Physiol. Chem. Phys. & Med. NMR (2006) *38*: 55–76

In Response to an Open Invitation for Comments on AAAS Project 2061's Benchmark Books on Science

Part 1: Documentation of Serious Errors in Cell Biology

Gilbert Ling

Damadian Foundation for Basic and Cancer Research
Tim and Kim Ling Foundation for Basic and Cancer Research
110 Marcus Drive, Melville, NY 11747
Email: gilbertling@dobar.org

Abstract: Project 2061 was founded by the American Association for the Advancement of Science (AAAS) to improve secondary school science education. An in-depth study of ten 9 to 12th grade biology textbooks led to the verdict that none conveyed "Big Ideas" that would give coherence and meaning to the profusion of lavishly illustrated isolated details. However, neither the Project report itself nor the Benchmark books put out earlier by the Project carries what deserves the designation of "Big Ideas." Worse, in the two earliest-published Benchmark books, the basic unit of all life forms — the living cell — is described as a soup enclosed by a cell membrane, that determines what can enter or leave the cell. This is astonishing since extensive experimental evidence has unequivocally disproved this idea 60 years ago. A "new" version of the membrane theory brought in to replace the discredited (sieve) version is the pump model — currently taught as established truth in all high-school and college biology textbooks — was also unequivocally disproved 40 years ago. This comment is written partly in response to Bechmark's gracious open invitation for ideas to improve the books and through them, to improve US secondary school science education.

KEY WORDS: AAAS, AAAS Project 2061, association-induction hypothesis, cells, cell biology, cell membrane, membrane permeability, potassium ion, semipermeability, sieve membrane theory, sodium ion, sodium pump hypothesis, solute distribution in cell

IN THE EARLY 1980's, thoughtful Americans began to suspect something seriously remiss in the educational system, especially in science education. Books after books were written. It began with "*A Nation at Risk: The Imperative for Education Reform,*" published 1983 by the National Commission on Excellence in Education — in response to an

urgent request from the then Secretary of Education of the United States, T. H. Bell. The following is an excerpt from the Commission's report:

"In many other industrialized nations, courses in mathematics (other than arithmetic or general mathematics), biology, chemistry, physics and geography start in grade 6 and are required of *all* students. The time spent on these subjects, based on class hours is about three times that spent by even the most science-oriented students (in the US), i.e., those who select 4 years of science and mathematics in secondary school."

As years went by, the concern began to focus more and more on biology teaching. Thus in 1990*, Fulfilling the Promise: Biology Education in the Nation's Schools* was published conjointly by three organizations: the Board on Biology, Commission on Life Sciences and the National Research Council. The authors of this volume first pointed out that in the widely adopted high school curriculum in the US, biology holds a pivotal position. It is at the start of the sequence of science courses. At its best, an inspiring biology course may invoke the student's interest in not just biology but other sciences as well. In most cases, this goal has not been reached, as a 1988 survey showed.

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

One major cause for the trouble, according to the authors of *Fulfilling the Promise*, is the poor quality of the biology textbooks. They de-emphasize the drama and excitement of discovery and "portray biology as the worst kind of literature — all characters and no story." Other studies reached a similar verdict.

On June 8, 2000 Anjetta MacQueen of the Associated Press made banner headlines in newspapers across the Nation with her article, "Group gives biology textbooks failing grade."

In brief, a study by Project 2061 of the American Association for the Advancement of Science (AAAS), revealed that 9th through 12th grade biology textbooks used in the United States, despite their enormous size and lavish illustrations, uniformly fail to convey "big ideas." Of all the ten most popular textbooks examined, none escaped the indictment.

Dr. George Nelson, the director of this arm of Project 2061, explains with a parable what the project meant by "big ideas". "*Providing bits of information about transmission, carburetors, fuel injectors, universal joints, and cooling systems doesn't convey a sense of a car as a mode of transformation*." What the parable suggests is that, like the disassembled auto parts, the biology textbooks also present the names, illustrations and other descriptions of bits and pieces of the living cell — to the exclusion of the "big ideas" how these parts work together to make a living cell living. Put it differently, what is completely missing in the high school textbooks is a valid coherent theory of living phenomena at the basic level.

The question now shifts to, Has Project 2061 itself uncovered these "Big Ideas? It seemed that Project 2061 was way ahead of me in asking this question.

Thus, Project 2061 did not begin and end with this survey of high school biology textbooks its ultimate goal. Much earlier, Project 2061 had involved several hundreds of scientists, mathematicians, engineers, physicians, historians and educators in producing a series of benchmark books on all sciences (Project 2061 1990, p. x.)

The first one of the series that came off the press in 1993 is entitled *Science for All American.* As pointed out by Project Director, Dr. F. James Rutherford in its Preface, the

purpose is to present what he regards as a valid expression of "the view of the scientific community on what constitutes literacy in science." Then on page 63 of the book, I found the following passage describing the basic unit of all life: the living cell:

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

Four years later, Project 2061 published the second book of the series, entitled *Benchmarks for Science Literacy*. On page 113, it describes once more the living cell:

> "*Every cell is covered by a membrane that controls what can enter and leave the cells. . . .*"

However, neither in these passages nor elsewhere in the books could one find what Dr. George Nelson might call the "big ideas." That is, ideas that would give coherence to, and make sense out of the unconnected bits and pieces of the living cell.

Worse, the idea reiterated in both volumes that *a cell membrane determines what can enter or leave the cell* is wrong. The idea that chemicals in the biological environment can be sorted out into two kinds — those that can enter and leave the cell and others permanently and absolutely left out — is implicit in van't Hoff's theoretical concept of *semipermeabilit* (van't Hoff 1887, 1888*.)* It is more commonly known under the name of the Sieve Membrane Theory of the living cell. Unfortunately, this too has been unequivocally disproved well over half of a century ago. However, even that is not all that has gone wrong and calls for prompt recognition and remedy.

The discovery of the fallacy of the Sieve version of the membrane theory in the 1930–1940's led to the adoption of the sodium pump version of the membrane theory. And soon it was taught in virtually all high school as well as college biology textbooks, including the ten most prominent biology textbooks Project 2061 scrutinized as well as four of the most prominent college biology textbooks I examined (for names, see Endnote 1 of article.)

Figure 1 is a cartoon of the elaborate four-color renditions of the sodium pump found without fail in every one of the heavy biology textbooks that I have examined.

The trouble is, this pump version of the membrane theory too was disproved — not last year, or the year before that, but fully forty (40) years ago — a solid fact that was, as if by magic, made invisible to Project 2061 and its advisors.

There is little question in my mind that Dr. George Nelson and Dr. F. James Rutherford as well as their staffs are highly capable and dedicated people. Nonetheless, the inability to see and recognize the proven fallacy of the (later) pump version of the membrane theory in all the textbooks scrutinized was Mystery 1.

The recommendation as a guideline for future textbook improvements, another version of the membrane theory that was disproved even earlier, the sieve version, was Mystery 2. Taken together, these two mysteries have created a grave crisis.

For if allowed to continue uncorrected, it would undermine the high-minded and noble purpose of Project 2061. For inaction would create the false impression that no one really cares about the future of all high school students — which literally means the future America — as well as the rest of the world that follows in the footsteps of America.

It is almost an anticlimax to point out how allowing proven falsehood to be taught universally year after year as established truth would also demoralize the teachers and

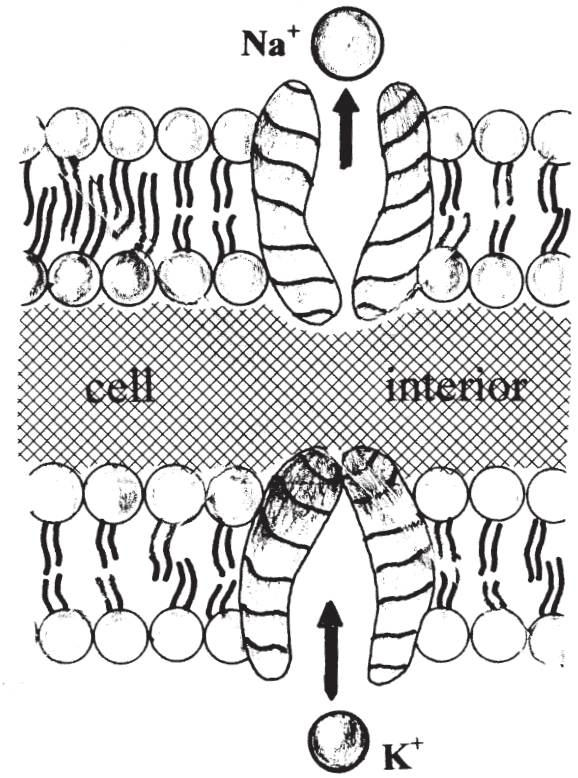

FIGURE 1. A diagrammatic illustration of what is given in most if not all current biology textbooks, representing of a phospholipid bilayer cell membrane traversed by one sodium (potassium) pump.

seriously tarnish the good name and credibility of Project 2961 in particular and AAAS in general.

