Life

at the **Cell** and **Below-Cell** Level

The **Hidden History** of a
Fundamental
Revolution in **Biology**

Gilbert N. Ling

Pacific Press
New York

Original edition, 2001

Grateful acknowledgment is made to the following for permission to reprint excerpts from: *Medicinal Chemistry*, 2nd Ed., edited by Alfred Burger. Copyright ©1960, Interscience Publishers, Inc. Reprinted by permission of John Wiley & Sons, Inc. *What is Life? With Mind and Matters and Autobiographical Sketches* by Erwin Schrödinger. Copyright ©1967. Reprinted with permission of Cambridge University Press. *Albert Einstein*: *Out of My Later Years* by Albert Einstein. Copyright © 1956 by the Estate of Albert Einstein. Permission granted by the Albert Einstein Archives, The Jewish National and University Library, The Hebrew University of Jerusalem. *Biological Symposia*, Volume 3. Copyright © 1941. Reed Elsevier Inc. Quote from Robert Dean excerpted with permission of R. R. Bowker, a Division of Reed Elsevier Inc. *Nerve, Muscle and Synapse* by Bernard Katz. Copyright © 1966. Reprinted by permission of the McGraw-Hill Companies. *Problems of Cell Permeability* by A.S. Troshin Copyright © 1966, Pergamon Press. Permission granted by the Estate of A.S. Troshin. *Journal of the American Chemical Society,* Volume 77. Copyright © 1955, The American Chemical Society. Quote from Bertil Jacobson, excerpted with permission from, the Journal of the American Chemical Society. *History of Physiology* by Karl E. Rothschuh. Copyright © 1966. Reprinted by permission of Krieger Publishing Co. Further permissions to reprint previously published material will be found in Figure and Table legends.

Library of Congress Cataloging in Publication Data.
Ling, Gilbert N.
Life at the Cell and Below-Cell Level: the Hidden History of a Fundamental Revolution in Biology:/Gilbert N. Ling. 1st ed.
p. cm.
includes bibliographical references and index
LCCN: 00-093519
ISBN: 0-9707322-0-1

1. Cell physiology. 2. Cytology. 3. Biophysics. 4. Biochemistry. I. Title.
QH631. L56 2001 571.6
QB101-200233

Manufactured in the United States of America
Typeset by Farm River Publications, Branford, Connecticut.
Printed by Cushing Malloy, Inc., Ann Arbor, Michigan
Cover design by G.N. Ling

To my wife, Shirley

Contents

Preface

MRI (Magnetic Resonance Imaging), now a household word, is a new medical technology that is saving human lives every day. Invented by Dr. Raymond Damadian and technologically improved upon by Dr. Paul Lauterbur, MRI originated from a physico-chemical theory of life called the *association-induction hypothesis*[107 p xxv n1]—first introduced in 1962 in a book entitled: *A Physical Theory of the Living State: the Association-Induction Hypothesis*.[98] Professor Ralph W. Gerard of the Department of Physiology at the University of Chicago wrote a Forward for this book, which ends with the following passage:

> "*...Thus there must be some very comprehensive and basic principles at the molecular level that underlie and illuminate all the special manifestations of living systems. Ling offers no less than such a general molecular theory of life phenomena.*" [98 p ix]

In addition, Professor C. N. Yang—my onetime university roommate, fellow winner of the Boxer Scholarship to further study in the US, Nobel Laureate of 1957 in physics, author of the Yang-Mills *non-Abelian gauge theory* (widely regarded as a contribution as important as Einstein's theory of relativity[551])—wrote another endorsement for my book:

> *"At a time when we look forward to the merging of the physical and biological sciences, this is a most stimulating book, distinguished by a bold and inquisitive attitude on the one hand, and careful experimental methods on the other."* [98 *dust jacket*]

(No one could have foreseen that the theory Yang endorsed was to spawn a medical technology (MRI), which forty years later would play a key role in saving the life of Yang's wife, Chili.)

Endorsements notwithstanding, the crucial question remains: "Is the association-induction hypothesis correct?" Fifty years after the 1952 publication of the embryonic version of the association-induction hypothesis known as Ling's Fixed Charge Hypothesis (LFCH), I can answer without hesitation: YES, in essence. Yet, this theory, as well as the results of its world-wide confirmation— it has been published in scientific journals here and abroad, article by article, spanning more than half of the 20th century—is known to only a few, unbelievable as this may seem. To break through this wall of unnatural opaqueness[247] and to reveal the shining truth, so-long hidden from so many, is the first goal of publishing and publicizing this volume, in which the most up-to-date version of the association-induction hypothesis and its key supportive evidence make up five of the seventeen chapters. Together they offer a bird's eye view of the entire history of Mankind's search for understanding of its priceless possession, life itself—at the most basic level.

However, philosophers and scientists did not always agree that life can be explained in physico-chemical terms. As late as the first half of the 19th century, *vitalism* still had a strong following.[3 pp 219–287] Its proponents argued, for example, that the latent equivalent of life or *causa vitae* can be recognized for what it does but cannot be explained (in physico-chemical or any other terms); just as gravity can be recognized for what it does but cannot be explained.

The ablest opponents to vitalism include: Hermann von Helmholtz (1821–1894); Carl Ludwig (1816–1895); Emil Du Bois-Reymond (1818–1896) and Ernst von Brücke (1819–1892). United as if they were one, the four firmly believed, and brilliantly defended their view, that the laws governing the inanimate world govern the living world also. Their success in their respective pursuits is witnessed by the fact that two of them were elevated to the rank of aristocracy —Helmholtz by the German Emperor, William I and Brücke by the Austrian Emperor, Francis Joseph. Still, the Reductionist Four did not succeed in explaining life in physico-chemical terms. The time was still not right.

First, the Reductionist Four (and most of their peers) were studying *organ physiology* while the foundation of life lies at the *cell and below-cell level*. And in their time, the study of cells and cell constituents had barely begun. Second, despite the great genius of Helmholtz as a physicist *and* physiologist, the physicochemical knowledge needed was not yet available.

Both of these handicaps were gone by the time my generation of cell physiologists appeared on the scene. Advances in basic physics and chemistry made possible the formulation of the association-induction hypothesis; advances in experimental biology furnished the isolated living cells and subcellular preparations on which to test the predictions of this hypothesis. The extensive confirmations of the association-induction hypothesis have, therefore, not only vindicated the Reductionist Four, they have also heralded the arrival of a new era of united physical and biological sciences into one single coherent SCIENCE. (See Epilogue of this volume also.) But, aside from fulfilling a long-sought philosophical goal, one may ask, What practical good can this unification produce that a segregated science cannot? The answer is, Probably more than what one can fathom at this time.

In the beginning, the separation of science into physics, chemistry, biology etc., was not bad. On the contrary, this simplifying approach made possible major progress in the last two centuries. Still, segregation means fragmentation. Long continued, fragmentation is a dead end. Thus without input from other branches of science, each of the isolated subgroups would sooner or later reach the end of the road. This is not just my private fear; there are already signs of this happening.

In 1996 the highly-respected Addison-Wesley Publishing Company introduced a book by John Horgan under the title: *The End of Science.*[380] As a reporter for the popular magazine: *Scientific American*, Horgan conducted exclusive interviews with more than forty *outstanding* scientists, including physicists (Freeman Dyson, Murray Gell-Mann, Stephen Hawkins), biologists (Stephen Jay Gould; John Eccles; Stanley Miller), science-historians (Thomas Kuhn, Karl Popper; Paul Feyerabend) and other illustrious scholars. On the reasonable assumption that Horgan had not misrepresented those he interviewed, they and Horgan apparently share the view given in the subtitle of the book: *Facing the Limits of Knowledge in the Twilight of the Scientific Age*. In my view, this belief in the imminent end of science from such an elite group of opinion-makers is a most dangerous misconception. And, if not corrected soon, it may inflict serious harm on many, if not everyone.

The belief in an impending end of science is the offspring of the union of two man-made artifacts: (i) the fragmentation of science and (ii) blindness to the association-induction hypothesis. The fragmentation of science produces the premature end of physics and chemistry. Oblivion to the

association-induction hypothesis falsely equates the dying of an obsolete but widely-taught (membrane) theory to the dying of biology itself (see below). In fact, the association-induction hypothesis has been steadily gathering support throughout the second half of the 20th century without major setbacks. This alone has demonstrated amply that not only biology but SCIENCE as a whole is steadily growing (though at a rate artificially made much slower than what could be[247]). But so far very few know this "good news;" many more know Horgan's (mistaken) "bad news."

For, if basic science is reaching its end, why should the public continue to support it financially? If basic science is reaching its end, why should young people choose basic science as their future career? With dwindling financial support and declining numbers of fresh recruits, even the healthiest science may very well perish as *The End of Science* has forecasted.

If perchance we lose basic science, (i) With what are we to sustain the prosperity and well-being of the modern society? (ii) With what are we going to provide for its ever-increasing population in the face of ever-diminishing natural resources, especially oil and natural gas[552]? (iii) With what are we to cope with the uncertain climatic environment that the burning of fossil fuels has helped to create[553]? Sure, we have marvelous and powerful technology but the existing technology was derived from basic science of the past. To cope with unfamiliar new crises, new technology will be needed (in addition to old technology) and truly new technology can only come from new basic science.

To help forestall this calamity (mistakenly shutting down basic science) is then the second goal for publishing and publicizing this book; but there are still other reasons. For, if I am not mistaken, the man-made blindness to real progress in basic biology over such a long period of time (see text and Reference 247) has produced still other damaging consequences to, for example, education.

On July 28, 2000 in major newspapers across the nation, Anjetta McQueen of the Associated Press reported[554] that a study by the American Association for the Advancement of Science (AAAS) (Project 2061 headed by Dr. George Nelson) had revealed something shocking: "Big Biology Books Fail to Convey Big Ideas." [555] Of the ten widely adopted textbooks reviewed, not one escaped the indictment.

Jean Baptiste Lamarck (1744–1829) invented in 1802 the word, *biology*.[3 p 144] Then and now, biology has one meaning: *the science of life*. Therefore, no idea in biology can be bigger than *what is life* at the most ba-

sic level and in scientific terms. With this in mind, one sees that the blame for the failure of the biology textbooks to convey "big idea(s)" could not be the fault of the textbook-writers, nor the textbook publishers, nor even school board members who decide on what to teach—for if individuals in one or the other of these categories were the culprits, some would certainly have done the right thing and produced a good textbook containing the correct "big ideas." The failure to produce a single good textbook with the correct "big ideas" indicates that the cause of failure must be deeper.

In my view, the trouble originates from the version of biology that is still widely if not universally taught, a version in which the truly "big idea" on life has never been developed (and probably never will be) and therefore is wanting (See Introduction.). In contrast, the association-induction hypothesis—which is, as pointed out above, largely hidden from public knowledge—offers a clear-cut description of life at the most basic level in terms of modern physics and chemistry. Thus, the third objective in publishing and publicizing this volume is to come to the aid of high-school and college biology students and teachers, future biology textbook writers as well as school-board members in overcoming this crippling deficiency. Through them and in time we hope that a new generation of young scientists will be adequately, indeed superbly, equipped to meet head on the new world that is soon to challenge us all as never before.

Now a few words on the association-induction (AI) hypothesis. In the wake of the earlier embryonic version (LFCH) mentioned above,[94; 96] the AI hypothesis was published in 1962.[98] However, it was not until 1965, after the subsidiary theory of cell water had been added, that the association-induction hypothesis became complete, and as such, history's first *unifying* physico-chemical theory of life at the cell and below-cell level was born.

The first word of the title, *association*, denotes that the three predominant components of the living cell—proteins, water and potassium ions—are in close contact or association with each other and are not free. Therefore, this contradiction of the widely accepted belief of free water and free ions is *revolutionary*. The second word, *induction*, indicates that the living cells and their constituent parts are *electronic* machines, where long-range information and energy transfer involved in the functional activities is achieved by repetitions of short-range propagation of electrical polarization and depolarization or *induction*. Since there is no counterpart of this arm of the AI Hypothesis in the conventional theory of the living cell, the inductive component of the association-induction hypothesis is

not revolutionary but—surprising as it may sound—"castle-building" in a frontier land that has not been explored before.

Further theoretical developments and the results of world-wide experimental testing of the predictions of the association-induction hypothesis culminated in the publication of a second book, *In Search of the Physical Basis of Life*[15] (Plenum Publ. Co., New York). Another eight years later, a third book announced the completion of *A Revolution in the Physiology of the Living Cell*[107] (Krieger Publ. Co., Malabar, Florida).

As they are, each of these three books has served a specific purpose. The 1962 book (no longer in print, though at the time I am writing this, a few copies are available at the Barnes and Noble website) launched the association-induction hypothesis. The 1984 book, 791 pages long, widened its scope and broadened its applications. The 1992 book, 378 pages long, rigorously details the completion of the revolution. Though each of these three books contains materials needed for the full understanding the AI Hypothesis not found in the other two, to serve the specific threefold objectives described above, a new (fourth) book is needed. And this volume is it.

To succeed in achieving the triple goals described, the book must be able to reach people from widely different backgrounds. Who are they? Foremost on my list of prospective readers are high school and college biology teachers and school board members, because it is in their hands the future lies. All are in one way or another committed to teaching the correct important ideas and facts in basic biology and to infusing students with a sense of wonder and excitement in exploring the *last* great frontier of unexplored relevant truth. So that one day the students of today and scientists of tomorrow would be able to, for example, design drugs to cure cancer, AIDS, mad-cow disease, Ebola etc., etc., as easily as a competent radio repairmen fixes a broken transistor radio.

Beside teachers and school board members, there are new and established research scientists who might have been frustrated and discouraged by observations that could not be fitted into the framework of textbook knowledge he or she had learned in earlier days and saw no future in continuing their work. Physicians who seek to understand illnesses but cannot succeed fall into another category. They might, for example, be puzzled by the success of alternative medicine while conventional approaches have failed. Then there are the especially motivated high-school students, college biology majors, premedical students, graduate and post-doctoral stu-

dents in addition to the still larger body of simple, intelligent people who are not satisfied with knowing little about what makes their own brains, muscles and kidneys work, and who want to know more. All of these people may benefit to varying but significant degrees from the new paradigm of the most basic aspect of the life sciences presented in this volume.

As pointed out above, this volume is presented as a story or history starting from the moment Mankind began to peek into the microscopic world of cells and microbes with the invention of microscopes—and even earlier, much earlier—continuing through landmark events of false starts and new insights put away for the wrong reasons etc., etc., culminating in the association-induction hypothesis of today.

To serve the needs of these and other diverse groups of potential readers, I have made a serious effort to insure mastery of the book by readers from widely diverse backgrounds—as explained in the immediately following section entitled: *Answers to Readers' Queries.*

Answers to Readers's Queries (Read First!)

The in-depth and extensive experimental confirmation of the essence of the physico-chemical theory of life, which this volume embodies, marks the beginning of a new era in biology (See Preface). To participate in this exciting adventure, however, one needs certain simple basic knowledge of biology as well as of physics and chemistry. For some, all the required knowledge may be old hat; for others, part, or even all of it, may not be. Since neither the required basic biology nor the required basic physics and chemistry is difficult to master, for those who need it, this volume provides an easy, do-it-yourself course centered around an attached customized dictionary which I call a *Super-Glossary*. This *Super-Glossary* will answer all the questions a reader is likely to raise and thus enable him or her to move ahead without difficulty. A guideline for this self-teaching course is given in the form of answers to questions raised by several readers.

(1) Is there some specific order that you recommend for a first-time reader?

Answer: Yes. For the first reading, I recommend that you start with the Epilogue (Chapter 17) at the end of the text, followed by the Summary Plus (Chapter 16) and then the Preface. This way you will start with a perspective for what is to follow. After the Preface, read this *Answer to Readers' Queries* once more before starting to read the main text.

(2) What can I do if I get stalled on a word or a concept but cannot find it in my dictionary or textbooks I have?

Answer: Make a light pencil mark on the incomprehensible item and leave a stick-on tag on the page on which it is found. And then read on until you come across another "roadblock." Repeat the same procedure—again and again—until you feel that you have covered

enough pages for this time. Stop and consult the *Super-Glossary* on p. 288 near the end of the book, which contains more than 900 (and thus virtually all) the technical words, terms, basic concepts etc. used in the writing of this volume. Again, make light pencil marks, this time on the incomprehensible items in the *Super-Glossary*. Armed with the explanations of the incomprehensible items, read the sections they appear in once more before moving forward again. If you follow this suggested routine, obviously you won't get stuck anywhere for any length of time (and thus become discouraged and stop reading.) The pencil marks and stick-on tags will also make the book a part of you and the next reading will be easier, until a full and complete understanding is achieved. In addition, you will be practicing a basic step of *self-education*—a vital skill if you plan to venture into totally unexplored new realms of knowledge in the future.

If even the *Super-Glossary* cannot offer you the answer to the question that puzzles you, explain to me your problem *via* an e-mail to gilbertling@dobar.org or a regular letter (110 Marcus Drive, Melville, NY 11747). I will try my best to respond to you as soon as possible and will thank you for helping me to launch a better *Super-Glossary* in the next edition of the book.

(3) Where can I find help if I come across an abbreviation like LFCH a second or third time but cannot remember where I first saw the one with an explanation attached, nor the explanation?

Answer: There is a *List of Abbreviations* (on p. 330 just after the *Super-Glossary*) where all the abbreviations used in the volume are listed and explained. The *Subject Index* may also be helpful.

(4) If I want to pursue further a subject mentioned in the book, can I find the original article in which the finding was first described?

Answer: Yes. A *Bibliography* with more than 550 single or multiple references is provided. The references are cited as superscripts in the text with the reference number followed by a page, figure or table number as the case may be. The information should guide you to the original article. For articles originating from my laboratory, you may send me a request card identifying the article. I will send you a reprint if I still have one.

(5) What does *Appendix 1* contain?

Answer: In *Appendix 1*, I have gathered together most of the equations in one way or another related to the content of the book. Familiarity with them is not necessary for the mastery of the book, but may be of interest to those wishing to know more.

(6) What does a symbol like [14.3(2)] stand for?

Answer: [14.3(2)] means subsection 2 under section 3 of Chapter 14. One easy way to locate it is to consult the *Table of Contents* and find the number of the page on which this specific subsection is located. The Chapter number indicated on the upper right corner of each right-hand (recto) page may also be helpful.

(7) I noticed that the legends to the figures and tables are often much longer than in an ordinary scientific article. Is there a specific reason for these lengthy legends?

Answer: Yes. Each legend has been expanded from the one originally found with the figure or table. More details are added to make the text presentation as short and succinct as possible.

(8) In what way can I benefit from the List of Figures, Tables and Equations?

Answer: In this volume, a figure (or table or equation) first introduced, say on page 10, may be referred to again and again on pages farther beyond. The List of Figures, Tables and Equations on p. 333 gives you the number of the page where that specific figure is presented (the first time) and thus saves you time and effort in locating that figure (or table, or equation).

Introduction

With no exception, each one of us began as a single cell. That tiny fertilized egg, scarcely larger than a speck of dust, then divided into two daughter cells. The daughter cells grew and divided into four cells. And this repeated itself many times over. Somewhere in the course of the development, the daughter cells ceased to be copies of each other through differentiation. Division, growth and differentiation over a period of nine months, transformed that single fertilized egg cell into trillions upon trillions of cells of diverse shapes and functions. Together they made up or will make up the baby that was you, I and anyone else who is living, had lived or will live in time to come.

Smiling, laughing and other normal physiological activities tell us that a baby is well. This is just a short way of saying that the trillions of cells making up the baby are well. Similarly, when the baby is sick, it is a short way of saying that some or all of the baby's cells are sick. When we give medicine to the sick baby (or sick grown-up), our hope is that the medicine will make these sick cells well again. But unfortunately we are not sure.

Why are we not sure? For an answer, let us consider one illness, cancer.[247]

From the time of her birth to her death, a female American child has approximately a 1 in 3 chance of contracting invasive cancer; a male child, 1 in 2.[358 table 5] In America alone, cancer kills 1500 men, women and children *everyday*, notwithstanding America has the best medicine of the world. Beginning in the 70's, a quarter of a century of "war on cancer" did not bring victory. On the contrary, the cancer death rate rose from 158 per 10,000 population in 1970 to 210 in 1997.[553] Part of the increase can be attributed to the aging of the population and the fact that older people are more cancer-prone. But that alone cannot explain why a Nation so proficient in other difficult scientific undertakings—be it the landing of a human on the moon, the creation of computer science and technology or the unraveling of the human genome—should be so bogged down in

attempting to control diseases from the common cold to cancer. There must be a special cause for this pointed tardiness. What can it be?

Prof. Alfred Burger offered a clue. In his monumental treatise on thousands of drugs, "Medicinal Chemistry," he wrote: "*Almost all the problems of medicinal chemistry would become more amenable if we had even an inkling of the reactions of any drug with body chemicals.*"[345 p 19]

By *body chemicals,* Prof. Burger referred to the chemical components of the living cells. The failure to gain an *inkling* of how *any* drug works reveals something few are aware of: an unbelievably poor understanding of how the living cell functions in general and its response to drugs in particular—as currently taught at virtually all levels of education under the heading of *cell physiology* or another name to the same effect..

Thus the magical formula, which gave us the "many-splendored" world of today, seems to have lost its magic in this specific quest for scientific truth. That magical formula was invented in the 17th century in Europe and is known as the *scientific method.*

The scientific method comprises four steps: (i) observation; (ii) introduction of an explanation of the observation, known as hypothesis or theory, which also generates testable predictions; (iii) experimental testing of these theoretical predictions; (iv) conclusion based on the result of the experimental testings, which confirm or refute the theory.

Not explicitly stated, but implied as a matter of course, is a crucial fifth step. *A theory that has been unequivocally disproved by extensive experimental testings must be replaced by one more promising. Or better, by one that has already been consistently confirmed.* It is my opinion that the current poor understanding of cell functions mentioned above reflects a failure to implement the fifth step when due.

The science of how living cells work (cell physiology), currently taught at different levels of education with varying degrees of detail, is founded on an old theory. Beginning well over a century ago, it is known as the *membrane theory*. Later it was modified and called the *membrane-pump theory*. Some 50 years ago I first published my suspicion that the cell may not have enough energy to operate the pumps postulated in this membrane-pump theory.[96] The initial response,[382] which was quickly answered,[550] did not continue, but petered out into total silence. As years went by, the contradictory evidence against the membrane-pump theory have grown in number and in consistency (see Chapter 12). Nevertheless, the membrane-pump theory continues to be taught as the truth by many

innocent teachers all over the world. And it still serves as the foundation for research on cancer, AIDS, and other deadly diseases by mostly innocent investigators.

By the same token, the great majority of teachers, students and researchers do not know that an alternative theory—already extensively tested and verified *in its essence*—has been in existence and steadily developing for nearly 40 years. It bears the name, the *association-induction (AI) hypothesis*. Unlike the membrane-pump theory, the AI Hypothesis does offer, from its very inception, a broad outline of a theoretical mechanism of how drugs work, based on state-of-the-art understanding of modern physics and chemistry (Section [14.3(3)]).

No one can tell how much closer we would be to conquering cancer, AIDS and other fatal diseases if, say, half of the money, talent and effort spent on research projects based on the membrane-pump model had been diverted to support projects based on the association-induction hypothesis. Nonetheless, history shows that useful inventions as a rule follow progress in pure science quickly. Thus 50 years after Michael Faraday discovered magneto-electric induction (1831), electric power plants came into being (1880).[54 p. 196] Thirty years after Maxwell introduced his unified theory of electromagnetic waves (1867–1873), Marconi obtained a British patent for the future radio industry (1900).[254] Only 15 years after the introduction of the association-induction hypothesis (1962), Raymond Damadian invented the medical breakthrough now known as magnetic resonance imaging or MRI[15 p vii; 98 p xxv] (1977) [11.3(1)]. (In contrast, despite a far longer history, neither the original membrane theory nor its pump version has produced anything useful to the best of my knowledge.)

Thus pure science, if wisely and unwaveringly nurtured, will continue to spawn new inventions, which in return will nurture and protect our species as well as our fellow living creatures who share this beautiful but fragile planet with us. That is, if we do not forget that to reach these goals it takes more than just money—though money is indispensable—but *human* effort and dedication as well. And over time, nothing can replace a continual flow of young talents who are eager to engage in this, the greatest adventure of all—the search for (relevant) scientific truth in the service of Mankind, living and yet to come.

This volume provides a summary of the (scientific) rise and fall of the membrane (pump) theory, the emergence and growth of the association-induction hypothesis, and important but nearly forgotten

alternative theories and their experimental support. It is written with the hope that a reader familiar with its contents knows what is, in my opinion, the *essence of all cellular and subcellular physiology*—from its beginning (and even earlier) to this day. In short, this is a story of the largely *hidden* saga of man's search for understanding of life at the most basic cell and below-cell level.

> "It is highly dishonorable for a Reasonable Soul to live in so Divinely built a Mansion as the Body she resides in, altogether unacquainted with the exquisite structure of it." (Robert Boyle, 1627–1691)[369]

> "...we have this further duty, the care for what is eternal and highest amongst our possessions, that which gives to life its import and which we wish to hand on to our children purer and richer than we received it from our forebears." (Albert Einstein) [365 p 151]

> "Unfortunately, school teaching keeps up certain traditional views , which have been out of date for many years and which obscure the understanding of the actual state of affairs." (Erwin Schrödinger on school teaching of physics in 1944)[363 p 58]

> "Science has become the most distinctive of human activities, and the indispensable tool for the survival of the billions who now inhabit the planet." (Charles Van Doren). [364 p xxiv]

1

How It Began on the Wrong Foot—Perhaps Inescapably

Around 1609 Zacharias Janssen and Galileo Galilei (1564–1642) independently invented the compound microscope.[367] Through such a microscope, Robert Hooke (1635–1703)—described as "a Person of a prodigious inventive Head, so of great Virtue and Goodness"[459, 3 p 295]—saw tiny air-filled pores in a piece of cork and called them *cells*. In the same volume, "Micrographia," in which Hooke published his microscopic observations in 1665,[368] he also used the same word, cells, to describe fluid-filled "pores" in the pith of carrot, fennel, fern and the like, believing that these "cells" represent avenues of communication in plants.

A decade later, Antony van Leeuwenhoek (1632–1723), seeking the cause of the hotness of pepper, discovered under a single-lens microscope, little animalcules (bacteria) in a watery extract of hot pepper.[367] He did not know that each of these animalcules comprised just a *single cell*. For, among other reasons, it was long before the word, "cell," acquired the meaning it has today.

In fact, between the later part of the 17th century and the early 19th, different views emerged on what were called cells. von Haller,[309 p 393] Grew[3 p 180] and later Brisseau de Mirbel[460] believed as Hooke himself had believed, that cells are fluid-filled cavities or spaces. Malpighi and Moldenhawer, on the other hand, contended that cells are closed sacs or utricles.[3 pp 180–184]

When G. R. Treviranus demonstrated in 1805 that the membrane between two neighboring cells in the buds of a buttercup plant is in fact double and that the neighboring cells could be torn apart without damaging either one,[461] the cells-as-entities theory gained ground. Treviranus's discovery undoubtedly accelerated the broad acceptance of cells as entities. Nonetheless, one hesitates to describe this event as a triumph of one view over another. It is possible that they were not dealing with the

same object.[462; 463] One group might have focussed on the cross-sections of what were later named xylem and phloem; the other group on true living cells as we now know them.

Between 1835 and 1840, two basic defining biological concepts were formally introduced: Theodor Schwann's Cell Theory,[1; 335] according to which cells are the basic units of all animals and plants and Felix Dujardin's sarcode,[2] as an even more fundamental building block of life. Their historical importance notwithstanding, neither concept arose *de novo* at the time (see Chapter 2 and Figure 72).

From Robert Hooke on, *mature plant cells* were a favorite material in early microscopic studies of living matter. Their large size, their well-defined boundaries, their ready availability and their good keeping quality offered compelling reasons for their choice in cell studies. And with it, a hidden pitfall—as will be made clear presently.

Schwann's focal interest was on (mature) plant cells.[1] Dujardin, on the other hand, studied the kind of *Infusoria*[323] known as protozoa today. These unicellular organisms, when crushed, spilled out a gelatinous, water-immiscible material, which Dujardin described as *gelée vivante* (living jelly) and named *sarcode.*[2]

Schwann believed that the containing membrane of a cell (and that of its nucleus) is of overriding importance over (what he believed to be) a homogeneous, transparent liquid filling in the space (Zwischenraum) between the nuclear and cellular membrane.[3 pp 193–194] True, a *mature* plant cell[4] like that shown in Figure 1A resembles Schwann's notion of "one hollow cell inside a hollow cell."[3 p 193] However, young plant cells—like all animal cells—are solid bodies. About a decade after Dujardin announced his sarcode, two botanists, Karl von Nägeli and Hugo von Mohl also described a viscous fluid in *young* plant cells, which von Mohl called *protoplasm.*[6,7] Following an extensive comparative study, Ferdinand Cohn concluded that the animal sarcode and the plant protoplasm are the same.[8] Robert Remak then suggested that both be called *protoplasm.*[9]

In the years following, improved microscopes have extended both the variety of living cells studied and the accuracy of the (reported) observations made. In consequence, new insights evolved on the nature of a *typical* living cell. In 1857 Franz Leydig announced: "Cell contents are of higher dignity (Webster's Dictionary:[404] a higher rank) than the membrane."[10] In 1861, Max Schultze (1825–1874) pronounced his famous "*protoplasmic doctrine.*" To wit, cells are "naked little lumps of

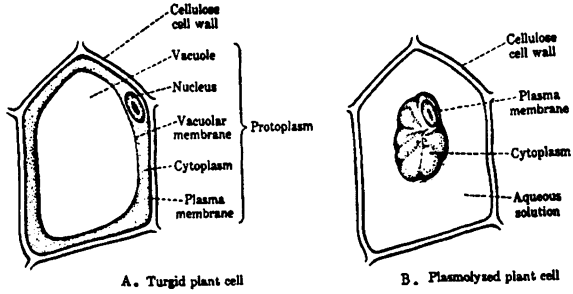

Figure 1. A mature plant cell undergoing plasmolysis. The central vacuole is marked as "vacuole" in A. In B, the shrunken cytoplasm, nucleus, etc. still enclosed in the plasma membrane makes up the "protoplast." (Glasstone[13])

protoplasm with a nucleus."[11] In the views of both Leydig and Schultze, cells do not possess a covering membrane chemically different from the protoplasm.[3 p 200]

By the turn of the century, there was agreement among microanatomists on the *solid* nature of most living cells. Thus, in introducing his monumental treatise, "The Cell in Development and Heredity," American cytologist, E.B. Wilson pointed out in 1928 that the name, *cell*, is a misnomer, because cells "do not, in general, have the form of hollow chambers as the name suggests but are typically solid bodies."[12 p 4]

2

The Same Mistake Repeated in Cell Physiology

Thus far, we have been discussing the structure or anatomy of the living cell. As already mentioned, the science describing how cells function is *cell physiology*. The understanding of cell physiology rests upon the understanding of *cell anatomy*. Obviously, a sound cell physiology cannot be built upon an unsound cell anatomy.

Under ideal conditions, cell physiological studies should begin *after* the anatomy of a typical living cell has been fully and accurately grasped. In reality, the studies of cell physiology began *long before* the recognition that a typical living cell is solid. Not surprisingly, the appeal of mature plant cells, which had captivated the early cell anatomists, was not lost on the early cell physiologists. Like the early cell anatomists, the early cell physiologists also made the mistake of generalizing what they discovered in the atypical mature plant cell as typical of all living cells. From such a viewpoint, a fluid-filled pig's bladder like the one described next could be seen as a model of all living cells.

Abbé Nollet (1700–1770), Preceptor in the Natural Philosophy to His Majesty Louis XV of France, and the scientific opponent of Benjamin Franklin (1706–1790) on electricity, was the first to record in 1748 an experiment on *osmosis*—even though the term, osmosis, was not yet invented (see below). Nollet immersed in water a pig's bladder filled with water and (grain) alcohol. He discovered that water moved across the bladder wall to the inside of the bladder containing both water and alcohol, but alcohol did not move to the outside containing only water.[13 p 651] Nollet thus discovered an unusual attribute of the bladder wall—an attribute later given the name *semipermeability* by van't Hoff (1826–1894).[14] Nollet's discovery also set the stage for the fateful study of artificial membranes of Moritz Traube. However, neither Nollet's work just described nor that of

Traube to be described in the chapter immediately following involved living cells.

Two early physiological investigators of the real *living* cell were René J. H. Dutrochet (1776–1847)[3 pp 184–188] and Wilhelm Pfeffer (1845–1920).[15 pp 10–11] They too were botanists. And they too worked primarily with mature plant cells. Like Jean Baptiste Lamarck (1744–1829)[308] and Lorenz Oken (1779–1851),[5] Dutrochet had also espoused the idea that cells are the basic units of life—all before Theodor Schwann's definitive study leading to the same identification {and that of Matthias Schleiden (1804–1881)[3 pp188–189]}.

Dutrochet believed that life is *movement* and its *cessation*, death. And he studied the movements of water in and out of mature plant cells and called them respectively *endosmosis* and *exosmosis.*[16] Later, the prefixes were dropped. Spontaneous movement of water in and out of solutions or living cells has been referred to ever since as *osmosis*.

As mentioned, the microanatomists had rather quickly discovered and corrected the error in describing a typical living cell as a fluid-filled chamber. The early cell physiologists did not make a quick correction on their own; nor did they respond to the revised view of the microanatomists. The same mistake committed by early microanatomists was repeated by the cell physiologists. Only this time, the mistake has endured to this very day. Under the banner of the *membrane theory*, or the modified version called the *membrane-pump theory*, the typical living cell is once again *a membrane-enclosed dilute solution.*

3

How the Membrane Theory Began

Moritz Traube (1826–1894), a Berlin tradesman and amateur research scientist, made an elementary but history-making discovery.[17] When a drop of copper sulfate solution is brought into contact with a drop of potassium ferrocyanide solution, a thin layer of reddish-brown *copper-ferrocyanide* precipitate forms at, and blankets, the entire boundary. After that, no further formation of precipitate occurs. Thus the thin layer of precipitated material formed between the two drops of solutions has halted further passage to the other side of the copper ion as well as the ferrocyanide ion.

Traube published his finding in 1867.[17] Its significance was promptly recognized by Wilhelm Pfeffer. By allowing the copper ferrocyanide precipitate to form within the wall of a porous porcelain cylinder, Pfeffer transformed Traube's fragile layer of copper-ferrocyanide precipitate into a practical experimental model strong enough to withstand not only routine handling but even unilateral application of mechanical pressure. Placing sucrose solutions of different strengths on either side of such a fortified copper-ferrocyanide membrane, he saw water moving from the dilute to the concentrated side[18]—reminiscent of what Abbé Nollet witnessed across his dead animal membrane and what Dutrochet observed in and out of living mature plant cells.

Pfeffer also found that this osmotic water movement could be brought to a stop by applying to the side containing the more concentrated sucrose solution a pressure of just enough strength (to be referred to as *osmotic pressure).* Now if one side contains sucrose solutions of different concentrations (represented as C) and the other side contains plain water, or if the temperature was varied while C is held constant, then the osmotic pressure, π, was shown to be proportional to C, and to the absolute temperature, T, respectively.[18]

Dutch botanist, Hugo de Vries (1848–1935), who introduced the important *mutation theory* to genetics,[363 p 34] brought Pfeffer's exciting findings to the attention of physico-chemist, J. H. van't Hoff—whose name was mentioned above. Not long after, van't Hoff discovered that an equation resembling the perfect gas law (i.e., PV = RT, where P is the pressure applied to a (perfect) gas occupying a volume, V, at the absolute temperature, T and R is the *gas constant*) could correctly predict both sets of relationships Pfeffer's meticulous work had brought to light:

$$\pi V = R'T, \qquad (1)$$

where V is the volume of solution containing one mole of sucrose and thus equal to 1/C. π, T have the meanings given above. R′ is a constant. Substituting Pfeffer's experimentally determined values of π, V (= 1/C) and T into Equation 1, van't Hoff obtained the constant R′, which in numerical value, is close to that of the gas constant, R (1.987 cal.deg.$^{-1}$ mole^{-1}).

For the origin of the osmotic pressure, van't Hoff introduced his *bombardment theory*. Pointing out the analogy of the perfect gas law and the van't Hoff equation shown above (Equation 1), he wrote: "In the former case, the pressure is due to the impacts of gaseous molecules on the walls of the containing vessel, and in the latter to the impacts of the molecules of dissolved substance on the semipermeable membrane."[13 p 664; 14] (For unexpected later developments, see [11.3 (7)].)

Thus Pfeffer's accurate study of osmotic pressure paved the way for what is often referred to as van't Hoff's *solution theory*. Continued investigations on both model systems and living (mature) plant cells led Pfeffer to ideas on the living plant cells, which were later referred to as Pfeffer's *membrane theory* (see below).

Pfeffer published his major work in 1877 in a monograph entitled: "Osmotische Untersuchungen" ("Osmotic Investigations").[18] In this volume, his main theme was that a peripheral layer of the protoplasm called *Plasmahaut* (*"protoplasm skin"* as one meaning of the word, plasma, is protoplasm)[404 p 879] or *plasma membrane* covers not only the outer surface of the cell but any exposed surface of protoplasm where it comes into contact with "*another* aqueous solution."[18 p 234] And that a plasma membrane exhibits properties similar to those of the copper-ferrocyanide precipitation membrane. And that it is the universal

presence of this enclosing plasma membrane that endows all living cells with its semipermeable properties and osmotic behaviors.

In "Osmotische Untersuchungen" Pfeffer made no specific mention of the physicochemical nature of the cell content, nor did he mention Schwann by name. Yet in this monograph one comes upon a phrase here and another one there, each suggesting that, like Schwann, he too believed that the cell content has the property of an aqueous solution. Thus by referring to an aqueous solution in contact with the protoplasm as "another aqueous solution" just cited above, he offered one hint. He offered a second hint in his Summary of the second and last section entitled "Physiological Part" in these words: "Since protoplasm is also delimited from the cell sap by a plasma membrane, the cell osmotically resembles a system formed out of two cells of different sizes filled inside each other"[18, 1985 p 235]—reminiscent of Schwann's "one hollow cell inside another hollow cell" imagery. Yet the smaller "cell" is unquestionably filled with an *aqueous solution* (the cell sap) and the larger one with *protoplasm*. If Pfeffer saw fundamental differences between the two contents, he made no mention of it.

