its equivalent NO-NO-NO systems have reduced activity far beyond that predictable

from the polymer's molar concentration. Thus a 30% solution of dialyzed PEO with MW of about 600,000 exhibits an osmotic activity equal to that of a 1 molar solution of sucrose and thus 2000 times higher than that anticipated on the basis of the molar concentration of the polymer. Furthermore, a similar PEO solution when confined within a dialysis bag will swell or shrink, much as living cells do, in solutions of Na-citrate of varying concentration, even though PEO carries no net electric charge (the total concentration of anionic charges due to accidental oxidation in the solution is less than  $5 \times 10^{-6}$  M) and even though the dialysis tubing is fully permeable to Na-citrate (Ling, in press).

In summary, the osmotic activity of resting cells appears only to a small extent due to free ions and molecules but primarily due to the extended matrix proteins.

10 to the minus 6th power

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## LEGENDS

Table 1 -  $\rho$ -values of  $\text{Na}^+$  in water containing native proteins (A), gelatin (B), PVP (C), and poly(ethylene oxide) (PEO) (D) ( $25^\circ\text{C}$ ) (from Ling et al, 1980 by permission of Physiological Chemistry and Physics)

Figure 1 - The  $\rho$ -value of sucrose (A and C) and the apparent minimum "non-solvent" water (B and D) of native and denatured proteins. C and D represent average of 15 proteins studied (from Ling et al, 1980 by permission of Physiological Chemistry and Physics)

Figure 2 - Plot of the permeability to 11 hydroxylic compounds ranging from water (1) to sucrose (17) at 3 different temperatures ( $0^\circ$ ,  $4^\circ$ ,  $25^\circ\text{C}$ ) of reversed frog skin against the permeability of heat-treated cellulose-acetate membrane. Straight line described by equation shown in graph was obtained by the method of least squares (from Ling, 1973 by permission of Biophysical Journal)

Figure 3 - The resting potential of isolated giant mitochondria in Cuprizone-fed mice in the presence and absence of valinomycin. Data points are from Maloff et al (1978). Solid lines are theoretically calculated from the equation of Ling (1979). The intrinsic equilibrium content for the exchange of unidentified cation (possibly  $\text{H}^+$ ) for  $\text{K}^+$  increased by a factor of 3 in response to valinomycin (from Ling, in press by permission of Physiological Chemistry and Physics)

TABLE 1

Group	Polymer	Concentration of medium (M)	Number of assays	Water content (%) (mean $\pm$ SE)	p-Value (mean $\pm$ SE)
(A)	Albumin (bovine serum)	1.5	4	81.9 $\pm$ 0.063	0.973 $\pm$ 0.005
	Albumin (egg)	1.5	4	82.1 $\pm$ 0.058	1.000 $\pm$ 0.016
	Chondroitin sulfate	1.5	4	84.2 $\pm$ 0.061	1.009 $\pm$ 0.003
	$\alpha$ -Chymotrypsinogen	1.5	4	82.7 $\pm$ 0.089	1.004 $\pm$ 0.009
	Fibrinogen	1.5	4	82.8 $\pm$ 0.12	1.004 $\pm$ 0.002
	$\gamma$ -Globulin (bovine)	1.5	4	82.0 $\pm$ 0.16	1.004 $\pm$ 0.004
	$\gamma$ -Globulin (human)	1.5	4	83.5 $\pm$ 0.16	1.016 $\pm$ 0.005
	Hemoglobin	1.5	4	73.7 $\pm$ 0.073	0.923 $\pm$ 0.006
	$\beta$ -Lactoglobulin	1.5	4	82.6 $\pm$ 0.029	0.991 $\pm$ 0.005
	Lysozyme	1.5	4	82.0 $\pm$ 0.085	1.009 $\pm$ 0.005
	Pepsin	1.5	4	83.4 $\pm$ 0.11	1.031 $\pm$ 0.006
	Protamine	1.5	4	83.9 $\pm$ 0.10	0.990 $\pm$ 0.020
	Ribonuclease	1.5	4	79.9 $\pm$ 0.19	0.984 $\pm$ 0.006
(B)	Gelatin	1.5	37	57.0 $\pm$ 1.1	0.537 $\pm$ 0.013
(C)	PVP	1.5	8	61.0 $\pm$ 0.30	0.239 $\pm$ 0.005
(D)	Poly(ethylene oxide)	0.75	5	81.1 $\pm$ 0.34	0.475 $\pm$ 0.009
		0.5	5	89.2 $\pm$ 0.06	0.623 $\pm$ 0.011
		0.1	5	91.1 $\pm$ 0.162	0.754 $\pm$ 0.015
(E)	Methylcellulose	0.1	4	83.4 $\pm$ 0.43	0.689 $\pm$ 0.008