However, like many other crises, it also brings with it an uncommon opportunity. For as the Chinese word for crisis (wei-chee) points to, it is a combination of the word for danger (wei) and that for opportunity (chee). Incidentally, former Vice-President Al Gore also called attention to this Chinese word combination in his immensely important book on global warming, *The Inconvenient Truth* — a title that, I think, may aptly be applied to what I am writing too.

This communication, the first installment of what I am writing on the crisis, was begun in the hope that Project 2061 was seriously looking for ways to improve their otherwise wonderful books when it offered at the end of Benchmark a gracious open invitation for comments.

This installment will concentrate on what has so far eluded Project 206: the disproof of both the sieve version and the pump version of the membrane theory. In a following installment, I shall examine how I can in other ways help Project 2061 in realizing its goal — by thinking aloud with my readers how major scientific progress had been achieved in the past and how learning science, especially the rapidly advancing life science, is not

only committing to memory some facts but in learning how to question and the mental skill to determine what is popular fallacy and what is truth.

In the end, I may be able to help by pointing out the direction where the "Big Idea" might be in existence all along but made invisible by the same magic factors that have blinded Project 2061 to part of the real-life history of sieve and pump.

The experimental unanimous disproof of the concept that the cell membrane determines what can enter or leave the cell

By the mid-19th century, it was already widely known that salts, like table salt, or sodium chloride (NaCl), do not exist as single entities but comprise two (or more) electric charge-bearing entities called ions. For sodium chloride, the two kinds of ions are the positively-charged *cation*, sodium ion, represented by the notation, Na^+ and the negatively-charged *anion*, chloride ion, represented by the symbol Cl^-. Similarly, the salt, potassium chloride (KCl) consists of the cation, K^+, and the anion, Cl^-.

When sodium chloride is dissolved in water, each ion goes its own way and in the process taking on a more or less permanent coat of water molecules. For that reason, the sodium cation in water or another aqueous medium is sometimes called *hydrated* sodium ion. Now, the force holding the Na^+ and water molecules together in the hydrated sodium ion is electrostatic in nature. As such, its strength depends on the "distance of the closest approach" separating the ion and water molecules. Now, a naked Na^+ has an atomic weight of 22.99; it is therefore smaller than an unadorned K^+ with an atomic weight of 39.10. As a result, the positive charge of the smaller Na^+ can come and stay closer to, and thus exercises a stronger attraction for, the surrounding water molecules than the larger K^+ and consequently the smaller Na^+ holds onto more water molecules around itself than around the larger K^+. The net result is that the hydrated Na^+ ends up being larger than the hydrated K^+. This difference in the hydrated ionic sizes is important for understanding the once popular theory on selective membrane permeability to be discussed below.

But before that, we must first turn our attention to two other salts: cupper sulfate ($CuSO_4$) and potassium ferrocyanide ($K_4Fe(CN)_6$). Like NaCl and KCl, when these salts dissolve in water, they too dissociate into their component ions, which include the copper cation, Cu^{++}, carrying two positive electric charges and the ferrocyanide anion, $Fe(CN)_6^{4-}$ carrying four negative electric charges. These two salts played a key role in a simple but fateful experiment carried out by a Berlin tradesman-scientist by the name of Moritz Traube some time before the year 1867.

In this experiment, Traube (1867) brought together one drop of a solution of copper sulfate and another drop of a solution of potassium ferrocyanide. He noted that at the surface of contact between the two drops, where the copper ion from one drop met the ferrocyanide ion from the other drop, a reddish-brown gelatinous precipitate of copper ferrocyanide was formed. And, once this precipitation membrane was formed, no further precipitate was formed beyond the membrane in either direction. The conclusion Traube reached was that this gel membrane is impermeable to the copper ion and the ferrocyanide ion.

So Traube had thus discovered the way to make a nearly perfect model of what the Dutch physical-chemist, J. H. van't Hoff (1887, 1888) envisaged theoretically as a *semi-permable membrane.* Namely, a membrane that permits the passage of water but not the substances or solutes dissolved in water.

By allowing the precipitation to take place not in open water as Traube did but in the fine pores of an unglazed porcelain thimble, Pfeffer succeeded in making a much sturdier copper ferrocyanide membrane that can be freely handled and manipulated (Pfeffer 1877, 1985.)

Now, if one immerses such a copper-forrocyanide membrane thimble in a vessel containing water and introduces a strong solution of cane sugar, or sucrose, inside the thimble, water outside the thimble will move to the inside of the thimble. However, this movement of water can be stopped or even reversed in direction if one applies a positive mechanical pressure to the inside of the thimble. The magnitude of the mechanical pressure required just to stop the water flow was referred to as the *osmotic pressure* produced by the sucrose solution used (Findlay 1919.) The demonstration of a positive osmotic pressure then becomes a way to determine whether or not a particular water-soluble substance like sucrose is able to permeate the copper ferrocyanide membrane. Soon a large number of solutes were tried, some pronounced as *permeant*, others *impermeant*.

Among the substances found unable to cross the copper ferrocyanide membrane are sucrose, potassium sulfate, calcium chloride, potassium ferrocyanide. In contrast, potassium chloride (KCl) was able to pass through the copper ferrocyanide membrane (Ostwald 1890.)

To explain the behavior of this nearly ideal semipermeable property, Traube introduced an "*atomic sieve theory*." In this theory, the copper ferrocyanide gel membrane has pores of such a diameter that only small molecules and ions can pass through the membrane. Larger molecules and ions are barred — indefinitely. (For the ultimate fate of this theory, see Ling 2001, p. 115, 133.)

Asymmetrical distribution of Na^+ and K^+ in living cells

Abderhalden was a talented and highly productive scientist. Once he studied under the great German chemist, Emil Fischer, to whom we owe much of the fundamental knowledge of proteins. In 1898 Abderhalden published the results of an analysis of the K^+ and Na^+ concentration in the blood plasma and in the red blood cells of rabbits. His data, reproduced here in Table 1, shows much more Na^+ than K^+ in the blood plasma. In sharp contrast, the red blood cells contain a high concentration of K^+ and no Na^+ at all (Abderhalden 1898.)

Other investigators went on to find out if this absence of Na^+ in (rabbit) red blood cell could be confirmed in other types of living cells that could also be isolated from the host animal in intact and healthy form. One type of cells they chose was the cell of the small voluntary muscles of frogs, notably the frog sartorius muscle, which can be easily isolated surgically in an intact form. In the 4th edition of his famous *Textbook of General Physiology*, which appeared in print in 1923, Sir William Bayliss noted that from all the evidence

TABLE 1. Abderhalden's historic data on the K^+ and Na^+ contents of the rabbit plasma and red blood corpuscles.

	plasma (mM)	Red blood cells (mmoles/kg)
Potassium	6.59	133
Sodium	193	0

The unit of concentration has been changed from parts per million in the original to milli-molarity or millimoles per gram. (data from Abderhalden 1898)

on hand, "it is almost certain that there is no sodium ion in frog muscle cells " (Bayliss 1927, p. 121.) From these historical "discoveries" many at the time believed that cells in general contain no sodium ion but at the same time an abundance of its chemically highly similar sister alkali metal ion, K^+.

In 1926 L. Michaelis, a very capable biologist, was aware of the difference in the sizes of the hydrated K^+ and hydrated Na^+. Thus armed, he introduced the idea that it was the smaller size of the hydrated K^+ that allows it to pass through the new kind of membrane model made of dried collodion (i.e., nitrocellulose or gun cotton.) And as he also observed experimentally, the larger hydrated Na^+ apparently could not do so. Two years later, a pair of German cell physiologists, R. Mond and K. Amson (1928) from Heidelberg adopted Michaelis's pore-size vs K^+/Na^+ selectivity idea and used it to explain the sustained high concentration of K^+ and absence of Na^+ (or low Na^+, see below) in frog muscle cells. Namely, the small K^+ can enter through small membrane pores and accumulates inside the cells while the larger Na^+ are kept out permanently.