The membrane theory has been widely attributed to Pfeffer, even though the term, membrane theory, was not cited in Pfeffer's "Osmotische Untersuchungen" published in 1877 nor in the second edition reissued unaltered in 1921, long after Pfeffer had "moved away" from the study of osmosis.[18 pp xxii–xxiii]

The long history of visualizing living cells as fluid-filled vesicles, buttressed by the elegant solution theory of van't Hoff, provided what appeared to be an unshakable foundation for the *membrane theory.* Additional evidence came from early osmotic studies and from investigators in other specialized fields of cell physiology. They include *Julius Bernstein*, author of the membrane theory of cellular electrical potentials[19] and *Frederick Donnan*, author of the theory of membrane equilibrium of ionic distribution and electrical potential.[20] That these theories and their corroborative evidence indeed offer support for the membrane theory, follows from the fact that they share the same fundamental assumption that living cells are membrane-enclosed dilute solutions.

The interweaving theories summarized above have also conjointly made the membrane theory the first coherent general theory of cell physiology. This theory can explain on the basis of the simple postulation of living cells as membrane-enclosed dilute solutions, four major subjects of cell physiology: (i) cell volume control, (ii) selective solute distribution, (iii) selective solute permeability and (iv) cellular electrical potentials.

In Chapter 4 immediately following, I shall examine the major supportive evidence for the membrane theory—focused primarily on the

existence of a semipermeable cell membrane—as well as what has become of them eventually. Supportive evidence for free cell water and free cell potassium ion (K^+) came later and will be presented in Chapter 5.

4

Evidence for a Cell Membrane Covering All Living Cells

The following is a summary of the evidence for the existence of a cell membrane covering all living cells.

4.1 From studies of cell volume changes and solute permeability

Cell volume change, the first subject matter of cell physiological experimentations and observations, reflects largely the movement of water in and out of cells. In the membrane theory, water movement in turn reflects the permeability of the cell membrane to water, and the permeability or impermeability to substances dissolved in water.

(1) A semipermeable diffusion barrier at the cell surface

Chambers and Chambers showed that non-injurious dyes, which could not penetrate the cell from the outside, diffused rapidly through the cytoplasm when micro-injected into the cell interior. They stopped on reaching the cell surface, unable to escape from the cell.[22]

(2) Plasmolysis

When a mature plant cell is exposed to a concentrated solution of table salt (sodium chloride, NaCl) or cane sugar (sucrose), the inside of the plant cell or *protoplast* (a name coined by von Hanstein,[27] Figure 1B) shrinks away from the containing cell wall (Figure 1). The degrees of shrinkage varies with the strength of the NaCl or sucrose solution used. This phenomenon was extensively studied by Hugo de Vries[28] and is known as *plasmolysis*.

In 1871 de Vries observed that the *protoplast* of beet roots remained shrunken for days in a concentrated solution of sodium chloride (NaCl).[24] This observation led him to conclude that the plasma (cell) membranes of these cells are *(absolutely) impermeable* to NaCl. In the context of the membrane theory, this observation is of life-death importance. For it sub-

stantiates a basic tenet of the membrane theory: a substance, which at high concentration causes sustained shrinkage, must be *absolutely* and *permanently impermeant.*

To explain the need for this tenet, consider the following. Many vertebrate cells spend their entire life-spans—which may be as long as 100 years—in tissue fluids containing NaCl as its main osmotically-active ingredient. If NaCl can enter the cell, even slowly, the cell would sooner or later be chock-full of NaCl, lose its osmotic equilibrium and probably die. Therefore, the cell membrane must be impermeable to NaCl, *permanently* and *absolutely.*

A high point in the experimental study on plasmolysis occurred in the year 1918, when K. Höfler invented a method for measuring *quantitatively* the volume of the (irregularly-shaped) shrunken protoplast[29] (Figure 1B). Applying this method, he showed that in the mature cells of the plant, *Tradescansia elongata*, the product of the volume of the protoplast, V, and the concentration of sucrose in the bathing solution, C, is close to being a constant. This invariance of the product, VC, was hailed as a decisive quantitative confirmation of the membrane theory. It shows that the living cell indeed behaves like a *perfect osmometer* in solutions containing different concentrations of an impermeant solute like sucrose.[30]

Further investigations, however, led Höfler to reverse his earlier stand.[31] Thus in later studies, he discovered that it is not the whole cell enclosed by the cell membrane that behaves like a perfect osmometer. Rather, it was only the central vacuole enclosed by the vesicular membrane (or *tonoplast*), which obeys the constant VC relationship: This new discovery poses a serious difficulty for the membrane theory.

At high concentrations, sucrose causes the shrinkage of the central vacuole. This is a fact. This shrinkage could not have happened without sucrose first entering into the cell's cytoplasm, which completely surrounds the central vacuole. Sucrose could not have entered the cytoplasm without first penetrating the plasma or cell membrane, which in turn completely surrounds the cytoplasm. Therefore, the cell membrane is *not* impermeable to sucrose—*in contradiction to the basic tenet that a solute that causes sustained plasmolysis like sucrose is impermeant to the cell membrane.*

Other studies showed that in concentrated solutions of an electrolyte (e.g., a salt of potassium ion, K^+), the surrounding cytoplasm may actually swell even as the central vacuole is shrinking.[32] This observation too cannot be explained by the membrane theory.

(3) Transient and sustained volume changes

As I have just pointed out above, sodium chloride (NaCl) is the major ubiquitous component of the tissue fluids of all vertebrates including frogs and humans.

Charles E. Overton—a distant cousin of Charles Darwin—showed in 1902 that an isolated frog *sartorius* muscles—a thin and flat muscle on the inside surface of the thigh, often highly developed in tailors of early days (sartor, Latin)—retained their normal weights in a 0.7% NaCl solution.[25] Such a weight-preserving 0.7% NaCl solution was described as *isotonic* by H. J. Hamburger; higher and lower concentrations of NaCl solutions were respectively called *hypertonic* and *hypotonic.*[26] When 5% methyl alcohol (methanol) was included in an isotonic 0.7% NaCl solution, the muscle immersed in the solution showed no change from its previous weight in the 0.7% NaCl solution alone. However, in a solution containing 3% ethylene glycol and 0.35% NaCl solution, the muscle shrank first followed by a slow return to a higher weight, close to that produced by the unadulterated 0.35% NaCl solution alone.

The explanations offered are as follows: Methyl alcohol is, like water, highly permeable to the cell membrane. Hence, its addition to an isotonic NaCl solution caused no change in the weight of the muscle (if one assumes that the dilution of NaCl thus brought about is trivial). Ethylene glycol is also permeant, but less so than methanol. Therefore the inclusion of ethylene glycol caused first a shrinkage followed by a return to normal and above normal weight as more and more ethylene glycol enters the cells. In NaCl solutions at a concentration higher than 0.7% or in a 0.7% NaCl solution containing also 3% glucose, the muscle shrank and stayed shrunken. These observations were seen as once more confirming the basic tenet of the membrane theory that *only concentrated solutions containing solutes like NaCl or glucose, which are absolutely and permanently impermeant to the cell membrane can cause sustained cell shrinkage.* Then something unexpected came up again.

In 1937 and 1938, D. Nasonov, E.I. Aizenberg[33] and I. Ye. Kamnev[34] from the former Soviet Union made a simple discovery. They first showed that in a normal isotonic Ringer's solution containing in addition 4% sucrose, frog muscle shrank until it reached and then sustained a smaller volume (Figure 2)—as one would have expected on the basis of the membrane theory. However, what one did not expect was that as the muscle was shrinking, *sucrose was actually entering into and accumulating inside the*

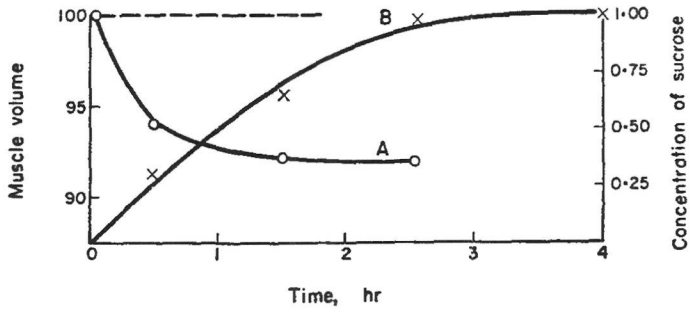

Figure 2. Time course of shrinkage of frog muscle cells in a Ringer's solution containing 4% sucrose (A). Muscle cell volume is given as percentage of the initial cell volume (left ordinate). Concentration of concomitantly-accumulated sucrose in the cells (B) is expressed as weight percent per 100 g. of tissue water (right ordinate). (Nasonov and Aizenberg[33] and Kamnev[34])

shrinking cells. This inward permeation of sucrose continued until a steady level—lower than that in the external medium—was reached and then maintained (For additional details of their experiments, see [8.2].)

These simple but highly important findings showed that sucrose, which causes sustained shrinkage, is in fact permeant. Thus, like Höfler's later observations, this discovery also refutes the tenet—vital to the membrane theory—that only impermeant solutes can cause sustained shrinkage. Permeant solutes can cause sustained shrinkage also.

With the advent of radioisotopes and associated technology in the late 1930's and early 1940's, cell physiology entered a new age of enlightenment. Indeed with the radioactive tracer technique, (true) permeability—the *sine que non* of the membrane theory—can now be studied with precision and without ambiguity, *often for the first time* {[13.3]; [15.2(1)]}.

Thus the data shown in Table 1 once more confirm the existence of a diffusion barrier to labeled sucrose and NaCl as the surgical amputation of the frog muscle cells doubled the rate of penetration of sucrose into the muscle cells.[23] However, the data also demonstrate that *before* the surgical amputation of the cell membrane, labeled sucrose *could* and did enter into (intact) muscle cells. Other studies have repeatedly and unequivocally established that solutes like sucrose[23, 35] and NaCl[36] are *permeant* to the cell's membrane, confirming that permeant solutes *can* cause sustained cell shrinkage, a truth already suggested in Höfler's later discovery, and established by Nasonov, Aizenberg and Kamnev. And, in consequence, the ele-

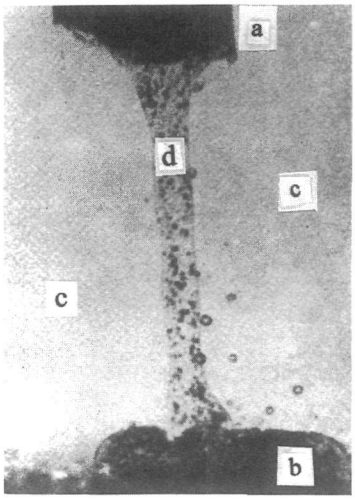

Figure 3. Outflow of protoplasm (endoplasm) (d) from the cut end of a *Nitella* cell (a) into a culture medium (c). The protoplasm collected as a flattened round drop (b) on the bottom of the cuvette. The picture was taken 5 minutes after the cut was made. These endoplasmic drops can survive 10–50 hours in the culture medium containing 80 mM KNO_3, 50 mM NaCl and 4 mM $Ca(NO_3)_2$. (Kuroda,[63] by permission of Academic Press)

mentary fact that living cells maintain steady and unchanging volumes in isotonic NaCl or sucrose solution can no longer be explained by the membrane theory.

(4) Membrane regeneration

In 1855, Swiss botanist, Karl von Nägeli crushed the root hairs of a water plant called *Hydrocharis.*[37] Escaping from the broken root hairs were little liquid balls or droplets (see b in Figure 3), which did not dissolve in the surrounding fluid or take up the vital dye present in the bathing solution. Furthermore, they exhibited osmotic shrinking and swelling like intact living cells. Similar water-immiscible little balls from the ciliate protozoa, *Stentor*, were described by Willy Kühne.[38] These investigators's observations were quoted by Pfeffer in his "Osmotische Untersuchungen" as evidence for his theory that *new cell membranes are promptly regenerated on the exposed surface of the protoplasmic droplets when they came into contact with an aqueous medium—an idea in fact first suggested by von Nägeli*[37] as Pfeffer pointed out.[18 p 141] And, according to Pfeffer, it is the regenerated cell membranes, which endow the droplets with osmotic behaviors normally seen only in intact cells.[18, 1st ed. p 127; 39 p 92]

In his "Osmotische Untersuchungen," Pfeffer did not quote by name Felix Dujardin, who saw that very same phenomenon—*as an escape of water-immiscible* "living jelly" or sarcode.[2] It is easy to understand Dujardin's theory: like oil, protoplasm is inherently water-immiscible. Pfef-

fer's model is more complex. New studies, which promised to shed light on the conflicting views, were reported by G. L. Kite in 1913.[40]

Kite injected with a micro-pipette dyes and other solutes into the cytoplasm of various types of living cells. In the eggs of the starfish, *Asterias*, for example, the injected acid dyes penetrated only the swollen injured area of cytoplasm inside the cell but stopped at the boundary of healthy cytoplasm. Distilled water injected into a starfish egg (or *Necturus* muscle) collected as a vesicle and was absorbed slowly. A drop of injected hypertonic salt solution actually increased in size with time.

From these and other observations, Kite suggested that the cytoplasm of cells like the starfish egg exhibits the same kind of osmotic properties as the intact cell surface does. And that *impermeability or partial permeability to dyes and crystalloids is not the exclusive property of the cell membrane but a property of all portions of the cell's protoplasm—in full support of Dujardin's and von Mohl's theory of water immiscible protoplasm.*

But the supporters of the membrane theory did not accept Kite's interpretation. They countered with the argument that a membrane has been regenerated, where the injection took place. And that it is this newly regenerated cell membrane, which gives the false impression that the naked protoplasm itself exhibits osmotic behavior.[41 p 125; 315] This was where the unresolved controversy remained for half of a century—until advanced technology opened the door to renewed study of this unresolved controversy.

As mentioned above, Table 1 shows that the rate of permeation of radioactively labeled sucrose into frog muscle cells—mounted in an *effectively-membrane-pump-less-open-ended* or *EMOC* setup {for description, see Figure 7 and [10.2(1)]}—was more than doubled after the intact end of the muscle was cut off, and the muscle cytoplasm directly exposed to the radioactively-labeled sucrose solution.[23] The cut end of a similarly amputated paired muscle was tested for its permeability to sucrose *24 hours after the cut*, and shown to have maintained the same enhanced permeability to sucrose measured immediately after the amputation (in the paired muscle). Similarly, after 51 hours of incubation, the cut end of the muscle showed the same enhanced permeability to radioactively labeled Na^+ as it did immediately after cutting. These findings show that no regeneration of cell membrane takes place at the exposed surface of bare

TABLE 1.

	Rate Before Cutting (μmoles/g)	Rate After Cutting (μmoles/g)	
		immediately after cutting	incubated after cutting
sucrose	0.073 ± 0.013	0.174 ± 0.031	0.168 ± 0.016 (24 hrs)
Na^+		22.7 ± 2.5	22.3 ± 5.1 (51 hrs)

Demonstration of the absence of *membrane regeneration* at the cut surface of frog muscle cells. Frog sartorius muscles were mounted on an EMOC setup illustrated in Figure 7 and the tibial ends protruding beyond the silicon rubber gasket were cut off with a pair of fine scissors. In one set, the cut end of the muscle cells was first exposed to a nonlabeled normal Ringer's solution for 24 hours before exposure to a fresh Ringer's solution containing 10 mM of radioactively-labeled sucrose for 2 hours at 25°C and analyzed for labeled sucrose in the cut muscle. The uptake of labeled sucrose was compared to their paired muscles, which were exposed to a similar labeled solution for a similar length of time immediately after cutting (without the 24 hour-long incubation.) The control rate of labeled sucrose permeation was determined by exposing a similar muscle preparation with *intact* tibial end to a similar labeled-sucrose-containing Ringer's solution also for two hours. Rates of labeled Na^+ uptake of the cut ends of frog sartorius muscles from a Ringer's solution containing 100 mM of labeled Na^+ were studied in a similar manner except that the exposure of the cut end of the muscle to labeled Na^+ lasted much longer (24 hours) and so was the time of preceding incubation (51 hours). (Data from Ling[23])

cytoplasm of frog muscle cells—a conclusion corroborated by studies using another new technology, electron microscopy.

Modern electron microscopy, in contrast to light microscopy, can see a 100 Å-thick cell membrane[269] when the cell preparation has been properly fixed and stained with electron-dense uranium and lead, for example. Using this technique I. L. Cameron made electron microscopic pictures of the cut edges of frog muscles cells both immediately and some time after amputation. The EM plates show no membrane regeneration at the cut ends.[42; 107 Figure 4.11]

In summary, *there is a diffusion barrier at the surface of the cell, which offers resistance to the passage of some substances at least* (see [13.3], however). *But the barrier is a relative one and not one of absolute permeability or impermeability. Cells as a rule do not behave like a perfect osmometer. The central tenet that only impermeant solute at high concentration causes sustained cell shrinkage has been disproved—throwing in doubt the membrane theory's classical explanations for the mechanism of cell permeability, cell volume control as well as solute distribution. Nor do frog muscle cells regenerate, instantly or more slowly, a new mem-*

brane at the cut surface of the (gel-like) muscle protoplasm. (Notwithstanding, one cannot forthwith infer from the muscle study that membrane regeneration does not occur on the surface of more fluid protoplasmic droplets (see [11.3(3)] for follow-up)). Out of the four physiological manifestations, which the membrane theory had at one time been able to explain, only the electrical potential remains intact at this point and it will be the subject of the following subsection.

4.2. From Bernstein's membrane theory of cellular electrical potentials

The copper-ferrocyanide membrane, though impermeable to copper and ferrocyanide ions as described earlier, allows the passage of both potassium ion (K^+) and chloride ion (Cl^-).[18 p 47] Studying the electric potential difference of solutions containing these and/or other ions across such a membrane, Wilhelm Ostwald suggested in 1890 that the electric potentials of muscle, nerve as well as electric eels may share a similar (membrane-based) mechanism.[43] Two investigators took up his suggestion: Julius Bernstein and Frederick Donnan (see also MacDonald[316]).

In 1902 Julius Bernstein postulated a theory, which he named "membrane theory."[19 p 542] In this theory, the steady electric potential difference measured across the normal resting nerve and muscle cell surface and historically known as the *resting or demarcation potential*[15 p 20] is a "membrane potential."[19; 232]

In naming his theory of resting potential "membrane theory," Bernstein made no reference to the "membrane theory" often attributed to Pfeffer [3]. This omission could be the result of the initial phase of the fragmentation of physiological science (see Epilogue) and the consequent isolation of plant physiologists represented by Pfeffer working with mature plant cells from animal physiologists represented by Bernstein working with muscle and nerves.

As such, Bernstein's membrane potential arises from the permeability of muscle cell membrane to K^+ and (presumed) impermeability to Na^+ and to all anions.[19] Bernstein's theory, diagrammatically illustrated in Figure 4B, predicts that the resting potential should be inside negative, the magnitude of the resting potential depending on the absolute temperature and on the logarithm of the external K^+ concentration (shown as C_2 in Figure 4B). As the techniques for measuring the cellular electric potentials improved,[253; 88; 441 to 443] all three predictions have been confirmed and confirmed repeatedly.[15 pp 68–73; 107 p 276]

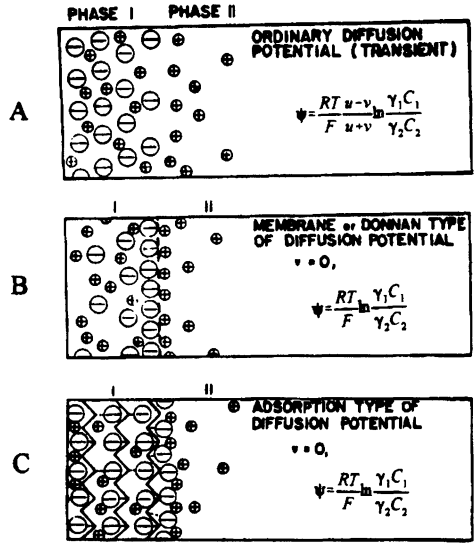

Figure 4. Diagrammatic illustrations of three types of electric potentials (ψ), one transient (A, ordinary diffusion potential) and two lasting (B, membrane potential or Donnan potential; C, adsorption potential). ⊕ represents free monovalent cation in A, B and C. ⊖represents respectively free monovalent anion in A and B and fixed monovalent anion in C. u and v are the mobility of (monovalent) cation and anion respectively. C_1 and C_2 are the concentrations of (monovalent) salt in phase I and II respectively; γ_1 and γ_2 are the corresponding activity coefficient of salt ions in the two phases. The equation describing the adsorption type of potential and cited in the illustration C is valid only if the fixed anionic sites are uniform throughout the cell including the cell surface. If the surface anionic sites are at a different concentration than in the bulk phase cytoplasm, a more correct equation would be equation 10 in the text (which is also listed as A22 in Appendix 1). (Ling,[96] reproduced from *Phosphoras Metabolism* by permission of The Johns Hopkins University Press)

However, Bernstein's membrane theory also predicts that the resting potential should vary with the logarithm of the total concentration of *intracellular* K^+ (shown as C_1 in Figure 4B). This has been confirmed in four laboratories[418] but not in ten others.[419]

In addition, Bernstein's membrane theory requires that the cell membrane be impermeable to Na^+, which, as pointed out above, turns out to be otherwise[36] [4.1(3)]. With the permeability of the cell membrane to Na^+ established, the parameters C_1 and C_2 of Figure 4B must represent the *sum* of intra- and extra-cellular concentration of K^+ and Na^+ respectively rather than the intra- and extra-cellular concentration of K^+ alone. Since these intra- and extra-cellular sums of concentrations are approximately equal and the logarithm of a ratio of unity is zero, the theory can no longer predict an inside-negative resting potential of some 90 mV. as observed,[95] but instead, no potential difference at all.

In response to this difficulty, Professor A. L. Hodgkin introduced in 1949 a modified membrane theory of cell potentials, which he calls "*ionic theory.*"[47]

In Professor Hodgkin's ionic theory, the segregation of ions into permeant and impermeant categories is replaced by one based on *varying* per-

meabilities. The more permeant K^+ exercises a stronger influence on the magnitude of the resting potential than the less permeant Na^+, for example. The ionic theory was able to explain quantitatively not only the *resting potential* but also the *action potential*—the physical basis of the nerve or muscle *impulse* (see [15.6] for a more detailed description). This was exciting news to many, especially graduate students like myself, then working on a Ph.D. thesis on the electric potential of frog muscle cells.

The ionic theory of Hodgkin is a modification of Bernstein's membrane theory. As such it too rests upon the basic tenets of free water and free K^+ in living cells. Experimental evidence in support of these tenets will be presented in Chapter 5 to follow.

4.3. From Donnan's theory of ionic distribution and membrane potential

In 1911, Frederick Donnan published an article entitled: "Theory of Membrane-equilibrium and Membrane-potential due to the presence of Non-dialyzable Electrolytes."[20] As the title indicates, his theory deals with the equilibrium distribution of ions (e.g., Na^+, Cl^-) in compartments separated by a semipermeable membrane—after an electrically-charged component, which is unable to pass through the semipermeable membrane, has been introduced into one compartment.

The main thrust of the Donnan theory is that the presence of such a non-dialyzable or *impermeant* electrolyte in one compartment causes *asymmetrical* distribution of the *permeant* electrolytes across the semipermeable membrane. In addition, a lasting electric potential difference, also called the *membrane potential*, is generated between the two compartments. Donnan's membrane potential is fundamentally not different from the membrane potential of Julius Bernstein presented above (see Figure 4B), except that Donnan's theory alone predicts a theoretical relationship between the magnitude and polarity of the membrane potential and equilibrium ionic concentrations.

Emil Abderhalden, a student of the great pioneer in protein chemistry, Emil Fischer (1852–1919), analyzed the contents of K^+ and Na^+ in human blood plasma and in red blood cells.[73] He found that the plasma contains little K^+ but a great deal of Na^+, but the reverse is true in the red blood cells.[74] pp 120–121 Abderhalden's data are in general agreement with the extensive data on the ionic contents of muscle tissues from Julius Katz published two years earlier.[75] Both sets of data portray *the strongly asymmetrical K^+ and*

Na^+ distribution across the surface of living cells[98 pp 232–233]*—despite the fact that chemically speaking, these two ions are much alike.*

In 1928, H. Netter from Heidelberg applied Donnan's theory of membrane equilibrium to the preferential accumulation of K^+ in frog muscle cells—in the belief that the muscle cell membrane is permeable to K^+ but impermeable to Na^+ and all anions present.[50] Also adopting the Donnan theory of membrane equilibrium were Boyle and Conway, who published in 1941 their version of the membrane theory with the membrane impermeable to Na^+ but permeable to both K^+ and the major external anion, Cl^-.[44; 45; 46]

In Boyle and Conway's version of ion distribution, the selective permeability of the cell membrane to K^+ and its impermeability to Na^+ were attributed to a sieve-like mechanism, permitting the passage of the smaller hydrated K^+ but barring the larger hydrated Na^+.

However, Boyle and Conway's sieve mechanism for cations is similar to an earlier one by Mond and Amson, in which Mond and Amson also postulated a muscle cell membrane with pores large enough to permit the passage of the smaller hydrated K^+ but impermeable for the larger hydrated Na^+.[51 p 78] Nor was Mond and Amson's theory entirely original, being an extension of L. Michaelis' theory for the selective permeability of smaller hydrated K^+ and impermeability to larger hydrated Na^+ ions through pores in dried collodion membranes.[401 p 42] Only Mond and Amson gave full credit to Michaelis. Sadly, Boyle and Conway gave no acknowledgment of Mond and Amson's idea, nor that of Michaelis, even though the very same paper in which Mond and Amson presented their (earlier) sieve idea was quoted but for a different reason.[44 p 6] For many years I had believed wrongly (and inadvertently helped to perpetrate) that this specific version of the sieve idea originated from Boyle and Conway.

As already mentioned in [4.1.(3)] and [4.2], the postulation that the cell membrane is in fact impermeable to Na^+ had been experimentally disproved years *before* Boyle and Conway's paper appeared in print in 1941.[36] The first publication on membrane permeability to Na^+ is that of P. Gérard in 1912.[36] Since then it has been confirmed again and again before 1941 (see Reference 36). *The failure of this latest version of the membrane theory to explain the asymmetrical distribution of K^+ and Na^+ set the stage for the adoption of the sodium pump hypothesis.*[49 pp 123–124]

Robert Dean has been frequently cited as the originator of the sodium pump hypothesis. In fact, what he did on this subject was not much beyond making the comment: "It is safer to assume that there is a pump of unknown mechanism which is doing work at a constant rate excreting sodium as fast as it diffuses into the fiber."[333 p 346] Others before him have made

similar comments. They include R. S. Lillie,[41 p 117] E. Overton[320 p 9] and Theodor Schwann.[3 p 194; 335] Thus Schwann postulated that the cell membrane possesses "metabolic power" by which the membrane regulates the chemical composition of fluids inside and outside the cells.[3 p 194]

The intensity of research on the sodium pump picked up dramatically after 1957, when Professor S.C. Skou of Denmark suggested that *an enzyme called Na, K-activated ATPase is the sodium pump.*[52] However, to introduce to the reader some other major relevant developments that had taken place before Skou's entry into this field, I shall keep the sodium pump hypothesis on hold until Chapter 12.

5

Evidence for the Cell Content as a Dilute Solution

The basic tenet of the membrane theory that the cell interior consists of a dilute aqueous solution can be resolved into the tenet of free water and the tenet of free K^+—since, as already mentioned, it was found at the turn of the century that K^+ is the main intracellular cation of most living cells.[73, 75, 98 pp 232–233] The experimental evidence for free water and free K^+ came in the early 1930's and late 1940's, and thus years after alternative concepts of what one may loosely refer to as "bound water" and "bound K^+" had already appeared (see Chapter 7). For this reason, I shall present only a brief sketch of the early evidence for free water and free K^+ here, and delay their follow-ups until [10.2(3)] and [11.3(5)]—after the opposing concepts have been reviewed.

5.1 Early evidence for free cell water

In 1930, A.V. Hill (1886–1977), Nobel Laureate, showed that urea distributes equally between muscle cell water and the surrounding medium.[81] This led him to conclude that all water in living cells is free and none "bound" or "nonsolvent." Confirmations followed soon.[82]

5.2 Early evidence for free cell K^+

In 1953, Professor Alan Hodgkin, Nobel Laureate and Professor Richard Keynes of the Cambridge University published their findings on the mobility of K^+ in the giant nerve fiber (axon) of the cuttlefish.[263] They concluded that K^+ which enters an axon is found in the axoplasm in much the same state as in a 0.5 M KCl solution.[263 p 526] The importance accorded this piece of work could be seen from a passage written by another Nobel Laureate, Professor Bernard Katz in "Muscle, Nerve and Synapse."[237]

> "Moreover, these authors (Ernst, Troshin and Ling) take the view that the potassium ions do not merely form counterions to the negatively charged colloidal structure but possess selective affinity and are chemically bound to the proteinates. {This sentence has not correctly represented my position. In my view, to be presented in Section [10.1(3)], cell K^+ is adsorbed primarily by *electrostatic forces* and cannot be regarded as "chemically bound." *Added by GL.*} It seems, however, very difficult to support this view in the face of the following pertinent observations by Hodgkin and Keynes (1953) (Reference 263, *added by GL*).... These results are discussed in detail because they are of crucial importance in the still persistent argument about the validity of the membrane concepts.... It was clear therefore that the labeled [K^+, *added by GL*] ions that had entered the axoplasm continued, inside cells, to behave as free ions with approximately normal mobility."[237 pp 42–44]

Sixteen years after the publication of Hodgkin and Keynes' work summarized above, Kushmerick and Podolsky presented new findings in support of Hodgkin and Keynes' conclusion. Kushmerick and Podolsky measured the diffusion coefficients of seven labeled solutes in the cytoplasm of 3- to 6-mm-long frog muscle segments with open ends.[264] The solutes were K^+, Na^+, Ca^{++} (calcium ion), SO_4^{2-} (sulfate ion), ATP (adenosine triphosphate), sucrose and sorbitol. They showed that all solutes (with the exception of Ca^{++}) demonstrated diffusion coefficients lower than their respective counterparts in free solution by a factor of two.

From these findings, they concluded that six of the solutes they studied, including K^+ and ATP, exist in the free state in the cell water. And it was mechanical barriers inside the muscle cell, which caused the impartial two-fold reduction of their respective diffusion coefficients. Thus it would seem that Kushmerick and Podolsky had not only confirmed the free-cell-K^+ tenet in particular but the broader free-cell-solute tenet of the membrane (pump) theory as well.

As mentioned above, further follow-ups for both the free K^+ and free water ideas will not be presented until Chapters 10 and 11 respectively. Nonetheless, it may be pointed out here that all five investigators and reviewer cited above had overlooked some earlier relevant information in the literature.

In 1913, Rudolf Höber reported on the *electrical conductance* of frog muscle measured at a frequency (0.9×10^7 Hz), at which the membrane resistance is short-circuited and thus no longer interfering. Höber found the

electrical conductance of muscle cytoplasm equal to that of a *0.1 to 0.2%* NaCl solution.[265] This finding contradicts the free K^+ tenet of the membrane theory, according to which the measured conductance should equal or at least approximate that of an *isotonic 0.7%* NaCl solution in equilibrium with a normal frog muscle [4.1(2)]. Höber's finding has been confirmed repeatedly by other investigators including Professor A.V. Hill—whose work was mentioned above—and myself.[266; 15 Table 8.1]

So far, we have focussed on the membrane theory and its underlying assumption that living cells are membrane-enclosed dilute solutions. In what follows, I shall examine theories and facts more closely related to the concept that living cells are solid bodies of protoplasm. I shall begin with the emergence of a new branch of chemistry, called *colloid chemistry*.

6

Colloid, the Brain Child of a Chemist

The discovery that cells are made of protoplasm, a finding of critical importance to cell physiology, was promptly seized upon and pursued with vigor and insight—not by a cell physiologist but by Thomas Graham (1805–1869), a chemist. Graham was at the time Master of the Mint of England,[53 p 183] an office once held by Isaac Newton toward the end of the 17th century.[464 p 229]

6.1 Colloid, the name-sake of gelatin— and a cogent model for protoplasm

Thomas Graham spent most of his life investigating the phenomenon of diffusion. He noted that substances like starch, gum and gelatin diffuse slowly and that they do not form crystals. Graham wrote in 1861: "As gelatine appears to be its type, it is proposed to designate substances of the class as *colloids* (from Greek κδλλη: glue, or gelatin, *added by GL*), and to speak of their peculiar form of aggregation as the *colloidal* condition *of matter.*"[53]

In naming the class of slow-diffusing substances colloids, Graham pointed out that "the plastic elements of the animal body are found in this class." By plastic elements he could only be referring to the soft tissues (in contrast to rigid elements like bones, horns, shells). These soft tissues are, according to Felix Dujardin, Hugo von Mohl, Max Schultze and others, made of *protoplasm*—even though Graham did not use this word.

However, by introducing colloid chemistry, Graham not only opened the door to investigating protoplasm from a chemical point of view, he also gave impetus to the deeper understanding of cell membranes. Thus Graham also invented *dialysis*, using a colloid-"sized"(stiffened) membranes for separating colloids from water and from substances readily dissolved in

water, including salts and sugars. Unlike colloids, salts and sugars diffuse much faster and do form crystals. For them, Graham gave the collective name, *crystalloids*.

In 1857, Michael Faraday introduced to the Royal Society a substance he called *colloidal* gold. (Interestingly enough, four years before Graham was to introduce the new word, *colloid.*[54 p 472]) Faraday showed that colloidal gold solutions are (like all other normal solutions) totally transparent "when a light is looked at through the fluid." However, "if a cone of sun's rays be thrown by a lens into the fluid, the illumination of the particles within the cone shows their presence as undissolved bodies."[54 p 472] This phenomenon is known as the Tyndall phenomenon, on the basis of which, an optical instrument called the *ultramicroscope* was constructed.[64 p 87] The ultramicroscope enables one to see otherwise invisibly small colloidal particles.

Martin Fischer, whose important contributions to cell physiology will be reviewed below, defined colloids in these words: "colloid systems result whenever one material is divided into a second with a degree of division coarser than molecular."[64 p 5] Ross Gortner, whose important work will also be presented below, offered a modification: "colloidal systems result where one material is divided into a second with a degree of subdivisions either (a) coarser than molecular or (b) where the micelles exceed 1–1.5 millimicra (10–15 Å) in diameter." Gortner further pointed out that an ultramicroscope makes visible colloid particles from 10 Å to 1000Å in diameter.[64 p 5] Wolfgang Ostwald set the limits of colloids between 10 Å and 10,000 Å.[65 p 24] But another definition given by H. Staudinger poses a special problem.

Staudinger believed that only molecules larger than 1250 Å can be considered true colloids (or *eucolloids*).[65 pp 23–24; 66] He also regarded colloids and macromolecules as synonymous. However, he was also the author of the *macromolecular hypothesis,* in which macromolecules are long chains of repeating units or monomers joined end-to-end by covalent bonds.[67]

Yet well-known colloids like Faraday's colloidal gold and Traube's copper ferrocyanide gel are not macromolecules but big aggregates of smaller units.They are definitely not small units joined together by covalent bonds. Thus notwithstanding the wide practice to equate the two, colloids and macromolecules are not the same. I shall return to this subject below in [11.3(2)].

By inventing colloid chemistry, Graham has brought together two substances outstanding in the history of cellular and subcellular physiology. They are copper ferrocyanide and gelatin. We already know how copper-ferrocyanide had launched the membrane theory. In the next section I shall review how colloid chemists have discovered more and more intimate relationships between gelatin and protoplasm. However, as will be made clear in [11.3(2)] following, the time to construct a (plausible) theoretical explanation for this intimate relationship was not to come until much later.

6.2. Coacervates

(1) History

In 1902, Pauli and Rona added neutral salts to a solution of gelatin at 30°C and observed the separation of the solution into two distinct layers. The bottom layer is rich in gelatin; the top layer is gelatin-poor.[58] In 1929 Bungenberg de Jong (1893–1977) and H. Kruyt coined the term *coacervation* for the phenomenon (from the Latin *acervu* meaning aggregation and the prefix *co*, meaning together).[59] *Coacervate* is also used to designate the colloid-rich phase of the separated liquid. In the case where *salt-linkage* formation between fixed anions and fixed cations of the colloids plays a significant role in the colloid structure, the coacervate is referred to as a *complex coacervate*.

Colloid chemists in the past had called all proteins colloids. With this in mind, one may think that all proteins can form coacervates. This is not true. Only what Bungenberg de Jong called *linear proteins* such as gelatin form coacervates.[61 p 185, p 239] Under conditions that promote the formation of coacervates from linear proteins, most *globular proteins form crystals* instead. This is a very important distinction to keep in mind. Most isolated native proteins are globular. Gelatin is therefore unique or almost unique in maintaining on a permanent basis a linear, or what I call *fully-extended conformation* [11.2]. Why does gelatin assume and sustain such a fully-extended conformation—a hitherto unanswered question—was given a possible explanation on the basis of new knowledge unknown until recently. And it will be reviewed in [11.3(2)] to follow.

(2) Bungenberg de Jong's two views on the physical state of water in coacervates

Bungenberg de Jong offered not one, but two theoretical interpretations for the structure of coacervates and the physical state of water in the coacer-

vate. In the old interpretation, individual small colloidal particles with a diffuse solvate coating first join together into larger particles with a clear boundary during the preparative process. When these larger particles in turn join together to form coacervate, their individual solvation shells merge to form an overall shell with a concrete outer boundary (though no explanation was given why a concrete boundary is formed). Note that in this (old) model, all or nearly all of the water in a coacervate is not normal liquid water but *hydration water.*[61 pp 245–246, p 249]

However, the new interpretation of coacervates, in Bungenberg de Jong's own words, "stand(s) diametrically opposed to this (old) original idea" of water in coacervates just described. Indeed, in the new model, "by far the larger part (of water in the coacervate) is to be regarded as *occlusion-water*"[61 p 249] which is "Not bound to the macromolecules,"[61 p 371] and therefore normal liquid water caught in between the network of macromolecules. This new definition leaves one with the impression that there is minimal interaction between the macromolecules and water in a coacervate—quite the opposite of the old model. Fortunately, Bungenberg de Jong and his coworkers have also left puzzled readers like myself some quantitative data, which permits a deeper look into the subject.