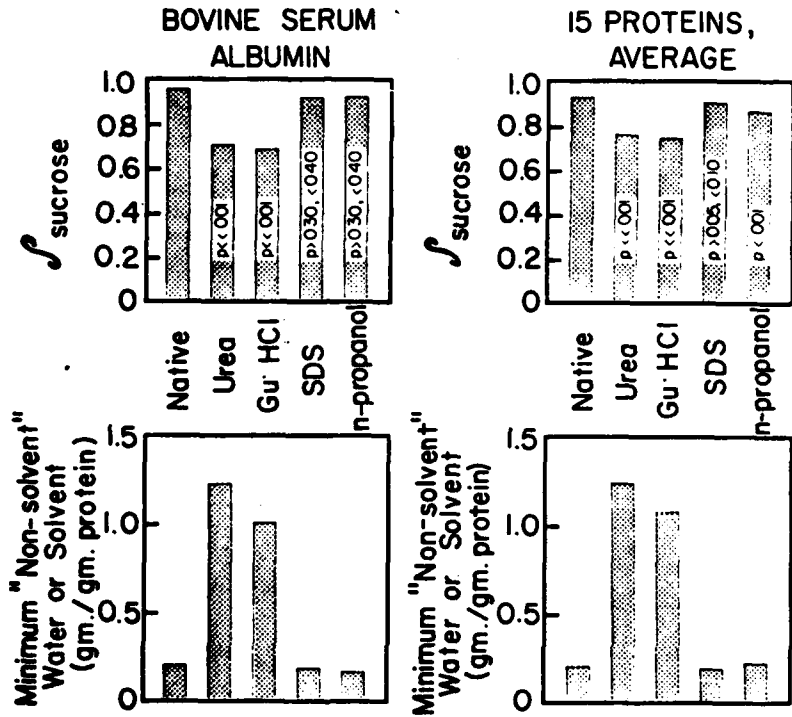


FIGURE 1

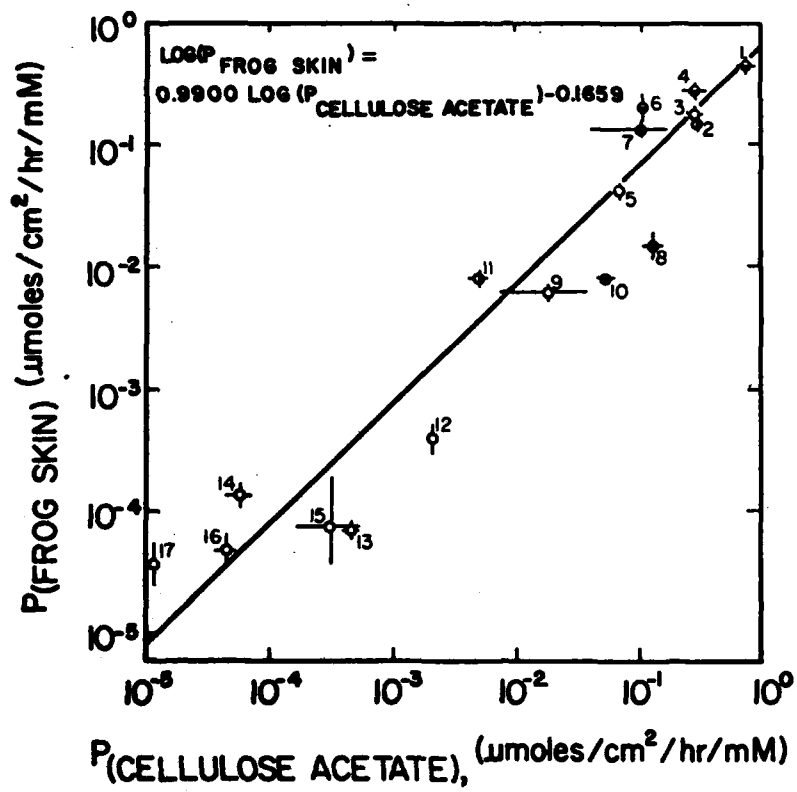


FIGURE 2

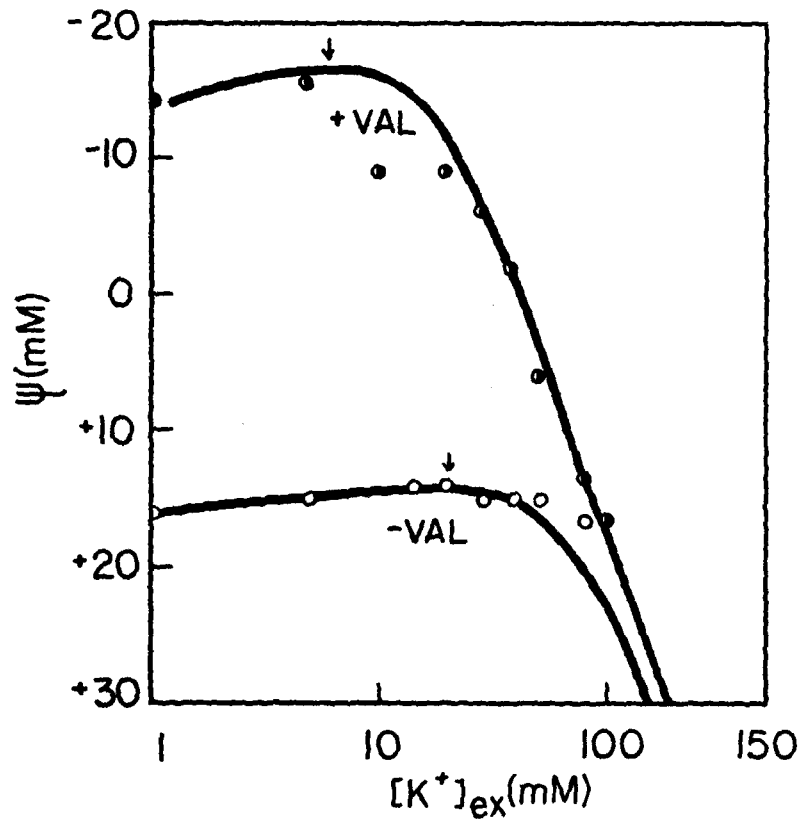


FIGURE 3

**END**

**FILMED**

**1-84**

**DTIC**