In 1941 P.J. Boyle and E.J. Conway from Dublin published a landmark paper beginning on page 1 of Volume 100 of the (English) Journal of Physiology (Boyle and Conway 1941.) The paper bears the title, *Potassium Accumulation in Muscle and Associated Changes*. (Regrettably, these authors, who have in fact elaborated on the theme of Mond and Amson gave no credit to the prior authors for expressing the same idea earlier — even though they knew about Mond and Amson's relevant paper, which in fact was in the reference list of Boyle and Conway's paper, but noted only for a minor side issue.) However, Boyle and Conway did add something new. While Mond and Amson believed that the muscle cell membrane is impermeable to chloride ion (Cl^-) as many others also believed at that time, Boyle and Conway believed and showed evidence supporting the opposite. This is important for their theory.

Table 2, reproduced from Boyle and Conway's article shows their quantitative theory for the sieve membrane idea. Based on the known mobility data in the literature, they were able to divide a gathering of ions into two categories: those supposedly able to traverse the cell membrane and others unable to do so. Examples of *permeant* ions include

TABLE 2. Boyle and Conway's quantitative sieve membrane theory.

Velocities of ions under gradient of I V./cm. or 0·5 V./cm. for divalent ions				Relative ion diameters (diameter of potassium ion= 1·00)			
Cations		Anions		Cations		Anions	
H	315·2	OH	173·8	H	0·20	OH	0·37
Rb	67·5	Br	67·3	Rb	0·96	Br	0·96
Ca	64·2	I	66·2	Cs	1·00	I	0·97
NH_4	64·3	Cl	65·2	NH_4	1·00	Cl	0·98
K	64·2	NO_3	61·6	K	1·00	NO_3	1·04
Na	43·2	CH_3COO	35·0	Na	1·49	CH_3COO	1·84
Li	33·0	SO_4	34·0	Li	1·95	SO_4	1·89
Ca	25·5	HPO_4	28	Ca	2·51	HPO_4	2·29
Mg	22·5			Mg	2·84		

The mobility data were from the International Critical Table and Chemiker Kalender. The relative ionic diameters were calculated on the basis of their diverse mobilities and on the assumption that K^+ has a relative diameter of one. (from Boyle and Conway, by permission of the *Journal of Physiology*, London)

the K^+ and Cl^-, which, being smaller are able to traverse the muscle cell membrane and accumulate inside the cell. The larger hydrated Na^+ and sulfate (SO_4^-), like the other larger ions magnesium (Mg^{++}) and calcium (Ca^{++}), are unable to go through the pores and are left out permanently and absolutely.

Boyle and Conway's 1941 paper gained worldwide attention. Nonetheless, before it had appeared in print, evidence refuting the the underlying concept that the muscle cell membrane is impermeable to Na^+ (and other large ions) began to appear and with time became a torrent of mutually-supportive, and highly consistent revolutionary findings.

Disproof of the concept that membrane impermeability accounts for the (once-believed) absence of Na^+ in living cells

The following are six examples of experimental findings, which unanimously testified to the permeability of the cell membrane to ***the*** classic membrane-impermeable cation, Na^+:

1. Wu and Yang (1931) reported experiments in which they injected NaCl solution into the veins of dogs and found significantly higher concentration of Na^+ in the muscle cells. They conclude that the muscle cell membrane is permeable to Na^+.
2. Kaplanski and Boldyreva (1933, 1934) kept carps (a large fresh water Cyprinid fish) for one month in a 1.5% solution of NaCl. In consequence, the Na^+ concentration of the fish muscle nearly doubled while that in the blood remained unchanged (Table 3.) They too concluded that the muscle cell membrane is permeable to Na^+.
3. Cohn and Cohn (1939) injected intravenously radioactive isotope ^{24}Na into veins of dogs. After varying lengths of time, blood was withdrawn, the red blood cells separated from plasma by centrifugation and then washed twice before their radioactivity was assayed. Their figure reproduced here as Figure 2, demonstrates steady accumulation of radioactive ^{24}Na in the red blood cells with time, leading the authors to the conclusion that the the cell membrane of the canine red blood cells is also permeable to Na^+.
4. L. Heppel fed rats on a low-potassium diet for 34 to 44 days, sacrificed some of the rats and analyzed the K^+ and Na^+ contents of rat tissues and found a decrease of K^+ level and an increase of Na^+ level. Heppel concluded that the muscle cell membrane is permeable to Na^+(Heppel 1939.) When radioactive isotope ^{24}Na was injected into the K^+-depleted rats, the ratio of ^{24}Na in rat muscle over that in serum reached the ratio of *total* Na^+ in muscle over that in serum in about 60 minutes as shown in Heppel's data presented here as Table 4 (Heppel 1940.)
5. B. Steinbach (1940) demonstated that frog sartorius muscle when incubated in a solution containing no K^+ but the normal amount of Na^+, lost K^+ and gained Na^+. These changes were reversed when the depleted sartorius muscles were transferred to a Ringer's solution containing a normal concentration of both K^+ and Na^+. After another period of incubation, the muscles regained its lost K^+ and extruded the extra Na^+ accumulated. Steinbach's table is reproduced here as Table 5. The author also concluded that the frog muscle cell membrane is permeable to Na^+.
6. Several tenths of a 1 mm in diameter and one or more centimeters in length, the giant inter-nodal cell of fresh water alga Nitella is diagrammatically illustrated in Figure 3. S.C. Brooks (1940) exposed Nitella internodal cells to radioactive ^{24}Na-labeled Na^+ and $^{86}Br^-$-labeled Br^- for different lengths of time. He then determined the concentra-

TABLE 3. The concentration of Na^+ in the blood and muscle of carps kept in a 1.5% NaCl solution for 70 days.

Tissue	Control	Experimental
Blood	123	129
Muscles	40	111·4

Data represent averages of many experiments and are in units of mg. per cent. (data of Kaplanskii and Boldyreva, 1934)

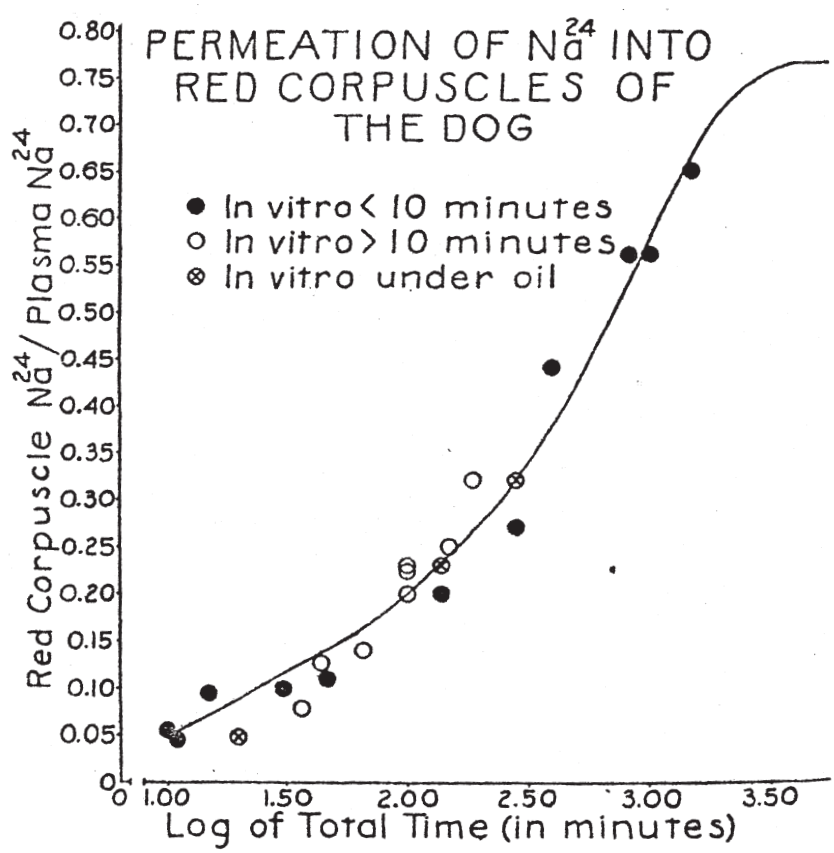

FIGURE 2. Radioactive ^{24}Na in isotonic saline was injected intravenously into normal healthy dogs. After varying lengths of time, blood samples were withdrawn, centrifuged and the red cells collected, washed and its radioactivity assayed. The red cell ^{24}Na contents expressed as a fraction of the plasma ^{24}Na concentration and shown on the ordinate and the total time after injection given as the abscissa. Different symbols refer to length of time between withdrawal of blood and radioactivity assay and whether or not the sample was kept under oil to prevent entry of CO_2. (from Cohn and Cohn, 1939 by permission of the Society of Experimental Biology and Medicine)

tions of these labeled ions in both the cytoplasm and the sap filling the large central vacuole. Figure 4 shows that labeled Na^+ entered the cytoplasm very rapidly while it took longer to reach the sap. Fast or slow, there is no question that both the cell membrane (*alias* plasma membrane) lining the outer surface of the cytoplasm and the vesicular membrane or tonoblast lining the inner surface of the cytoplasm are permeable to Na^+ (and Br^-.)