Holleman, Bungenberg de Jong and Modderman studied the equilibrium distribution of sodium sulfate (Na_2SO_4) in a simple coacervate of gelatin + Na_2SO_4 at 50°C.[70] At a gelatin concentration of 27.2%, the concentration ratio of sodium sulfate in the coacervate water and in external solution is 0.62. This partial exclusion of Na_2SO_4 indicates that of the $1 - 0.272 = 0.728$ or 72.8% of water in the coacervate, $1 - 0.62 = 0.38$ or 38% has no solubility for sodium sulfate. Dividing the total amount of this water (equal to $0.728 \times 0.38 = 0.277$) by the percentage of gelatin, one obtains $(0.277 / 0.272) = 1.02$ grams of "non-solvent water for sodium sulfate" per gram of dry gelatin. This figure is between *3 to 4 times* larger than the conventionally accepted (total) *hydration water* on native globular proteins (i.e., 0.2 to 0.3 grams/gram of dry protein).[155 Table 5] Nonetheless, there is also a difference between this realistic 38% "hydration water" and the 100% "hydration water" as implied in the old model. On the other hand, if the hydration water has higher than zero solvency or *q-value* for Na_2SO_4 {see [11.3(4)]}, the departure from the old model could become smaller. We shall return to this interesting subject in [11.3(3)] below.

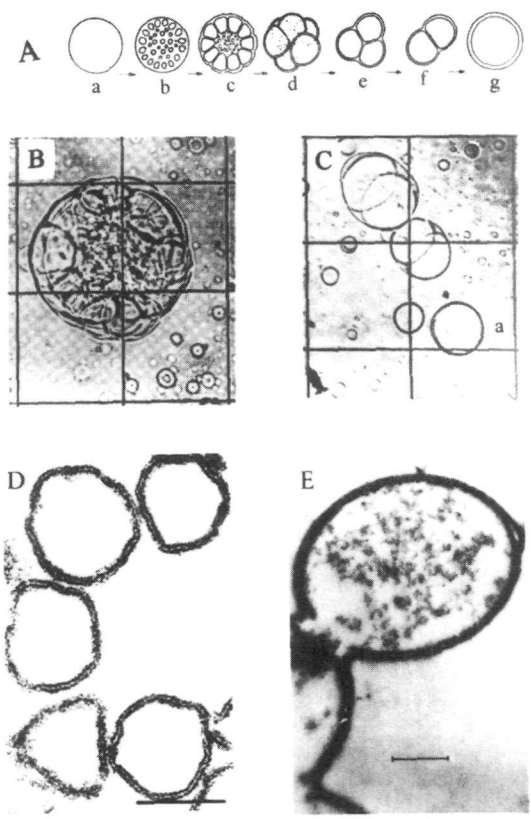

Figure 5. The step-by-step illustration of the *in vitro* evolvement of hollow vesicles with bilayered membrane and several outstanding examples
A. Diagrammatic illustrations of the morphological development of the hollow sphere with bilayer membrane (g) from a negatively charged (solid) coacervate drop of the complex gelatin-gum arabic type (a). b shows primary vacuolation. c and d are in the form of foam bodies as they are progressing through successive stages toward the hollow sphere (g). B. Actual photograph of coacervate at the foam-body stage corresponding to stage c in A. (203 × lin.)
C. Actual photograph of foam bodies in stage f. Note the double-layered wall in hollow vesicle marked a in this picture (as well as in the diagrammatic illustration g in A.) (196 × lin.) D. Partial reproduction of an electron micrograph of phospholipid vesicles reconstituted from pure renal (Na^+ and K^+)-ATPase by the cholate dialysis method. (× 290,000). Diameter of vesicle close to 900 Å. E. Electron micrograph of proteinoid "microsphere," subjected to elevated pH. Scale bar: 1 μm or 1000 Â. Note that membrane can be formed from gelatin + gum arabic (A,B,C), from (Na^+, K^+) ATPase + cholate (D), or from pure proteinoids obtained by heating mixtures of pure amino acids (E). The only common denominator among these three preparations is proteins of one kind or another. (A, B and C from Bungenberg de Jong,[61 p 460] D from Skriver *et al.*,[487] reproduced from *The Journal of Cell Biology* by copyright permission of The Rockefeller University Press; E from Fox[216] by permission of Springer-Verlag)

(3) Coacervate and protoplasm

If one mixes in the right proportion gelatin and *gum arabic*—a highly water-soluble, large complex polysaccharide from Acacia trees[60 p 98]—and allows the mixture to stand, two layers also separate out. If the test tube containing the layers is shaken, the gelatin-rich coacervate breaks up into

many little balls or droplets[61 p 438] (see also Figure 5A), which stay undissolved in the surrounding colloid-poor phase.

In 1926 W.W. Lepeschkin reported that the protoplasm oozing out from broken (young) cells of the plant, *Bryopsis plumosa* can also be shaken and broken up into many little balls.[62 p 75] They too stay undissolved in the surrounding aqueous medium. As judged by these strikingly similar characteristics, the gelatinous materials emerging from crushed protozoa by Felix Dujardin and Willy Kühne and from broken plant cells by von Nägeli, von Mohl, Lepeschkin as well as Kuroda (who produced Figure 3) must all be coacervates. This is not a new idea. Lepeschkin was among the firsts to suggest that protoplasm is a coacervate.[324]

(4) Coacervate and the living cell

In a review written for the journal, *Protoplasma*,[71] Bungenberg de Jong cited nine similarities between coacervates and what he called a *static model of the living cell*, including (i) water immiscibility, (ii) tendency to form vacuoles, (iii) tendency to engulf solid particles, (iv) behaviors under the influence of a direct-current (DC) electric field.

Bungenberg de Jong then pointed out that the most basic difference between the living cells and his static model lies in the possession of membranes in the living cells but not in the coacervates ("Der wesentliche Unterschied der lebenden Zelle gegenüber unserem statischen Modell bezieht sich wohl auf das Vorhandensein von Filmen oder Membranene in ersteren, die grundsätzlich Ungleichgewicht ermöglichen.").[71 p 164]

Why Bungenberg de Jong made this distinction is a mystery because he himself has shown how, under the right conditions, coacervate can form membranes too (Figure 5A and 5C and legend). In a following section I shall present the work and ideas of A. S. Troshin, who saw perhaps even greater significance in Bungenberg de Jong's work on coacervates than Bungenberg de Jong did himself.

7

Legacy of the Nearly-Forgotten Pioneers

The mistake of regarding all living cells as membrane-enclosed dilute solutions was fully recognized by micro-anatomists in the 1860's. Max Schultze announced his *protoplasmic doctrine* in 1861.[11] Thomas Huxley lectured on protoplasm as the *physical basis of life* in 1868.[72] Yet not until the turn of the century did a new generation of cell physiologists begin rising to the challenge. The research efforts of these early protoplasm-oriented cell physiologists centered around two subjects: (i) cell swelling, and (ii) the selective accumulation of potassium ion (K^+) over sodium ion (Na^+) in most living cells—a subject already briefly introduced in [4.3].

To explain the asymmetrical distribution of K^+ and Na^+ in red blood cells, the supporters of the membrane theory contended at first that the red blood cell membrane is impermeable to both ions.[76]

Benjamin Moore of the University of Liverpool objected briefly in 1906 to this impermeability interpretation.[77] Two years later, he and Herbert Roaf further elaborated on their stand.[77] First they reasoned that the content of K^+ in the cell is more or less constant in the cell's life cycle. It would be very difficult to see, they argued, how the cell could maintain a constant K^+ concentration inside the cell as it grows and multiplies, if at all times its membrane is impermeable to this ion.

As an alternative, Moore and Roaf suggested that *the cell protoplasm possesses a special affinity, or adsorbing power for K^+, but it has no adsorbing power for Na^+*. In support, they mentioned the preferential uptake of oxygen by living red blood cells (erythrocytes), and, even more to the point, selective uptake of K^+ over Na^+ by (inanimate) soils. However, they did not offer a molecular mechanism for the preferential adsorption in either the living cells as they hypothesized, or in soils as a known fact {see [10.1(3)]}.

C. E. Overton, better known for his *lipoidal membrane theory* (to be discussed in [13.1(1)]), discovered in 1902 reasons to question that living cells are truly membrane-enclosed dilute solutions. Thus when he transferred a frog sartorius muscle from an isotonic 0.7% NaCl solution to a hypotonic NaCl solution at half of its strength (0.35%), the muscle did not swell to twice its initial weight as expected on the basis of the membrane theory. Instead an increase of only a third of its initial weight was observed. Overton concluded that *at least* a part of the cell water must be *Quellungswasser* (swelling water, imbibition water).[25 p 273]

In 1907 and 1909 Martin Fischer, then a professor of medicine in the Oakland School of Medicine in Oakland, California, argued that the swelling of living cells is not an osmotic membrane phenomenon as widely believed then [4.1], but a result of the strong affinity of protoplasmic colloids for water—seen also in fibrin (the protein of blood clots) and gelatin.[546; 78] Along this line of reasoning, he proposed a *theory of oedema* and published a lengthy thesis on the subject.[78] In this 1909 document *he also offered briefly some new insights on the asymmetrical distribution of ions and other solutes in cells, which the distribution of* K^+ *and* Na^+ *exemplify.*

Fischer pointed out that the dissolved substances may be either at higher or lower concentration in the colloidal mass (protoplasm) than in the surrounding medium. *Adsorption* could then account for its being at a higher concentration; the *law of partition* (also known as the Berthelot-Nernst distribution law,[420] of which Henry's law for the distribution of gases in a liquid solvent is a special case—GL's addition) may account for its presence at a lower concentration.[78 pp 545–546] But Fischer did not further exploit these important ideas.

His major scientific contribution to cell physiology aside, Martin Fischer should also be remembered for his kindliness and generosity, giving support out of his own pocket to even his scientific opponents in Germany at the end of World War I.[79] Quoting Schopenhauer, colloid chemist Wolfgang Ostwald wrote on Fisher's 60th birthday: "As torches and fireworks pale and become invisible in the sunlight, so the mind and even genius and beauty are outshone and overshadowed by (the) goodness of heart."[79 p 441]

W. W. Lepeschkin also rejected the concept that living cells are membrane-enclosed dilute solutions. As mentioned earlier, when he crushed young cells of the plant, *Bryopsis*, in sea water, many water-immiscible little balls of protoplasm emerged[62 pp 289–290]—not unlike that

shown in Figure 3. On diluting the surrounding sea water with distilled water, these little balls swelled prodigiously and vacuoles formed within them. When these little balls were returned to sea water, they regained their original size and the vacuoles disappeared.

Lepeschkin estimated that the surface of these little balls created by shaking could be increased a thousand fold from the surface area of the intact cell. Because the total amount of (postulated) lipoids in the original membrane of the intact cell is limited, it could not be expanded to 1000 times its original dimension.[62 p 275] Accordingly, he concluded that these findings contradict the idea—emerging from a synthesis of the lipoidal membrane theory of Overton[21] and the theory of membrane regeneration [4.1(4)]—that each of these little balls is covered with a continuous lipoid membrane as postulated by Overton for all living cells. By the same token, the surface of these little balls must be covered with materials that are abundant in the protoplasmic droplet as a whole, i.e., protoplasm[62 p 276]—in harmony with Franz Leydig's, Max Schultze's belief that the cell surface is made of similar materials that make up the bulk of the cell's protoplasm. Surprising as it might seem, Wilhelm Pfeffer shared a similar view.[18 p 156]

In further support of Lepeschkin's idea, one may quote the observation of Pauli and Rona mentioned in [6.2]. They showed that a salt solution of (pure) gelatin warmed up to 30°C forms immiscible coacervate. Since there is no other structure-forming material than gelatin here, the membrane of the coacervate—if one calls the surface layer of coacervate a membrane—can only be made of the same material, which makes up the bulk of the coacervate, i.e., gelatin.

As mentioned above, Lepeschkin was among the earliest in maintaining that protoplasm is a coacervate.[324] He also offered a theory of the fundamental substance of living matter, a loose chemical complex of proteins and lipids, which he called *vitaproteids* or *vitaids*.[325] This specific hypothesis was severely criticized[332; 92 p 62] and not further defended.

Then there was Ross Gortner (1885–1942), for more than a quarter of a century the Chief of the Division of Agricultural Biochemistry at the University of Minnesota and a strong advocate for the existence of *bound water* in living cells. In an address given at the Faraday Society meeting to a group of scientists with diverse first-hand knowledge on various aspects of water—the printed version of which bears the title: "The State of Water in Colloidal and Living Systems" and was published in the *Transactions of the Faraday Society*[80]—Gortner presented his view that at least a part of

the water in living cells is not normal liquid water and is referred to as bound. As one of the criteria that sets bound water apart from normal water, Gortner suggested that bound water does not dissolve substances soluble in normal liquid water—hence an alternative name for bound water in his and his coworkers' vocabulary is "non-solvent water."

In anticipation of what follows in Chapter 11, I mention that Gortner referred to experimental evidence in the literature for the existence of multilayers of adsorbed water in inanimate systems. However, Gortner then backed away in these words: "Unfortunately the properties of water in oriented adsorption films have not been sufficiently characterized to enable us to state whether or not this may be the type of water which the biologist is coming to call bound water."[80 pp 684–685]

Gortner's paper presented at the Faraday Society meeting was well received. A majority of the participants expressed interest, some very enthusiastically. However, there was one notable exception, i.e., the same Professor A. V. Hill, whose evidence for free cell water has already been briefly presented in [5.1].

Not mentioned earlier is the fact that A. V. Hill almost single-handedly extinguished the enthusiasm generated for the "bound water" concept, and put to rout the entire colloidal approach to cell physiology. He achieved this feat by presenting just a single set of experimental data demonstrating that water in frog muscle accommodates the same concentration of *urea* as in the surrounding fluid.[81] Accordingly, there is no "nonsolvent" water. (In years following, others showed that *ethylene glycol* also distributes equally across the cell membrane of rectus muscle and of red blood cells.[82]) Since cell water is thus proven to be normal liquid water, the components in the cells high enough in concentration to match osmotically the free Na^+ and Cl^- in a Ringer's solution, can only be free K^+ and free cell anion(s). *Ergo, no bound water, nor bound K^+ in living cells*[81]—in affirmation of the clinching twin tenets of the membrane theory.

Hungarian biophysicist, Professor E. Ernst, a witness to these events, recalled how the opinion makers of the day, including Rudolf Höber (despite his own electric conductance study pointing to the contrary [5.2]), W. O. Fenn, and F. Buchthal all renounced the concept of bound water and bound K^+, and subscribed *in toto* to the idea that living cells are membrane-enclosed dilute solutions following van't Hoff's osmotic law.[83 p 112] Each acknowledged that his new position was based on Hill's crucial experimental finding of equal urea distribution and his compelling logic.

In 1940 the major English-language *Journal of Colloidal Chemistry* was combined with the *Journal of Physical Chemistry*. For a few years, the combined journal bore the new title *Journal of Physical and Colloidal Chemistry*. Then the words "*and Colloidal*" were quietly dropped. The demise of the main English-language colloid magazine did not signal the end of colloid chemistry *per se*. Other journals like *Zeitschrifts für Kolloid Chemie*, *Kolloid Beihefts* and even *Protoplasma* continued publication. It was only the fledgling colloid- or protoplasm-oriented cell physiology that suffered an (undeserved) near-fatal blow.

8

Aftermath of the Rout

The long-term outcome of this rout of the protoplasm-oriented cell physiologists by A. V. Hill was devastating. The iconoclastic and often brilliant ideas and courageous voices of Martin Fischer, Herbert Roaf, Benjamin Moore, Ross Gortner, W.W. Lepeschkin vanished from the scene. It is in this "purified" silence that my career as a cell physiologist began in Chicago.

Only many years after receiving my Ph.D. degree did I discover piece by piece that there were alternatives to the membrane theory. And still later, did I realize how virtually all protoplasm-oriented cell physiologists in America and Europe had raised their heads brightly for a moment and then vanished. That is, with the exception of two isolated groups in far away Communist eastern Europe. One was led by E. Ernst in Pécs, Hungary; the other by Dmitrii Nasonov in Leningrad of the former Soviet Union.

8.1 The tiny Hungarian enclave under E. Ernst

Like Fischer, Moore, Roaf and others before him, Eugene Ernst (Ernst Jeno) (1895–1981) also believed that the membrane theory was wrong.[83] Nor did he believe that cell water is normal liquid water and that cell K^+ is free.

Instead, he believed that cell K^+ is *largely unionized, undissociated and unhydrated.*[83 p 152] Ernst subscribed to the view of Macallum and others [10.2(5)] that K^+ in striated muscle cells is not evenly distributed but located in the A-bands.[84] And that a substantial part of cell water is "swelling water" (*Schwellungswasser, Quellungswasser*) associated with muscle cell proteins.[399] In support, Ernst and his coworkers showed that both NaCl and gelatin lower the relative vapor pressure of water, but gelatin does it many times more effectively than NaCl. From this perspective, they argued

that frog muscle more closely resembles gelatin-gel than a solution of NaCl.[83 p 112; 399]

In anticipation of work to be described below [11.2], I may mention that Ernst's study of water sorption of frog muscle and gelatin was at a much lower range of relative vapor pressure than that of the normal physiological environment. In fact, most of their data points on frog muscle correspond to a final water content 10% or less of the total water content. The few data points corresponding to higher vapor pressures are too scattered to assess their true significance. This scattering could reflect too short an equilibration time (2 to 3 days),[399] (see end of [11.2]).

Professor Ernst died in 1981. His work was continued by his student, Professor Joseph Tigyi and later by Professor Miklós Kellermayer and his group at the University of Pécs.[85] In 1994, the University of Pécs kindly awarded me an honorary doctoral degree.

8.2. The Leningrad school under Nasonov and later, Troshin.

Dmitrii Nasonov (1895–1957) was born in Warsaw, the son of a zoology professor. Nasonov started out as a microanatomist, studying at one time at Columbia University in New York under cytologist E. B. Wilson, mentioned earlier. Nasonov was a decorated war hero in the siege of Leningrad during World War II. After the war, he returned to science and held the position of the Director of the Institute of Cytology in the same city.

With a solid foundation of cell anatomy, Nasonov's career as a cell physiologist was distinguished by his unwavering conviction that a sound cell physiology must rest upon a correct cell anatomy. And that a correct cell anatomy is impossible without taking into account that the cell is solid and made of protoplasm. His *protein theory of cell damage and excitation* illustrates the general direction of his own and his immediate associates' research.[86]

Nasonov also introduced his *phase theory* of permeability and bioelectrical potentials—contending that cells do not possess a cell membrane with varying permeability [86 p 164] and that an electrical potential difference occurs only across the surface of injured protoplasm but not across the surface of normal resting cells. This is how Nasonov described it in his own words: "According to our theory, the electromotive force arises only at the moment of injury or excitation when the electrodes (electrolytes ?, *query of G.L.*) are released from their linkage with the protein substrate. In this re-

spect our theory resembles the *alteration theory* of Hermann (1885)."[86 p 178; 87; 15 p 20]

I disagree to varying degrees with Nasonov on these two specific concepts. Thus the two-fold increase of permeability to sucrose following cell amputation offers one piece of evidence for the existence of a diffusion barrier (or cell membrane) at the normal cell surface (Table 1, see also [13.6]). The exploration with the Gerard-Graham-Ling capillary glass microelectrode[88] makes it hard to deny the existence of a potential difference across the healthy normal resting cell membrane—in harmony with the "pre-existence theory" of cell potentials first offered by L. Hermann's teacher, Emil DuBois-Reymond.[89] I shall return to the question of why Nasonov believed that there is no cell membrane.

Differences in some specific issues notwithstanding, I have great admiration for the courage and originality of this remarkable investigator. His, Aizenberg's and Kamnev's demonstration that permeant solutes can cause sustained shrinkage of living cells described in [4.1(3)] is but one of their landmark discoveries. Other major contributions from the Soviet school of cell physiology are described in his monograph, "Local Reaction of Protoplasm and Gradual Excitation"[86] and in his student, A.S. Troshin's monograph, which will be reviewed next.

The Institute of Cytology at Leningrad survived Nasonov's death in 1957 and its directorship went to his prize pupil, Aphanasij S. Troshin, or simply A.S. Troshin.

9

Troshin's Sorption Theory for Solute Distribution

In 1956, A. S. Troshin published the Russian version of his monograph,[90] which was to be translated into three other languages (German,[91] Chinese, and English). The English version bears the title: "Problems of Cell Permeability."[92] Interestingly enough, the contents of these monographs do not focus on what the title of the book tells us, permeability, which is a *rate process*. Instead, they deal primarily with solute distribution, which in the view of Troshin and others including myself, represents an *equilibrium phenomenon*. A possible interpretation of Troshin's choice of the title may be as follows: Troshin knew how the proponents of the membrane (pump) theory—who are by far the majority in this field—misinterpretated these equilibrium distribution problems as problems of membrane permeability, but he chose to refer to these underlying phenomenon as "permeability" problems to be in touch with a larger audience.

In the Preface to the original Russian edition and reprinted in the English edition of Troshin's book, Nasonov wrote: "We have come to the conclusion that this theory (the membrane theory) gives a completely false idea of the structure of the cell and the nature of the substance contained in the protoplasm. Furthermore, as a result of the apparent simplicity of the scheme it offers in explanation of the many problematic phenomena, the membrane theory has acquired great popularity among physiologists and has, in our opinion, induced them to follow a false line in their theoretical researches."[92 p xiii]

And this is how Troshin himself introduced his own book: "According to the theory developed by Lepeschkin, Nasonov and Fischer, and also certain other workers, the greater or lesser permeability of cell for any substance is to be explained not by the greater or lesser penetration of the substance through the cell membrane, but by the difference in the solubility of the substance in the protoplasm and the surrounding aqueous medium

and by the adsorption or chemical binding by the cell colloids of the matter penetrating the wall."[92 p 3]

This self-effacing declaration notwithstanding, I believe that Troshin deserves the lion's share of credit for the "Sorption Theory" for solute distribution in living cells. It is true that Moore, Roaf, Fischer, Lepeschkin, Nasonov and others had pronounced these basic ideas earlier—which is certainly of prime importance and each of these pioneers should be acknowledged for their priority—but it was Troshin who transformed these ideas summarily expressed into quantitative data expressed in a rigorous equation form. To introduce Troshin's work, I shall begin with the earlier work of I. Ye. Kamnev.

In 1938 Kamnev published in the Russian-language journal, *Archives of Anatomy, Histology and Embryology*, an article entitled, "The permeability for sugars of striated frog muscle."[34] This is a simple but defining paper. When frog muscles were immersed in a Ringer's solution containing sucrose or galactose, both sugars readily entered the muscle cells and eventually reached constant levels, which were below their respective levels in the bathing solutions (Figure 2). In dead cells, the level of sucrose and galactose rose to levels similar to those in the bathing medium.

Kamnev's conclusion that sucrose and galactose enter the muscle cells rests squarely upon the accuracy of his estimate that frog sartorius muscle has an extracellular space equal to 9% of its total muscle weight[86 p 114] (and that sucrose or galactose found beyond what is contained in this extracellular space has entered the muscle cells). Since extracellular space as high as 35% has been reported in the literature,[see 336 p 677] confirmation of the 9% figure is vital to establishing the validity of Kamnev's conclusion.

Work from my own laboratory supports Kamnev. Thus between 1967 and 1975, my associates and I obtained from *five* independent methods, four of which were entirely new (low concentration inulin probe method, 10.3%; poly-glutamate probe method, 8.9%; single fiber sucrose space method, 9%; Br^{86} efflux-analysis method, 8.2%; the centrifugation method, 9.4%)[49 p 136] an average value of 9.2% ± 0.69% (mean ± S.D.) for the extracellular space of frog sartorius muscle, which lies close to Kamnev's 9% figure. My evidence that sucrose enters frog muscle cells as shown in Table 1 above support Kamnev's conclusion from a different angle.

Kamnev concluded that the steady level of sugar reached in the muscle cells does not depend on a membrane mechanism but on the solubility of

these sugars in the muscle sarcoplasm, extending the idea Martin Fischer first suggested [7]. Kamnev expressed the belief that the sarcoplasm behaves as a phase with different solvent properties from water in the surrounding medium.

Ten years after Kamnev's publication, Troshin resumed this line of study, indicating that various other nonelectrolytes behaved just like galactose and sucrose in reaching steady levels in cell water lower than in the surrounding medium.[93] It is the rapid and seemingly equal rates of entry of these solutes into frog muscle and other living cells {see [16.6(3.2)]}, which apparently led Troshin's mentor, Nasonov to the conclusion that there is no cell membrane.[86 p 164] (As pointed out earlier, I do not agree with Nasonov on this specific point. I also believe that to no small measure is this disagreement a consequence of my access to the powerful radioactive-tracer technology, apparently unavailable at their times to Nasonov and Troshin.)

Following Bungenberg de Jong,[321] Lepeschkin,[324] Duclaux, Guilliermond, Oparin and others,[824; 92 p 58] Troshin suggested that living cells are *complex* coacervates. Quoting work from Bungenberg de Jong's laboratory and his own, Troshin showed that, like water in living cells, the water in a simple gelatin coacervate accommodates various solutes at levels lower than that in the surrounding medium. Neither Kamnev nor Troshin offered a molecular explanation how water in living cells differs from normal liquid water, nor a molecular mechanism why sucrose, galactose have low solvency in cell water. Nor did they offer an explanation why urea and ethylene glycol distribute equally across the muscle cell surface [5.1].

On the role of metabolism on solute distribution, Troshin wrote: "Thanks to metabolism, the sorptional activity of the protoplasm is maintained in a definite level.....Upon breakdown of metabolism, this level changes: the solubility of substances in the protoplasm increases and the binding by cell colloids of some substances is depressed...."[92 pp 373–374] No mechanism was offered why breakdown of metabolism leads to increased solubility and decreased binding. Four years earlier, Nasonov concluded his section on "phase theory of bioelectric potentials and cellular metabolism" in these words: "energy is evidently necessary for the maintenance of certain labile chemical compounds (including the structure of proteins)."[86 p 202] But he too offered no explanation of how energy maintains the structure of proteins and other labile compounds.

In harmony with the ideas first introduced by Martin Fischer (and partially also by Moore and Roaf), Troshin believed that solutes inside the cells in general fall into two categories: *adsorbed* (or otherwise bound) and *dissolved in cell water*. He then introduced a two-term equation (Equation A1 in Appendix 1), containing a linear term representing solute dissolved in cell water following Henry's Law (or more correctly, in my opinion, the Berthelot-Nernst Distribution Law of which Henry's Law is a special case for solubility of *gases* in solvents[13 pp 696–697]), and a "hyperbolic adsorbed" fraction following Langmuir's adsorption isotherm.[117] Troshin also showed how the equilibrium distribution of a variety of solutes in living cells as well as coacervate models can be described by the two-term equation. I have repeatedly suggested that this two-term equation be named "Troshin equation" in honor of the author, who died of cancer in 1982.[173 p 20; 174 p 285]

After Troshin's death, the spirit and philosophy of protoplasm-oriented cell physiology at the Leningrad Institute of Cytology began to wane, flicker. That is, until Professor Vladimir Matveev, formerly at the Institute of Marine Biology, Academy of Science USSR at Vladivostok, arrived. Ever since he has been valiantly doing everything possible to bring that spirit and philosophy back again.[554] However, to resume our story, we must turn back the clock to the year 1951 when young Troshin published a series of five short papers on solute distribution in coacervate models and in living cells.[93] In the same year, I published a short article describing the essence of what was to be known as Ling's Fixed Charge Hypothesis.[94]

10

Ling's Fixed Charge Hypothesis

As mentioned earlier, I began my training as a cell physiologist under Professor Ralph W. Gerard in the world-famous Department of Physiology at the University of Chicago. Like virtually all my peers, I was totally immersed in the belief that the membrane theory was the one and only guiding light. Naturally my first full-length publications (coauthored respectively with Prof. Gerard and W. Woodbury) are on the subject of "membrane potential,"[95] a name and concept taken straight from the membrane theory.

Following a presentation of the "Sodium Pump Hypothesis" I gave at a departmental seminar[49 p 124]—based solely on information gathered at the library—I carried out some simple experiments of my own. My goal was to find out if the combined action of metabolic poisons and low temperature (0°C) would promptly lower the concentration of K^+ in frog muscles as the hypothesis would have predicted. The result was unforeseen, but also exciting. No change at all in the K^+ concentration at the end of a five-hour-long experiment (see Table 8.4 and pp. 176–177 in Appendix 1 of Reference 49 for results of later more extensive confirmatory work). As I became more and more drawn into the new direction of research thus started, my doubts about the sodium pump hypothesis grew.

In the years following, I spent much time trying to dream up an alternative energy-saving mechanism to replace the hypothetical sodium pump. It was tough going. Years went by and I was getting absolutely nowhere. Then all of a sudden a possible solution dawned on me while I was browsing and mulling in the basement of the Welch Library of the Johns Hopkins Medical School in Baltimore. This new seminal idea, the centerpiece of what was later called Ling's Fixed Charge Hypothesis (LFCH),[96] was also the first step toward the construction of the unifying theory of cell physiology, called the *association-induction hypothesis* (AI Hypothesis; AIH).[98]

The essence of this hypothesis will be presented here and in Chapters 11, 14 and 15.

The idea that intracellular K^+ could be selectively adsorbed over Na^+ was, to the best of my knowledge, first suggested by Herbert Roaf and Benjamin Moore in 1908. However, as pointed out once already (and until the LFCH appeared), neither they nor anyone else had offered a quantitative molecular mechanism for this striking discrimination between a pair of ions so closely similar. Nor had anyone else suggested an explanation why proteins—the most likely candidates inside the cells to offer the sites for the adsorption of K^+—can do the job in the living cell, but repeated attempts in the past to reproduce the phenomenon *in vitro* had all failed.[99; 41 p 120] Nor had a molecular explanation been offered why this ability of selective adsorption (if correct) is promptly lost upon cell death. Ling's Fixed Charge Hypothesis represents the outcome of my first attempt to answer these questions.

10.1 A theory of selective accumulation of K^+ over Na^+

Three new theoretical concepts were introduced in the construction of a coherent theory of selective adsorption of K^+ over Na^+ and the closely related phenomena just mentioned—a theory to be referred to later as Ling's Fixed Charge Hypothesis (LFCH). However, only one of the theoretical concepts described under (3) below represents what I mentioned above as the *seminal idea.*

(1) Enhancement of counter-ion (or neutral molecules) association with site fixation

This is a major new concept introduced in 1952—not in the recognition of the existence of fixed charges, which had occurred long before[100]—but in the *theory of full associations* of these fixed charges with free counterions like K^+ and Na^+. The incorporation of this basic concept distinguishes LFCH (and the association-induction hypothesis) from other "fixed charge hypotheses" of the past and present.[101] Indeed, without full counter-ion association, selective adsorption of K^+ over Na^+ by the mechanism proposed below under (3) or by others suggested elsewhere later [14.1]—*would have been impossible.*

In the wake of the great theories of ionic dissociation of Arrhenius[102] and of Debye and Hückel,[103] it has been widely believed that monovalent ions of one electric charge in a dilute aqueous solution are fully dissociated

from monovalent ions bearing the opposite charge—regardless if one species is fixed in space. (For illustrations of this deep-seated belief as late as 1961, see Figure 1 in Reference 104, Figure 5 in Reference 100; Figure 1 in Reference 105.) In harmony with this view of full ionic dissociation, the influential protein-chemist, K. U. Linderstrøm-Lang of the Carlsberg Laboratory in Stockholm described a protein molecule as an ellipsoid with electric charge uniformly smeared over its surface. Counterions in number matching the excess charges of the opposite polarity hover over the protein as a diffuse ion cloud.[467] In his opinion, direct contact between proteins and counterions does not exist. My view to the contrary, to be reviewed next, was definitely running against the tide.

For the enhancement of counterion association in consequence of the *spatial fixation* of one species of ion,[96 p 769] one of the two causes I gave in 1952 is the overlapping of the attractive electric field of neighboring fixed charges—this field-overlapping is the microscopic foundation of what is known as the *Law of Macroscopic Neutrality.*[97 pp 330–331] The result of this field-overlapping is not merely an increase of the *adsorption energy* of the ion [14.2(1)], enhancing its association with the fixed ion, but also the confinement of a dissociated counterion to within a much smaller space surrounding the fixed ions.[96 p 769] This confinement reduces the *entropy of dissociation* of the counterion and strengthens its association with the fixed ion also.

The second cause I gave in 1952 is kinetic in origin (and nothing much more beyond that). Indeed, its *specific detailed mechanisms are given for the first time here and now*: While the chance of an anion and cation meeting and pairing may be assumed to be about the same whether or not one species is fixed in space, the number of effective collisions received from surrounding water molecules, which would tear apart the associated pair, is at least halved if one species is fixed and thus unmoved by the collision. The result is also an enhancement of association. Note that the first (field-overlapping) cause is restricted to particles carrying net electric charges (i.e., ions), the second (kinetic) cause does not suffer such a restriction and may therefore underlie all localized *adsorption* including the adsorption of ions and water.

The theory of enhanced counterion (and neutral adsorbent) association has been reiterated and discussed in my publications in years following.[98 pp 17–28; 106 pp 152–155; 107 pp 39–41] Its importance in compelling *close-contact association* in living phenomena is put center-stage by the title of the general

theory, *association*-induction hypothesis. Experimental confirmations on inanimate models of the predictions of this theory as applied to ions[469; 98 pp 17–22; 107 p 40] and to water[470] had been already in the literature—before the (only) theory for its explanation known to me (i.e., LFCH just reviewed) was published. One of these experimental studies will be summarized here.

Kern showed that the activity coefficient of Na^+ in 0.0125 to 0.2 M Na salts of isobutyric acid ($CH_3CHCOOHCH_3$) ranged from 0.90 to 1.00, indicating that 90% or more of the Na^+ is free. When these isobutyric-acid monomers are joined end-to-end into the linear polymer, polyacrylic acid $(–CH_2CHCOOHCH_2–)_n$, and the carboxylic groups thus (not completely but substantially) *fixed in space*, the activity coefficient of Na^+ at the same concentration range, fell to 0.168 to 0.315, indicating that from 68% to 83% of the Na^+ is now associated with the carboxyl groups.[469]

(2) The salt-linkage hypothesis and a critical role for ATP

According to the LFCH, the negatively-charged β- and γ-carboxyl groups of isolated native proteins are largely engaged in *salt-linkages* with fixed cations (e.g., positively-charged ∈-amino groups and guanidyl groups belonging respectively to lysine and arginine residues of intracellular proteins)[108] and thus not free to adsorb cations like K^+. In the LFCH, these β- and γ-carboxyl groups can be made available for adsorbing K^+ ions by *adenosinetriphosphate* or ATP, when ATP occupies key controlling *cardinal sites* [14.3(3)].

In this early model, ATP serves its role by the same mechanism proposed earlier by Riseman and Kirkwood for keeping the contractile proteins like myosin from collapsing upon themselves and shortening, i.e., long-range electrostatic repulsion (transmitted through space).[109] Later, in the association-induction hypothesis, I introduced a new (though related) mechanism (see [14.3] below). ATP is, of course, the end product of energy metabolism. Its critical role in maintaining selective K^+ adsorption explains the loss of this ion upon cell death, when metabolism ceases and ATP regeneration comes to an end.

3) The 1952 electrostatic model for the selective accumulation of K^+ over Na^+ in living cells

Taking into account *dielectric saturation*[110] (rapidly declining *dielectric constant* as one approaches an electrically charged site or ion, as illustrated in the inset of Figure 6) in the electrostatic interaction between a fixed an-

ion and a free monovalent counter-cation, one can calculate the statistical probability of finding the counter-cation at different distances from the center of the negatively charged oxygen atom (of a β- or γ-carboxyl group) (Curve 2 in Figure 6).

The bottom part of Figure 6 also shows the diameters of the smaller hydrated K^+ and the larger hydrated Na^+ ion.[98 p 548; 111] Note that only the center of the smaller hydrated K^+ can enter the "shell of high probability of association" around the fixed anion and become selectively adsorbed, but the hydrated Na^+ is too large to do the same and is thus largely left out.

What is illustrated in Figure 6 is the first-of-its-kind *quantitative molecular mechanism for the selective adsorption of K^+ over Na^+*. Anticipating new developments to be described below in [14.1], I recast this mechanism in an adulterated but easier-to-parley lingo: Due to the different distances separating (the center of) the fixed anion and (the center of) the smaller hydrated K^+, the *electrostatic field strength* experienced by a K^+ is stronger than that by the larger hydrated Na^+. The resulting preferential adsorption of K^+ over Na^+ on the β- and γ-carboxyl groups provides an energy-conserving, quantitative molecular mechanism for the selective ac-

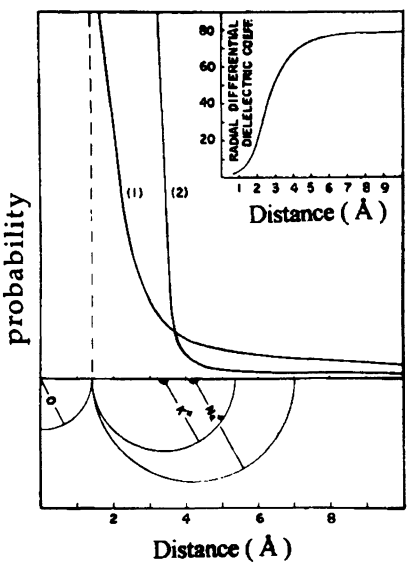

Figure 6. A theoretical model for the selective adsorption of K^+ over Na^+ on a fixed oxyacid site presented in 1952 as part of Ling's Fixed Charge Hypothesis (LFCH) Computation takes into account the decrease of the dielectric constant of water (referred to in the Inset as "radial differential dielectric coefficient") when approaching an ion as illustrated in Inset. Theoretical curve (2) shows the probability of finding a monovalent cation (e.g., K^+, Na^+) associated with the fixed oxyacid anion—partially represented at extreme left of bottom section of the figure—at distance (away from the center of the oxygen atom of the oxyacid group) indicated on the abscissa in Angstrom units. Note that only the hydrated K^+ with its smaller radius shown in the bottom figure can enter the "shell of high probability of association" around the oxygen atom of the negatively-charged oxyacid group and becomes selectively adsorbed over the larger hydrated Na^+ (the center of which stays largely out of the shell of high probability) also shown in the bottom part of the figure. (Ling,[96] reproduced from *Phosphorus Metabolism* by permission of The Johns Hopkins University Press)

cumulation of K^+ over Na^+ in living cells. (For a more detailed presentation of this theoretical model, see pp. 54–57 in Reference 98.)

In summary, proteins like myosin in muscle cells carry many β- and γ-carboxyl groups. Adsorption of ATP on the controlling *cardinal site(s)* {[14.3(3)]} on myosin enables these β- and γ-carboxyl groups to associate with (or adsorb) either K^+ or Na^+. Since the hydrated K^+ is smaller than hydrated Na^+, it is energetically more favorable for K^+ to be adsorbed. Accordingly, K^+ is selectively accumulated in the cell over Na^+.

That said, I must add that this was how the LFCH (and the association-induction hypothesis) began in 1952. The proposed mechanisms are as valid today as then, as will be made clear in the next Section.

10.2. Experimental verifications of LFCH (and parts of AIH)

Experimental testings of the postulations of LFCH took more than 40 years to complete; the results are summarized next.

(1) Cytoplasm rather than the cell membrane as the seat of selective K^+ accumulation (and Na^+ exclusion)

Figure 7 illustrates an *Effectively Membrane-Pump-Less-Open-ended Cell* or *EMOC* preparation of a frog sartorius muscle.[112; 113] A sartorius muscle comprises some 1000 hair-like single muscle cells; each measures about 0.006 cm wide but 2.5–3.0 cm-long; all run in parallel and uninterrupted from one end of the muscle to the other end. With the pelvic end of the muscle tied to an anchoring string (f), the tibial end of the muscle is drawn through a tight-fitting, Vaseline-filled slit (g) in a silicone-rubber gasket at the end of the glass tube. A transverse cut of the muscle is then made at a point just beyond the silicone-rubber gasket and proximal to the tapering tibial end of the muscle protruding outside the gasket. This cut exposes the cytoplasm of all the 1000-some individual muscle cells uniformly and simultaneously to the radioactive K^{42} and Na^{22} in the Ringer's solution (e) bathing the cut end of the muscle. The intact portion of the muscle is suspended in part in vaseline but mostly in moist air. As mentioned above, electron microscopy and radioactive tracer studies have established that the cut end of a muscle cell does not regenerate a new cell membrane.[42; 113] [4.1(4)]

Since neither vaseline nor moist air contains K^+, nor can they accommodate Na^+, the sodium pump—if it exists—cannot function here. There-

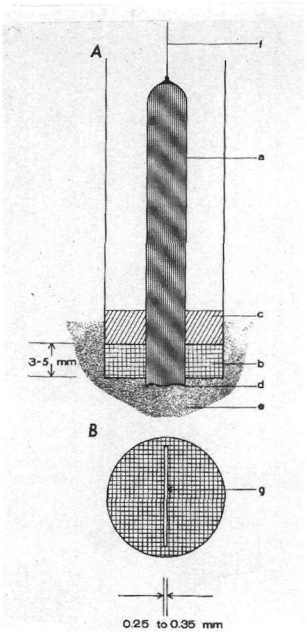

Figure 7. *Effectively membraneless open-ended cell (EMOC)* preparation of a frog sartorius muscle. (A) side view; (B) bottom view. Only the cut end (d) of the sartorius muscle (a) is in direct contact with the radioactively labeled Ringer's solution (e). Cut muscle is secured on one end by the tight-fitting slit (g) of the silicon rubber gasket (b) and on the other end by an anchoring piece of string (f). Vaseline (c) originally filling the entire lower end of the glass tube including the gasket slit effectively prevents seepage of labeled solution into and out of the gasket slit, through which the tibial end of the muscle passes. (Ling,[112] by permission of The Journal of Physiology(London))

fore, the entire cut muscle in an EMOC preparation does not have a functional (hypothetical) sodium pump. Accordingly, the membrane pump theory predicts that as incubation continues, the K^+ and Na^+ concentrations throughout the muscle cells will gradually approach those in the Ringer's solution bathing the cut end (source solution).

The observed distribution of these labeled ions was different. Thus in the intact portion of the muscle, the concentration of labeled K^+ continued to rise with increasing duration of incubation to levels much higher than that in the source solution, but that of labeled Na^+ rose only to approach the same low level found in normal cells (0.1–0.2 of that in the source solution) and rose no further (Figure 8A).

The labeled Na^+ content of the fluid collected from within the *extracellular space* between the muscle cells indicates a labeled Na^+ concentration virtually the same as that in the source solution (e in Figure 7). This equal concentration of labeled Na^+ in the extracellular space and in the source solution—hence an absence of a *diffusion head* along the length of the muscle toward the source solution—indicates that the failure of labeled Na^+ level

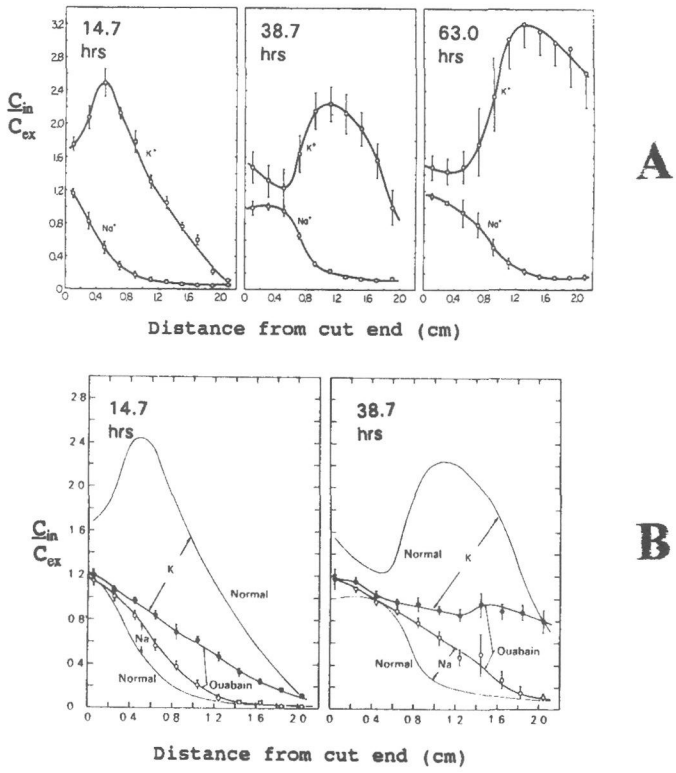

Figure 8. Distribution of labeled K^+ and Na^+ at different distances from the cut end (abscissa) of a frog sartorius muscle installed in an EMOC setup (illustrated in Figure 7). Duration of incubation are indicated in the figures (25°C). Ringer's solution bathing the cut end of the muscle contained no ouabain in (A) but (initially)10^{-4} M ouabain in (B). Ordinate represents the concentration of labeled K^+ or Na^+ in cell water expressed as a ratio to their respective concentration in the Ringer's solution bathing the cut end at the conclusion of the experiment. (Ling,[112] by permission of The Journal of Physiology (London))

to rise in the intact portion of the muscle cells was not due to a persistent backward transport of Na^+ *via* the extracellular space to the source solution from where the labeled Na^+ had originally come.

All these findings contradict the expectations based on the membrane-pump hypothesis. In contrast, they are in full harmony with the LFCH (and the AI Hypothesis), according to which the concentrations of K^+ and Na^+ in living cells reflect the attributes of the cytoplasm and have little to do with the cell membrane and nothing at all to do with a postulated sodium pump.

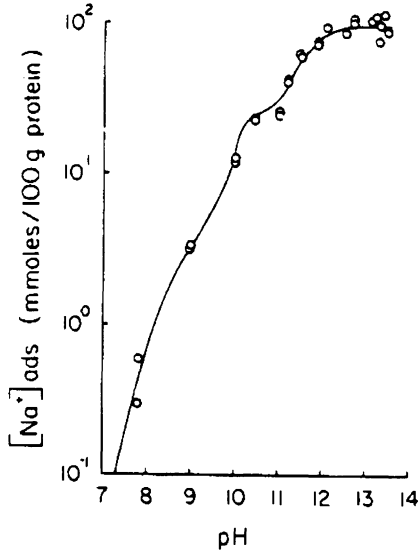

Figure 9. The quantitative relation between the number of fixed cations of hemoglobin neutralized and the number of Na^+ adsorbed. Both the number of fixed cations and of Na^+ are given in millimoles per 100 g. of hemoglobin. Points represent experimentally- measured number or concentrations of Na^+ adsorbed on 10% bovine hemoglobin at different pH's. The solid line going through or near most of the experimental points is a composite of the theoretically calculated titration (or neutralization) curves of all the α-amino groups, ∈-amino groups and guanidyl groups present in 100 g. of bovine hemoglobin. (Ling and Zhang[114])

(2) Confirming the salt-linkage hypothesis for past failures to demonstrate selective adsorption of K^+ on isolated proteins

The fact that isolated native proteins do not adsorb K^+ selectively (or Na^+) at a high level[99] had played a major role in the gradual adoption of the membrane pump concept.[41 p 120] However, this failure might not be the result of an inherent incapacity of proteins to adsorb K^+ and other alkali-metal ions; but, as I first suggested in 1952, the result of the engagement of the (would-be-K^+-adsorbing) β- and γ-carboxyl groups in forming with fixed cations what are known as *salt-linkages*.[108; 96 pp 774–781; 107 p 37 n4]

Thirty-six years later, Ling and Zhang confirmed this "salt-linkage hypothesis." Using a Na^+-selective glass electrode,[114] they demonstrated stoichiometric adsorption of Na^+, (or of other alkali-metal ions) on *all* the β- and γ-carboxyl groups of native bovine hemoglobin—after the fixed cations had been neutralized and thus eliminated from competition by the step-by-step addition of NaOH.

As shown in Figure 9, the experimentally measured *adsorbed Na^+* (empty circles) faithfully follow the theoretically calculated *titration (or neutralization) curves of the various fixed cations*. Thus, for each fixed cation eliminated by neutralization, one Na^+ binds onto the fixed anionic β- and γ-carboxyl group thus liberated—from the clutch of its former salt-linkage partner. This demonstration establishes that β- and γ-carboxyl

groups *can* indeed adsorb alkali-metal ions, and do so selectively, when the condition is right. The selectivity order for the alkali metal ions on the liberated β- and γ-carboxyl groups is $Na^+ > Li^+ > K^+ > Rb^+ > Cs^+$. There is also strong *auto-cooperative* interaction among the adsorption sites for Na^+ [14.3(2)]. $-\gamma/2 = 0.824$ Kcal/mole, corresponding to a Hill coefficient, n, of 4.05 {see [14.3(2)] on Hill coefficient and *cooperativity*}. Parallel studies revealed that what we have demonstrated in hemoglobin applies to all (five) native proteins examined.[114]

In human red blood cells, hemoglobin makes up 97% of its total intracellular proteins.[140 p 389] They also contain 96 to 100 mmoles of K^+ per liter of fresh red cells. If all this K^+ is adsorbed on β- and γ-carboxyl groups (for evidence, see subsection (4.2) following), hemoglobin must provide most of them. *In vitro*, however, native hemoglobin does not adsorb K^+ at neutral pH;[484] even though alkalinity does lead to its adsorption. Since the interior of living cells is neutral,[505] something else in living cells must serve the role of alkalinity in liberating β- and γ-carboxyl groups for the selective adsorption of cell K^+. According to the LFCH (and AIH), that something else is ATP. Subsection (6) following and Section [15.1(1)(2)] still later provide experimental support for this key theoretical postulation.

(3) The great majority of cell K^+ is not free

Three major sets of independent experimental evidence, to be presented next, demonstrate in a mutually-supportive manner that the bulk of cell K^+ is "tethered" within the cells. Together, these data have, on one hand, reversed the earlier conclusion of free cell K^+ described under [5.2] and, on the other hand, affirmed the still earlier work of Höber cited in the same Section.

(3.1) Mobility of intracellular K^+

In [5.2] I have reviewed the work of Hodgkin and Keynes, which led them to conclude that K^+ in squid axons is free as in a 0.5 M KCl solution.[263] Hodgkin and Keynes' conclusion is supported by results from the intracellular diffusion study of muscle segments by Kushmerick and Podolsky.[264] However, both conclusions are in conflict with the results of the (repeatedly confirmed) conductance measurement of R. Höber.[265, 266, 15 p 244] One asks: Is there some procedural difference between the studies of Höber (and his supporters) and those of Hodgkin-Keynes-Kushmerick-Podolsky (HKKP)?

There is. Höber and his supporters consistently used *intact* cells; HKKP just as consistently used cells *with open ends*.

Subsequent studies using the EMOC preparation have established that the cytoplasm near the cut ends of amputated muscle cells deteriorates rapidly as shown by the (local) loss of K^+ and gain of Na^+ (Figure 8A) (see also Figure 6 in Reference 112). Thus Kushmerick and Podolsky, who used short 3- to 6-mm length muscle segments with *both ends cut open*, were at risk of unknowingly studying injured or even dead cytoplasm.[264]

Hodgkin and Keynes too used giant cuttlefish axons with cut ends. To monitor the health of their cuttlefish axons under study, Hodgkin and Keynes tested from time to time the axons' electric excitability. Of the eleven axons studies, three lost their excitability before the end of the experiments. But since the mobility of labeled K^+ measured in all eleven experiments are closely similar, the authors stated: "There is, in any case, no evidence to suggest that loss of excitability caused any large change in mobility."[263 p 523]

The data presented in Figure 8 show that cutting the (muscle) cells open causes a spreading deterioration from the cut end (see also Reference 86, pp. 42–43). In contrast, the data of Figure 8 also show that the intact end of the muscle cells had remained normal for days on end as judged by its normal K^+ and normal Na^+ concentrations.[112 Figure 6] Thus one may reasonably expect making a more accurate determination of the mobility of cell K^+, if one can somehow *focus on the intact end of muscle* and keep away from the cut end. Indeed, it was based on this idea, that Ling and Ochsenfeld carried out their study of the diffusion coefficient of radioactive K^{42}-labeled K^+ (and of radioactive tritiated water) in frog sartorius muscle cells in EMOC preparations.[312]

Here we first exposed, under physiological conditions, an intact isolated frog sartorius muscle to radioactive K^{42} (with or without also tritiated water, H^3HO) until all or at least a major share of the cell K^+ had reached diffusion equilibrium with the labeled K^+ in the special incubation medium (Solution 731) developed for the long-term preservation of isolated frog muscle.[381] We then mounted the muscle in an EMOC setup like that shown in Figure 7, cut off the tibial end of the muscle and started washing the cut end in a large volume of vigorously stirred non-radioactive normal Ringer's solution. Representative data are reproduced in Figure 10.[312]

The points in Figure 10 are experimental and the lines are best-fitting theoretical curves from (well-known) theory of diffusion.[277] In some cases,

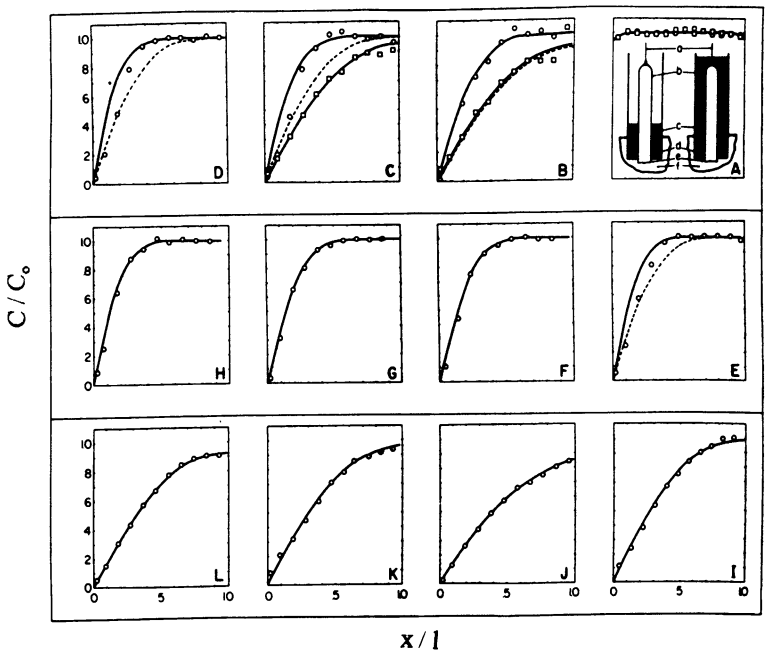

Figure 10. Diffusion profiles of labeled K^+ from surviving (B to H) and dead (I to L) frog sartorius muscles. Ordinate represents concentrations (C) of labeled K^+ (A to L) or of tritiated water (in A–C only) as fractions of their respective initial concentration in the muscle (C_o). Abscissa represents the distance x from the cut end of the muscle expressed as a fraction of the total length, l, of the (amputated) muscle. Circles, $^{42}K^+$; squares, tritiated water. Circles, squares (and lines going through or near most of the circles or squares) at top of A represent respectively ^{42}K and tritiated water concentration along the length of an intact unwashed normal frog sartorius muscle. For more details of EMOC preparation used (shown sketchily here in A), see Figure 7. Solid lines are theoretical diffusion profiles that fit all the points with a single ($D_K t/l^2$) value (F to L), where D_K is the diffusion coefficient of K^+, t is the duration of the experiment and l is as defined above, the total length of the (amputated) muscle. In composite figures C to E, each dotted line and the accompanying solid line fit only part of the data points. Muscles in I to L were killed by prior exposure to the metabolic poison, iodoacetate, and were in full rigor before being mounted on the EMOC setup but were kept at their initial normal relaxed lengths at all times. (Ling and Ochsenfeld,[312] with permission from *Science* copyright 1973. American Association for the Advancement of Science)

the whole set of data can be fitted with a single diffusion coefficient (H to F). In others, the data points in the region near the cut end can be best fitted with a faster diffusion coefficient; but a slower one can fit best the data points in the region close to and including the intact end of the muscle (B to E).

From a total of seventy-two (72) sets of independent experiments, the diffusion coefficient of K^+, D_K, in the healthy part of the muscle cytoplasm

away from the cut end is only *one eighth* (1/8) of that of a 0.1 M KI solution. In muscles deliberately killed by prior exposure to the metabolic poison, sodium iodoacetate (IAA), until rigor was fully developed while being held to their respective normal resting length (I to L), D_K is about three quarters (3/4) of, and thus close to, that of free K^+. In injured cytoplasm, D_K lies in-between, roughly one third (1/3) of that of free K^+.

The diffusion coefficient of tritiated water estimated from the intact ends of the same muscle preparations, where the D_K's were determined, is only reduced from that in normal tritiated water by a factor of two, a far cry from the eightfold reduction in the diffusion coefficient of cell K^+. This great divergence stands in sharp contrast to the uniformly two-fold reduction of the diffusion coefficients of the six solutes reported by Kushmerick and Podolsky.[264]

Our data led us to the conclusion that the fast D_K measured by HKKP may be that from either seriously injured or nearly dead cytoplasm. At a D_K value only one eighth of that of free K^+, by far the greatest majority of cell K^+ in frog muscle cells must be in a "tethered" state.

A subsequent suggestion from Krushmerick that the diffusion coefficient we measured was artificially slowed down by an inside negative electrical potential (i.e., the remnant of the resting potential)[270] has been refuted both by experimental observations and on theoretical grounds.[271]

Experimentally, I have shown that the rate of diffusion of radioactively-labeled, electrically-charged ions from the cut end of a sartorius muscle (in an EMOC preparation like that shown in Figure 7) remained *unchanged* after the electric potential difference across the open end of the muscle cells (regardless of its magnitude) had been eliminated by soaking the muscle before and during the study in an isotonic KCl solution[271]—which promptly and completely depolarized the (residual) electrical potential.[292] Thus whatever the electric potential difference that might exist across the cut end of muscle cell, it has no influence on the rate of outward diffusion of a charged ion through the cut end in complete agreement with theory shown next.

The Law of Macroscopic Electric Neutrality forbids any measurable quantity of the positively charged K^+ to move out of the cut end of the muscle by itself alone.[97 pp 330–331] Instead, all measurable quantity of K^+ diffusing out of the muscle must be accompanied by an equivalent amount of anions, or in exchange for an equivalent amount of cation(s) from the outside, or a combination of both. *These escorted and exchange movements*

are electrically neutral, and therefore indifferent to the presence of an electric potential difference across the cut end.

(3.2) X-ray absorption-edge fine structure

When a monochromatic X-ray beam shines through a thin layer of an aqueous solution, different proportions of the energy of the X-ray are absorbed, depending on the photon energy of the beam and the nature of the atoms or ions present (e.g., K^+). By expressing the ratio of the intensity of the incident beam over that of the emerging transmitted beam at different photon energy, an *X-ray absorption-edge fine structure* can be constructed. And this structure is a sensitive probe of the local environment of the energy-absorbing atoms or ions. Huang and coworkers demonstrated that the absorption edge fine structure of K^+ in frog erythrocytes is quite different from that of K^+ in a solution of a potassium salt of similar concentration.[115] They concluded that their data indicate binding of K^+ in the frog erythrocytes.

(3.3) K^+ activity measured in living cells with a K^+-specific ion-sensitive microelectrode

If intracellular K^+ is really free like that in a dilute KCl solution as postulated in the membrane (pump) theory, a K^+-specific microelectrode inserted into one kind of living cell should register a K^+ activity close to that obtained in all other kinds of living cells from a similar animal—both close to the K^+ activity in a free K salt solution of similar ionic strength.

In fact, studies reported on a variety of living cell types between 1972 and 1981 gave *widely different* K^+ activities ($a_K = \gamma_K [K^+]_{in}$, where a_K represents the *activity* of cell K^+, γ_K represents its *activity coefficient* and $[K^+]_{in}$, its *concentration*) corresponding to activity coefficients (γ_K) ranging from substantially below unity (e.g., 0.27 from *Amphioma* intestinal cells) to above unity (e.g., 1.20 from bullfrog intestinal cells). (For a compilation of the published data up to 1981 and their sources, see Reference 15, Table 8.2.)

These widely different measured activity coefficients once more contradict the free K^+ tenet of the membrane theory, which, as just mentioned, predicts a *uniform* value in all cells. In contrast, all the divergent data can be readily understood on the basis of self-evident facts and several derived concepts of LFCH and the AI Hypothesis: (i) that the electrode can only "see" the activity of K^+ in a microscopically thin layer of liquid in immediate contact with the electrode surface; (ii) that adsorbed cell K^+ is not

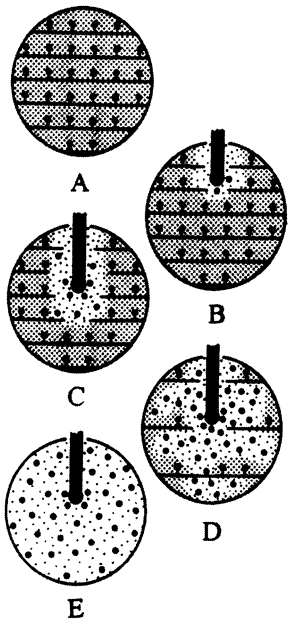

Figure 11. Diagrammatic illustration of how widely different *activity* of K^+ (a_K) in living cells can be registered by an intracellular K^+-specific microelectrode—shown as a vertical black bar with a spherical lower end—in consequence of varying local and spreading injury produced by the intruding microelectrode. Solid small black circles attached to the horizontal (protein) chains represent adsorbed K^+. Regular arrays of tiny dots in the background represent polarized-oriented cell water molecules. Unattached black circles and irregularly distributed small dots represent respectively free K^+ ions and free liquid water molecules. (A) Normal cytoplasm with polarized water and adsorbed K^+. No intruding electrode. Low a_K. (B) Relatively stable cytoplasm after electrode impalement. Modest K^+ liberation and water depolarization in regions immediately in contact with the electrode surface. a_K higher than in normal cells but lower than total K^+ concentration, C_K, ($a_K < C_K$). (C) Moderately labile cells. Extensive local K^+ liberation and water depolarization-disorientation but no change in regions farther away. ($a_K = C_K$). (D) More labile cells. Extensive local K^+ liberation and water depolarization-disorientation, accompanied by modest or no water depolarization-disorientation but more extensive K^+ liberation in regions away from the electrode ($a_K > C_K$). (E) Final stage of trauma. All water depolarized and all K^+ liberated. ($a_K = C_K$).(Ling[15] by permission of Kluwer Academic/Plenum Publishers)

"seen" and thus undetectable by the microelectrode; (iii) that water in healthy living cells is *polarized* with reduced solvency for K^+ {for evidence see [11.3(5)]}; (iv) that *depolarized* cell water has solvency for K^+ near or equal to that of normal liquid water, depending on the degree of depolarization; (v) that the intrusion of the microelectrode produces varying degree of protoplasmic damage,[129 pp 1220–1222] leading to from total desorption of K^+ near and far, to no desorption, and from total depolarization-disorientation of cell water near and far, to no depolarization-disorientation.

Figure 11 and its legend provide a diagrammatic illustration of how the wide spectrum of measured γ_K may all be understood on the basis of different combinations of the five factors mentioned above.

For yet another set of corroborative evidence for the tethered state of K^+ in frog muscle cells based on NMR studies of Na^+, which has stoichiometrically replaced the K^+, see [15.1(3)].

With the "tethered" state of cell K^+ established, I proceed to demonstrate next how and where the tethering takes place.

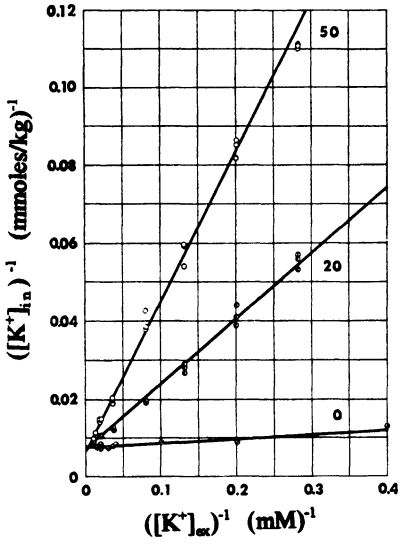

Figure 12. Double reciprocal plots of the equilibrium intracellular labeled K^+ concentration, $[K^+]_{in}$, against the external labeled K^+ concentration, $[K^+]_{ex}$, in the presence of 0, 20 and 50 mM of non-labeled K^+ (both labeled and non-labeled K^+ were in the form of acetate) (23–26°C). Incubation lasted 26 hours. The reciprocal of the equilibrium intracellular labeled K^+ concentration shown as $([K^+]_{in})^{-1}$ is in units of reciprocal millimoles of the ion in 1 kilogram of fresh muscle and shown as $(\text{mmoles/kg})^{-1}$; the reciprocal of the extracellular labeled K^+ concentration shown as $([K^+]_{ex})^{-1}$ is in units of reciprocal millimolarity or $(\text{mM})^{-1}$. Each point represents data from a single frog sartorius muscle. Lines obtained by the method of least squares. The total concentration of K^+-adsorbing sites in the muscle computed from the experimental data ranges from 137 to 154 mmoles per kg of fresh muscle. The average apparent adsorption constant of K^+ calculated is 665 $(\text{moles/liter})^{-1}$. (Ling and Ochsenfeld[116] reproduced from *The Journal of General Physiology* by copyright permission of The Rockefeller University Press)

(4) Cell K^+ are adsorbed on β- and γ-carboxyl groups one-on-one and in close-contact

According to the LFCH, most cell K^+ is tethered in consequence of its adsorption in an one-on-one, close-contact manner on the β- and γ-carboxyl groups of cell proteins. We tested this hypothesis in two ways.

(4.1) K^+ adsorption follows the characteristics of a Langmuir adsorption isotherm

Ling and Ochsenfeld determined the equilibrium concentrations of radioactively labeled K^+ in frog muscle, represented as $[K^+]_{in}$, in bathing solutions containing different concentrations of labeled K^+, represented as $[K^+]_{ex}$, and a fixed concentration of a competing alkali-metal ion, e.g., non-labeled K^+. We then plotted the reciprocals of $[K^+]_{in}$, against the reciprocal of $[K^+]_{ex}$ and obtained straight lines with slopes varying with the concentration of non-labeled competing K^+ but the same intercept on the ordinate (Figure 12).[116]

These characteristics of the double reciprocal plots are, of course, familiar features of *competitive inhibition* in enzyme kinetics.[446 pp 171–181] But

we are not dealing with a *rate* phenomenon as in enzyme activity (or in ion permeation, [13.2]), but with an *equilibrium* phenomenon of ion adsorption. Nonetheless, both the Michaelis-Menten formulation of enzyme kinetics and our own equation for solute distribution (Equation A11 in Appendix 1) incorporate the same Langmuir adsorption isotherm,[117] which in turn was derived on the basis of a one substrate (or ion)-one enzyme site (or adsorption site) assumption and, which gives rise to the observed characteristics of the double reciprocal plots.

Ling and Ochsenfeld also pointed out, however, that a converging double reciprocal plot like that shown in Figure 12 does not prove by itself *one-on-one, close-contact* adsorption; they could also be demonstrated (though less rigorously) for free ions participating in a Donnan equilibrium distribution.[116 p 822] To prove one-on-one, close contact adsorption, we need to demonstrate different impacts on the equilibrium distribution of the ion under study, (i.e., labeled cesium ion, Cs^+) by two or more monovalent cations with *different short-range attributes*, say (non-labeled) *large* cesium ion (Cs^+) and (non-labeled) *small* K^+.

Thus if these ions are not adsorbed one-on-one, in close-contact according to LFCH, but are fully dissociated counter-cations, the impact exercised by non-labeled K^+ and non-labeled Cs^+ would be the same, since they share the same *long-range attributes* of a monovalent cation. On the other hand, if they are engaged in *close-contact* adsorption, their differences in size and other *short-range attributes* will be "felt." And quantitatively different degree of competition for the accumulation of labeled Cs^+ will follow.

As shown in Figure 13 non-labeled K^+ and non-labeled Cs^+ indeed depress labeled Cs^+ accumulation in frog muscles to different degrees, showing that these ions are adsorbed *one-on-one and in close contact*—in agreement with the theory of enhanced countercation association through fixation of anionic groups on proteins [10.1(1)]. These and other data (to be shown in Figures 15 and 58 and work of Menten[378]) also demonstrate that Rb^+ (rubidium ion), K^+, Na^+, Cs^+ and Tl^+ (thallium ion) all bind onto the same anionic sites in frog muscle cells—opening the door to further studies on K^+ adsorption in living cells with the aid of *surrogate* monovalent cations, Tl^+ and Cs^+ (see subsection (5) following). Our immediately next step is, however, to determine the nature of the K^+-adsorbing sites in muscle cells and thus to find out if they are indeed β- and γ-carboxyl groups as predicted by the theory.

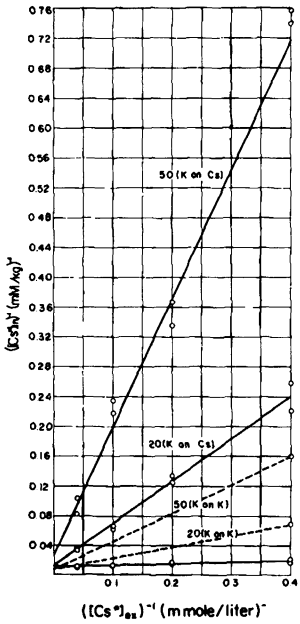

Figure 13. Double reciprocal plots of the equilibrium intracellular labeled Cs^+ concentration, $([Cs^+]_{in})^{-1}$, against the reciprocal of external labeled Cs^+ concentration, $([Cs^+]_{ex})^{-1}$, in the presence of 0, 20 and 50 mM of non-labeled K^+ (all as acetate) (23–26°C). Incubation lasted 45 hours. Each point represents data from a single sartorius muscle. Lines obtained by the method of least squares. Dotted lines are taken from Figure 12 for comparison. Taken together, the two sets of data show that the same concentration of (non-labeled) Cs^+ produces quantitatively sharply different impacts on the adsorption of labeled K^+ and of labeled Cs^+ respectively. The apparent adsorption constant for Cs^+ from the single set of data shown is 488 (moles/liter)$^{-1}$. For additional explanation of units and symbols used, see legend of Figure 12. (Ling and Ochsenfeld[116] reproduced from *The Journal of General Physiology* by copyright permission of the Rockefeller University Press)

(Since we have already established in subsection (1) above that the cell surface (membrane) is *not* the seat of selective ionic accumulation, it is no longer necessary to consider the fixed anionic sites at the *cell surface* as the seats of selective accumulation of intracellular ions demonstrated in Figures 12 and 13 above.)

(4.2) K^+ is adsorbed on β- and γ-carboxyl groups

To identify the cytoplasmic sites adsorbing K^+, Cs^+, Tl^+ and Na^+ in frog muscle as β- and γ-carboxyl groups, we took two approaches. (a) we exposed the muscle cell interior to a carboxyl group-specific chemical reagent, (a water-soluble carbodiimide,[118] which destroys carboxyl groups in the cell) anticipating a loss of adsorbed K^+-surrogate, labeled Na^+. (b) we determined the acid dissociation constants or pK values of the labeled Na^+-adsorbing sites in muscle cells, anticipating their agreement with the known pK values of β- and γ-carboxyl groups.

Owing to the obstruction caused by the cell membrane, neither method can be applied to *intact* cells. Cutting open the muscle cells would effectively overcome this drawback; unfortunately it also destroys the cells' ability of selective ionic accumulation near the cut edges (Figure 8). There-

fore, a prerequisite for this dual project is a modified Ringer's solution, in which 2–4 mm muscle cell segments with open ends can retain their ion accumulation mechanism long enough to complete the study. After many trials (and errors), such a modified Ringer's solution was found; its major ingredient is poly(ethylene glycol) or PEG 8000 (Mol. wt.. 6000–9000).[119] This modified Ringer's solution was used throughout the twin projects.

In theory, treatment with the carboxyl reagent, carbodiimide, should remove all β- and γ-carboxyl groups and hence all adsorbed K^+ or its surrogate ion, Na^+. In fact, the harmful effects of carbodiimide on the (open-ended) muscle segments limited the exposure time to just long enough to remove about half of the adsorbed Na^+ (Table 4.2 on p. 63 in Reference 107). ^{22}Na-labeled Na^+ was used in this study because it was the only alkali-metal radioisotope we had; and no funds, nor real need to purchase others. The principle demonstrated would have been the same, if we had used labeled K^+ instead. As pointed out in subsection (4.1) immediately above, all the alkali-metal ions and Tl^+ compete for the same sites in frog muscle and are therefore interchangeable surrogates of one another [see also (5) below].

Then there is a question about the specificity of carbodiimide action. It is true that carbodiimide destroys *all* kinds of carboxyl groups, not merely β- and γ-carboxyl groups. However, in muscle cells only the β- *and* γ-*carboxyl groups* are numerous enough[116 pp 841–842] to provide a uniform population of adsorption sites for the 80-odd mM of adsorbed Na^+ (or K^+) found in muscle cells (Figure 14). So the carboxyl groups demonstrated with carbodiimide can only be the β- and γ- types.

By varying the pH of the bathing solution and determining the pH, at which the labeled Na^+ adsorption is reduced to one half of its full value, the pK value of the adsorbing anionic sites was also determined in open-ended muscle segments. As shown in Figure 14, a *single inflection point*—indicating uniformity in the population of ion-binding sites—is observed at pH 3.85, which falls within the range of the pK values of β- or γ-carboxyl groups of proteins (3.65–7.30).[120, 121 p 112]

In summary, both the carboxyl specific reagent and the pK-value study have confirmed that β- and γ-carboxyl groups of intracellular proteins adsorb virtually all the alkali-metal ions in frog muscle cells in agreement with LFCH (and AIH).

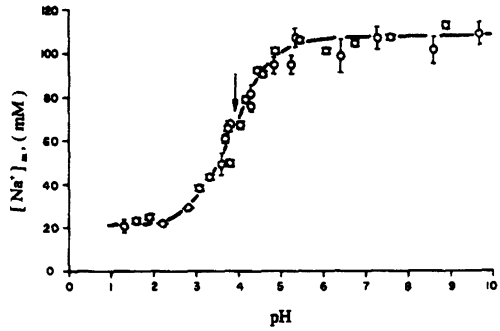

Figure 14. Uptake of labeled Na^+ by 2-mm wide muscle segments with both ends open at different pH's (0°C). Incubation solution contained 16.7% (w/v) PEG-8000. Each point represents the mean ± S.E. for 4 samples. For pH 7.5 and lower, H_2SO_4–Na_2SO_4 buffers were used. For pH above 7.5, glycine-NaOH buffers were used. Ordinate represents molar labeled Na^+ concentration in tissue water. Note that at pH 2.3 and lower, the curve levels off, indicating that at this and still lower pH (and corresponding *higher* H^+ concentration), all adsorbed labeled Na^+ have been chased away by the H^+ and the only labeled Na^+ in the muscle segments is *free* labeled Na^+ found in cell water. The downward arrow indicates the midpoint of the titration curve and hence the pK of the acidic group after the free fraction in cell water has been subtracted from the total labeled Na^+ uptake. (Ling and Ochsenfeld[122])

(5) In striated muscle cells, the K^+-adsorbing β- and carboxyl groups belong mostly to myosin

I pointed out in 1952 that in frog (striated) muscle, the intracellular protein, myosin, alone provides "sufficient or nearly sufficient" β- and γ-carboxyl groups to adsorb all the cell K^+.[96 p 774] In 1966, Ling and Ochsenfeld estimated that 60% of the β- and γ-carboxyl groups in frog muscle cells belong to myosin,[116] a figure they later revised to 47%.[122] Now, a striated muscle cell or fiber shows alternating dark or A-band and light or I-bands (Figure 15A). It has also been known that myosin is found exclusively in the A-bands.[123] Armed with these two sets of facts, LFCH predicts that a good part of muscle cell K^+ (or its surrogates Cs^+, Tl^+, Na^+) is adsorbed on the A-bands.

Once more, experimental observations in harmony with this prediction of a later theory had been reported (by Macallum[84] and Menten[378]) before the theory was introduced. I shall return to these older observations after presenting some new ones.

To test the prediction that K^+ is concentrated in the A bands of striated muscle, Ludwig Edelmann and I each took advantage of one special feature shared by the pair of surrogate ions, Cs^+ and Tl^+ (for K^+): their possession of long half-life, inexpensive radioisotopes (Cs^{134} and Tl^{204}) not available for K^+—hence, the promise of autoradiography; their heavy atomic weights—hence, the promise of direct visibility under an electron microscope.