TABLE 4. Penetration and equilibration of ^{24}Na into K^+-depleted rats fed on a low K^+ diet for 34–44 days.

Time between injecting ^{24}Na solution and sacrifice of animal (min)	$\frac{^{24}Na \text{ in muscle}}{^{24}Na \text{ in serum}}$ $\left(\frac{cpm/g}{cpm/g}\times 100\right)$	$\frac{Na^+ \text{ in muscle}}{Na^+ \text{ serum}}$ $\left(\frac{mmoles/kg}{mmoles/kg}\times 100\right)$
5	14	39.2
10	17	23.5
10	15	26. 8
20	23	36.4
31	31	34.4
60	33	32.7
60	28	35.0
182	38	31.1
187	38	41.2
215	32	30.2
260	33	32.6

(Data from Heppel 1940)

TABLE 5. K^+ and Na^+ exchange in frog muscle

Frog No.	Muscle No.	Na	K	Cl	Change
1	1	5.95	3.60	2.82	Na –2.67, K +25, Cl none
	2	3.28	6.05	2.94	
2	1	5.35	3.75	2.97	Na –1.93, K +1.55,
	2	3.42	5.30	2.78	Cl none
Pooled analyses[†]	1	5.04	3.44	3.79	Na –1.00, K +1.55,
		(4)	(8)	(8)	Cl +0.31
	2	4.04	4.99	4.10	
		(4)	(8)	(8)	

Muscles were soaked for 17 hours in K^+-free Ringer solution. One muscle was taken out for analysis while a second muscle was returned to a Ringer solution containing 10 mM K^+ and 6 to 8 hours before analysis. The data are not corrected for ions in the extracellular space. (from Steinbach, by permission of the *Journal of Biological Chemistry*.)

[†]The figures in parentheses give the number of separate analyses making up the average figure. The figures are not corrected for extracellular space.

In summary, from 1931 to 1940 scientists using a variety of experimental methods and animal tissues — including mammalian red blood cells and isolated frog muscle — have unanimously demonstrated that the cell membrane in general is permeable to Na^+. Thus, the once-popular idea that there is no sodium in living cells and that this absence of Na^+ is due to an absolute impermeable cell membrane with pores too narrow for the large hydrated Na^+ to squeeze through are unequivocally disproved.

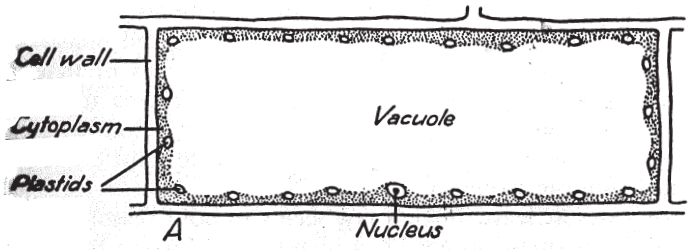

FIGURE 3. A diagrammatic illustration of a mature plant cell. (from Miller, 1936?)

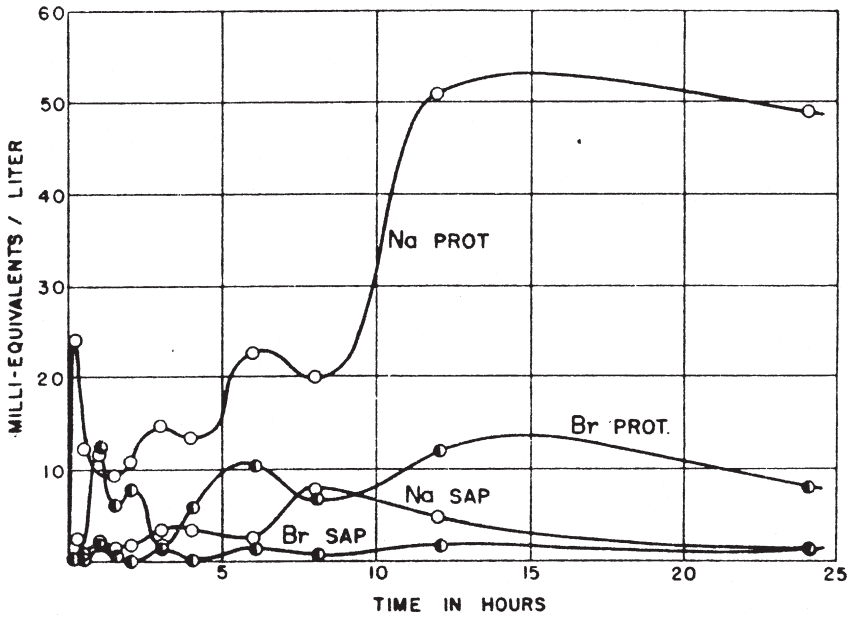

FIGURE 4. The time course of the changing concentrations of radioactively labeled Na^+ and Br^- in the cytoplasm and in the vacuolar sap of internodal cells of Nitella *clavata*. Cells were incubated in a solution containing 0.02 M labeled NaCl or 0.01 M labeled NaBr for up to 24 hours. Plants used in the Na^+ experiments had been pretreated by a prior incubation for 15 hours in a 0.01M non-labeled NaCl solution. pH of the media used about 6.0. Procedures of isolating the sap, the determination of the weight of cytoplasm (protoplasm), the intensity of illuminations etc. can be found in Brooks' original article. (from Brooks 1939, by permission of *Journal of Cellular and Comparative Physiology*.)

However, by itself, this set of evidence could not be seen as having disproved in general the (atomic) sieve membrane theory — which was introduced first by Traube, reaching its peak development in the work of Michaelis, Mond and Amson and Boyle and Conway. We will come back to this subject later.

I have reproduced in Table 1 Abderhalden's data on zero sodium concentration in rabbit red blood cells mostly for historical reason. Later work using more precise methods showed that there is Na^+ in rabbit red blood cells after all — though at a concentration much lower than either that of K^+ in the red blood cell or that of Na^+ in the bathing plasma. For example, Ponder's book, *Hemolysis and Related Phenomena* gave an intracellular K^+ concentration of 90 mmoles per liter of rabbit red blood cells and an intracellular Na^+ concentration of 16 mmoles per liter (Ponder 1948, p121.) This high intracellular/extracellular ratio of K^+ and low intracellular/extracellular ratio of Na^+ are by no means limited to rabbit and other red blood cells. Rather, they are the rules for K^+-Na^+ distribution in most living cells examined.

Thus, Table 6 taken from Ling (1962) shows that the rule holds for all 14 kinds of rat tissues listed. However, the data are given in micro-molarity per gram of fresh tissue. No correction was made for $Na^+$and other ions trapped in the extracellular space.

TABLE 6. The K^+ and Na^+ (as well as Rb^+ and Cs^+) concentration in the plasma and 14 rat organs fed a K^+-free diet containing Rb^+ and Cs^+ for a total of 14 days.