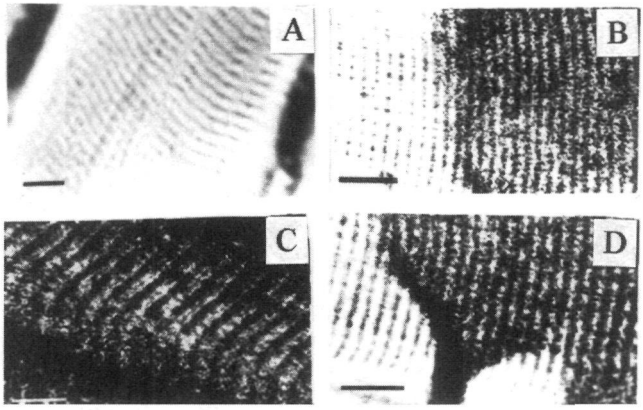

Figure 15. Autoradiographs of dried single muscle fibers. (A) Portion of a single muscle fiber processed as in all the other autoradiographs shown here but not loaded with radioisotope. (B),(C) and (D) were autoradiographs of dried muscle fibers loaded with radioactive Cs^{134} while living and before drying. (B) and (D) were partially covered with photoemulsion. (B) was stretched before drying. Bars represent 10 micrometers. Incomplete coverage with photoemulsion in B and D permits ready recognition of the location of the silver grains produced by the underlying radioactive ions to be in the A-bands. Careful examination suggests that the silver grains over the A-bands are sometimes double and that a faint line of silver grains can also be seen sometimes in the middle of the I-bands, corresponding to the position of the Z-line. (Ling[124])

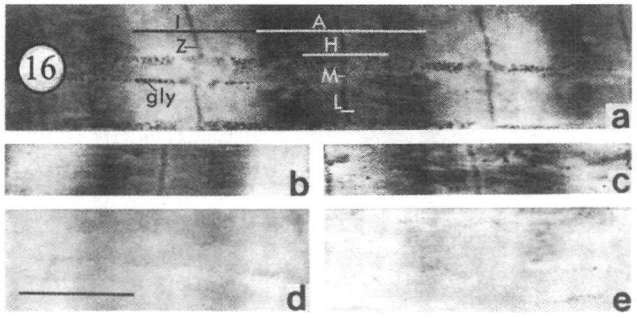

Figure 16. Electron micrographs of dry-cut, unstained section of freeze-dried frog sartorius muscle fibers. Muscles were loaded physiologically with Cs^+ (a) and Tl^+ (b,c) before freeze-fixation, freeze-drying and embedding. (c) was obtained after exposure of a cut section to room temperature for 1 hr. (d) is the central part of (a) after storage of 2 days in distilled water. (e) is from a normal "K^+loaded" muscle. A, A-band; H, H zone; M, M-line; L, L zone; Z, Z-line; gly, glycogen granules. Scale bar: 1.0 micrometer. (From Edelmann[125])

Autoradiographs of air-dried muscle fibers, in which the K^+ had been largely replaced (physiologically) by Cs^{134}-labeled Cs^+ before drying, are shown in Figure 15B, C and D. Note that the silver grains produced by the underlying radioactive Cs^+ are located primarily at the A-bands. This point is best shown in B and C, in which the muscle fibers were *partly* covered with photoemulsion.[124]

Figure 16 is from the electron-microscopic studies of Ludwig Edelmann, using shock-freezing, freeze-drying-embedding and dry-cutting

procedures. Here we see more clearly that the K^+ surrogate ions, Cs^+ and Tl^+ are found mostly at the edges of the A bands, which is predicted, but to a much lesser extent at the Z-line, which is not.[125]

Since then Edelmann has made much progress in affirming and expanding his early work. Most importantly, ion localization has been affirmed in (ultra rapidly-frozen) *hydrated preparations*, which has eliminated artifacts that might have occurred in air-drying, in freeze-drying and even in freeze-substitution methods.[392]

Still other confirmatory work came from *dispersive X-ray microanalysis* of Trombitás and Tigyi-Sebes,[431] of Edelmann[432] and of von Zglinicki[433] (for the different view of Somlyo *et al.*[434] and its rebuttal, see Reference 432 and Reference 107 p. 48). Based primarily on von Zglinicki's work on thin section of heart muscle, Ling and Ochsenfeld estimated that from 67% to (more likely) 80% of the K^+-adsorbing β- and γ-carboxyl groups may belong to myosin.

Having thus completed a summary of the more recent work, we are ready to return to the earlier work of Macallum and of Menten mentioned above.

Macallum introduced a K^+-specific chemical reagent, hexanitrite of cobalt and sodium, $CoNa_3(NO_2)_3$,[84] which forms an orange-yellow precipitate with K^+. The precipitate is made more conspicuous by further interacting with ammonium sulfide, whereupon the precipitate turns black. With this method, Macallum visualized the K^+ precipitate in muscle tissue at the A-bands but not in the I-bands. Using the same technique, Menten[378] demonstrated that the K^+ precipitates were not evenly distributed in the A-bands but only on their edges, and on the thin Z-lines.

Taken as a whole, the extensive and precise work of Edelmann,[125; 126] the single set of autoradiographic study of mine, the dispersive X-ray microanalysis of Trombitás and Tigyi-Sebes, of Edelmann and of von Zglinicki as well as the earlier work of Macallum and of Menten unanimously *confirm the theoretical prediction that K^+ in striated muscle cells is found primarily in the A-bands*. By the same token, Edelmann, I and the others cited above have also fully confirmed the earlier discovery of localized distribution of K^+ (*per se* and not its surrogates) in striated muscle of Macallum and Menten.

Menten also commented on the changing location of K^+ in insect muscle fibers and its relation to muscle contraction: "That this redistribution (of K^+) is intimately connected with the activity of the muscle seems apparent...where a wave of contraction is advancing up-

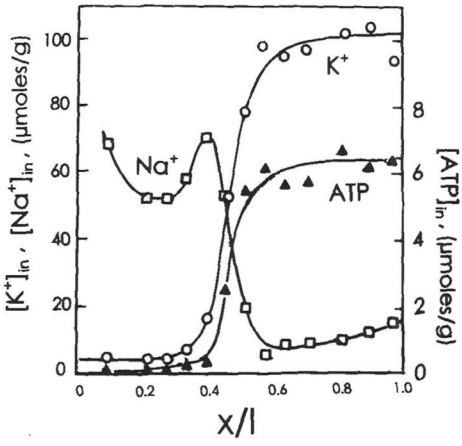

Figure 17. Distribution profiles K^+, Na^+ and ATP along the length of a frog sartorius muscle at a location x cm from the cut edge of the muscle, which measures l (lower case of L) cm in total. Data were obtained from a cut muscle in an EMOC preparation after 50 hours of sterile incubation at 25°C. The Ringer's solution bathing the cut end of the muscle contained initially 100 mM of Na^+, 2.5 mM of K^+ and 1 mM 2,4-dinitrophenol. K^+ and Na^+ were determined by atomic absorption spectroscopy. ATP concentration by the firefly enzyme method. (Ling and Blackman, unpublished. Figure is new and drawn after another version first published in Reference 107; permission to reproduce that specific original version was granted by Krieger Publ. Co.)

ward over the muscle. In the lower part of the figure the potassium is isolated in the dim band, in the upper contracted part it occurs in the vicinity of the light band and between the two extremes the intermediate grades are obtained."[378 pp 413–414]

The gist of this comment is in harmony with the model of muscle contraction first fully described as part of the AI Hypothesis in 1962.[98 pp 437–454; 15 Chapt 16; 122] In this model, fixed cations displace adsorbed K^+ from β-and γ-carboxyl groups, thereby forming salt-linkages seen as the central event in muscle contraction. The more recent conceptual development linking water-depolarization-disorientation with the formation of salt-linkages {[15.3(2)], [16.6(5.3)]} would suggest that the K^+ displaced by the fixed cations in forming salt-linkages during contraction temporarily accumulates in the I-bands as water in the I bands depolarizes momentarily.

Factually, Menten's idea has also been confirmed by: (i) the demonstration of reversible K^+ liberation during each contraction cycle of a beating turtle heart muscle (Wilde & O'Brien[390; 142 p 157]) and during bursts of contractions in perfused dog gastrocnemius muscle (Wood *et al.*[412]) even though parallel studies revealed no increase of membrane permeability to labeled K^+ during the contraction (Noonan *et al.*[458]) and (ii) the electron-microscopic demonstration of similar redistribution of the K^+-surrogate, Tl^+, during tetanic contraction of frog muscle fibers (Edelmann[327]).

(6) Quantitative relationship between adsorbed K^+ and ATP

The data presented in Figure 17 were obtained by Ling and Blackman with the aid of the EMOC technique (Figure 7);[107 p 198; 128] they demonstrate the distribution of K^+, Na^+ as well as of ATP in a cut sartorius muscle after 50 hours of sterile incubation at 25°C. Note that the injury-, and poison-induced changes in K^+, Na^+ and ATP concentration have already spread half way cross the muscle cells.

Corroborating the findings shown in both Figure 8 and Figure 17 are the electron microscopic studies of similarly cut frog sartorius muscle by Edelmann[129] and presented here as Figure 18. Frames (c) and (d) in this figure are photos of EM sections, which, in the location in the cut muscle and in the duration between amputation and specimen-freezing, are similar to (a) and (b) respectively. Only sections in (c) and (d) were not stained with the standard uranium-and-lead before embedding and cutting as in (a) and (b). Instead, they were exposed to, and thereby "stained," with electron-dense Cs^+ *only after* the muscle had been freeze-substituted, embedded and cut into ultra-thin sections (0.2 micrometer)—by means of a remarkable new technique introduced by Edelmann, which he calls "*adsorption staining method.*"[282]

Note that the section taken from a region 0.4 mm away from the cut end of the muscle (d) and stained with Edelmann's "adsorption staining method" looks like the corresponding section stained with uranium and lead (b). Both appear well-stained with electron dense materials at the two edges of the A-bands and the Z-line—resembling those shown earlier in Figure 15 (a) and 15 (b). Yet in the sections from near the cut end, there is poor staining with lead and uranium as shown in Figure 18 (a) and even poorer staining with Cs^+ as shown in Figure 18 (c). Taking into account the difference in the time of incubation, I estimate that the state of preservation of the muscle at 0.4 mm away from the cut here (Figure 18 b, d) is roughly equivalent to a location in Figure 17 at a relative distance (x / l) of 0.6 or even farther away from the cut end.

From data presented in Figure 17 and Figure 18, one reaches the following conclusions: (i) in region of injury, the ability of adsorbing K^+ or Cs^+ at the edges of the A-bands and Z-line is weakened or lost; (ii) in region of injury, the ability of partially excluding Na^+ is also weakened or lost; (iii) in region of injury, the concentration of ATP has fallen to near-zero values or zero.

(i) and (ii) when considered together are in harmony with the notion first suggested in 1952 in the LFCH that the level of ATP *per se* (not its rate of hydrolysis) is stoichiometrically related to the concentration of K^+ adsorbed on the β- and γ-carboxyl groups belonging mostly to myosin.[96] The high binding constant of ATP on myosin to be cited in [14.3(4)] leaves no doubt that within detectable limits, all ATP in a resting living cell is adsorbed.

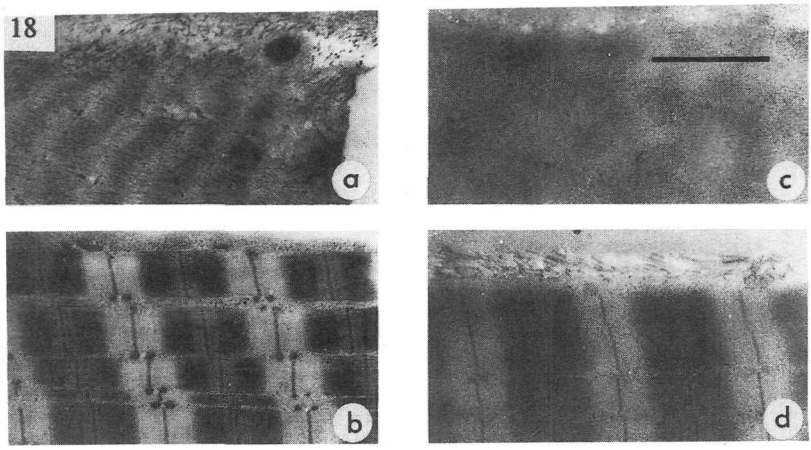

Figure 18. Electron micrograph of part of a frog muscle cell near and far away from its cut end. (a) an ultrathin section stained with uranium and lead by conventional procedure, showing the cut surface of the muscle fiber at the top of the figure; (b) a similar section made at 0.4 mm away from the cut end. (c) and (d) are from 0.2 mm-thick wet-cut section (not stained with uranium and lead but) stained with electron-dense Cs^+ by Edelmann's "adsorption-staining method" in which the cut section is exposed to a solution containing 100 mM LiCl, and 10 mM CsCl. (c) obtained from the cut edge of the muscle, (d) from a section 0.4 mm away from the cut edge. Note poor or no uptake of uranium/lead in (a) and of Cs^+ in (c), but normal uptake in both (b) and (d). (From Edelmann[129] by permission of Scanning Microscopy International)

A more extensive study of this stoichiometric relationship between ATP concentration and K^+ concentration in frog muscle was carried out by Gulati *et al.*[130] and the results shown in Figure 19. Regardless of their respective toxicological actions, 10 different poisons produced more or less the same quantitative relationship. *For each ATP adsorbed, roughly 20 K^+ are selectively adsorbed.* How such a one-on-many control over distance can be achieved will be the subject matter of Section 14.3 to come.

(7) A summary of Section 10.2

(i) The seat of selective accumulaton of K^+ over Na^+ is not at the cell membrane (and postulated pumps in the cell membrane) but in the protoplasm within the cells; (ii) formation of salt-linkages prevents the β- and γ-carboxyl groups of the so-called "native" globular protein from selectively adsorbing alkali-metal ions *in vitro;* (iii) in resting living cells, the electric conductance, the mobility, the X-ray adsorption edge fine structure as well as the activity coefficient of K^+ measured with a K^+-selective microelectrode unanimously confirm the *tethered* state of the cell K^+; (iv) competition studies in the presence of monovalent cations with different

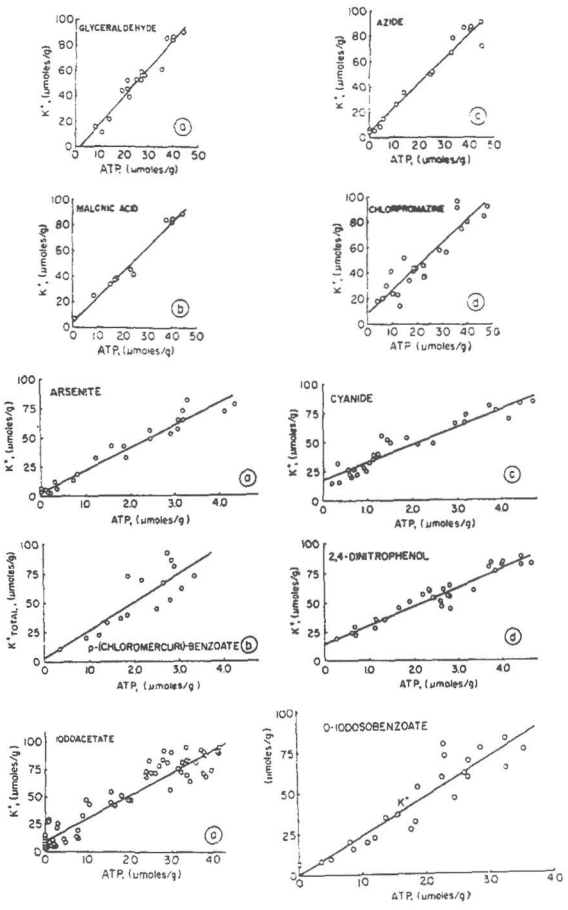

Figure 19. The quantitative relation between the equilibrium concentration of K^+ and of ATP in frog muscles exposed for varying lengths of time to different poisons as indicated in the figures (25°C). Erlenmeyer flasks containing the muscles and incubation solutions were steadily shaken at 150 rpm. To vary the ATP contents, the exposure time to the poisons was varied. To insure that diffusion equilibrium of K^+ had been reached, the muscles were routinely shaken for an additional 2 to 4 hours at 1°C before the muscles were fished out, blotted dry and analyzed. (Gulati *et al.*[130] by permission of Biophysical Society)

short-range attributes establishes that it is one-on-one, close-contact adsorption, which does the *tethering;* (v) with the aid of carbodiimide, which destroys the (β- and γ-)carboxyl groups, and the pH profile of the adsorbed ion, one concludes that the intracellular adsorption sites for cell K^+ are indeed the β- and γ-carboxyl groups as postulated in the LFCH (and AIH); (vi) autoradiography, transmission electron microscopy—on a variety of cell preparations including *frozen-hydrated* thin muscle sections—dispersive X-ray microanalysis as well as earlier chemical staining techniques establish conclusively that the majority of K^+ in striated muscle cells are adsorbed on the β- and γ-carboxyl groups respectively carried on the aspartic and glutamic residues of the contractile protein myosin located at the

two edges of the A-bands. A much smaller amount of cell K^+, though at high concentration, is adsorbed on the Z-line proteins located at the Z-line; (vii) the K^+ concentration profile along a single frog sartorius muscle with one end cut closely parallels the concentration profile of ATP. Similarly the equilibrium K^+ concentrations in different frog muscles after having been exposed to 10 different poisons for varying length of time parallel the ATP concentrations in the corresponding muscles. When the ATP concentration approaches zero, so does the K^+ concentration, confirming the postulation of the LFCH (and AIH) that it is the presence of (adsorbed) ATP *per se*, which determines the level of selectively adsorbed K^+ on the β- and γ-carboxyl groups of myosin and other intracellular proteins.

As mentioned above, the LFCH is an embryonic version of what eventually became the association-induction (AI) hypothesis, the central part of which, to be referred to as the AI Hypothesis proper, will be presented in full in Chapters 14 and 15. Next I present another subsidiary hypothesis of the AI Hypothesis, the *polarized multilayer theory of cell water*, which was added to the AI Hypothesis three years after the publication of the AI Hypothesis proper.

11

The Polarized Multilayer Theory of Cell Water

11.1 Background

Before presenting the polarized multilayer (PM) theory, it behooves one to examine earlier ideas of water structuring around proteins and other macromolecules (for a more extensive review, see Reference 155).

Briefly in 1953, in more detail in 1955, B. Jacobson offered a "water structure hypothesis" to explain the dielectric behaviors of aqueous macromolecular solutions. He said: "If the macromolecule has many oxygen and nitrogen atoms on the surface in such positions that they fit into the ideal water lattice, a very pronounced ordering effect is obtained and result in an almost ideal four-coordinated structure."[334 p 2923; 384] Jacobson described the hydration shells thus formed as "ice-like" to distinguish it from ordinary liquid water, which also has a four-coordinated structure [385; 386] but not nearly as perfect.

Two years later, Nobel Laureate, Albert Szent-Györgyi took the notion of ice-like water one step further. In Szent-Györgyi's "iceberg theory," *crystalline ice* surrounds protein molecules in living cells.[387 pp 32–37] Ice-like hydration water on proteins was also advocated by Klotz.[388] However, there is serious contradictory evidence against the iceberg theories.

Chambers and Hale studied freezing in supercooled frog muscle.[389] They showed (and Miller and Ling confirmed[297; 107 p 103]) that ice crystals form promptly at the points of contact when (real-world) ice crystals are brought in touch with the exposed cytoplasm of a *supercooled* frog muscle. And these crystals grow rapidly toward the intact ends of the muscle cells as unbranching spikes, which may be straight or twisted depending on whether the muscle fiber is allowed to stay straight or is twisted. If ice crystals truly exist in living cells at all times (as the iceberg theories contend), supercooling would have been impossible—as those pre-existing ice-crystals would have initiated ice formation spontaneously.

Contradictory evidence notwithstanding, both Jacobson and Albert Szent-Györgyi deserve praise for presenting enough good reasoning and intriguing experimental discoveries to bring attention back to such an important subject, which, it seems, has been pushed into the limbo largely as a result of Hill's urea-distribution finding (Chapter 7).

11.2 Polarized multilayer theory of cell water and its world-wide confirmation

The *polarized multilayer (PM) theory of cell water*, an integral part of the association-induction (AI) hypothesis,[154; 15 pp 271–310; 107 pp 69–110] was published three years *after* the publication of the AI Hypothesis (AIH) proper.[98] Notwithstanding, I have chosen to present the PM theory here first and the AIH proper later, because like LFCH, the PM theory also deals with the *static* aspects of the living cell. In contrast, the *dynamic* or *inductive* aspect—built upon the foundation of the static aspects—will be presented as part of the AI Hypothesis proper later.

In the PM theory all or virtually all the water in resting living cells assumes a dynamic structure different from normal liquid water, owing primarily to its interaction with a matrix of "fully-extended" protein chains present in all living cells.

By *fully-extended*, I mean that all the backbone NH and CO groups of the protein are not engaged in inter- or intra-macromolecular H-bonds. The fully-extended conformation is not to be confused with the "extended" conformation, which is sometimes used to denote the "β-pleated sheet" conformation.[485 p 501] Under certain conditions, the fully-extended conformation corresponds to what is called "*random coil*" and what Bungenberg de Jong called *linear* conformation [6.2(1)]. But according to the PM theory, the fully-extended proteins in living cells and the water they dominate are not randomly distributed [15.1(1)].

Fully-extended protein chains carry on their polypeptide chains or *backbones* a repeating sequence of *properly-spaced* negatively charged CO (referred to as N sites) and positively charged NH groups (referred to as P sites), both directly exposed to the bulk-phase cell water. Such a matrix of fully-extended proteins chains constitutes an NP-NP-NP system.[155] A two-dimensional checkerboard of N and P sites represents an NP system. NO-NO-NO system refers to chains carrying properly-spaced N sites alternating with vacant O sites. Conversely, a PO-PO-PO system refers to chains carrying properly-spaced P sites alternating with vacant O sites.

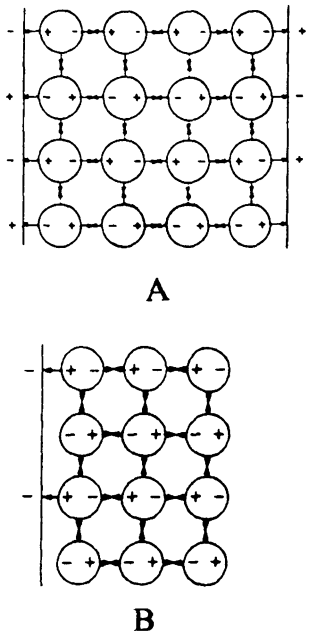

Figure 20. Diagrammatic illustrations of how arrays of alternatingly positively-, and negatively-charged fixed sites (on a matrix of linear chains [NP-NP-NP system], not shown or) on juxtaposed surfaces (NP-NP system) (A), or of alternatingly negatively-charged and neutral sites (NO system) (B) can produce the *dynamic structure* of polarized-oriented multilayers of water molecules. A stable three-dimensional cooperatively-linked assembly of water molecules follow from charge-dipole interactions with the fixed charged sites and from both radial and lateral dipole-dipole interaction with oppositely orientated neighboring water molecules. (From Ling[155] by permission of John Wiley & Sons, Inc.)

Note that having a vacant site and having no site between each pair of nearest neighboring N or P sites are not the same, owing to the different distances separating the nearest-neighboring N or P sites.

Now, water molecules are special because the two positively-charged hydrogen atoms are not symmetrically placed on either side of the negatively-charged oxygen atom, but are asymmetrically placed, not unlike those shown in the formula, H_2O. Accordingly, each water molecule may be seen as a *dipole* with a positive end and a negative end and a well-defined (permanent) *dipole moment*, equal to 1.86 debye.

On the properly-spaced, alternating N and P sites of a fully-extended protein chain are oriented and polarized a layer of *oppositely-oriented* water dipoles. This first layer of polarized-oriented, adsorbed water molecules in turn adsorbs, orients and polarizes a second layer of oppositely-oriented water dipoles. And this continues until a *dynamic structure* of multiple layers of polarized and oriented water are built up. Figure 20, intended to illustrate this dynamic structure, is unfortunately itself a static picture; and, as such, exaggerates the orderliness and stability of the dynamic water structure.

In a living cell like frog muscle, the polarized-oriented water averages some six molecules deep between adjacent fully-extended protein chains. Though such a number sounds small, it is all that is needed to orient and polarize all the cell water of frog muscle into the *dynamic structure* of polarized multilayers.[156 p 201] Then one asks: What is a dynamic structure?

The Taj Majal does not move; it is a *static* structure. A formation of migrating snow geese represent another structure; each element of the flock is constantly shifting back and forth around a central position; together, they constitute a *dynamic* structure. So is cell water according to the PM theory. Our next task is to test the prediction of this theory experimentally and find out if the predictions can be confirmed.

To develop and confirm a theory in the complex science of cell physiology, a highly useful tactic is to find a good inanimate model and find it early. Indeed, discovering a cogent inanimate model which, though much simpler than its living counterpart, behaves like its complex living counterpart, may itself be an indication that the theory is on the right track. In contrast, failure to find an inanimate model after extensive search is a warning sign that the theory may be wrong—as has happened in the theory of membrane potentials to be told in greater detail in [15.5(1)] below. The PM theory, in contrast, has had an altogether different story to tell. A whole collection of cogent inanimate models has been discovered early in its history.

For reasons to be presented in greater detail in [11.3(2)] below, gelatin, urea- and NaOH-denatured proteins as well as linear polymers carrying properly-spaced oxygen (or nitrogen) atoms with *lone-pair electrons*[425 pp 131–133]—including poly (ethylene oxide) (PEO), polyvinylpyrrolidone (PVP), polyvinylmethylether (PVME) and poly (ethylene imine) (PEI)—are all fully-extended. As such, they "reach out" to interact with bulk-phase water, and are designated *extrovert models*.

Poly(ethylene oxide) (PEO) $(-CH_2CH_2O-)_n$, in particular, offers insight into the importance of *proper-spacing* between a pair of nearest-neighboring oxygen (or nitrogen) atoms. PEO with two methylene groups in between two nearest-neighboring oxygen atoms, is highly soluble in water and polarizes and orients water molecules. However, the reduction of one, or the addition of one (or more) methylene group(s) between two nearest-neighboring oxygen atoms makes the polymer altogether insoluble in water,[425 p 132; 481 p 113] let alone polarizing the bulk-phase water as PEO does.

Most native globular proteins and proteins denatured by SDS and n-propanol have their backbone NH and CO groups locked in α-helical or β-pleated sheet conformation, and are classified as *introvert models*.

Worldwide studies of the outstanding physicochemial attributes of water in the living cell and in model systems were in part from my own laboratory. And in part from other laboratories, where the study of living cells and models were sometimes carried out separately by workers unaware of each other. In those cases, they are "wedded" through my intervention.

The cell-physiological attributes, which have been thus far investigated include, but not exclusively, the following: (1) osmotic activity: models;[160] cells;[107 p 101; 296] (2) swelling and shrinkage: models;[161] cells;[98 pp 246–247; 159; 199] (3) freezing point depression of water: models;[162; 389] cells;[297; 298; 107 pp 102–103] (4) vapor sorption at near-saturation vapor pressure: models;[163] cells;[159] (5) NMR rotational correlation time $\{\tau_r\}$: models;[164; 107 pp 93–95] cells;[299; 300; 301; 302] (6) Debye dielectric reorientation time $\{\tau_D\}$: models;[303] cells;[165] (7) rotational diffusion coefficient from quasi-elastic neutron scattering: models;[304] cells;[166] (8) solvency for solutes: models;[154; 155; 156; 168; 170; 172; 175; 306] cells.[131 Fig. 6; 154; 156; 190; 307; 107 Chapt 8]

These comparative studies have consistently shown that water dominated by extrovert models shares characteristic features of water in normal resting living cells, but introvert models do not or do so weakly—in agreement with the predictions of the PM theory. Regretfully there is no space to go into greater details on all the subjects listed. Nonetheless, I shall devote some space here to answer two specific questions. (i) How much free water exists in a typical living cell like frog muscle? (ii) How much of the water in the frog muscle cells exists in the form of polarized, oriented multilayers? In designing experiments to answer these questions, we were in fact putting to test the PM theory, according to which all or nearly all cell water exists as polarized multilayers and there is little or no free water.

The answer to the free-water question rests on two pieces of information. First, frog muscle cells do not regenerate a new cell membrane at the cut surface. This we already know [Section 4.1(4)]. Second, Ling and Walton have developed a simple *centrifugation method* (4 minutes of spinning at 1000 g in a hermetically-sealed packet), which quantitatively removes all the (free) water in the extracellular space of a frog sartorius muscle without removing any water from within the muscle cells.[516] Thus armed, Ling and Walton cut sartorius muscles into 2 mm and 4 mm long segments with both ends open and subjected them to 4 minutes of centrifugation at 1000 g by the established procedure. What we found was that the percentage of water extracted is equal to that in the extracellular space of normal intact sartorius muscle.[296] We conclude that there is no free water in frog

muscle cells; for otherwise, it would have been spun out along with all the (indisputably) free water in the extracellular space.

To answer the question of how much cell water exists as polarized multilayers, I begin by briefly reviewing the historical backgrounds on gaseous sorption.

The amount of a gas sorbed on solids, when plotted against the partial vapor pressure of the gas, may assume the shape of a lying-down Z. An initial steep uptake is followed by a region of flat uptake to be followed by a second steep uptake.[532 pp 364–369] In earlier times, the large uptake at the high end of the partial vapor pressure had been often explained as due to *capillary condensation.*[532 p 444] In 1934, de Boer and Zwikker offered an alternative interpretation based on their *polarization theory.*[533] This theory, in turn, rests on the idea that on a checker-board of charged surface sites, a first layer of gaseous molecules is adsorbed. The induced electric dipoles of the adsorbed gas molecules in turn polarize a second layer and this continues until multilayers of gas molecules are adsorbed.

In 1952, Brunauer, Emmett and Teller severely criticized de Boer and Zwikker's theory, pointing out that the electrical polarization cannot proceed beyond a single layer.[534] They then introduced their own model, known as the BET theory (after the initial letters of their respective names). In the BET theory, only the first layer of gas molecules is adsorbed according to a Langmuir isotherm. Additional gas molecules are collected on top of the adsorbed layer by simple condensation and, as such, not different from ordinary liquid form of the gas.

However, in their attack on de Boer and Zwikker's theory, Brunauer *et al.* pointed out specifically that their criticism is limited to adsorption of gas molecules like the noble gases, which do not possess *permanent dipole moments*. For gas molecules, which do possess a substantial permanent dipole moment (like water)—a case which has been treated by Bradley[278]—Brunauer *et al.* made it clear that their criticism does not apply.

In presenting the PM theory of cell water in 1965, I pointed out that the BET theory can only explain the uptake of water at the very low vapor pressure on sheep's wool and on collagen.[154] In contrast, Mellon, Korn and Hoover have shown how their water sorption data on various proteins and polypeptides are quantitatively explained over the entire range of water vapor pressure by the Bradley isotherm.[535]

Although Brunauer *et al.* found no objection to the Bradley treatment of gaseous molecules with permanent dipole moments, they did not elaborate on why. Recently I have looked into this problem a little more closely and plan to publish my finding before too long. However, this much I can say here. The success of multilayer adsorption of molecules with large permanent dipole moments (like water) is due to the combined *polarization* and *orientation* of neighboring water molecules by the N and P sites. This polarization-orientation produces properly-oriented permanent-and-induced dipole moments of the adsorbed water molecules, which in turn polarize and orient another layer of neighboring water molecules and so it continues on and on. Indeed, under ideal conditions of sufficiently high charge density at the N and P sites and the temperature of absolute zero, the extra water-to-water interaction energy due to the polarization-orientation stays constant *as one moves farther and farther away from the polarizing surface or chains.* Although this ideal condition is not real-

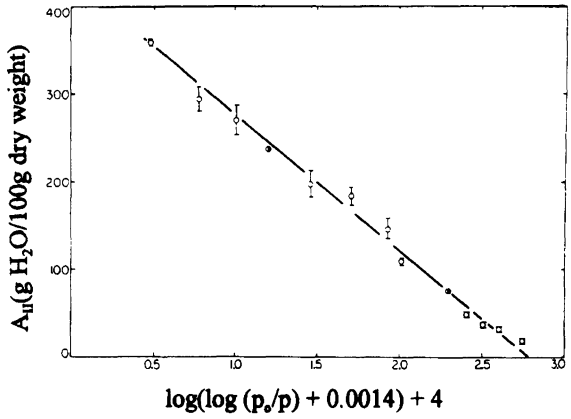

Figure 21. Water sorption of surviving isolated frog-muscle-fiber bundles at various relative vapor pressure (p/p_o) where p is the vapor pressure and p_o, the saturation vapor pressure under the same condition (25°). Data plotted refer to 95% of the total muscle water. The remaining 5%, which follows a Langmuir adsorption isotherm, has been subtracted. Straight line going through or near most experimental data points indicates obedience to the Bradley polarized multilayer adsorption. isotherm. Half circles represent data points from separate time course studies. (Ling and Negendank[159])

ized in the real world, ultra-long distance propagation by the combined polarization and orientation is thus theoretically feasible and has been reported[15 pp 279–280] (though not required in the PM theory of cell water).

As pointed out earlier, R.S. Bradley derived a theoretical adsorption isotherm for polarized multilayers of (gaseous) molecules with permanent dipole moments—like water (Equation A2 in Appendix 1[278]). The PM theory predicts that most if not all the water in resting and healthy living cells (as well as of extrovert models like gelatin) should follow the Bradley multilayer adsorption isotherm.

To test this prediction, Ling and Negendank studied under *sterile* conditions at 25°C, equilibrium water sorption of small muscle-cell-bundles dissected from isolated frog sartorius muscles.[159] Equilbrium time was 7 to 8 days. Solutions of NaCl and sulfuric acid of different concentrations provided relative vapor pressures ranging from near zero (0.043) to near saturation (0.996). As shown in Figure 21, 95% of frog muscle cell water does indeed follow the Bradley isotherm as predicted by the PM theory. The remaining 5% follows a monolayer Langmuir isotherm and is very tightly bound, apparently on polar side chains of cell proteins.[426]

Data of water sorption of gelatin from J. R. Katz published in 1919 also covers the whole range of humidity.[194] I analyzed the data and found that it too follows the Bradley isotherm.[15 pp 288–289]

In Section [8.1], I pointed out the earlier work on equilibrium water sorption of frog sartorius muscles of E. Ernst, J. Tigyi and their coworkers.[399] The bulk of their data points are confined to muscles retaining 10% or less of their normal water content, and thus may correspond to a considerable extent with the tightly-bound water mentioned above.

Having presented a bird's-eye-view of the extensive experimental verification of the PM theory, we now turn our attention to what have further evolved from the root PM theory, both theoretical and practical.

11.3 Theoretical and practical extensions of the PM theory (and confirmations)

(1) Invention of MRI

In the dynamic structure of polarized multilayers, water molecules are moving about and exchanging positions all the time. Nonetheless, if one takes a snapshot at any instantaneous moment, one finds that most of the water molecules are attached somewhat more strongly to their neighboring water molecules and indirectly to their ultimate anchoring CO and NH sites than in normal liquid water. Attaching to its neighboring water molecules restrains and slows down the movement of the water molecules, both translational and rotational. Motional restriction of water molecules and its constituent parts (e.g., H atoms or protons) is therefore an important implication of the PM theory.

Freeman Cope, who received his training as a physicist at Harvard and his medical education at Johns Hopkins Medical School, recognized that motional restriction of water in living cells implied in the PM theory, if true, should be demonstrable with the aid of an invention of the physicists,[339] the *nuclear magnetic resonance* (NMR) spectrometer.

Using this instrument, one can assess the *rotational correlation time* (τ_r) of the hydrogen atoms or protons of the water molecules or their relaxation times, T_1 and T_2. Without going into the details, one may say that spatial fixation and orientation of the water molecule, hence their two constituent protons, *increases* the efficiency of the protons in losing their magnetization, by a process known as *relaxation*. The PM theory of cell water dictates that cell water protons being less mobile than those of normal liquid water should have shorter T_1 and T_2 than protons of pure liquid water. Cope soon confirmed this expectation.[300] But he was not alone.

Another young cell physiologist, Carlton Hazlewood, also from Johns Hopkins Medical School (and coworkers) made the same discovery at the same time.[301] (For additional confirmations and *qualifications* on the significance of the findings, see Reference 340). Then a third young scientist appeared on the scene. His name is Raymond Damadian. With a borrowed instrument and some malignant-tumor-carrying rats, which he persuaded the Sloan-Kettering Institute to give to him, he was *en route* to make a landmark discovery.[302; 339 p 611]

Damadian showed that the T_1 and T_2 of the water protons of normal tissues are different among themselves. But the water protons in the malignant tumors (cancer) he studied have consistently longer T_1 and T_2 than those of the respective normal rat tissues from which the cancer cells were derived. Thus water protons in liver cancer or *hepatoma* have much longer T_1 and T_2 than those of water protons in normal liver tissue.

From the very beginning, Damadian had the vision that one day this difference in T_1 and T_2 of water protons will be the basis of a new medical tool—the like of which never existed before—to visualize and detect cancer without surgery.[302]

Longer T_1 and T_2 of the water protons in cancer cells are also in harmony with Albert Szent-Györgyi's prophetic announcement in 1957 in a footnote[387 p 136] that cancer has "less water structure." His concept of "less water structure" in cancer cells is compatible with the PM theory. However, subsequent work including work yet to be published, has also shown that "less water structure" is only *one* of the causes of the longer T_1 and T_2 of cancer tissues observed.[340]

Even before the deriding laughter of his many cynical critics had died out, Damadian and two graduate students, Larry Minkoff and Michael Goldsmith, had hand-wound the ninth largest superconducting magnet of the world, and in the twinkle of an eye, built the first NMR machine, christened "Indomitable"[305] —now keeping company with other history-making inventions in the Smithsonian Institute of Washington, D.C.

On July 15, 1988, Damadian and Paul Lauterbur—who had introduced a more sophisticated technique for visualizing the NMR picture than the one Damadian first introduced and successfully employed—conjointly received from President Ronald Reagan the National Technological Award for the invention of what is now widely known as *magnetic resonance imaging* or MRI. On February 12, 1989, Damadian was inducted into the National Inventors' Hall of Fame. Damadian deserved all these and much

more. But the following letter, which he wrote me on November 9, 1977 tells more of the inner nature of this visionary and generous man:

> "On the morning of July 3, at 4:45 A.M....we achieved with great jubilation the world's first MRI image of the live human body. The achievement originated in the modern concepts of salt water biophysics, on which you are the grand pioneer with your classic treatise, the association-induction hypothesis."[15 p. vii; 107 p xxv]

Not very many people would write such a letter to a friend (but also inevitably, a scientific competitor). But the generosity shown here has materialized into even more vital help, when he and his MRI-machine manufacturing company, Fonar, offered support and shelter for me and my associates, Margaret Ochsenfeld and Zhen-dong Chen, when my scientific opponents and their NIH allies forced the closing of my laboratory at the height of its productivity.[247] That took place in the year 1988, when we said Goodbye to Pennsylvania Hospital in Philadelphia, the Nation's First Hospital, home of our research for 27 years .