Animal No.		9070806		9070807		9071713		9071714	
Duration of feeding (days)		6		8		14		15	
Organ	Ion	Concentration, µM/g	[p] tissue / [p] plasma	Concentration, µM/g	[p] tissue / [p] plasma	Concentration, µM/g	[p] tissue / [p] plasma	Concentration, µM/g	[p] tissue / [p] plasma
Plasma	Na	142.84	-	128.89	-	150.98	-	151.00	-
	K	8.22	-	5.26	-	5.03	-	4.03	-
	Rb	0.22	-	0. 24	-	0.12	-	0.31	-
	Cs	0.56	-	0.30	-	0.30	-	0.67	-
Brain	Na	59.84	0.42	44.84	0.35	38.53	0.26	53.72	0.36
	K	90.42	11.00	85.41	16.24	63.86	12.70	72.29	17.00
	Rb	0.64	1.14	2.56	8.53	2.49	8.30	-	-
	Cs	-	-	0.64	2.67	0.71	5.92	-	-
Lens	Na	-	-	-	-	28.06	0.19	36.41	0.24
	K	-	-	-	-	32.50	6.46	59.71	14.80
	Rb	-	-	-	-	2.83	9.43	6.64	21.40
	Cs	-	-	-	-	0.71	5.92	3.67	5.48
Gastroeneimus	Na	30.74	0.22	23.52	0.18	41.90	0.28	30.32	0.20
	K	88.15	10.72	85.04	16.17	83.80	16.66	71.54	17.80
	Rb	1.79	6.27	4.34	14.47	7.29	24.30	8.80	13.20
	Cs	1.38	3.20	2.39	9.96	4.86	40.50	7.64	24.60
Diaphragm	Na	41.42	0.30	29.71	0.23	31.50	0.21	46.30	0.33
	K	93.75	12.41	78.63	14.95	31.49	6.26	69.44	17.20
	Rb	2.16	3.86	4.10	13.67	4.81	28.75	8.47	18.20
	Cs	1.70	7.72	2.65	11.04	3.45	16.03	12.22	27.30
Heart	Na	59.07	0.41	62.05	0.48	50.53	0.34	49.18	0.33
	K	72.80	8.86	54.29	10.32	30.85	6.14	158.24	14.47
	Rb	1.15	2.05	3.70	10.53	3.12	17.50	8.15	12.16
	Cs	1.48	6.59	1.39	8.92	2.11	10.40	3.57	11.50

TABLE 6. (continued)

Animal No.		9070806		9070807		9071713		9071714	
Duration of feeding (days)		6		8		14		15	
Organ	Ion	Concentration, µM/g	[p] tissue / [p] plasma	Concentration, µM/g	[p] tissue / [p] plasma	Concentration, µM/g	[p] tissue / [p] plasma	Concentration, µM/g	[p] tissue / [p] plasma
Spleen	Na	35.82	0.25	32.53	0.25	30.02	0.20	49.63	0.33
	K	95.56	11.63	84.86	16.13	62.99	12.52	70.59	17.50
	Rb	3.01	5.37	3.56	11.87	6.64	20.58	11.65	17.40
	Cs	1.12	5.09	2.04	8.50	2.47	22.13	3.97	12.80
Erythrocyte	Na	32.32	0.23	24.77	0.19	30.41	0.20	32.01	0.21
	K	70.41	8.57	68.96	13.11	67.70	13.45	61.96	15.40
	Rb	1.21	2.16	2.46	8.20	4.16	13.86	5.88	8.78
	Cs	0.42	1.91	0.48	2.00	0.59	4.92	1.25	4.03
Kidney	Na	80.00	0.56	60.89	0.47	98.56	0.65	68.31	0.45
	K	58.00	7.06	52.19	9.92	45.67	9.08	44.33	11.00
	Rb	1.22	2.19	3.36	11.20	8.46	28.20	8.98	13.40
	Cs	1.32	6.00	2.78	11.58	5.38	44.83	5.84	18.80
Liver	Na	38.25	0.27	30.63	0.24	62.22	0.41	43.99	0.29
	K	74.84	9.10	76.86	14.61	63.55	12.63	47.25	11.75
	Rb	3.14	5.61	5.62	18.73	8.90	29.83	12.32	18.40
	Cs	1.00	4.54	2.38	9.92	8.26	68.83	6.91	22.30
Stomach	Na	63.40	0.44	45.31	0.35	67.54	0.45	-	-
	K	65.21	7.93	45.31	8.61	24. 88	4.95	54.34	13.40
	Rb	2.28	4.07	3.36	11.20	6.74	22.47	16.17	24.10
	Cs	1.85	8.41	2.72	11.33	4.73	39.42	9.20	29.70
Duodenum	Na	66.66	0.67	53.19	0.41	86.79	0.57	67.26	0.45
	K	93.33	11.35	72.69	13.82	75.67	15.04	65.22	16.20
	Rb	2.60	4.64	4.89	16.30	10.42	34.74	11.86	28.40
	Cs	2.20	10.00	4.04	16.83	7.48	62.33	8.80	17.70
Large intestine	Na	54.32	0.38	45.63	0.35	44.94	0.30	58.14	0.39
	K	76.04	9.25	66.37	12.62	44.94	8.93	68.18	16.90
	Rb	3.30	5.89	3.48	11.60	5.39	17.97	10.29	15.40
	Cs	2.51	11.41	3.29	13.71	4.36	36.33	6.91	22.30
Adrenal gland	Na	66.16	0.46	-	-	78.09	0.52	63.83	0.42
	K	73.79	8.98	-	-	78.09	15.52	56.23	14.00
	Rb	-	-	-	-	9.37	31.23	6.66	9.90
	Cs	-	-	-	-	6.65	55.42	5.65	18.20
Testis	Na	-	-	-	-	-	-	51.41	0.34
	K	-	-	-	-	-	-	58.97	14.60
	Rb	-	-	-	-	-	-	6.77	10.10
	Cs	-	-	-	-	-	-	3.30	10.70

Data are given in µmoles per gram of fresh tissue or plasma. The tissue/plasma ratios are given under $[p]_{tissue}/[p]_{plasma}$. Note that in all cases, the $[p]_{tissue}/[p]_{plasma}$ for Na^+ are consistently below unity. A substantial fraction of the tissue Na^+ is in the extracellular space, which in this case was not subtracted. To obtain a rough idea how much of the tissue Na^+ is in the cells and how much in the extracellular space, see Table 7 following. (data from Ling 1962)

Table 7, in contrast, presents a more elaborate set of data on the K^+ and Na^+ distribution in 11 (or 12) types of frog tissues and cells beside Achilles tendon and skin. Based on data obtained by the Ling and Walton's *centrifugation method* for determining the amount of water (and solute) in the extracellular space, Table 7 presents both the weight percentage of extracellular-space water designated as *centrifugation-extractable water* and the K^+ and Na^+ content of unadulterated living cells. Without exception, all 11 (or 12) types of frog tissue cells demonstrate the same high intracellular/extracellular ratio for K^+ and low intracellular/extracellular ratio for Na^+ as seen in rabbit red blood cells. None of them, however, shows zero Na^+ content.

TABLE 7. The K^+ and Na^+ contents of 11 types of tissues and cells in addition to skin and Achilles tendon of normal North American leopard frogs (*Rana pipiens pipiens*, Schreber.)

Tissue	Total tissue water content (%)	Centrifugation extractable water (%)	Cell water content (%)	$[Na^+]_{in}$ µmoles/ gram wet wt	$[K^+]_{in}$ µmoles/ gram wet wt
Plasma				103.8 (mM)	2.5 (mM)
Achilles tendon	82.5 ± 0.40	18.4 ± 3.64	78.3 ± 1.31	65.8 ± 2.50	52.7 ± 2.43
egg	48.7 ± 0.48			40.3 ± 2.33	49.9 ± 1.94
heart (ventricle)	83.4 ± 0.35	15.7 ± 0.72	80.3 ± 0.32	33.4 ± 2.14	80.6 ± 2.32
intestine	81.4 ± 0.55	12.7 ± 1.31	78.7 ± 0.39	55.7 ± 2.20	71.2 ± 2.20
kidney	81.0 ± 0.50	17.6 ± .44	77.0 ± 0.33	43.7 ± 0.81	83.0 ± 0.72
lens	63.8 ± 0.61			61.8 ± 2.94	23.6 ± 2.81
liver	73.3 ± 0.46	15.9 ± 1.38	68.3 ± 0.36	24.0 ± 1.66	73.5 ± 0.43
muscle (sartorius)	79.3 ± 0.49	8.20 ± 0.32	77.4 ± 0.53	22.6 ± 3.17	106.3 ± 2.65
oviduct (with eggs)		10.8 ± 1.44	75.8 ± 0.46	14.9 ± 1.28	28.1 ± 3.16
oviduct (without eggs)	79.6 ± 0.67	24.0 ± 1.02	73.2 ± 3.65	47.3 ± 1.06	69.0 ± 7.47
skin	75.7 ± 1.10	15.1 ± 0.96	71.4 ± 1.02	73.0 ± 8.0	47.5 ± 2.18
spleen	77.1 ± 2.98	7.94 ± 1.60	75.1 ± 0.40	20.3 ± 0.87	107.9 ± 1.31
stomach	82.0 ± 0.69	11.2 ± 1.22	79.7 ± 0.53	65.7 ± 1.76	54.9 ± 1.77
testis	85.4 ± 1.14	18.5 ± 2.10	82.1 ± 1.10	30.5 ± 1.09	95.4 ± 0.81

To determine the percentage of water in cells and in the extracellular space, we used the centrifugation method of Ling and Walton (1975.) The method consists of weighing the tissue after following a standard procedure of blotting on wetted filter paper, followed by wrapping and hermetically sealing the tissue-semi wet filter paper stack in paraffin film (Parafilm) before centrifugation for 4 minutes at 1000 g. It is absolutely necessary to prepare the so-called semi-wet filter paper correctly and not allowing it to dry. To prepare semi-wet filter paper, a stack of Whatman No. 1 filter paper is soaked overnight in a regular frog Ringer' solution (Formula 731, see Ling and Bohr (1969) for detailed composition) and then centrifuged exactly the same way as the tissue (4 minutes at 1000 g) while the Parafilm wrapped package sits at the bottom of a 25 ml metal shield in an International Centrifuge and spun for 4 minutes at 1000 g. Once the extracellular water content, listed as centrifugation extractable water, is obtained, the K^+ and Na^+ contents of the tissue cells were then calculated from the total tissue K^+ and Na^+ contents and listed respectively as $[K^+]_{in}$ and $[Na^+]_{in}$ in µmoles / gram of fresh cell weight. (from Ling and Ochsenfeld, unpublished)

The failure of the atomic sieve theory is not limited to the asymmetric distribution of Na^+. Rather, it includes the intra-, extracellular distribution of all of the larger solutes that have been studied in adequate detail (Ling 1952 pp. 761–763; Ling 2006, Table 1 on p. 7.) Three technical advances have made it possible for us to say that we have so far not encountered a single water-soluble chemical substance that we can say without hesitation to be absolutely and permanently unable to go through a cell membrane or non-living model of the cell membrane. (That said, I must also admit that an elephant will not be able to enter a living cell. Anything less than an elephant but say almost as big as a living cell may be able to enter it, but only if we can verify that by waiting long enough.)

The three technical advances are: (1) a way of keeping isolated tissues *in vitro* in physiological condition for 9 days at 25° C and a month or more at 0° C (Ling and Bohr 1969); (2) the availability of a wide range of ions and chemical, big and small in the radioactively labeled form; and (3) the introduction of 5 independent methods for the accurate determination of the extracellular space of frog muscle and other tissues (for rough description and sources, see Ling 1997, p.136.)

Thus equipped, we were able to study in detail the permeation into, and accumulation in frog muscle cells, a wide variety of chemical substances. They include 21 sugars, sugar alcohols and other nonelectrolytes as listed in Table 8. The same table also shows that, without exception, everyone being able to penetrate and reach diffusion equilibrium within 48 hours — some in much shorter times — even though the study was conducted at the low temperature of 0° C; even though a solute like raffinose has a molar volume of almost half of a liter (499 cc.)

Next, we review another page of the history of cell physiology in the aftermath of the crisis produced by the collapse of the atomic sieve theory as applied to Na^+ distribution in living cells. To salvage the membrane theory, according to which a living cell is a membrane-enclosed solution of free water and free ions and other solutes, a hypothetical device called the sodium pump was installed to replace the atomic sieve now out of the picture. There is a striking difference, though, between the two models. A sieve performs its function without the steady energy expenditure; a pump cannot.

The sodium pump

In 1946, I came to the United States from China to study cell physiology at the world-famous Department of Physiology at the University of Chicago. It was a day in the second spring after my arrival in Chicago, on which I gave a Monday afternoon seminar at the Department. The subject of the talk was the Sodium Pump — based solely on a library research I made in the preceding weeks. At the outset of my talk, I told my audience that only thing I felt confident in sharing is that nobody seems to know what it is beyond the name.

To show that there was objective cause for my failure to find anything worth reporting, I can now cite from two scientists from the Physiological Laboratory of the Cambridge University of England. They apparently encountered a similar problem more than 25 years after me. Thus in the 1975 edition of the Annual Review of Physiology and under the title, *The Sodium Pump*, Glynn and Karlish wrote:

> "If the great mass of work that has been done led to the general acceptance, even provisionally and even in outline, of a hypothesis accounting for the working of the pump, we could

TABLE 8. Time for the 22 non-electrolytes (including labeled water) to reach diffusion equilibrium in frog muscle cells.

Solute	Equilibration Time (hours)
water	<< 1
methanol	<20
ethanol	<20
acetamide	<10
urea	<24
ethylene glycol	<10
1,2-propanediol	24
DMSO	< 1
1,2-butanediol	24
glycerol	<20
3-chloro-1,2-propanediol	24
erythritol	<20
D-arabinose	<45
L-arabinose	<45
L-xylose	<45
D-ribose	<24
xylitol	24
D-glucose	<15
D-sorbitol	<10
D-mannitol	<24
sucrose	< 8
raffinose	10

The sequential order in the list roughly follows the decreasing size or molecular volume of each compound, the first one being the smallest, the last one, the largest. The data as a whole shows that with the exception of the three pentoses, an incubation period of 24 hours at 0° C is adequate for all the other non-electrolytes listed. For other data presented in the same publication, the final *equilibrium distribution coefficients* or q-values for all solutes equal to or larger than those of erythritol (Molecular volume 130.27 cc), have q-values equal to, or smaller than 0.29. In other words, these larger non-electrolyes distribute themselves with an asymmetry quantitatively equal or very close to that of the Na^+ in the same frog muscle cells. (from Ling *et al* 1993)

have described the hypothesis and then consider the evidence for it. Unfortuinately, *no such hypothesis exists...*" (*Ital. mine*.)

However, what I did not expect occurred at the end of my 1948 talk. It was the extremely kindly but equally startling response of two of my much respected and beloved professors. Each took me aside and told me in private and in almost identical words that the sodium pump is a *sacred cow* and I should leave it alone. There is nothing gained by making yourself a martyr.

I sincerely thanked each of them for their kindness but asked myself, Why should anyone in power care about what a lowly graduate student thinks about a purely scientific theory? I could not just leave the sodium pump alone since it was directly in the path of my Ph.D. thesis on the electrical potential of single frog muscle cells.

So, the next thing you know, I was doing an elementary experiment that anyone in the same situation would have thought of immediately. That is, an experiment, which could allow me to find out if the K^+ and Na^+ in an isolated frog muscle would change dramatically when I cut off its energy supply by exposing it to metabolic poisons.

The very first experiment I did was mind absorbing. Nothing happened at all to either the K^+ content nor the Na^+ content — long after the cessation of all its active metabolism.

In the weeks and months following, I repeated this simple experiment again and again — without fail, each experiment reiterated to me the same story. As an illustration, the results of one of these repetitions was what I published in 1952 (Ling 1952, p. 765) and reproduced here as Table 9.

Disproof of the pump version of the membrane theory on the basis of energy insufficiency

As anyone who has pumped, say, water from a wet basement knows, pumping requires a constant supply of energy. The moment one turns off the electricity, pumping stops. This is, of course, an expression of the First Law of Thermodynamics, the Law of the Conservation of energy (Fermi 1936.).

Pumping water out of a basement performs work because water is lifted from a low place to a high place up a gravitational gradient. Pumping Na^+ out of the living cells also

TABLE 9. Effect of Na iodoacetate (IAA) and pure nitrogen and low temperature upon the K^+ concentration of frog muscle and nerves.

Type of Tissue	Muscle No.		Weight (gms.)	mM. K+/l. of intracellular water
Sartorius	1	Control	0.0870	60.7
	2	Expt'l	0.0750	69.8
Semitendinosus	1	Control	0.0710	72.6
	2	Expt'l	0.0795	81.8,
Tibialis anticus longus	1	Control	0.0938	71.1
	2	Expt'l	0.0900	79.2
N. ischiadicus +	1	Control	0.0300	38.1
N. tibialis + N. peroneus	2	Expt'l	0.0260	39.5
Sartorius	1	Control	0.0730	73.4
	2	Expt'l	0.0700	78.0
Semitendinosus	1	Control	0.0660	83.0
	2	Expt'l	0.0730	77.4
N. ischiadicus +	1	Control	0.0260	42.8
N. tibialis + N. peroneus	2	Expt'l	0.0242	40.0
			Muscles	Nerves
Average		Control	100.0%	100.0%
		Expt'l	105.2%	98.5%

All muscles and nerves were incubated for 5 hours at 0° C, the experimental series in a Ringer's solution containing 0.5 mM IAA in an atmosphere of pure nitrogen, the controls in a plain Ringer's solution in air. (from Ling 1952, by permission of the Johns Hopkins University Press, Baltimore, MD)

performs work. Only here it is done against two gradients, a concentration gradient and an electric gradient. The minimum energy that must be spent to pump one mole of Na^+ from inside a frog muscle cell to the outside can be calculated when one poisons isolated muscle cells with an atmosphere of pure nitrogen (plus 0.5 mM sodium cyanide) — , which suppresses aerobic respiration — and the poison, sodium iodoacetate (IAA), which suppress anaerobic metabolism, or glycolysis.