Soon little Fonar is up to its ears in battles for survival with the world's greatest industrial giants, General Electric, Siemens, Phillips, Hitachi, and Toshiba, to mention just a few. But the US also has the world's finest *patent system*, which not only protects and rewards the inventors small or big, but just as important, ensures the disclosure of the invention to the world so that continued progress can be made on the basis of the new knowledge (see Reference 326 for danger of losing or seriously hobbling by unnecessarily altering the US patent system). Thus, in 1997 the Supreme Court of the United States, in affirming the verdict of the lower Court and the Appellate Court, ordered General Electric Co., the giant of the industrial giants, to pay Fonar Co. 128.7 million dollars for infringement on Fonar's pioneering cancer-detection patent and another patent.[305] However, much of this money went to the Minneapolis law firm, Robins, Kaplan, Miller and Ciresi, which had courageously and ably championed the Fonar cause on a contingency basis.

But to bring us to the present status of the understanding of cell water, other scientists have also made major contributions. As one of the pioneer workers in the NMR study cell of water, Carlton Hazlewood suffered persecution and severe hardship in standing firm on his scientific beliefs. Jim Clegg, who, among his many other contributions, affirmed the restricted motional freedom of cell water by means of ultra-high frequency dielectric

studies.[165] The late Bud Rorschach quarter-backed the study of rotational diffusion coefficients with the aid of quasi-elastic neutron scattering methods.[166; 304] Freeman Cope's suicide in 1982, following the loss of support for his scientific work, is unspeakably tragic.[247]

(2) What makes gelatin unique leads to a new definition of colloids

Earlier in [6.2(4)], I pointed out why colloids need a new definition. Not only is the old macromolecular definition misleading, that definition has nothing specifically to do with gelatin. Yet gelatin not only gives colloids its name, it also stands out as historically the most prominent colloid. Undoubtedly the failure to offer a self-consistent definition of colloid has contributed to the declining interest in, and low esteem of, colloid chemistry as a science by some members of the scientific community at least (see end of [16.3] below).

The opportunity to recognize the underlying causes of gelatin's uniqueness improved in the wake of two developments: (i) the recognition of the unique amino-acid composition of gelatin, which insures that at least 56% of its polypeptide chain[508; 157] exists permanently in the fully-extended conformation: {13% of proline and 10% hydroxyproline residues, which are unable to form α-helix or β-pleated sheet, because they do not have a proton on their pyrrolidine nitrogen atom; 33% of glycine, which being a strong "helix-breaker" [see Section 14.1(3.1)] are averse to do so}; the absence of tertiary-structure-stabilizing disulfide bridges (-S-S-)[151] in gelatin also enhances the fully-extended conformations. (ii) the introduction (and affirmation) of the PM theory according to which properties typical of colloids are exhibited by polarized-oriented water interacting with fully-extended proteins or other extrovert. Merging of these two new sets of knowledge with part of the old definition reviewed earlier led to a new definition of *colloids:*

> "A colloid is a cooperative assembly of fully-extended macromolecules (or large aggregates of smaller units) carrying properly-spaced oxygen, nitrogen or other polar atoms and a polar solvent like water, which at once dissolves and is polarized and oriented in multilayers by the macromolecules or equivalents" (for an older and not-yet-fully-developed version, which the present one supersedes, see Reference 158).

Note that according to this definition, an extrovert model is a colloid; but an introvert model is not, or less so. Since most "native"[107 p 37 n4] globu-

lar proteins are introverts, they too are not *bona fide* colloids. In harmony with this delineation Thomas Graham[53] stated that substances that form crystals are not colloids (but are *crystalloids)* and native golublar proteins do form crystals (under conditions which promote the formation of *coacervate* from *linear* or *fully-extended* proteins) [6.2(1)]. As will be made clear in [14.3] following, living cells would be, according to this definition, colloidal, but dead ones would be less so or not at all.

All these new efforts notwithstanding, one cannot help feeling that the name colloid might have had a history too long and too confused to allow a new definition to be broadly adopted. Despite this, *the new definition can still serve a useful purpose for it tells that colloids include water (or other polar solvent) in polarized multilayers rather than just big molecules by themselves.*

(3) A new hypothesis of coacervation based on the PM theory

Coacervation involves the separation of a single homogeneous aqueous solution into two phases with a clear boundary between them. The key ingredient of the solution is an adequate concentration of fully-extended chains like gelatin (but not globular proteins). As a rule, a rise of temperature triggers the coacervation process. Despite its high popularity at one time,[321; 324; 325] thus far there is no explanation of what a coacervate really is or why it is formed. The two mutually exclusive definitions of coacervates given by Bungenberg de Jong—the *master* of colloidal chemistry and coacervation—and his own experimental data contradicting one or both, make plain the unsettled state.

In the new hypothesis I am putting forth, coacervation involves an *auto-cooperative transition,* during which three simultaneous events take place: (i) a condensation and reorientation of the fully-extended extrovert molecule like gelatin into parallel arrays with "chains" of more intensely polarized-oriented water molecules bridging water-polarizing polar groups (e.g.,CO and NH) of neighboring extrovert chains, leaving no "free ends" of water chains "dangling;" (ii) a "squeezing out" of part of the water, which forms a separate extrovert-poor phase; (iii) at the surface of the coacervate phase, the fully-extended extrovert molecules are oriented perpendicular to the surface with chains of attached water molecules in the "fully-satisfied state" (i.e., polarized and oriented from both ends of the water-molecule chain), thereby creating a clean and clearly-delineated

boundary separating the extrovert-rich coacervate phase from the extrovert-poor solution phase.

The overall effect of an elevated temperature in bringing about coacervation is to drive enough of the least polarized water molecules in the coacervate phase to outside the coacervate phase. At this temperature, the entropy gain associated with water molecules moving from the condensed phase to the external free solution phase exceeds the loss of entropy and gain of (negative) energy from the "fusing" of the loose ends of the water chains to reach a higher degree of polarization and orientation in the coacervate phase—until a new equilibrium is established. Auto-*cooperativity* gives rise to the sharp *all-or-none* character of the transition. (For more on cooperativity, see [14.3(2)] and Section 4 in Appendix 1.) (A rigorous treatment of the three-dimensional cooperative transition phenomena, to which coacervation in the present hypothesis belongs, is one of the most difficult goals in theoretical physics (see Yang[541 p 1]) and far beyond the degree of sophistication that the present qualitative model calls for.)

In the past, we have represented a two-dimensional checkerboard of positive P sites and negative N sites as an NP system. Two juxtaposed NP systems as an NP-NP system. What I now suggest for a coacervate is an NP-NP-NP system "juxtaposed" to more than one parallel chain. It is not easy to express this in the old way. Accordingly, a new nomenclature system is introduced, in which what is formerly represented as an NP-NP system will be represented now as $(NP)^2$. The gelatin chains in a coacervate as I suggested above will be written as $(NP\text{-}NP\text{-}NP)_n$ where the superscript n represents the average (undetermined) number of nearest-neighboring chains arranged around and in parallel with one fully-extended-protein chain. A coacervate of PVME, which carries vacant O sites rather than P sites, would be adequately represented as $(NO\text{-}NO\text{-}NO)^n$.

The theoretical model of coacervation outlined above offers some new insights: (i) a possible explanation for the sharp boundary of a coacervate, hence its immiscibility with the surrounding normal liquid water. No other molecular mechanism known to me has been offered before for this immiscibility; (ii) a possible explanation of why *only* fully-extended-protein chains (or what Bungenberg de Jong called *linear* proteins) can form coacervate [6.2(1)], because (according to the PM theory and its extensive supporting evidence) only fully-extended protein chains can effectively polarize and orient multiple layers of water; (iii) a possible explanation of why protoplasms emerging from cut cells—ranging from Dujardin's Infusoria to Lepeschkin's *Bryopsis* cells—are all water-immiscible: because they are coacervates—as suggested by Lepeschkin and others long ago[321; 324] (but without suggesting a physico-chemical mechanism of why).

The reader may recall that radioactive tracer studies in conjunction with electron microscopy have established that in cells like frog muscle with rigid gel-like protoplasm, membrane regeneration does not occur [4.1(4)]. Nonetheless, I cautioned that this finding may not rule out the possibility of membrane regeneration on more fluid protoplasm like that shown in Figure 3. With a new definition for coacervate on hand, we are now in a position to resume that line of thinking.

One may also recall that the central theme of Pfeffer's *Osmotische Untersuchungen* deals with this very same subject of membrane regeneration.[18] Pfeffer strongly believed that protoplasm has the innate property of forming a water-immiscible new membrane when it comes into contact with "another aqueous solution."[18 p 234] And it was with this argument that proponents of the membrane theory attempted to deflect Kite's claim of water-immiscible protoplasm inside living cells. However, we now know that a pure 50% solution of poly-(vinylmethylether) or PVME, like gelatin, also undergoes coacervation at a moderately-raised temperature (34°C)[15 p 357] but without the need of added salts or gum arabic as in the case of gelatin. Cooling brings the PVME coacervate back into solution again. These observations show that it is not the contact with an aqueous solution that brings about the formation of a water-immiscible surface, because PVME was in intimate contact with, indeed a part of, the aqueous solution *before* the formation of water-immiscible coacervate phase.

As I will make clear in [14.3] following, all the protoplasms in resting living cells, according to the PM theory, exist as more or less *parallel-oriented* NP-NP-NP chains,[06 p 8; 425 156 p 202] and as such are already water-immiscible—as witnessed by the demonstration by Lepeschkin of the formation of isolated protoplasm droplets from crushed *Bryopsis* plant cells to form *vacuoles* anywhere in the droplet when the droplets were placed in a hypotonic solution [7]. Again the formation of the new immiscible interface lining each "puddle" of vacuolar fluid is not created when that part of protoplasm comes into contact with "another aqueous solution,"[18 p 234] which does not exist. Rather, it is water from, and passing through the protoplasm that makes up the aqueous solution inside each vacuole when the protoplasmic droplet is transferred from an isotonic to a hypotonic environment.[62 pp 289–290]

Equally informative is the additional observation of Lepeschkin that these vacuoles in *Bryopsis* protoplasmic droplets vanished when salt-rich sea water is added to the surrounding hypotonic solution.[62 pp 289–290] Thus being in contact with an aqueous solution does not insure the (continued) existence of a water-immiscible protoplasmic surface either. Instead, in both the PVME model and the *Bryopsis* protoplasm, it is the physical environment which determines the coacervation or its reversal. Raising and lowering the temperature and raising and lowering of environmental water activity respectively causes the coacervation and its reversal in PVME and *Bryopsis* protoplasm.

This inborn water-immiscibility of coacervate does not mean that there would be no secondary rearrangement at a newly exposed protoplasmic surface. Indeed, secondary rearrangement as a rule occurs in response to the new surface formation of *Nitella* protoplasmic droplets as the remarkable work of Ueda, Inoue and their coworkers at the Hokkaido University of Japan has demonstrated.[411] Only that rearrangement is not producing the water immiscibility. Were the protoplasm itself truly a simple water solution, *some* of it would have escaped into the surrounding water when the protoplasm is vigorously shaken. And in-

stead of thousands of little water-insoluble globules actually obtained (Lepeschkin), we would most likely get a soup of dissolved protoplasm.

Summarizing this digresssion, I would now conclude that membrane regeneration is not the cause of the formation of protoplasmic globules like those shown in Figure 3. *As coacervate, protoplasm, fluid or rigid, is inherently water-immiscible—in harmony with the original view of Felix Dujardin when he described the living jelly from protozoa and called it sarcode.*

Since this new hypothesis of coacervation and coacervate is presented for the first time here, rigorous experimental testings belong to the future. Nevertheless, confirmation can be deduced from some experimental observations made before this hypothesis was put forth.

The ρ *value* (the *apparent* equilibrium distribution coefficient) of Na^+ (as citrate) in solutions containing different concentrations of two extrovert models, polyvinylmethylether (PVME) and gelatin are shown respectively in Figure 22A and Figure 22B.[172] Note that at a PVME concentration of 10%, the ρ-value is about 0.4, telling us that *at least* 60% of the water must have profoundly altered its solvency due to interaction with this extrovert model. Figure 21B shows that gelatin solution at room temperature yielded similar results but the ρ-values for Na citrate and Na-sulfate are higher.

One recalls that years ago Holleman, Bungenberg de Jong and Modderman had studied the equilibrium distribution of sodium sulfate (Na_2SO_4) in a gelatin + Na_2SO_4 system[70] [6.2(2)]. Only our study presented in Figure 22B was conducted at 25°C, theirs at 50°C. As alluded to in [6.2(1)], only at the higher temperature does the gelatin system exist as a *coacervate*. Yet both sets of data show exclusion of sodium salts. So do the solutions of PVME cited above, which studies were also carried out at a temperature of 23°C and thus below the coacervation temperature (34°C). Thus existence in the state of a coacervate is not necessary for the partial solute exclusion observed. The existence of a matrix of fully-extended protein or polymer chains is enough to do that—in harmony with the PM theory. Nor is the existence in a *gel state* a condition for solute exclusion as I myself had once (erroneously) suggested.[155 p 691] Although this is not to deny that protoplasm may exist (transiently or permanently) in a gel state as seen in the ectoplasm of *Nitella*,[63] myoplasm of muscle and axoplasm of nerve.[342]

The introduction of the new theory of coacervation above leads to new predictions. In the past, it has been noted that, though qualitatively speaking extrovert models and the living cells share similar ability of partially excluding solutes like Na^+ salts and sugars, quantitatively speaking, the degrees of exclusion are as a rule quite different. The q-values tend to be con-

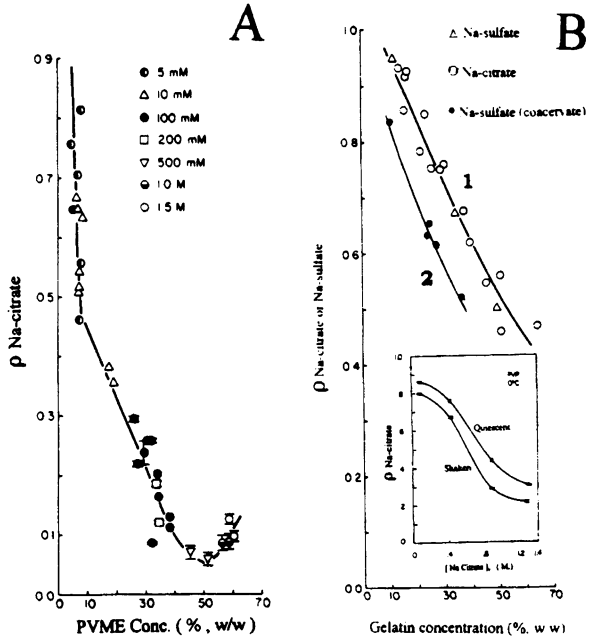

Figure 22. The *apparent equilibrium distribution coefficient* (ρ-value) of Na citrate or Na sulfate in water in dialysis sacs vs. water in the surrounding medium. Sacs contained also different concentrations of poly (vinylmethylether) (PVME) (A), and of gelatin (B1). Symbols in A denote the concentrations of the Na citrate in the bathing media. In B2, five solid circles, taken from Hollemann *et al.*,[70] represent the ρ-values of Na sulfate in a simple *coacervate* of gelatin maintained at a temperature of 50°C. In contrast, the data points along line 1 are taken from Ling & Ochsenfeld[172] and were obtained from simple homogeneous viscous solutions of gelatin maintained at 25°C. Note that the ρ-value at the same concentrations of gelatin in the coacervate state is consistently lower than that of gelatin in the state of a simple solution. The inset in Figure 22B, taken from Ling, Walton and Bersinger,[425] shows the influence of *one-dimensional* stirring in lowering the ρ-value of Na citrate in a solution of the extrovert model, polyvinylpyrrolidone (PVP). One-dimensional stirring was achieved with a moving, silicon-rubber-coated lead shot, which traveled at regular intervals from one end to the other end of quarter-inch wide, long and taut dialysis sacs containing the PVP solutions (and the coated lead shot). The dialysis sacs were in turn immersed in Na-citrate solutions in screw-cap tubes, which were mounted radially on a rotator-shaker so that the capped end of each tube was pointing vertically upward at one time and vertically downward a little later. Note that at the same Na citrate concentration (hence the same PVP concentration) the ρ-values for Na citrate at equilibrium are also consistently lower in the stirred than in the unstirred PVP solutions. (partly from Ling and Ochsenfeld[172])

siderably lower in living cells than in the model systems studied. In explanation I have suggested that "maximal water polarization will occur only when there is a more or less parallel orientation of the protein chains."[306 p 8; 425; 156 p 202]

In the new hypothesis of coacervaton just presented, an enhancement of parallel orientation of the NP-NP-NP chains is also postulated—leading to the new prediction that the exclusion of solutes should be more intense and the q-value for excluded solutes *lower* in a coacervate than in a similar but non-coacervate NP-NP-NP system. A comparison of the ρ-values of Na-sulfate in gelatin solution (line 1 of Figure 22B) with that in gelatin coacervate at the same concentration (line 2 of Figure 22B) bears this out. Thus line 2 in Figure 22B is consistently below line 1, showing lower ρ-values for Na-sulfate in gelatin when it is in a coacervate state than as a simple solution.

However, the data of line 1 was obtained at a lower temperature (25°C) than that of line 2 (50°C). Could this temperture difference be the cause of the difference in ρ-value? Studies of both Gary-Bobo and Lindenberg[351] and of Ling *et al.*[425 p 127] showed that higher temperature *increases* the ρ-values of partially excluded Na^+ salts. So the lower ρ-value of Na-sulfate in gelatin coacervate could not have been the result of the temperature difference. On the other hand, that the lowering of the ρ-value in the coacervate model could arise from a *more parallel orientation* of the protein chains, as suggested in the new hypothesis, is supported by other independent data from Ling *et al.*[425] and shown in the inset in Figure 22B.

This inset shows that the ρ-value for Na-citrate in solutions of another extrovert model, polyvinylpyrrolidone or PVP, indeed *decreases* at both 0° and 25°C, when the polymers are made to orientate themselves in parallel arrays *by unidirectional stirring*[425] (see legend of Figure 22 for details). Woessner and Snowden studying the high-molecular-weight bacterial polysaccharide, Kelzan® observed enhancement of the long-range ordering of the polymer in response to stirring.[437]

A further look into additional underlying cause(s) for the *still* lower q-values for Na-salts and other probe molecules *in living cells* will resume in the subsection (5) after a quantitative theory of solute distribution has been presented in the subsection immediately following.

(4) A quantitative theory of solute distribution (exclusion) in cell water and model system and its experimental verification

At the outset, I like to point out that in my opinion the phenomenon of *solute exclusion from cell water is one of the most revealing of the inner workings of the living cell. In contrast to some of the quantitative data obtained by other experimental methods, quantitative data from solute exclusion*

studies is model-independent and thus incisive in testing and in verifying the PM theory, as I will demonstrate below.

Carl Ludwig, often regarded as the father of modern physiology, conducted an experimental study on pig's bladder in 1849 and thus almost exactly 100 years after Abbé Nollet made history's first recorded osmotic study on the same bio-material.[167]

Ludwig started out with a *dried* bladder and followed its uptake of water and of sodium sulfate from an aqueous solution of that salt. He found that, relatively speaking, the dried bladder took up much more water than sodium sulfate. At a loss for an explanation, he wrote: "The smallest part of the membrane has a pronounced affinity for water—whether it is chemical or adhesive will be explained to us one day by Chemistry when it has lifted itself out of its theoretical misery." (Die kleinsten Theilchen der Membran haben eine ausgesprochene Verwandshaft—ob chemische oder adhäsive wird uns die Chemie erläutern, wenn sie sich aus ihrem theoretische Elend erhoben hat—zu dem Wasser).[167]

The inadequacy of basic physics and chemistry to explain complex biological phenomena was again put on record by Martin Fischer another 60 years later. Thus in the concluding page of his thesis, "The Oedema," Fischer wrote: "When we speak as we have done throughout this paper of the "affinity" of the colloids for water, we have not used this word unthinkingly. An affinity is not a clearly defined force, but we have chosen it to cover a present lack of knowledge .... Physical chemistry has not yet settled for us what this is, but toward the answer to this question it is now striving. When it is obtained we will have to strike out this mysterious word "affinity" and write into its place the names of such clearly defined forces as physical chemistry may choose to dictate."[78 p 657]

It is astonishing that not until almost the very end of the 20th century, and thus one century and half after Ludwig's complaint, has a quantitative molecular mechanism for the unequal uptake of water and sodium sulfate been offered for the first time (see below). In answer to Fischer's quest, multilayer orientation and polarization introduced in [11.2] also offer a clearly-defined physico-chemical mechanism for the *affinity* of colloids for water. Experimental verifications for both proposed theoretical mechanisms follow.

In the PM theory of solute distribution in cell water (and model systems), there are two mutually complimentary causes for the lower levels of Na^+ (and its anion) or sucrose in cell water than in the surrounding medium.

An *energy* cause (or more precisely, *enthalpy* cause, which differs little) and an *entropy* cause, both originating from the stronger water-to-water interaction in the polarized multilayered cell water than in normal liquid water.[168]

Thus the energy needed to excavate a hole in the cell water to accommodate a Na^+ ion or sucrose molecule from the external medium is greater than the energy recovered in filling up the hole left behind in the external normal liquid water. *This disparity between energy gained and energy spent is the basic energy cause of solute exclusion.*

The entropy cause also originates from the stronger and hence "stickier" water-to water interaction in the cell. As a result, the rotational motion is more restrained and the rotational entropy of the solute in the cell water is lower than in the external medium. Since for polyatomic molecules like sucrose or *de facto* multiatomic solute like hydrated Na^+ the rotational entropy constitutes the major part of their total entropy, the reduced entropy and reduced energy combined make the *free energy* of distribution for (larger) solutes in cell water less favorable, and the equilibrium concentration of the (larger) solutes low.

Both the energy factor and the entropy factor for reduced solubility in cell water increase with the size of the solute. Hence the "*size rule,*" according to which *the (true) equilibrium distribution coefficient (or q-value) for a solute between cell water (or water dominated by extrovert models) and the external medium varies inversely with the molecular volume of the solute.*[168, 169, 170] This is one of the theoretical predictions of the PM theory, which the experimental studies described next were designed to test.

In Figure 23 the equilibrium concentrations of 9 nonelectrolytes in dialysis sacs containing 39% (w/v) native bovine hemoglobin, are plotted against the external concentration of the nonelectrolytes.[170] Note that the slopes of the best-fitting straight lines, which equal the *true equilibrium distribution coefficients* or *q-values*, are all close to unity. Thus the presence of native hemoglobin, at even this high a concentration, did not alter materially the solvency of water for solutes as large as the trisaccharide, raffinose (molecular volume, 500 cc). Now, native hemoglobin is an introvert model. Most of its backbone sites are locked in α-helical and other intra-macromolecular H-bonds and thus unable to interact strongly with the bulk-phase water in the dialysis sac. Its minimal effect on the solvency of the bulk-phase water notwithstanding, when the molecular volume of

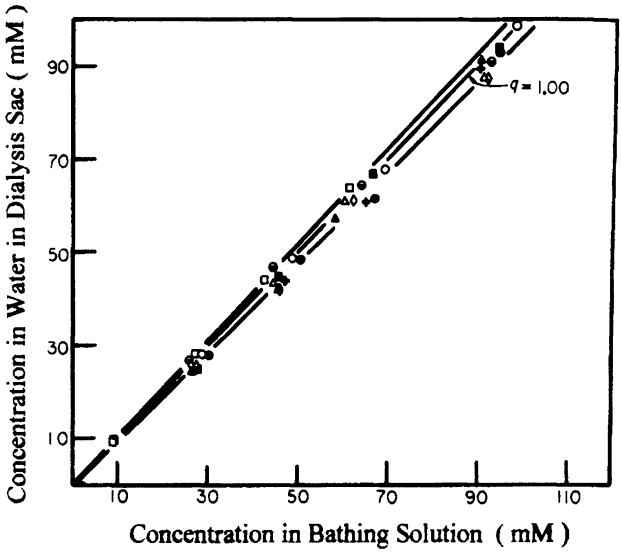

Figure 23. Equilibrium distribution of various nonelectrolytes in a neutral solution of 39% native bovine hemoglobin solutions in dialysis sacs. Incubation lasted 5 days at 25°C. Bathing solution contained 0.4 M NaCl. Ethylene glycol ●, glycerol Δ, erythritol □, D-xylose +, sorbitol ▲, mannitol ◊, trehalose ■, raffinose O. (Ling and Hu[170])

the probe solute reaches the thousands (cc), partial exclusion from concentrated solutions of native proteins does become demonstrable.[171]

Figure 24 shows the equilibrium distribution of the same 9 nonelectrolyes in a much less concentrated bovine hemoglobin solution (18%) (w/v).[170] Only this is not native hemoglobin any more but bovine hemoglobin *denatured* with NaOH (0.4 M). Each best-fitting curve remains a straight line but the slope now varies. By the method of least squares, one determines from the slope of each straight line the q-value of that particular solute in the water within the dialysis sac. (Remember that this is in essence the same NaOH-treated bovine hemoglobin that was made to liberate all its β- and γ-carboxyl groups from salt-linkages and thus to adsorb Na^+, Figure 9.)

In Figure 25 line b, where the q-values of the 9 nonelectrolytes obtained from the data of Figure 24 are plotted against their respective molecular weights, a Z-shaped line is obtained, indicating decreasing q-value for solutes with increasing molecular weight—in agreement with the "size rule." In contrast, the q-values of the same solutes in the native-hemoglobin solution obtained from the data of Figure 23 hover around a flat straight line

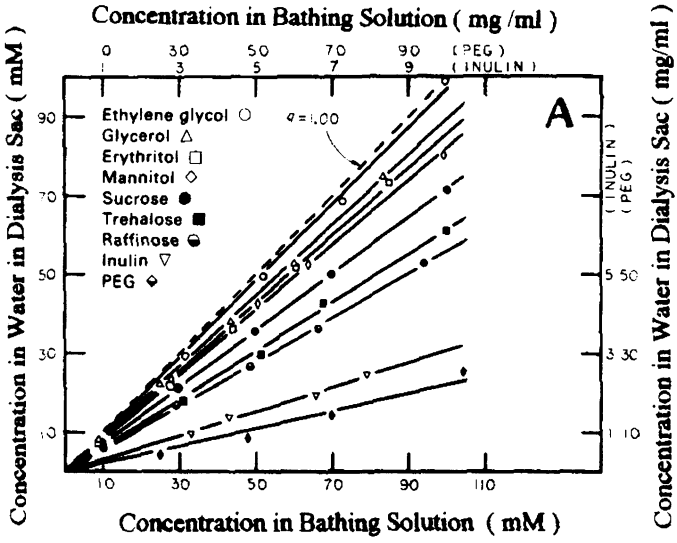

Figure 24. Equilibrium distribution of nine nonelectrolytes in dialysis sacs containing bovine hemoglobin (18%) dissolved and incubated in, and denatured by, 0.4 M NaOH. Incubation lasted 5 days at 25°C. Symbol for each nonelectrolyte as indicated in figure. (Ling and Hu[170])

with a q-value of approximately one (Figure 25 line a). Plotted this way, these data more clearly demonstrate how, in agreement with the PM theory, only extrovert models exercise strong influence on the solvency of water, but introvert models do not or do so weakly. So far, the subject matter are inanimate models. I now proceed to the central subjects of our studies, the living cells.

Ling, Niu and Ochsenfeld studied the equilibrium distribution profiles of 21 nonelectrolytes in *isolated frog muscles* at 0°C.[156] At this temperature, none of the sugars and other nonelectrolytes studied are being metabolically degraded or otherwise altered.[156 Table 1] It is also a temperature well tolerated by the North American leopard frogs from Vermont (*Rana pipiens pipiens*, Schreber), which povided the muscle tissues. Parts of the experimental results are shown in Figures 26 and 27.[156] Again the plots of all 21 solutes studied, shown and not shown, are all rectilinear in agreement with theory. The q-values of each of the solutes from the slopes of the straight lines are plotted against the logarithm of their respective molecular weights in Figure 28.

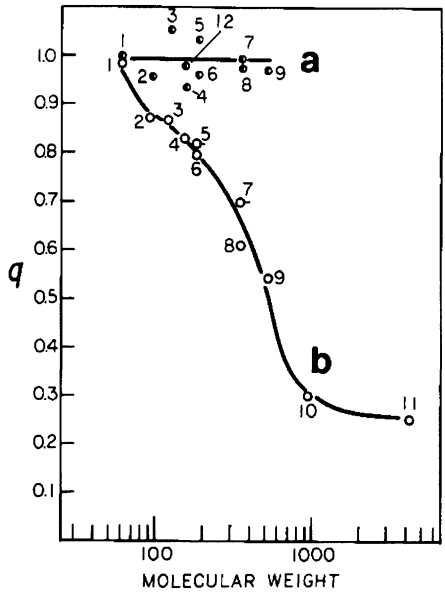

Figure 25. The (true) equilibrium distribution coefficients (q-values) of various nonelectrolytes in a 39% solution of native bovine hemoglobin (**a**) and in an 18% of NaOH-denatured bovine hemoglobin solution (**b**) plotted against the molecular weights of the respective solutes. The q-values are obtained by the method of least squares from the slopes of the equilibrium distribution curves similar to, and including those shown in the two preceding figures (Figures 23 and 24). (1) ethylene glycol, (2) glycerol, (3) erythritol, (4) D-xylose, (5) D-mannitol, (6) sorbitol, (7) sucrose, (8) trehalose, (9) raffinose, (10) inulin, (11) polyethylene glycol, PEG-4000. (Ling and Hu[170])

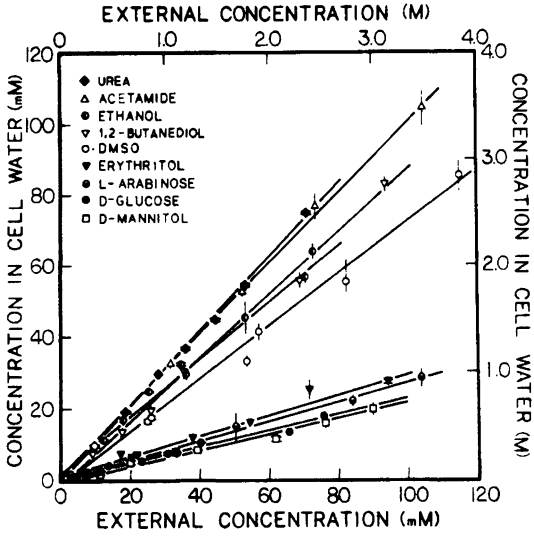

Figure 26. The equilibrium distribution of 9 nonelectrolytes in frog muscle cell water at 0°C. Each point represents the mean ± S.E. of at least four samples. Incubation time (enough or more than enough to insure diffusion equilibrium in all cases) was 6 days for L-arabinose and L-xylose but 24 hours only for all others. Each of the straight lines going through or near the experimental points is obtained by the method of least squares, its slopes yielding the respective (true) *equilibrium distribution coefficient* or q-value of that solute. (Ling *et al.*[156])

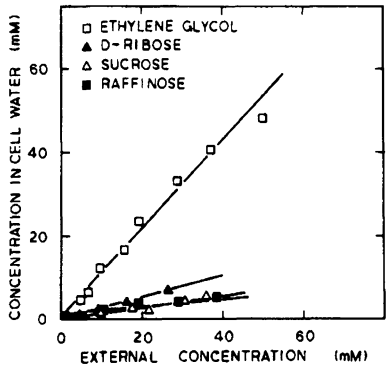

Figure 27. The equilibrium distribution of 4 non-electrolytes in frog muscle cell water at 0°C. Incubation time was 24 hours in all cases and long enough to reach diffusion equilibrium. For more details, see legend of Figure 26. (Ling *et al.*[156])

Again the majority of points fall on or near a Z-shaped curve in agreement with the "size rule," just like that of the NaOH-denatured hemoglobin and two other extrovert models, gelatin and PEO shown as insets in the same figure. (The data on gelatin are taken from Gary-Bobo and Lindenberg.[351] They are not q-values from slopes of straight-line plots as our own data are, but values obtained at a single concentration of the solutes studied, and are thus strictly speaking *apparent equilibrium distribution coefficients or* ρ*-values* though they may be very close or equal to their respective q-values.) Seven points of the frog muscle data fall above the curve, which fits the majority of points.

In 1993, using *statistical mechanical* methods, I derived an equation for solute distribution in living cells and model system[168] (Equation A3 in Appendix 1). The same data on the distribution of 21 nonelectrolytes in frog muscle shown in Figure 28 is presented once again in Figure 29. Only here the q-values are plotted against the logarithm of the respective *molecular volume*s of the nonelectrolytes rather than their molecular weights. And the curves going through or near the experimental points are theoretical according to Equation A3. Note that the excess water-to-water interaction energy in frog muscle cells shown as the *exclusion intensity*, U_{vp} , is 126 cal/mole.

In Figure 29 the seven aberrant points shown in Figure 28 now fall on a separate theoretical curve, which differs from the "normal" curve below it (only) in having a 30% higher U_s, *the surface component of the polarization energy*. In other words, the surface of each of these seven nonelectrolytes has stronger than usual interaction with the surrounding (polarized

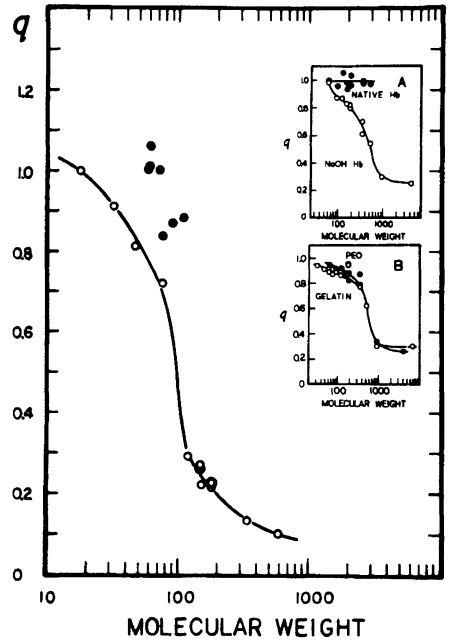

Figure 28. The (true) equilibrium distribution coefficients (or q-values) of 21 non-electrolytes in muscle cell water shown on the ordinate plotted against the molecular weights of the respective non-electrolytes. For comparison, q-value *vs* molecular weight plots of similar non-electrolytes in solutions of NaOH-denatured bovine hemoglobin (18%), gelatin gel (18%), poly (ethylene oxide) or PEO (15%) and native bovine hemoglobin (39%) are shown in the insets. Data on gelatin gel represent ρ- rather than q-values (see text) and were taken from Gary-Bobo and Lindenberg.[351] (Ling *et al.*[156])

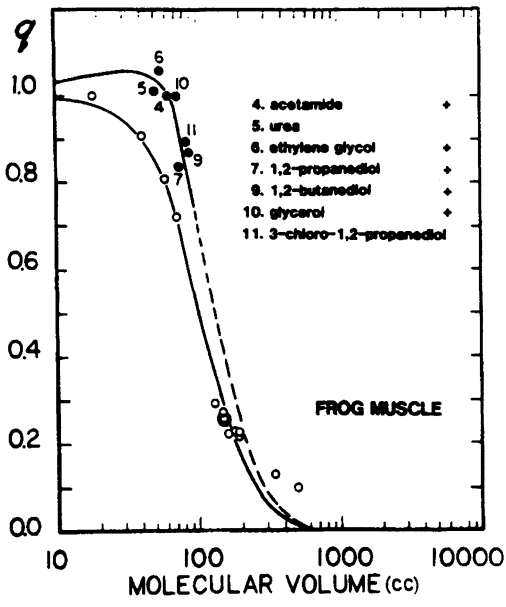

Figure 29. The equilibrium distribution coefficients (or q-values) of 21 non-electrolytes in muscle cell water plotted against the *molecular volumes* of the respective non-electrolytes. The points are experimental and are the same as shown in Figure 28. The lines going through or near the experimental points are theoretical according to Equation A3 in Appendix 1. For both the lower and upper theoretical curves, U_{vp}, the "exclusion intensity" for one mole of water, is the same at 126 cal/mole. U_s, the "surface polarization energy," on the other hand, is 119 cal/mole of water for the lower curve and 156 cal/mole for the upper curve. Chemicals marked with the + signs are established cryoprotective agents. (Ling *et al.*[156])

and oriented) water molecules—in harmony with the fact that five of them (marked with plus signs) are known *cryoprotectants*, which protect living human embryos and other surviving cells and organs from freezing and thawing injury. One may even speculate that a sixth, urea, might also be a cryoprotectant, were it not also a *protein denaturant*.

Some of the quantitative data presented above in Figures 24, 26 and 27 allow estimation of the average number of layers of polarized water between nearest neighboring water-polarizing sites.[156 p 201] *The results show that in frog muscle as well as the extrovert models, multiple layers of polarized water are involved, in agreement with the polarized multilayer (PM) theory* [11.2].

Verifying the central issue of the PM theory is a significant event of major proportion. Some other new knowledge that has come to light in the course of verifying the PM theory also deserves attention.

Firstly, as mentioned above, the average site-to-site space in frog muscle cells is spanned by about six layers of polarized water. These six layers of water molecules, when taken together make up the whole water content of the muscle cell. This conclusion is in harmony with an earlier conclusion from centrifugation experiments on muscle segments that in a normal typical living cell like frog muscle, there is no free water.

Secondly, in the PM theory of solute distribution, the exclusion intensity, U_{vp} (and surface component of interaction energy, U_s,) are assumed to be constant throughout the bulk-phase cell water. In theory, this agrees with the unchanging excess water-to-water interaction beyond the first layer of adsorbed water at the N and P sites (see end of [11.2] in fine print). In fact, agreement of the experimental data with the theoretical-derived curves demonstrated in Figure 29 support the postulated quasi-constancy of water-to-water interaction in bulk-phase cell water.

Thirdly, a striking feature of this quantitative theory is that it has generated an extraordinarily sensitive method to measure *minute* excess water-to-water interaction energy *beyond* the enormous normal *latent heat of vaporization* (9.7171 kcal/mole[436]). In this extraordinary capability, the method resembles that of a fine analytical balance, which can accurately assess the weight of a minute sample on weighing pans five orders of magnitude heavier.