Of relevance here is the fact that in a frog muscle fully poisoned with pure nitrogen and IAA, the only *possible* energy source are the content of the so-called high energy phosphate bond compounds, creatine phosphate (CrP) and ATP present in the muscle at the moment the poisons applied terminated all active metabolism. The difference in the contents of CrP + ATP at this moment and at the conclusion of the experiment gives the maximally available energy (see Ling 1952, pp. 765–766, Ling *et al* 1973, pp. 11–12 for evidence against the existence of some as yet unknown energy source.)

Now, we need a Gerard-Graham-Ling (*alias* Ling-Gerard) microelectrode (Ling and Gerard 1948) to monitor the cellular resting potential (about 85 mV inside negative), a γ-scintillation counter to monitor the rate of outward flux of radioactively labeled Na^+ from the poisoned muscle cells and a cold room to accommodate the whole setup and keep it at 0° C. It needs be mentioned that given the membrane theory's doctrine of free water and free ions in the cell, virtually all the measured Na^+ coming out of the poisoned cells must be due to pumping (Ling 1997, p. 155.)

The first set of data comparing the maximally available energy and the minimum required energy was reported briefly in 1952 (Ling 1952.) Assuming that all the energy available has just one purpose, i.e., pumping Na^+, and that all processes involved in utilizing energy is 100% efficient, the minimum energy need was found to be 400% of the maximally available energy.

However, rather crude methods of analysis and measurements were employed. So in the course of the following five years, I spent much time improving the methods and making more and more, better and better experiments. Results of the last three sets of fully completed experiments in 1956 are presented in Figure 5. It shows that the minimally needed energy is at least from 15 to 30 times (or 1500% to 3000%) of the maximally available energy (Ling 1962, p. 211.) Thus from energy consideration alone, the sodium pump hypothesis was experimentally disproved.

One may add that the essence of the finding has been first confirmed by Jones in mammalian smooth muscle (1965) and then by Minkoff and Damadian (1973) in the bacteria, E. coli.

That said, I must add that this 15 to 30 times figure, overwhelming by any standard, is not an accurate estimate. It still grossly *under*estimates the energy disparity.

The causes for this underestimation are many and no effort will be made to count them all. Only two would suffice here. First, Na^+ is but only one among a countless number of solutes that are in the same position: *being able to cross the cell membrane and found in the cell at the concentration lower than that in the bathing medium.* In a by no means comprehensive search, Ling *et al* (1973) found a minimum of 18 pumps already postulated by the year 1968. Some of these postulated pumps are not single pumps but conglomerates of pumps, like the sugar pumps and free amino acid pumps (Table 10.) None of the scientists postulating these pumps paid attention to the already bankrupt state of energy supply from one sodium pump alone. The isolated living cells have nowhere to borrow energy.

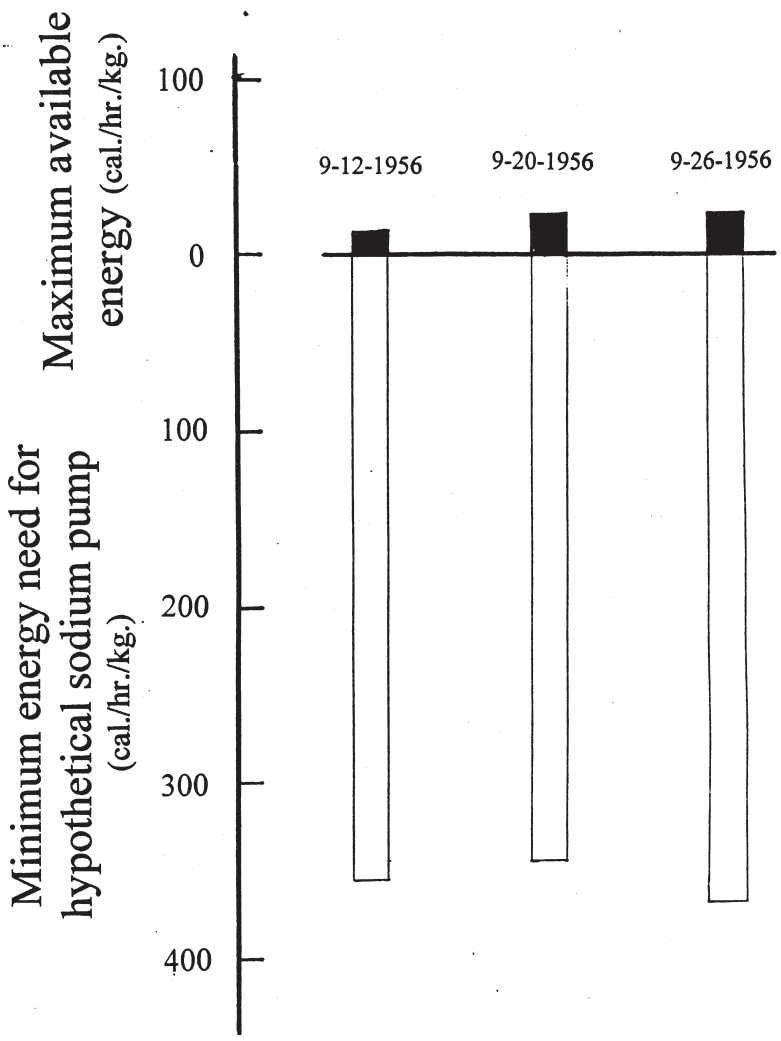

FIGURE 5. A comparison of the maximally available energy of (poisoned) frog sartorius muscle cells at 0°C (upward black bars) and the minimum energy need to pump Na^+ against both (measured) electric potential gradient and a concentration gradient. Duration of the experimental observation for Experiment (9-12-1956) lasted 10 hrs.; Experiment 9-20-1956, 4 hrs.; Experiment 9-26-1956, 4.5 hrs. Active oxidative metabolism was suppressed by exposure to pure nitrogen (99.99%, in addition to 0.001 M NaCN); glycolytic metabolism, by sodium iodoacetate and doubly insured by actual lactate analysis before and after the experiment. Other detailed studies reported in 1952 (Ling 1952, Table 5 on page 765) and in 1962 (Ling 1962, Table 8.4) showed respectively that under similar conditions of 0° C temperature and virtually complete inhibition of active energy metabolism, the K^+ and Na^+ concentrations in frog muscle, nerves and other tissues remain essentially unchanged for as long as the experiments lasted (5 hrs. for the 1952 reported experiment, and 7 hrs. and 45 min. in the 1962 reported findings. (For additional details, see Ling 1962, Chapter 8 and Ling 1997.) In the computations, it was assumed that the frog muscle cell does not use its metabolic energy for any other purpose(s) than pumping sodium ion and that all energy transformation and utilization are 100% efficient. (from Ling 2004)

TABLE 10. A partial list of pumps already postulated in 1968

Solute	Direction	System	Reference*
Na, K	coupled	many cells	169
Ca^{++}	outward	RBC, striated muscle	170, 171
Mg^{++}	outward	frog sartorius	172
$Choline^{+}$	inward	RBC	173
Amino acids	inward	RBC, muscle, tumor	174–176
D-xylose	inward	rat diaphragm	177
D-xylose	outward	rat diaphragm	178
Na^{+}	inward	frog sartorius -	179, 180
Noradrenaline	inward	vascular smooth muscle	181
Prostaglandins	inward	mammalian liver	182
Curarine	inward	mouse diaphragm	183
Br^{-}, I^{-}, ReO_4^{-}, WO_4^{-}	outward	Ascites	184
CU^{+2}	inward	Ascites	185
Aminopterin	inward	Yoshida sarcoma	186
Cl^{-}	inward	squid axon, motor neurons	187, 188
Mn^{++}	inward	*E. coli*	189
Cl^{-}	outward	*E. coli*	189
Sugars	inward	*E. coli*	189
Amino acids	inward	*E. coli*	189
Tetracycline	inward	*E. coli*	190

* For references to the sources of these publications see Ling *et al.* 1973. (Ling *et al.* 1973, by permission of the New York Academy of Sciences)

Second, the list of permeant solutes with below unity intra-, extracellular distribution ratios include compounds that had not existed on this planet until some chemists synthesized them for the first time in history. And, there is no limit to the number of these man-made chemicals that would require pumps. For that reason, the number of required pumps as a whole has no limit.