Fourthly, *even more striking is the demonstration* of *how a small difference in the water-to-water interaction energy of 126 cal/mole can produce such high degrees of exclusion of the larger solutes like sucrose and* Na^+

from cell water. Yet the underlying principle could not be simpler. Take sucrose as an example. It has a molecular weight of 342.3 and a molecular volume of 338.8.[156] The molecular volume of water is 18.02. Dividing 18.02 into 338.8, one obtains the number of water molecules each sucrose molecule displaces, 18.8. Multiply 126 by 18.8 one obtains 2369 cal/mole or 2.37 kcal/mole, which is no longer a tiny free energy difference but one of the same order of magnitude as the latent heat of vaporization. As a result, a substantial exclusion of sucrose is predicted and has been recognized for as long as the history of cell physiology.

(5) A possible cause for the lower q-value for Na^+ (salts) in cell water than in extrovert models

Equipped with a quantitative theory of solute exclusion (Equation A3 in Appendix 1), we can now resume our inquiry begun in [11.3(3)] on the question, why frog muscle cell water has much lower q-value for sodium salts than in a simple solution of an extrovert model or even in a coacervate of the same extrovert model. As just pointed out above, the main parameter that determines the q-value of a solute in the new theory is the *exclusion intensity,* U_{vp}.

Now, frog muscle cells contain 20% proteins. The q-value of NaCl in frog muscle cell water is about 0.15 (see legend of Figure 54.) The ρ-value of Na-sulfate in gelatin solution at a similar 20% concentration is 0.85 (line 1 in Figure 22B); that of gelatin coacervate is 0.7. In agreement, the exclusion intensity or U_{vp} is only 16.5 cal/mole for gelatin solution[168 p 166] but that of living frog muscle is 7.6 times higher at 126 cal/mole.[156 p 196]

The U_{vp} for *gelatin coacervate* is as yet unknown. It is reasonable to expect its value to fall between that of simple gelatin solution and that of the living frog muscle cells. A straight-line extrapolation yields a U_{vp} of about 40 cal/mole for gelatin coacervate. This admittedly *very* rough estimate suggests that in converting a simple gelatin solution into a gelatin coacervate, the excess water-to-water interaction due to multilayer polarization and orientation has significantly increased. This idea is in harmony with the theory that more regular parallel orientation of the extrovert chains reduces the q-value for larger probe molecules in both inanimate coacervate and in living cells as discussed in [11.3(3)].

Having said that, I must emphasize that the figure obtained by extrapolation is no better than an educated guess. Definitive testing of the new hypothesis of coacervation as well as the role of parallel orientation of water-polarizing proteins in living cells awaits future investigations.

(6) Answer to A.V. Hill's once-widely accepted proof of free cell water and of free cell K^+

A. V. Hill's two papers published in 1930, by himself and in conjunction with Kupalov, dealt a near-fatal blow to those advocating a protoplasmic approach to cell physiology.[81] By demonstrating that urea (and later, ethylene glycol as well) distribute(s) equally between water in frog muscle cells and the surrounding medium, and by demonstrating that frog muscle is in vapor equilibrium with an isotonic solution of free Na^+ and free Cl^-, they convinced many influential cell physiologists that cell water and cell K^+ are both free ([5] and [7]).

Now, A. V. Hill's historical affirmation of these two basic tenets of the membrane theory rests ironically upon the correctness of the theory he attacked, i.e., *bound water is non-solvent*—meaning that bound water does not dissolve *any* solutes. If this concept of *categorically* non-solvent water itself is wrong, then what Hill's attack had really done was to demolish a faulty theory. The question now boils down to simply this. Is the concept of categorically non-solvent water wrong?

Figures 26 and 27 show that the very same water can have normal solvency for some substances but reduced solvency for others.[156] Thus the q-values for *urea* and *ethylene glycol*—the very same pair of key probes in Hill's and his supporters' history-making studies—are both *close* to unity (at respectively 1.05 and 1.02) confirming what Hill and his supporters had found, but the q-values for sucrose and NaCl *in the same kind of water* are way *below* unity.

Indeed, this inequality of the q-values of different solutes is precisely that described by the "size rule." Namely, the q-value decreases with increasing molecular volume. However, urea and ethylene glycol are not just small when compared to sucrose and hydrated Na^+, they also have surface structures that fit snugly into, and hold tightly onto, the surrounding water structure {[11.3(4)], Figure 29}. In other words, small size and high *surface polarization energy* (U_s) raise their q-values in the polarized-oriented cell to unity, or even beyond as the top curve of Figure 26 and of Figure 27 respectively indicate.

Further corroboration of this interpretation came from studies of the equilibrium distribution of pairs of radioactively labeled solutes (one H^3- and the other C^{14}-labeled) in the same extrovert-dominated water in the same dialysis sac. Thus in solutions of 10 urea-denatured proteins, the *apparent equilibrium distribution coefficient* or ρ-value—which differs from

the *true equilibrium distribution coefficient*, or q-value, in that the q-value represents only solute dissolved in the solvent (e.g., water) of both internal and external phase; the ρ-value *might (or might not)* include adsorbed components in the internal phase—is 1.001 ± 0.008 for H^3-labeled urea but only 0.732 ± 0.019[175 p 28] for C^{14}-labeled sucrose. Similarly, in solutions of NaOH-denatured bovine hemoglobin, the q-value for H^3-labeled ethylene glycol is 0.998; but that of C^{14}-labeled sucrose is only 0.627 (Figure 24).

In summary, Hill's demonstration of equal distribution of *urea* in cell water and confirmatory findings of equal distribution of *ethylene glycol* have successfully demolished only the mistaken theory of (categorically) "non-solvent" water in living cells. It has not proved that all cell water is normal liquid water; nor has it established that the bulk of cell K^+ is free—as the reader familiar with the contents of [10.2(3)] and [11.3(4)] may have realized earlier.

Having pointed out the mistaken *categorically non-solvent water*, I hasten to add that this idea is linked to only a small part of Professor Ross Gortner's (and his associates') important and valid work on water in colloids and in living cells. A consultation of Reference 80 and especially of Reference 64 would readily set straight misconceptions that might have been inadvertently introduced. Nor is there a need to reiterate what is obvious. Making a mistake does not reflect adversely on the integrity (and even skill) of a scientist, unwillingness to give up a (proven) mistake may.

(7) Osmotic cell volume control

We now return to the subject that had launched the earliest cell physiological investigations in the 18th century: to wit, the control of cell volume. Since *permeant* solutes are now known to cause *sustained* cell shrinkage too (Figure 2), the van't Hoff-Pfeffer explanation of volume control based on the assumption of absolute impermeability to sucrose and sodium chloride is no longer tenable. In the next section, I shall present a new explanation of cell-volume control based on the PM theory.

At one time, van't Hoff's *bombardment theory* was widely accepted [3]. It was eventually shown to be wrong.[13 pp 664–670] Osmotic pressure is now known to be an expression of the *relative vapor pressure* of the solution according to the following equation:[13 pp 668–669]

$$\pi V_1 = RT \ln\left(p^\circ / p\right), \qquad (2)$$

where π, as before in Equation 1, is the osmotic pressure, V_1, the partial molar volume of the solvent in the solution (water in the present case), R

and T are the gas constant and absolute temperature respectively, p^o/p is the reciprocal of the *relative vapor pressure* (p/p^o) represented by the ratio of the existing vapor pressure, p, divided by the saturation vapor pressure, p^o under the same conditions. *In living cells the replacement of the (incorrect) bombardment theory with the (correct) vapor pressure model shifts volume control from the cell membrane to the bulk-phase cell water.*

Since the relative vapor pressure is a measure of the *activity* of water, the osmotic activity of sucrose and NaCl, once considered to arise from their continual bombardment on the semipermeable cell membrane, in fact originates from their capacity in reducing the *activity* of water. A 0.7% NaCl solution keeps frog muscle cells at their normal volume, because the combined action of the dissociated Na^+ and Cl^-, each at a concentration of 0.118 M (approximately equal to 0.7% together), reduces the relative vapor pressure of water to match that of the physiological vapor pressure of amphibian tissues, i.e., 99.64% of saturation vapor pressure.

This new perception brings to focus another important question: What inside the living cells act(s) as the counterpart(s) of the free external Na^+ and Cl^- in an isotonic NaCl or Ringer's solution, so that the partial vapor pressures in and out of the cells are kept equal?

(7.1) What reduces the intracellular water activity to match that of a Ringer's solution?

After Julius Katz reported in 1896 that a large variety of vertebrate muscles all contains a high concentration of K^+ and phosphates,[75] many thought that (free) intracellular K^+ and (organic) phosphate ions are the main "osmotically active" agents inside living cells like frog muscle.[44]

Subsequent work described in [10.2] above have left little doubt that the bulk of cell K^+ (and intracellular anions) are *adsorbed* (e.g., ATP,[287] creatine phosphate;[310] see also Menten[378] for evidence of (localized) adsorption of muscle chloride and phosphates). In an adsorbed state, these intracellular ions do not lower the vapor pressure as *free* ions do. Therefore, something else in the living cell must serve this role instead. According to the AI Hypothesis, that something else is primarily the *fully-extended* intracellular proteins. *As such, they polarize and orient the bulk of intracellular water—thereby reducing its activity or relative vapor pressure.*

One prediction of this theory is that *extrovert models, which simulate the postulated water-polarizing fully-extended intracellular protein(s) in*

living cells [11.2], *should adsorb enough water at physiological vapor pressure to match that of living cells.* However, this prediction calls for a drastic departure from the conventional belief on the quantitative aspect of protein hydration.

Frog muscle, for example, contains 20% proteins and 80% water. If all that water is evenly adsorbed by *all* the cell proteins, the average amount of water adsorbed by one gram of dry proteins would still amount to 4.0 grams—a far cry from the widely accepted value of 0.2 to 0.3 gram of water per gram of native protein.[155 pp 673–674]

To put the new theory to a test, we must find out experimentally just how much water is adsorbed at equilibrium at physiological vapor pressure by extrovert models like gelatin, and PEO. One recalls that Ernst had already made an attempt in this general direction by comparing water sorption of frog muscle and gelatin. However, he and his coworkers worked at relative vapor pressure far below that of physiological vapor pressure, their results are of lesser importance here. To study vapor sorption at physiological vapor pressure, however, poses a technical difficulty.

Although studying water sorption of isolated proteins has long become commonplace, the higher limit of relative vapor pressure is in almost all cases 95% (of saturation).[192] In contrast, the relative vapor pressure of a frog Ringer's solution (and frog cells with which it is in vapor equilibrium) is much closer to saturation at 99.64%. A big gap exists between the influence of 95% and of 99.64% relative vapor pressure on the water content of living cells. Thus at a relative vapor pressure of 95%, the equilibrium water content of frog muscle is only 0.88 gram per gram of dry weight and thus less than a quarter of the normal water content maintained in equilibrium with a relative vapor pressure of 99.64% , i.e., 4.0 grams/gram dry weight.[159]

Indeed, until the publication of Ling and Hu's work in 1987,[163] the only published data carried to saturation or near-saturation vapor pressure that I could find were the study on sheep's wool by Speakman and others published in 1944 and later,[193] and that on a variety of biomaterials by J. R. Katz published in 1919.[194] The difficulty, which has kept many others from working at saturation or near-saturation vapor pressure—notwithstanding its strategic physiological importance—is most likely the *slow attainment of vapor equilibrium* between samples and the vapor phase at this high a relative vapor pressure.

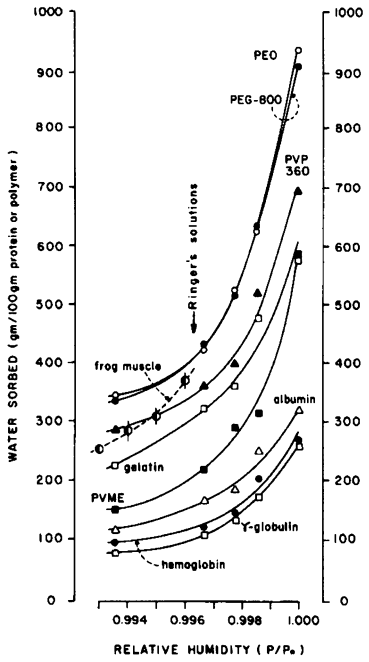

Figure 30. Equilibrium water sorption of 5 extrovert models (gelatin, PEO, PVP 360, PVME, PEG-800), and 3 introvert models (native bovine serum albumin, native bovine hemoglobin and native bovine γ-globulin) (25°C). Data were obtained by the *Null Point Method* introduced by Ling and Hu[163] (see text). Arrow points to the *relative vapor pressure* of a normal frog Ringer's solution (0.9966). Dashed line indicates the equilibrium water vapor sorption of living frog muscle taken from Ling and Negendank.[159] (Ling and Hu[163])

J. R. Katz's experiments lasted as long as several months. Yet Ling and Hu[163] showed that after 319 days at a relative vapor pressure of 0.99858, the weight of the extrovert model, polyvinylpyrrolidone (PVP-360), was still creeping upward. To overcome this difficulty, Ling and Hu introduced a new method. Called the *null point method,* it shortens the time for each run to 5 days and has enabled Ling and Hu to obtain the data described next.

Figure 30 shows that at the relative vapor pressure of a frog Ringer's solution (0.9964), the extrovert models PEO, PEG-800, PVP-360 and gelatin each sorbs from 300 grams of water per 100 gram to over 400 grams per 100 grams of polymer or protein—which approaches or equals the 400 grams per 100 grams of proteins of normal living frog muscle as shown by the dashed line in Figure 30. Even *introvert* models like native hemoglobin and bovine serum albumin take up from 90 to 150 grams of water per 100 grams of proteins, equivalent to 0.9 to 1.5 grams per gram of proteins, considerably higher than the figures often cited (e.g., 0.2 to 0.3 g/g).[155 p 673]

In neither the extrovert nor the introvert models thus studied *in vitro*, were special efforts made to align in parallel the polymer or protein molecules as in studies on PVP shown in the inset of Figure 22. Therefore, these polymers or proteins studied are not arranged in *parallel array*—as the (postulated) fully-extended proteins in living cells in the PM theory. Parallel orientation enhances the intensity of water polarization and orientation as experimentally demonstrated already in subsection (3). An increase in the intensity of water polarization and orientation might bring the accord even closer between the water content of living frog muscle cells (4.0 grams per gram of cell protein) and what were shown for the extrovert models (from 3.0 to 4.0 grams per gram of protein or polymer) (Figure 30).

Having confirmed in essence the prediction that gelatin and other extrovert models can indeed exert sufficient influence on the activity of water to match or at least approach that of the (postulated) fully-extended extrovert proteins in living cells, or that of a Ringer's solution, I shall next raise the question if sacs of solutions of extrovert models can demonstrate *in vitro* swelling or shrinkage in solutions containing solute(s), to which the enclosing membrane is permeable. Such a demonstration, if successful, would account for two types of observations, which the membrane-pump theory can no longer explain: (i) intact living cells like frog muscle cells can sustain swollen or shrunken volumes in solutions of ions or others solutes, to which the cell membrane is fully permeable [4.1(3)] (Figure 2), and (ii) open-ended frog muscle cell segments can also sustain swollen volumes in similar solutions, quantitatively matching those of intact living cells, even though these muscle segments do not regenerate new membranes at the exposed cut surfaces.[98 p 246; 296]

(7.2) Reversible osmotic shrinkage of solutions of extrovert model in dialysis sacs immersed in concentrated solutions of substances to which the sac membrane is fully permeable

A fundamental tenet of the membrane theory is that only impermeant solutes can cause sustained cell shrinkage. Nasonov, Aizenberg and Kamnev disproved that. Even earlier, Martin Fischer demonstrated swelling and shrinkage of (membraneless) gelatin as well as fibrin.[78; 546] The PM theory has gone a step further in providing not only an explanation of why gelatin behaves like living cells [11.3(2)], it also provides a quantitative molecular theory for the equilibrium volume of both gelatin and the living cell (Equation A12 in Appendix 1).

In the PM theory, that a concentrated solution of permeant solute can cause sustained cell shrinkage results from a combination of three contending forces:

(i) the tendency of cell proteins to adsorb additional (layers of polarized and oriented) water and thus to cause swelling;

(ii) the restraining force against swelling by salt-linkages formed within and among intracellular proteins;

(iii) the tendency of the cell to shrink owing to the *low q-values of, and hence* lower concentration of Na^+ and Cl^- (or their osmotically-active equivalents) in the cell water than in the external medium. Lower intracellular Na^+ and Cl^- concentrations reduce the water activity in the cell less than in the external medium, making the activity of water in the cell higher than in the bathing medium. Since water moves from higher activity to lower activity, the cell shrinks. Taking into account the "size rule," one finds that the larger the (major) solute(s), the greater the shrinkage.

In Figure 22, I have shown how a dialysis sac containing PVME demonstrates reduced solvency for Na sulfate or citrate. Other studies show that poly(ethylene oxide) or PEO behaves like PVME. Now, if one transfers a PEO-filled dialysis-sac from a sodium citrate solution of one concentration to another at a lower or higher concentration, the sac will undergo and sustain swelling or shrinkage respectively even though the dialysis sac is fully permeable to sodium citrate (Figure 31). Thus impermeability to sodium citrate is not the foundation for the *sustained* shrinkage a concentrated solution of sodium citrate brings about—contrary to the prediction of the membrane theory but in full harmony with the later observations of Höfler[31] and of Nasonov, Aizenberg and Kamnev[33, 34] [4.1(2) and (3)].

Indeed, all the dialysis sac does is to prevent the PEO from dispersing in the medium. Similar reversible swelling and shrinkage occur in all the extrovert models examined, including gelatin, urea- and NaOH-denatured bovine serum albumin.[161] Other studies show that non-charged solutes like D-glucose and D-sorbitol also produce similar volume changes like sodium citrate does on solutions of extrovert models.[161] Since we have presented extensive (though indirect) evidence that some or all proteins in frog muscle, red blood cells and, by inference, all other living cells exist in the fully-extended conformation [11.3(4)], osmotic shrinkage of frog muscle

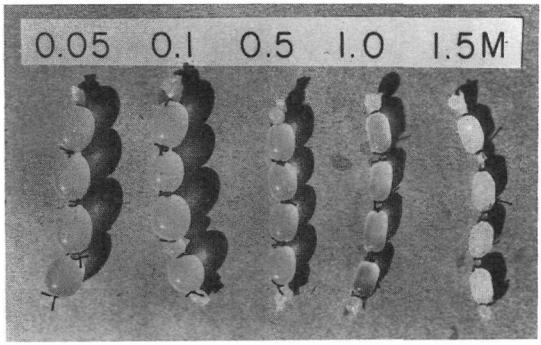

Figure 31. Sustained and reversible swelling and shrinkage of dialysis sacs filled initially with a 30% (w/v) solution of poly(ethylene oxide), or PEO, in solutions of sodium citrate at concentrations indicated in the photograph. PEO is a neutral polymer; its swelling and shrinkage have nothing to do with macroscopic electrostatic (or Donnan) effect. (Ling[15] by permission of Kluwer Academic/Plenum Publications)

in the presence of concentrated solutions of sucrose and galactose as observed by Nasonov, Aizenberg and Kamnev can now be understood.

In contrast, dialysis sacs containing solutions of *native* bovine serum albumin, (an *introvert* model), do not undergo swelling or shrinkage respectively in concentrated and dilute sodium-citrate solutions[161]—also in harmony with the prediction of the PM theory.

So far, we have been dealing with what historically has been called osmotic swelling and shrinkage and how they involve the interplay of two of the (three) contending forces (i) and (iii) listed above. In [15.3] I shall demonstrate how cell volume changes can also be produced by perturbing the restraining force of salt linkages as described under (ii).

As mentioned above, I have derived on the basis of the PM theory, an equation for the equilibrium cell-water content, 'a', in grams of water per 100 grams of dry protein (Equation A17 in Appendix 1). This equation can not only account for observations of Nasonov, Aizenberg and Kamnev[33, 34] that permeant solutes can initiate and sustain shrinkage of living cells,[195] it has also generated theoretical curves describing the equilibrium water content, a, of living cells when immersed in solutions of permeant solutes with widely different q-values (Figure 32). At high concentrations, solutes of low q-value like sucrose and NaCl cause water to move out of the cell and shrinkage occurs. In contrast, solutes like ethylene glycol with high q-value causes uptake of water, hence swelling from initial weights in Rin-

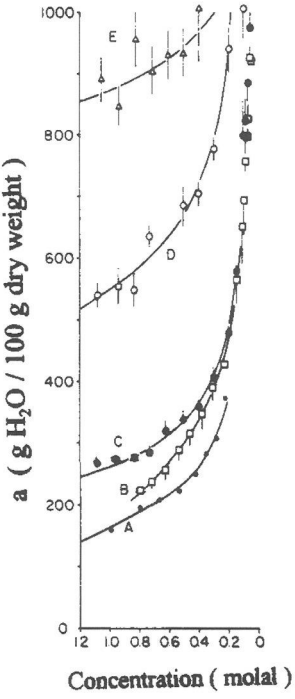

Figure 32. Water contents of frog muscles equilibrated in solutions of various sugars or sugar alcohols at concentrations given on the abscissa (25°C). In contrast, the data of Curve A are taken from Ling and Negendank[159] which show the water contents of frog muscle suspended in moist air and not in direct contact with NaCl or sulfuric acid solutions of different concentrations providing the different relative vapor pressure. In A, the water vapor uptake is plotted not against the partial vapor pressures as in the original publication but against different sucrose concentrations giving respectively the indicated partial vapor pressures. Curve B represents D-mannitol; Curve C, erythritol; Curve D, glycerol; and Curve E, ethylene glycol. (Ling[195])

ger's solution or tissue fluid. Note that the *total volume* of the cells, not merely that of cell water, can also be readily obtained from the values of 'a' with the aid of Equation A13 in Appendix 1 and values of other parameters indicated.

Having presented a bird's eye view of the opposing theories to the membrane theory ranging from that of Benjamin Moore to LFCH/PM theory and their respective supportive evidence, we resume our interrupted discussion in Section [4.3] on the sodium-pump hypothesis, or the broader membrane-pump hypothesis.

12

The Membrane-Pump Theory and Grave Contradictions

By now the reader has become thoroughly familiar with the subject of selective accumulation of K^+ over Na^+ in living cells, mostly in the context of the ideas of Moore and Roaf, Fischer, the LFCH and the PM theory.

The explanation offered by the membrane theory for this major cell physiological phenomenon has gone through several changes. First, the cell membrane was regarded as impermeable to both K^+ and Na^+ ions[76]—it was this version of the membrane theory that provoked the criticism of Moore and Roaf[77] cited in Chapter 7. Then the membrane was considered as permeable only to K^+ but not to Na^+ as the Mond-Amson-Boyle-Conway's version of the membrane theory suggested [4.3]. Finally, it was recognized that the cell membrane is also permeable to $Na.^+$ [(36)] When both K^+ and Na^+ can traverse the cell membrane, their intracellular concentrations should be the same as in the outside solution, contrary to facts.

It was in the emerging crisis thus created that the sodium pump hypothesis materialized. As mentioned above, the same or a broader category of metabolic pumps had been brought up from time to time throughout history.[49; 333] In this specific version of the metabolic pump hypothesis, the low level of cell Na^+ is maintained against its constant inward diffusion by the activity of a postulated outward pump located in the cell membrane. Like all pumps, *ceaseless* activity requires a *ceaseless* supply of energy.

Biochemical studies of energy metabolism in the 1930's led to the discovery that the final end-product of metabolism is *adenosine triphosphate* (also known as *adenyl pyrophosphate)* or *ATP.*[273] Meyerhof and Lohmann measured the *heat of dephosphorylation of ATP* (to ADP) and obtained a value of –12.0 Kcal/mole.[318] Based upon this and other relevant data, Lipmann then offered a new major cell physiological hypothesis,[132] in which each one of the two terminal phosphate bonds in the ATP molecule contains an extra amount of free energy. This energy can be liberated to per-

form biological work with the aid of an ATP-splitting enzyme known as ATPase. These unusual phosphate bonds are given the name, "*high energy phosphate bonds*" and represented as ~P.

Danish biochemist, Jens C. Skou found that the "membrane fraction" of a crab nerve homogenate contains a special kind of ATPase, which requires both K^+ and Na^+ for maximum activity—hence the name *Na,K-ATPase.* Skou then postulated that this and similar enzymes located in other cell membranes can liberate, and use the "high energy" in the high-energy-phosphate bonds of ATP to transport Na^+ out of the cell, and K^+ into the cell, both against concentration gradients. In other words, *this membrane Na,K-activated ATPase is the postulated Na pump.*[52]

A vast amount of time, talents and resources have been spent in research related to Skou's hypothesis, culminating in the award of the 1997 Nobel Prize for Chemistry to Prof. J. C. Skou specifically for his work on the sodium pump. It seems to me that the decision-making majority of some committee laid an egg.[247]

My reason for this view is not hard to understand. Each piece of the experimental evidence supporting the LFCH [10.2] and the PM theory [11] is often sufficient (i) to reverse the evidence once considered to be in favor of the sodium pump hypothesis; (ii) and/or raise serious questions about the validity of the sodium pump hypothesis. However, to simplify the matter, I cite four additional sets of evidence focused specifically on questions that have direct bearing on the validity of the sodium pump hypothesis.

(1) The minimum energy need of the postulated sodium pump in frog muscle under rigorously controlled conditions has been shown to be *at least* 15 to 30 times the maximum available energy[49, 98 pp 189–212] (Table 2). For more than ten years after its publication, *none* have disputed my conclusion that the sodium pump is energetically in violation of a basic law of physics, i.e., the Law of Conservation of Energy. Meanwhile, the essence of my finding has been *twice* confirmed.[274; 275]

(2) The sodium pump is one of the pumps needed to keep the cell afloat. In an admittedly incomplete list, Ling, Miller and Ochsenfeld counted, in 1973, 18 pumps that had been proposed and published, some of which are not single pumps but each a multitude of pumps including the various sugar pumps and various free amino acid pumps.[131 Table 2; 49 Table 1]

These are, however, only pumps at the cell membrane. Still other pumps are needed across the membrane of various subcellular particles. Due to their enormous surface area, the sodium pump at the sarcoplasmic

Table 2.

Date	Duration (hr)	Rate of Na^+ exhange integrated average (moles/kg per hr)	$\psi + E_{Na}/\mathcal{F}$ integrated average (mV)	Minimum rate of energy required for Na^+ pump (cal/kg per hr)	Maximum rate of energy delivery (cal/kg per hr)	$\left[\frac{\text{Minimum required energy}}{\text{Maximum available energy}}\right]$
9-12-56	10	0.138	111	353	11.57 (highest value, 22.19)	3060%
9-20-56	4	0.121	123	343	22.25 (highest value, 33.71)	1542%
9-26-56	4.5	0.131	122	368	20.47 (highest value, 26.10)	1800%

Comparison of the *minimal energy need* of the postulated sodium pump in frog muscle cells with the *maximum available energy* after both respiration and glycolysis were stopped. Metabolic inhibition was achieved by a combination of low temperature (0°C), pure nitrogen (99.99% pure nitrogen further purified by activated copper at high temperature), 1 mM NaCN and 1 mM sodium iodoacetate. Under these conditions, the only energy available[131 pp 11–12] to the muscle cells were from the store of ATP, ADP and creatine phosphate at the time when the poisons had begun to exercise their full effect and a minute trickle of glycolysis, which had escaped the iodoacetate blockage (but was accurately determined and taken into account in the energy balance calculation from analysis of the total lactate formed and analyzed). Rate of sodium pumping (according to the sodium pump hypothesis) was determined from small muscle-fiber bundles isolated from semitendinosus muscles of North America leopard frogs (*Rana pipiens pipiens*, Schreber). Pumping was against both an electric potential gradient (i.e., the resting potential measured with Gerard-Graham-Ling microelectrodes) and a concentration gradient (computed from the labeled Na^+ in the cells and in the bathing solution) under similar experimental conditions described above. For more details and supportive evidence for the experimental procedures adopted, see a recent review.[49] (From Ling[98] with permission)

reticulum (alone) in voluntary muscle cells, for example, requires 50 times more energy than a similar pump at the cell membrane.[49 pp 130–133] Yet as shown in (1), just one postulated pump alone at the cell membrane would demand for its operation more energy than what the cell has at its disposal.

(3) One year before Skou presented his sodium pump hypothesis, Podolsky and Morales demonstrated that the high-energy-phosphate-bond concept was in serious trouble.[133] To wit, the so-called "high energy phosphate bond" *does not contain high energy*. Podolsky and Morales's conclusion was based on the more precise measurement of the heat of hydrolysis of ATP and a (judicious) correction for the heat of neutralization of the acid liberated during the hydrolysis (Table 3). Thermodynamic analyses led George and Rutman[134] to a similar conclusion: that there is no high energy in the so-called high-energy phosphate bond.

Table 3.

Hydrolysis					Neutralization					Net
Temperature	Myosin	*n*ATP	*h*	ΔH_{obs}	Temperature	*n*HCl	*h*	$\frac{h}{n\text{HCl}}$	ΔHp(ioniz)	$\Delta H_{obs} + \Delta H_p$ (ioniz)
°C	gm. per 100 ml.	μmoles	milli-calories	kilo-calories per mole	°C	μmoles	milli-calories	kilo-calories per mole	kilo-calories per mole	kilo-calories per mole
19.2	0.28	6.83	–45.9	–6.7	19.4	4.47	–8.7	–1.94	2.0	–4.7
19.2	0.28	6.93	–46.2	–6.7	19.4	4.52	–8.9	–1.97	2.0	–4.7
19.3	0.28	6.63	–43.6	–6.6*	18.3	4.52‡	–8.8	–1.94	1.6	–5.0
18.5	0.09	6.83	–45.0	–6.6	18.7	4.57‡	–8.95	–1.96	2.0	–4.6
Mean										–4.75
σ										0.2

Heat content (enthalpy) change in myosin-catalyzed hydrolysis of ATP in phosphate buffer. Purified myosin labeled Sample 48 was dissolved in 0.60 M KCl. Total orthophosphate concentration was 0.05 M; pH, 8.00. n_{ATP} represents the number of moles of ATP hydrolyzed; n_{HCl}, the number of moles of HCl in the 0.6 M KCl neutralized. The last column represents heat content change with hydrolysis of one mole of ATP after correction for the heat of neutralization of the H^+ produced. Parallel studies with other buffers yielded similar results. σ is the standard deviation. (Podolsky and Morales,[133] by permission of *Journal of Biological Chemistry*)

With the truth about the "high energy phosphate bond" concept brought to light, even the 1500% to 3000% energy disparity figures for the postulated sodium pump in frog muscle cited under (1) appear to be serious *underestimations.*[49]

Since there is no utilizable free energy stored in the phosphate bonds of ATP, the Na,K-activated ATPase or any other ATPase cannot by hydrolyzing ATP provide energy for pumping Na^+ and K^+ ions.

(4) The technique of removing the cytoplasm or axoplasm of squid nerve axon was perfected in two laboratories in the year 1961.[135] The healthy condition of the "axoplasm-free" squid-axon membrane thus obtained has been established by its normal electric activities. When the open ends of such a hollow membrane sheath are tied, after sea water containing essential nutrients have been introduced into the sac, it provides an *ideal preparation* to test the membrane pump theory. There is every reason to expect it to pump Na^+ out and K^+ in against concentration gradients—that is, if the membrane-pump theory is fundamentally sound. In fact, active transport of K^+ or Na^+ against concentration gradients could not be demonstrated in these ideal preparations by some of the most skilled workers in the field.[136 p 95; 137; 138]

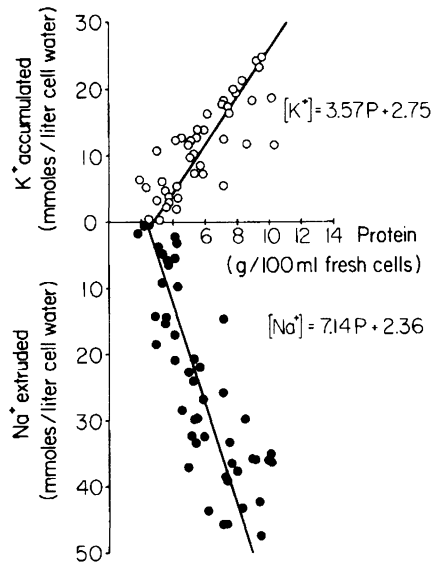

Figure 33. Reuptake of K^+ and extrusion of Na^+ from red-blood-cell "ghosts" prepared from washed human red blood cells. The study followed rigorously a procedure described in Reference 471. Freshly drawn blood was obtained (mostly) from different donors. When blood from the same donors was used, it was drawn at at least 6-weeks intervals. Each data point represents the difference of K^+ or Na^+ concentration in samples of the ghosts at the beginning of incubations and after 18 hours of incubation in the presence of ATP (37°C). Straight lines corresponding to the two equations shown in the graph were obtained by the method of least squares. Total protein content represented as P in the equations was obtained by subtracting the sum of the weights of lipids, phospholipids, salt ions, and sucrose from the dry weights of the ghosts. (Ling, Zodda and Sellers[140])

Similarly, resealed red blood cell ghosts also freed of all cytoplasmic proteins and supplied with ATP do *not* transport K^+ or Na^+ against concentration gradients.[139, 140, 107 pp 25–27] In contrast, similarly-treated, resealed red cell ghosts supplied with ATP *do* re-accumulate K^+ and extrude Na^+ *if and only if*, a measurable amount of the cytoplasmic proteins remains in the ghosts.[107 pp 25–27] Indeed, the levels of K^+ re-accumulated is *directly* proportional to the level of proteins remaining in the ghosts (mostly hemoglobin), as shown in the upper plot of Figure 33. And the level to which Na^+ is brought down is *inversely* related to the concentration of residual intracellular proteins also[140] as shown in the lower plot of Figure 33.

Since Nature's own membrane—when freed of cytoplasm—cannot do what the membrane pump theory has predicted, it is not surprising that claims of success in demonstrating K^+ and Na^+ transport against concentration gradients by man-made "membrane pumps" of isolated Na,K-ATPase are themselves mistakes. The mistakes appear to have come from an overlooked *isotope leakage* from the isotope-loaded vesicles while passing through a Sephadex column—in order to separate the vesicles from the isotope-loading solution—as pointed out in some detail by Ling and Negendank in 1980.[138 p 224; 107 pp 22–25]

Parenthetically, Ling and Negendank also pointed out[138 pp 234–235] that the data perhaps prematurely interpreted as indicating ATP-energized pumping (in the wrong direction) are not without value. For they suggest that water in the vesicular membrane (see Figure 5D for an EM picture of similar vesicles) might have become polarized and oriented by the Na,K-activated ATPase, which assumes at least in part the fully-extended conformation under the influence of ATP as a cardinal adsorbent [15.1]. The reduced rate of permeation of labeled Na^+ through the polarized-oriented water may then slow down the loss of labeled Na^+ from the vesicles during their passage through the Sephadex column, and account for the higher level of labeled Na^+ found in these vesicles than in the otherwise similarly-treated vesicles but without ATP.

When all the above evidence is taken into account, I have reached the conclusion that *the selective accumulation of K^+ over Na^+ in nerve, muscle, erythrocytes and other cells with a uniform cell membrane, and called unifacial cells, is not at all likely the consequence of the ceaseless activity of a postulated pump in their cell membranes*. The sodium pump hypothesis is thus no longer a useful model, and it is high time for its retirement.

In 1976, two of my former graduate students and an inexperienced fledgling reporter for *Science* magazine attempted to convince readers that the sodium pump hypothesis was still viable. A careful analysis of their output showed that there is little merit in it as it was based on some non-existent "crucial experiments" and on not telling the whole truth—as was made clear in an article entitled "Debunking the Alleged Resurrection of the Sodium Pump Hypothesis" I published in 1997.[49]

As was made clear in [4.3], the "pump" was installed as the last resort to keep the membrane theory afloat. The retirement of the sodium pump hypothesis, therefore, also spells the end of the tenure of *the paradigm of living cells as membrane-enclosed dilute solutions,* a paradigm that has dominated the field of cell physiology throughout its entire history. However, not everything introduced in the membrane theory has been proven wrong. Thus, there is strong evidence that living cells are covered with a diffusion barrier of some sort, as originally postulated in the membrane theory. This barrier variously called the *protoplasmic skin*, *plasma membrane* or *cell membrane* is the subject matter of the following chapter.

13

The Physico-Chemical Makeup of the Cell Membrane

13.1 Background

There is extensive evidence that a (relative) diffusion barrier exists at the surface of most, if not all, living cells [e.g., 4.1(1)]. In my view Pfeffer's Plasmahaut (protoplasm skin) is a more appropriate name for this relative barrier. Yet the term, "cell membrane" has been so long in usage that one can only accept it reluctantly—as one accepts reluctantly the name, "cell." The key question we address in this chapter concerns the physico-chemical makeup of this cell membrane. There are two opposing views on the subject: one based on the membrane-pump-theory and one based on the LFCH (and AI Hypothesis). Following a brief description of both, I shall review the results of experimental investigations aimed at finding out which model is closer to the truth.

(1) The membrane theory

Traube's study of the copper ferrocyanide precipitation membrane launched the membrane theory. Unlike Thomas Graham's starch-sized paper, but more like Abbé Nollet's pig bladder, copper-ferrocyanide membrane is not only impermeable to colloids but also "impermeable" to crystalloids like copper ion, ferrocyanide ion and sucrose. To explain this selective permeability, Traube suggested in 1867 his *atomic sieve theory,* in which pore size determines permeability or impermeability[17]—long before similar ideas were reintroduced again and again by a succession of investigators including Michaelis,[401] Mond and Amson[51] and Boyle & Conway.[44]

Traube's sieve idea proved wrong, however. Thus Bigelow, Bartell and Hunter demonstrated distinct osmotic activities of copper-ferrocyanide membranes with pore diameters as wide as 0.5 micron (5000Å).[55; 56 p 96; 13 pp 656–657] A membrane with 5000 Å-wide pores cannot act as a sieve to bar

the passage of (virtually impermeant) "large" solutes like sucrose with a molecular diameter of only 8.8 Å

However, earlier in 1855 L'Hermite demonstrated how *solubility* (rather than pore size) may underlie semipermeability or more correctly, *differential permeability* to various solutes[57; 56 p 98] (see also Liebig[482]). In support of his solution theory, L'Hermite carried out an ingenious and convincing experiment. He filled a cylinder with chloroform (heaviest and stays at bottom), water and ether (lightest and stays at the top) in that order. Now ether is (slightly) soluble in water; chloroform is not. Accordingly, ether passes through the water layer to enter the chloroform layer, but chloroform cannot go through the water layer to reach the ether layer. Water in this case acts as a *semipermeable* medium, permeable to ether but impermeable to chloroform.