Since the full documentation of my energy study of frog muscle was first published in my 1962 book*, A Physical Theory of the Living State: the Association-Induction Hypothesis* (Ling 1962), and this book has been long out of print, I decided in 1997 to reproduce the entire section dealing with the subject as an appendix to a publication, entitled "*Debunking the Alleged Resurrection of the Sodium Pump Hypothesis*" (Ling 1997, Appendix 1.) (Other than directly going to the printed journal of the listed URL of the paper itself, you can also reach it by going to the website (www.gilbertling.org) and find Debunking as a link.)

The 41-page lone main text of the 75-page long Debunking was an in depth review of the disproof of the pump theory. It was written in response to a claim that there was enough energy for the sodium pump after all. The Debunking article shows how this alleged resurrection was based solely on a *non-existent* crucial experiment and on knowingly tellng half truths. Why did my once starry-eyed student sink so low just to earn a living, is what made me shed tears — not only for their loss but also for other would-be scientists in the world they find themselves. What is spelled out in Debunking is one

additional reason that had prompted me to take on the challenge of writing this document and its sequel.

I thank Dr. Raymond Damadian and all the members of his Fonar Corporation for their continued support, Margaret Ochsenfeld and Dr. Xhen-dong Chen for their dedicated and skilful help and valuable scientific contrituions.

Endnote 1.

The list of ten biology textbooks that Project 2061 has examined and found rich in details but short on "big ideas."

(1) *Biology* (Miller-Levine) Prentice Hall, 1998; (2) *Biology: A Community Context*, South-Western Educational Publishing, 1998; (3) *Biology: Principles and Explorations*, Holt, Rinehart & Weston, 1996; (4) *Biology: The Dynamics of Life*, Glencoe McGraw-Hill, 1996; (5) *Biology: Visualizing Life*, Holt, Rinehart & Weston, 1998; (6) *BSCS Biology*: A Human Approach, Kendall Hunt, 1998; (7) *BSCS Biology*: *An Ecological Approach*, Kendall Hunt, 1998; (8) *Health Biology*, D.C. Health & Co., 1991; (9) *Insights in Biology*, Kendall Hunt, 1998; (10) *Modern Biology*, Holt, Rinehart & Weston, 1999.

Four college biology textbooks examined by GL:

(1) *Molecular Biology of the Ce*ll, 3rd ed., Garland Publishing, Inc. 1994; (2) *Biology*, 5th ed., Addison, Wesley Longman, Inc. 1999; (3) *Essential Cell Biology*: *An Introduction to the Molecular Biology of the Cell*, Garland Publishing, Inc. 1998; (4) Molecular Cell Biology, W. H. Freeman and Co., 2000.

References

Abderhalden, E. (1898) *Z. physiol. Chem. 25:* 65 (p. 100).

Bayliss, W.M. (1927) *Principles of General Physiology* 4th edition, Longman,Green and Co., London.

Boyle, P.J. and Conway, E.J. (1941) *J. Physiol. 100:* 1.

Brooks, S.C. (1939) *J. Cell. Comp. Physiol.* 14: 383.

Cohn, W.E. and Cohn, E.T. (1939) *Proc. Soc. Exp. Biol. Med. 41:* 445.

Fermi, E. (1936) *Thermodynamics,* Dover Publications, Inc. New York, p. 11.

Findlay, A. (1919) *Osmotic Pressure* 2nd edition, Longmans, Green, Inc., London.

Glynn, I.M. and Karlish, S.J.D. (1975) *The Sodium Pump* in *Ann. Rev. Physiol. 37:* 13.

Gore, A. (1962) *An Inconvenient Truth; the Planetary Emergency of Global Warming and What We Can Do about It*, Rodale, Emmaus, PA.

Heppel, L. (1939) *Amer. J. Physiol. 127:* 385.

Heppel, L. (1940) *Amer. J. Physiol. 128:* 449.

Jones, A.W. (1965) Ph. D. Thesis, Appendix, University of Pennsylvania, Philadelphia.

Kaplanskii, S. Ya. and Boldyreva, N. (1933) *Fiziol. Zh. 16:* 219.

Kaplanskii, S. Ya. and Boldyreva, N. (1934) *Fiziol. Zh. 17:* 96.

Ling, G.N. (1952) In *Phosphorus Metabolism* (W.D. McElroy and B. Glass, eds.), Vol. II , p. 748, Johns Hopkins University Press, Baltimore, Maryland.

Ling, G.N. (1962) *A Physical Theory of the Living State: the Association-Induciton Hypothesis,* Blaisdell Publishing Co., Waltham, MA.

Ling, G.N. (1997) *Physiol. Chem. Phys & Med, NMR 29:* 123. Available via http://www.physiologicalchemistryandphysics.com/pdf/PCP29-2_ling.pdf

Ling, G.N. (2001) *Life at the Cell and Below-Cell Level: The Hidden History of a Fundamental Revolution in Biology,* Pacific Press, New York.

Ling, G.N. (2006) Chapter 1, A Convergence of Experimental and Theoretical Breakthroughs Affirms the PM Theory of Dynamically Structured Cell Water at the Theory's 40th Birthday in: *Water and the Cell* (Pollack, G.H., Cameron, I.L. and Wheatley, D.N., eds.) Springer Verlag, Berlin, New York.

Ling, G.N. and Bohr, G. (1969) *Physiol. Chem. Phys. 1*: 591.

Ling, G.N. and Gerard, R.W. (1948) *J. Cell. Comp. Physiol. 34:* 383.

Ling, G.N. and Walton, C. (1975) *Physiol. Chem. Phys. 7:* 215.

Ling, G.N., Miller, C. and Ochsenfeld, M.M. (1973) *Ann. N.Y. Acad. Sci. 204:* 6.

Ling, G.N., Niu, Z. and Ochsenfeld, M. (1993) *Physiol. Chem. Phys. & Med. NMR 25:* 177.

Ling, G.N., Walton, C. and Ling, M.R. (1979) *J. Cell. Comp. Physiol. 101:* 262.

Ling, G.N., Walton, C. and Ochsenfeld, M.M. (1983) *Physiol. Chem. Phys. 15:* 379.

Michaelis, L. (1926) *Naturwissenschften 14:* 33.

Miller (1936?) *Plant Physiology* McGraw Hill Publ. Co. New York

Minkoff, L. and Damadian, R. (1973) *Biophysical J. 13:* 167.

Mond, R. and Amson, K (1928) *Pflhgers Arch. ges. Physiol. 220:* 69, p. 78.

National Academy Press (1990) *Fulfilling the Promise: Biology Education in the Nation's Schools.*

National Commision on Excellence in Education (1983) *A Nation at Risk: the Imperative for Educational Reform.*

Nelson, G. (2000) *Big Biology Books Fail to Convey Big Ideas: Reports AAA's Project 2061*. http: project2061.aaas.org.newsinfo.press.r1000627.htm

Ostwald, W. (1890) *Z. phys. Chem. 6:* 71.

Pfeffer, M.F. (1877, 1st ed.) (1921, 2nd ed.) *Osmotische Untersuchungen: Studien zur Zell-Machanik,* Engelmann, Leipzig.

_____. (1985) *Osmotic Investigation Studies on Cell Mechanics* (English transl. by G. K. Kepner and Ed. J. Stadelmann, Leipzig), van Nostrand, Rheinhold, New York.

Ponder, E. (1948) *Hemolysis and Related Phenomena,* Grune and Stratton, New York .

Project 2061, AAAS (1990) *Science for All Americans*, Oxford University Press, Oxford, New York.

Project 2061, AAAS (1993) *Benchmarks for Science Literacy* Oxford University Press, Oxford, New York.

Steinbach, H.B. (1940) *J. Biol. Chem. 133:* 695.

Traube, M., (1867) *Arch. Anat. Physiol. u Wiss. Med.* 87–128; 129–165.

van't Hoff, J.H. (1887) *Z. phys. Chem. 1:* 481.

van't Hoff, J.H. (1888) *Phil. Mag. 26:* 81.

Wu, H. and Yang, E.F. (1931) *Proc. Soc. Exp. Biol. Med. 29:* 248.

Received November 10, 2006;
accepted December 19, 2006.