A well-known extension of L'Hermite's solution theory is Charles E. Overton's *lipoidal membrane theory*.[21] Following an earlier suggestion of Quinke, who postulated the existence of an oil layer over the surface of living cells,[542] Overton reiterated this postulation, adding that the different permeability of the cell membranes to various non-electrolytes is attributed to their different solubility in that membrane oil.

Experimental support for Overton's lipoidal membrane theory came from R. Collander[320] and is shown as Figure 34. Here the ordinate represents the product of experimentally measured permeability, P, of a particular substance into *Nitella* cells multiplied by the molecular weight, M, of this substance raised to the power of 1.5 (see legend for justification). The abscissa represents the olive oil/water distribution coefficient of the 69 compounds studied. The good correlation demonstrated suggests that solubility in a lipoid cell membrane determines the relative permeability of a nonelectrolyte. Behind this good appearance, there are serious unresolved problems. Three will be mentioned.

Firstly, the permeability was measured by first exposing the *Nitella* cells to a solution containing the nonelectrolyte under study for a length of time, before samples of the fluid of the central vacuole (see Figure 1A) were collected and analyzed. Now, the reader may recall that Höfler had demonstrated in his later studies that the diffusion barrier to sucrose in the mature plant cell he studied is the (inner) vesicular membrane (or tonoplast) and not the (outer) plasma or cell membrane (Figure 1). Collander did not address this problem. Therefore, a distinct possibility exists that the diffusion barrier, which the data of Figure 34 represent, is not of the cell

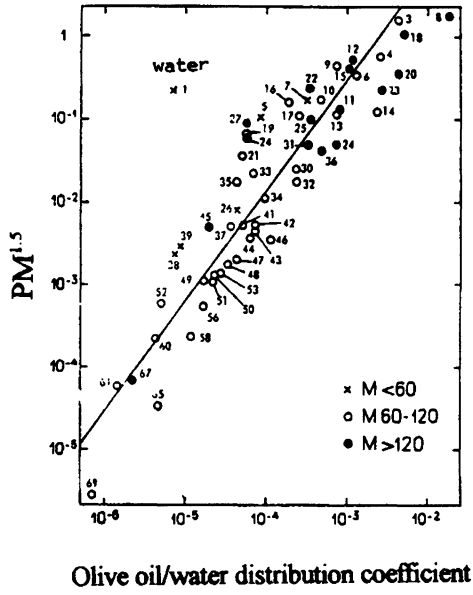

Figure 34. Correlation between the permeability of *Nitella mucronata* cells to 69 nonelectrolytes and their respective olive oil/water distribution coefficient. Note that the ordinate represents the product of the permeability constant of each solute multiplied by its molecular weight raised to the power of 1.5. ($M^{1.5}$ factor was introduced apparently to improve the linearity of the curve only.) The numbers near the data points represent the nonelectrolytes: 1, water (HD); 7, ethanol; 8, paraldehyde; 52, ethylene glycol; 61, urea; 69, glycerol. (Collander,[320] by permission of Academic Press)

membrane but that of the tonoplast. Tonoplasts are special structures found in mature plant cells and cannot be seen as a model of all cell membranes.

Secondly, the distinguishing feature of a *semipermeable* membrane is its overwhelmingly higher permeability to water than to solute dissolved in water. Indeed, the first report on the subject by Abbé Nollet showed the pig bladder to be permeable to water but impermeable to grain alcohol or ethanol. Yet a look at Figure 34 reveals that the olive oil/water distribution coefficient of ethanol is not lower than that of water but more than 100 times *higher* than that of water. Thus the *Nitella* membrane should be 100 times more permeable to ethanol than to water, contrary to facts underlying the very concept of semipermeability.

Thirdly, the interfacial tension of oil and water is at least a hundred times higher than that actually measured at the cell surface (see [13.4] for more details).

Since both the atomic sieve model and the lipoidal membrane model have encountered serious difficulties, a succession of investigators attempted to combine the two models into one. Nathanson's mosaic membrane theory,[438] Ruhland's ultrafilter theory,[422] Collander and Bärlund's lipoidal-filtration theory,[403] Danielli-Harvey's paucimolecular theory[483] as well as the currently accepted Singer-Nicolson fluid-mosaic membrane[200] (in which phospholipid bilayer has replaced Overton's oil layer) are examples. In all these models, a layer of oil or phospholipids forms a continuous barrier between the cell interior and the external medium. In all these models the cell membrane is a true membrane with a distinct boundary facing both inward and outward. There is, however, one exception. That is the membrane model of Wilhelm Pfeffer, often cited as the originator of the membrane theory. Actually, in Pfeffer's model, proteins make up a part or the entirety of the plasma membrane.[18 p 156] And there may not be a distinct boundary separating the Plasmahaut from the cytoplasm.[18 p 139] Therefore, the (alleged) founder of the membrane theory has, in fact, much in common with Franz Leydig's and Max Schultze's thoughts on the chemical nature of the cell membrane [1].

(2) LFCH (and AI Hypothesis)

In presenting Ling's fixed charge hypothesis (LFCH) in 1951 and 1952, I suggested that what is true in the bulk-phase cytoplasm should be true also at the cell surface.[96 pp 781–782] More specifically, I suggested that fixed anions at the cell surface should also selectively adsorb K^+ over Na^+ as they do in the cytoplasm. Based on this idea I introduced a theory of selective K^+ permeability on the one hand, and later, in 1955, a new theory of the resting potential on the other hand (see Figure 4C). Both subjects will be discussed in detail in Chapter 15 to come.

When the PM theory was introduced 13 years later, the concept that the bulk of cell water exists in the dynamic structure of polarized multilayers was introduced. Again extending the concept of fundamental similarity of the bulk-phase protoplasm and cell-surface protoplasm, a more complete model of the cell membrane emerged.

Thus, like the bulk-phase cytoplasm, by far the largest area of the cell membrane is occupied by polarized-oriented water. In other words, the cell membrane is primarily a (polarized-oriented) water membrane, dominated by fixed anions (or fixed cations) and/or more specialized sites mediating the traffic of free amino acids, sugars and even proteins.[15 pp 426–435]

Having made clear the *ubiquity* of polarized-oriented water as the continuous phase of the bulk-phase protoplasm as well as that of the cell membrane, I must also stress the *versatility* of this model. This versatility originates from the variability in the proportions of surface area covered respectively by polarized water, surface proteins as well as phospholipids. Just as important, this versatility also comes from the degree of polarization of the membrane water and the polarity and electrical charges as well as steric characteristics of the functional groups carried by the membrane proteins. Other characteristics will be described later after the *AI Hypothesis proper* has been presented.

It may be added that, although in the new model polarized-oriented water has displaced phospholipids as the continuous barrier, phospholipids are, nonetheless, important. They may, for example, stabilize the protein-water system at the cell surface.

In summary, the continuous phase of the cell membrane is polarized-oriented water. The most consistently present solid component of the cell membrane is protein. The model as such does not require, nor does it exclude, a clear-cut boundary separating the membrane from the cytoplasm like that facing the external medium.

Having provided a rough sketch of the two opposing models of the physico-chemical makeup of the cell membrane, we move on to examine which one is favored more consistently by the results of experimental studies designed to test their respective relative proximity to the truth.

13.2 Ionic permeation

In 1953, I showed that the rate of entry of labeled Rb^+ entry into frog muscles is competitively inhibited by the presence of external K^+.[147; 218; 219] Similar quantitative relationships had been reported the year before by Epstein and Hagen for the entry of Rb^+ into barley roots and interpreted on the basis of a *carrier* theory,[220] as did Conway and Duggan later from their study of the effect of K^+ on the rate of entry of Na^+ into fermenting yeasts.[221]

To test the hypothesis that it was the presence of *fixed anions* obeying the rule of full counterion association [10.1(1)], three non-living models were chosen: ion exchange resin sheets bearing fixed sulfonate groups, oxidized and dried collodion membrane bearing carboxyl groups, and sheep's wool bearing β- and γ-carboxyl groups. We then studied ionic per-

meation into each one of these models in the same way Epstein and Hagen, Conway and Duggan and we ourselves had studied ionic permeation into living cells. We anticipated that similar results should be obtained. They were.

Thus labeled Cs^+ entry into each of the three inanimate models indeed follows "Michaelis-Menten kinetics" for enzyme activities[446 Chap 4] similar to that describing alkali-metal ion entry into living cells.[147, 222] Later a fourth model of an isolated actomyosin gel from rabbit muscle was added, again producing similar results.[223] The question arises, Has anyone found an inanimate model for the carrier theory?

The phospholipid bilayer is extremely impermeable to ions.[209] For ions to traverse such a bilayer at physiological rate, a ferry-boat or *carrier* is a must. So when valinomycin and other similar substances were first discovered to act as a highly K^+-specific carrier or *ionophors* (see [13.6] below), many started to look for their natural counterparts. A great deal of time, money and effort has been spent on this collective effort. In 1975, Paul Müller, who, with Don Rudin at the Eastern Pennsylvania Psychiatric Institute (EPPI), initiated the intense research efforts on phospholipid bilayers at the time when Eisenman, I myself and most of the other senior members were leaving EPPI (for backgound, see [14.1] to follow), made the closing comment: "A lot of us have spent a wasted ten years or so trying to get these various materials into bilayers...."[224] Tragic as it is, this statement is of a kind rarely seen in the science literature and, in my view, to be viewed with respect, even admiration for its forthrightness and honesty.

I now focus on more details of the theoretical model based on the LFCH (and the AI Hypothesis), now made plausible by the studies on four types of inanimate models. Figure 35 diagrammatically illustrates a microscopic portion of a cell surface facing the external solution.[222] The continuous phase represents polarized and oriented water, in which are shown four β- and γ-carboxyl groups—but not the rest of the protein molecule(s) bearing these carboxyl groups.

As pointed out in Figure 35, an external monovalent cation, say K^+, may enter the cell *via* either the *saltatory route* (Route 1) or the *adsorption-desorption route* (Route 2). Low solubility in the polarized-oriented cell surface water lends resistance to the entry of external cations *via* the saltatory route. In addition, a would-be entrant cation *via* this route also encounters a *continuous* repulsive energy barrier. This repulsive mechanism is electrostatic in nature and similar to that offered by the cotton plug on a

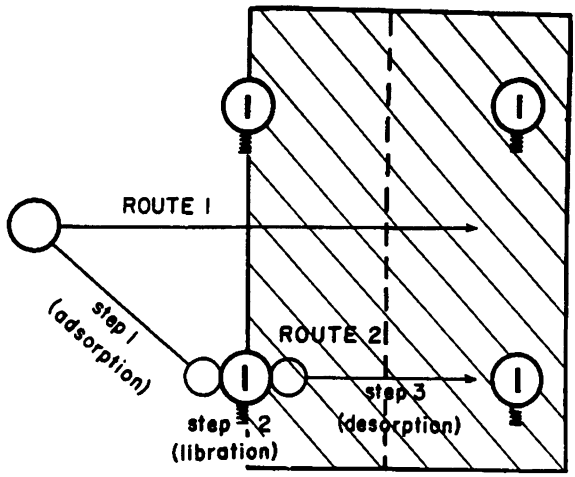

Figure 35. Two alternative routes of entry of a monovalent cation like K^+ into an inanimate anionic fixed charge system or living cell with anionic surface sites. Four fixed anions are represented but not the remainder of the proteins carrying these anions. Route 1 is the *saltatory route*. It involves wading through the polarized water between the fixed anions. Route 2 is the *adsorption-desorption route*, involving a sequence of three steps: adsorption, libration around the fixed anion, desorption. The adsorption-desorption route involves two ions, the fixed ion and the entrant ion, and is therefore referred to as the *doublet route*. (Ling and Ochsenfeld[222] by permission of The Biophysical Society)

sterile culture flask of bygone days. Though the meshes of the cotton plug are many times the size of a bacterium, the plug effectively bars the (electrically charged) bacteria from entering the flask.

The alternative route of entry is the *adsorption-desorption route*. It is energetically more favorable for an external cation to slide into the vicinity of a fixed anion. Once adsorbed onto a fixed anion, a prospective entrant cation may undergo a *libration* step to circle around the fixed anion before desorption and entry into the cell interior. This is called the *doublet (adsorption-desorpton) route* and may represent the most probable route of entry for ions that are not too strongly adsorbed onto the fixed anion (see below).

For a cation that is very strongly adsorbed, the desorption step may be slow. But it may be accelerated by a second cation, *via* what is called *triplet adsorption-desorption route* as illustrated in Figure 36. Note the difference between what is called a *billiard* type and a *pin-wheel* type of the triplet route depends on which side the *activating* cation comes from, the outside solution or the cytoplasm inside the cell.

In summary, the saltatory route, and the two modes of adsorption-desorption route—the doublet route and (the billiard type and the pin-wheel type of) the triplet route—predict a variety of interactions by which

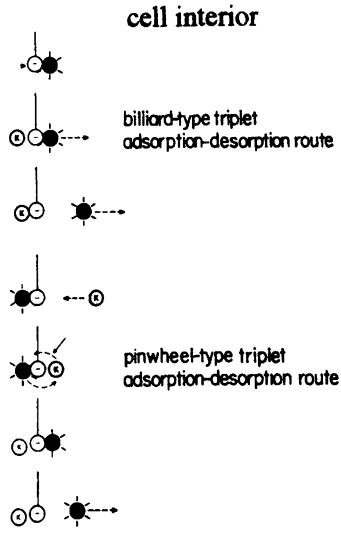

Figure 36. The *triplet adsorption-desorption route* is a variant of the doublet adsorption-desorption route shown in Figure 35. And it plays a more important role in the permeation of ions that are very strongly adsorbed. Depending on the origin of the third "activating" cation shown as an encircled K, it is called either the "*billiard type*" in which case, the third activating ion comes from the outside or a "*pinwheel type,*" if the activating cation comes from within the fixed-charge system or cell. Open circles containing a negative sign represent fixed anions. Solid circles represent the cation entering the cell. (Ling[15] by permission of Kluwer Academic/Plenum Publications)

one external cation may affect the entry of another cation into a living cell, including (i) indifference, (ii) competition and (iii) facilitation. All these predicted relationships have been experimentally confirmed.[222] Thus, while external Rb^+ competitively inhibits the entry of K^+ into frog muscle cells, K^+ only facilitates the entry of Rb^+, in agreement with theory and the established fact that Rb^+ is strongly adsorbed on the β- and γ-carboxyl groups on the cell surface as well as in the bulk-phase cytoplasm of frog muscle cells.

Using a high concentration of strongly-adsorbed external competing cation, one can assess by what proportion a cation enters, by the saltatory route or by the adsorption-desorption route. The data presented in Figure 37 show that some 85% of the K^+ entry is inhibited by 45 mM external Rb^+, but only half of the Na^+ entry is inhibited by 30 mM external K^+. These data suggest that the great majority of K^+ entry is *via* the adsorption-desorption route, but only half of Na^+ entry is *via* that route. The remaining K^+ and Na^+ are most probably entering by the saltatory route.

Thus far, we have been concentrating on the *inward permeation* or *influx* of cations into living cells. The same model shown in Figures 35 and 36 can also account for the *outward permeation* or *efflux* of cations from within the cells to be discussed in [15.2] to come.

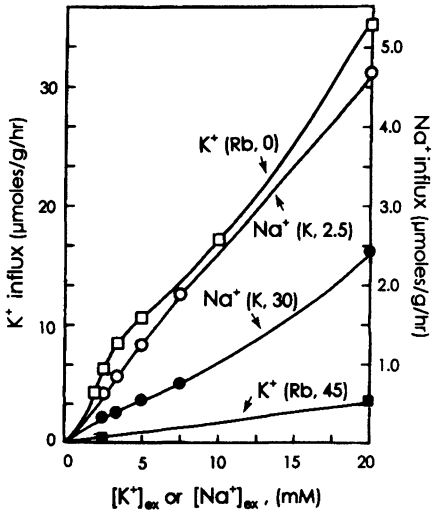

Figure 37. Influence of a high concentration of a strongly-adsorbed competing ion Rb^+ or K^+ respectively on the influx rate of labeled K^+ and of labeled Na^+ (shown simply as K^+ and Na^+ in the figure) into frog sartorius muscles. □ represents rate of influx of K^+ in the absence of external Rb^+; ■, the rate of K^+ influx in the presence of 45 mM of competing external Rb^+. ○ represents the rate of Na^+ influx in the presence of the normal K^+ concentration of 2.5 mM; ●, the rate of Na^+ influx in the presence of 30 mM of external K^+. (Ling, unpublished)

13.3 Water traffic into and out of living cells is bulk-phase limited

Membrane permeability is the cardinal feature of a living cell in the membrane theory. Yet permeability to a substance as important as water was not amenable to *direct measurements* until radioactive-tracer technology became available in the late 1930's and early 1940's.

The recognition that most living cells are solid bodies rather than membrane-enclosed dilute solutions raises the theoretical possibility that, for fast-moving substances like water, the rate of diffusion through the cytoplasm may approach or equal its rate of diffusion through the cell membrane.

To put this idea to a test, Ling, Ochsenfeld, Reisin and Karreman studied the water traffic in and out of two types of unusually large cells: frog ovarian eggs[201] and giant barnacle muscle cell.[202] Radioactive tracer techniques made it possible to obtain accurate and detailed data on the time course of exchange of labeled tritiated water between an individual cell and its environment. Since the mathematics of both membrane- or *surface-limited diffusion* and *bulk-phase-limited diffusion* have long been worked out,[277; 486] there is little difficulty in distinguishing between the two by examining the theoretical *influx profiles* diagrammatically illustrated in Figure 38. *Membrane- or surface-limited* diffusion shows typically an

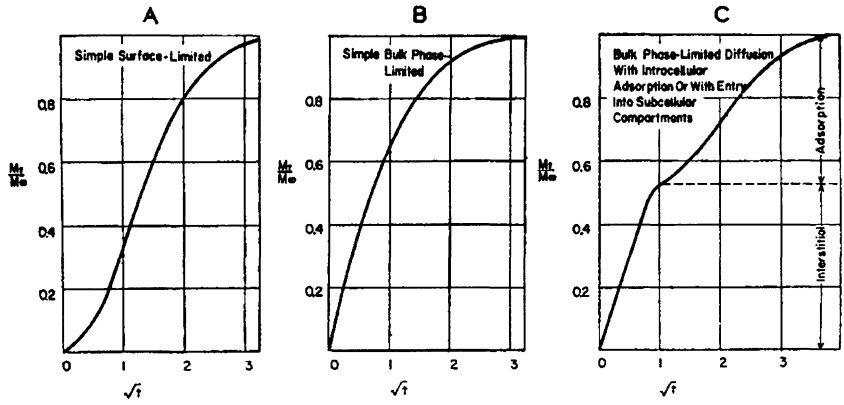

Figure 38. The time course of influx of a labeled substance into systems with different rate-limiting steps as indicated in each figure. The "influx profiles" are theoretically calculated. The ordinate represents the uptake, M_t, of the labeled material at time t as a fraction of the total amount of the material in the system eventually reached (M_∞). The abscissa represents the square root of the time, t. Note that in the simple surface- or membrane-limited profile (A), the initial uptake is conspicuously *concaving upward.* In contrast, in bulk-phase-limited diffusion, simple (B) or otherwise (C), the initial profile is *straight or slightly convex* regardless of adsorption or entry into subcellular compartments. When there is adsorption or entry into subcellular compartments and their rates are significantly slower than the rate of diffusion as in C, an inflection point in the later part of the profile may be seen. (Ling *et al.*,[201] reproduced from The Journal of General Physiology by copyright permission of The Rockefeller University Press)

S-shaped profile when the fractional uptake at diffusion time t is plotted against the square-root of t. In contrast, *bulk-phase-limited* diffusion with or without subsequent adsorption or entry into subcellular compartments exhibits an initial profile respectively concaving downward (B) or almost straight (C).

In Figure 39, I present six samples of the experimentally determined *influx profiles* of tritiated water into frog ovarian eggs (for details, see legend).The conclusion we drew from this and the companion study on giant barnacle muscle cell is that the rate of exchange of labeled water between frog ovarian eggs and its environment and between giant barnacle muscle fiber and its bathing medium are—contrary to conventional belief—both *bulk-phase limited* like that theoretically illustrated in Figure 38 B and C. That is, the rate of diffusion of radioactively labeled water throughout the interior of these cells is *equal* to the rate of diffusion of radioactively labeled water through their respective cell membranes.

As a special bonus, the quantitative data collected on the basis of the theory of bulk-phase limited diffusion provides an *internal* check on the

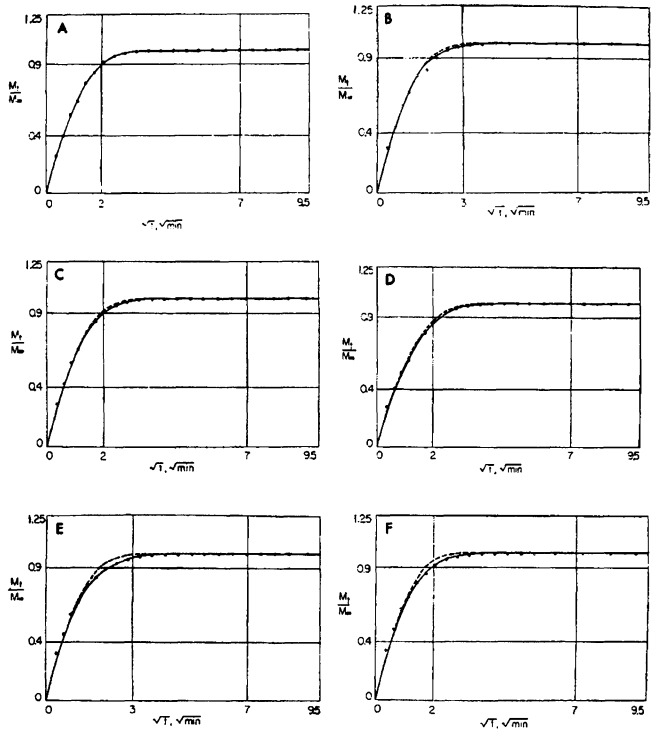

Figure 39. *Influx profiles* of tritiated water into single frog ovarian eggs (D, E and F) or small *clusters* of a few eggs (A, B and C). No difference could be detected between these two groups. In all the data obtained, the *diffusion is bulk-phase-limited.* Solid lines going through or near most data points are theoretical for simple bulk-phase diffusion (A) or for bulk-phase-limited diffusion with adsorption or entry into subcellular compartment (B to F). Dashed lines in B to F are theoretical profiles for *simple* bulk-phase limited diffusion (as in B of Figure 38) and are included for comparison. The diffusion coefficients obtained from eight sets of studies yield 1.00 ± 0.10 (S.E.) in units of 10^{-5} cm^2/sec. in comparison with that of tritiated water in normal liquid water at the same temperature (25°C) equal to 2.44 in the same unit. (Ling *et al.*[201] reproduced from The Journal of General Physiology by copyright permission of the Rockefeller University Press)

validity of the conclusion that the water diffusion in these cells is indeed bulk-phase-limited.

For, being bulk-phase-limited, the data of efflux (or influx) of labeled water provides a *new* way of measuring the *diffusion coefficient* of water in the cytoplasm. The validity of our overall conclusion that the exchange of water in these cells is bulk-phase-limited would be confirmed if good accord could be demonstrated between the diffusion coefficient of cell water thus obtained and the diffusion coefficient of water obtained by other dif-

ferent methods in the same type of cells. Accords were found for both sets of data.[107 p 224] But I shall discuss in detail only the giant barnacle muscle data here, because more published data from other laboratories are available on water diffusion in this type of cells.

The diffusion coefficient of labeled water in giant barnacle muscle cells was determined by Ling and Reisin from its bulk-phase-limited rate of diffusion. In units of 10^{-5} cm^2/sec, the diffusion coefficient from eight sets of data is 1.35 ± 0.125 (s.d.).[202] This agrees with the average of four other sets of data from the literature obtained by alternative independent methods: 1.36 ± 0.13 (s.d.) [from that of Caillé and Hinke at 1.34;[203] that of Abetsedarskaya *et al.* at 1.56;[204] that of Finch *et al.* at 1.20;[205] and that of Walter and Hope at 1.35[206]].

The only known data in the literature not included in the average shown is that of Bunch and Kallsen, who gave a value of 2.42.[207] We decided against including their data because they made their measurements on short muscle segments with two cut ends. Our EMOC studies shown in Figures 8, 17 and 18 have demonstrated that cut muscle segments deteriorate rapidly near the cut end even when only one end is cut. It is logical to expect that deterioration would be more rapid and extensive in short muscle segments with both ends cut like those employed by Bunch and Kallsen.

In concluding this section, I want to point out that water exchange being bulk-phase-limited does not mean that the exchange of all other solutes are also bulk-phase-limited. On the contrary, parallel studies reveal that permeation of a variety of solutes studied is *surface-limited.*[15 pp 404–417] Nonetheless, the two detailed studies cited above show that in as far as water is concerned, the cell behaves as if it were a "naked lump of protoplasm" without a special membrane covering, as stated in Max Schultze's *protoplasmic doctrine* over 140 years ago.

A continuous lipid or phospholipid bilayer cannot produce a bulk-phase-limited diffusion of water as we have demonstrated because the rate of permeation of water through phospholipids is exceedingly slow (see subsection immediately following). The established bulk-phase-limited diffusion of water offers one piece of serious evidence against the lipid layer or phospholipid bilayer theory of cell membranes.

With lipid layer and phospholipid layer out of contention, the only other building material of the living cell, which can form a continuous layer (and yet act as a quasi-impermeable barrier to virtually all solutes dissolved in water but not to water itself [11.3(4)]), can only be (polarized-oriented)

water itself. More evidence *against* the lipid or phospholipid membrane model but *for* the water membrane model follows.

13.4 Permeability of living cells to water is orders of magnitudes faster than that of phospholipid bilayers

Cited from time to time in favor of the phospholipid bilayer theory is that the permeability of living cell membrane to water matches that of an artificial phospholipid bilayer.[344 Table 4.1] In fact this claim is wrong in a strange way. A seriously faulted experimental procedure has fortuitously produced the expected quantitative data predicted by an incorrect theoretical model.[208; 107 p 225–230]

The serious experimental error originates from grossly underestimating the permeability of water though the living cell membrane.[107 p 228] A part of this underestimation originated from mistaking a *bulk-phase-limited* diffusion of water (demonstrated in the preceding subsection) for a *membrane-limited* one. This error is a serious one. But an even grosser error came from comparing the *properly-measured* water permeability across phospholipid bilayers (where the solutions on both sides of the lipid bilayer were stirred during the permeability measurement) to the *improperly measured* water permeability of the living cell membrane (where only the external bathing medium was stirred). The result is that the time needed for the labeled water to diffuse within the unstirred cell water—a process by nature very slow—is lumped together with the time needed to traverse the cell membrane and the sum ascribed exclusively to the time needed to traverse the cell membrane.

The correct water permeability of living cells is in fact *orders of magnitude faster* than the water permeability of phospholipid bilayers, which are extremely impermeable to water just as they are to (hydrated) ions[209] and to nonelectrolytes.[435]

13.5 Interfacial tension of the living cell is too low to match that of a phospholipid layer

The interfacial tension of oil and water is equal to about 10 dynes/cm or higher.[344 p 91; 197 p 2197] The interfacial tension of phospholipid bilayers (from sheep red cells) against water is lower but still in the range of 1 to 2 dynes/cm,[405] e.g., brain lipids, 1 dyne/cm;[406] egg lecithin plus cholesterol, 1 dyne/cm.[407] On the whole, the interfacial tension of simple lipids or phos-

pholipid bilayers measured against water are respectively more than one to two order(s) of magnitude higher than that of the living cell most carefully studied: sea urchin eggs measured against sea water by Prof. K. S. Cole[408] at 0.08 dyne/cm.

Harvey also studied the interfacial tension of sea urchin eggs. He gave an upper limit of 0.2 dynes/cm.[409] Cole pointed out, however, that Harvey used a deformed egg, in which "the surface was considerably stretched" and the measured interfacial tension thus artificially increased. From this, Cole reached the conclusion that his and Harvey's data are essentially the same at the 0.08 dyne/cm value. However, even in Cole's study, he had assumed that "rigidity and viscous effects of the surface and interior" of the egg to be negligible—which may not be correct in view of the solid nature of cell protoplasm—thus the true interfacial tension of sea urchin eggs may be even lower than 0.08 dyne/cm.

The view of a very low interfacial tension of living cells is in harmony with the (maximum) interfacial tension measured (against water) on *protoplasmic droplets* obtained from *Nitella* cells (0.04 dyne/cm.).[410 to 416] The relevance of this data is enhanced by the fact that these protoplasmic droplets not only exhibit an inside-negative *resting potential* of from 70 to 90 mV, equal to those seen in many normal excitable living cells,[411] but they are also electrically excitable, generating an *action potential* in response to a short pulse of electric current—again like normal excitable living cells.[411; 413; 414; 416]

Ueda, Inoue and their coworkers, who produced this beautiful series of work, suggested that a phospholpid membrane is first formed on the surface of the droplet, into which proteins then moved later. (In support, they showed that phospholipase A rapidly destroys the integrity of the droplets.)[415] On this specific point, I disagree—for the same reason cited above against a phospholipid cell membrane: the interfacial tension of their protoplasmic droplets is far too low to match that of a phospholipids-water interface. Indeed, with increasing temperature, the interfacial tension of the isolated protoplasmic droplets continues to fall until it reaches values well below 0.001 dyne/cm. With a lowering of the temperature, the value returns to 0.04 dyne/cm. It is not easy to reconcile these low interfacial tension values with that of a continuous phospholipid bilayer.

Like the protoplasmic droplets from *Nitella*, simple coacervate of gelatin, or a complex coacervate of gelatin and gum arabic, also have very low interfacial tension, ranging from 0.0015 to 0.0023 dyne/cm.[61 p 434 Fig 1] These coacervates do not contain phospholipids at their surfaces or elsewhere. According to the PM theory—including the subsidiary hypothesis

on coacervation just introduced—they have exceedingly low interfacial tension because the coacervate surface contains a great deal of water (albeit polarized and oriented in parallel arrays).

The low interfacial tension of living sea-urchin eggs (0.08 dyne/cm or even lower), of *Nitella* endoplasm droplets (0.04 dyne/cm) and of gelatin-gum arabic coacervate (0.0023 dyne/cm), when seen together, suggest that the living cell membrane is just like the bulk-phase protoplasm comprising in the main fully-extended proteins and multilayers of polarized-oriented water.

However, as also pointed out earlier, phospholipids may play a stabilizing role in maintaining the normal cell membrane structure. That may be the reason why *Nitella* protoplasmic droplets disintegrate on exposure to phospholipase A.[411] However, Zwaal *et al.* found that exposure to phospholipase A did *not* disrupt red blood cells.[417] Maybe this stability reflects the fact that red blood cell membrane contains the highest phospholipid content (47% of dry membrane weight) when compared to 13 other cell types.[344 Table 9–2] Still more evidence against the existence, and a barrier function of a continuous phospholipid bilayer at the cell surfaces, follows next.

13.6 Ionophores strongly enhance K^+ permeability through authentic continuous phospholipid bilayer but have no impact on the K^+ permeability of cell membrane of virtually all living cells investigated

As mentioned above, the phospholipid bilayer is extremely impermeable to ions including K^+[209] [13.2]. Yet, as shown by Andreoli and coworkers, the K^+ permeability of a bilayer of phospholipids (isolated from sheep erythrocytes) increases 1000-fold on exposure to 10^{-7} M valinomycin.[210] Ling and Ochsenfeld saw in this remarkable discovery another rare opportunity to test the validity of the phospholipid bilayer model in general and the fluid-mosaic model in particular.

We reasoned that if all cell membranes truly contain a continuous phospholipid bilayer barrier, exposure of the cells to 10^{-7} M *valinomycin* (or to two other similar K^+-specific ionophores, *nonactin* and *monactin* also at 10^{-7} M) should increase the cell's K^+ permeability to a degree quantitatively matching that demonstrated on man-made membranes, whose composition was unquestionably that of pure or virtually pure phospholipids.

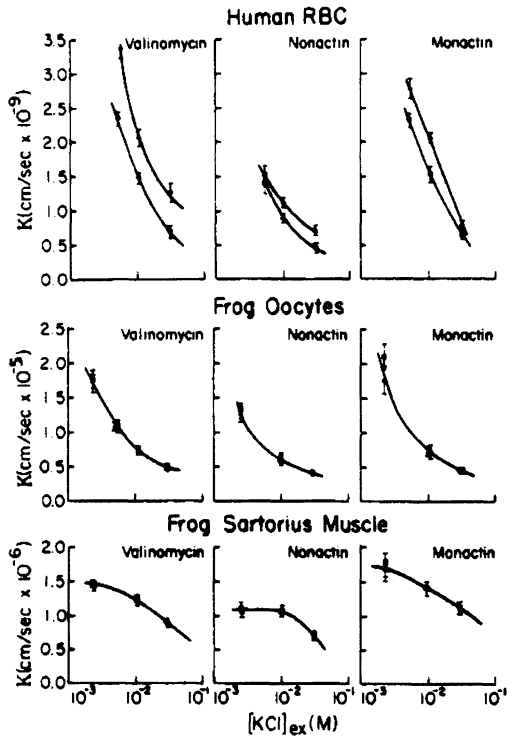

Figure 40. Effects of three antibiotics (also referred to as "ionophores"): valinomycin, nonactin, and monactin, all at 10^{-7} M, on the inward K^+ permeability of respectively freshly-drawn human red blood cells (RBC), frog ovarian eggs (or oocytes) and frog sartorius muscles at different total external KCl concentrations indicated on the abscissa. K^+ permeability given in cm/sec. ▲, antibiotic-treated. O, control. (Ling and Ochsenfeld[211])

What we observed did not confirm this expectation.[211] As shown in Figure 40, neither the K^+ permeability of frog eggs nor that of frog muscles shows any detectable response to the presence of any one of the three ionophores. Human erythrocytes, in contrast, show a consistently higher K^+ permeability in response to each one of the three ionosphores. The increase is 20% for monactin, 28% for nonactin and 80% for valinomycin—in contrast to the expected 1000-fold or 100,000% increase of K^+ permeability across an authentic phospholipid bilayer in response to the same concentration of valinomycin (10^{-7} M). This enormous quantitative difference shows that even in the (exceptional) red blood cell membrane, the phospholipid component cannot be very high.

We are not alone in finding an absence of detectable influence of valinomycin on K^+ permeability. Stillman *et al.*, Maloff *et al.* and Negendank & Shaller have respectively shown that neither squid axon[212] nor mouse liver mitochondria inner membrane[213] nor human lymphocytes[69] show increased K^+ permeability in response to valinomycin. Taking all together, we reached the conclusion that *physiologically active cell membranes like those of frog egg, frog muscle, squid axon, human lymphocytes and liver mitochondria inner membrane do not possess a continuous diffusion barrier in the form of a phospholipid bilayer*, confirming and extending what we have already demonstrated in [13.3] to [13.6] above.

In the next subsection, we present evidence of a *non-living* "polarized water membrane," which, in its permeability to labeled water and to six labeled sugars and sugar alcohols, quantitatively matches those of a *living* cell membrane—at three different temperatures.

13.7 Strongly polarized-and-oriented water in lieu of phospholipid bilayer

Let us also not forget that removal of 95% of the phospholipids from the inner membrane of rat liver mitochondria and from other membranes did not eliminate the trilaminar membrane structure.[214; 15 pp 382–384] Nor let us forget that a *lipid-free* membrane of the proteinoid (and water) microsphere shows a trilaminar membrane structure similar to the cell membrane (Figure 5E).[216] These facts add more support for the notion of (i) *domains of strongly polarized-and-oriented water at the cell surface forms a continuous barrier;* (ii) the cell surface proteins, which polarize this water, also provide β- and γ-carboxyl (and other polar) groups that control the traffic of ions and other solutes (see Chapter 12 in Reference 15) and (iii) cell surface proteins and water *together work as the selective diffusion barrier to the passage of water, nonelectrolytes* [217] [16.6(3.2)], *ions* [13.2 above] and other substances—as the following findings further substantiate.

In Figure 41 is presented the permeability through an inverted frog skin to water and six nonelectrolytes at three different temperatures, plotted against the permeability of the same substances at the same temperatures through a heat-activated, water-loaded cellulose acetate membrane. That the permeability to water is higher than to ethanol and everything else studied in both the inverted frog skin and the cellulose acetate membranes agree with the first recorded observation on osmosis by Abbé Nollet

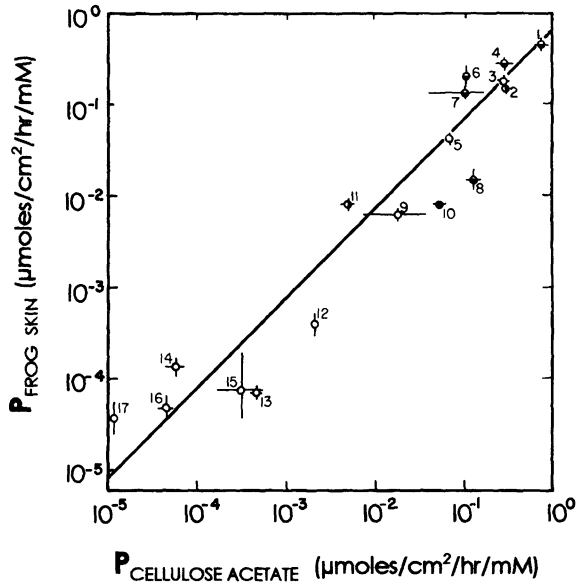

Figure 41. The permeability of reversed *living frog skin* to labeled water (THO), alcohols, sugar alcohols and sugars at three different temperatures plotted against the permeability of *heat-activated, hydrated cellulose acetate* membrane to the same labeled water and nonelectrolytes at the same temperatures. In most cases the data points represent means ± S.E. In the exceptional cases, where only two data points were available, no S.E. were given. (1) THO, 25°C; (2) THO, 4°C; (3) THO, 0°C; (4) methanol, 25°C; (5) methanol, 0°C; (6) ethanol, 25°C; (7) n-propanol, 25°C; (8) ethylene glycol, 25°C; (9) ethylene glycol, 0°C; (10) glycerol, 25°C; (11) glycerol, 4°C; (12) glycerol, 0°C; (13) erythritol, 0°C; (14) xylitol, 0°C; (15) sorbitol, 0°C; (16) L-glucose, 0°C; (17) sucrose, 0°C. Empty circles represent data obtained at 0°C; circles with right half filled, at 4°C; circles with bottom half filled at 25°C. (Ling[217] by permission of The Biophysical Society)

[Chapter 2]—whereas, as pointed out earlier, Overton's lipoidal membrane model with its 100 times higher solubility for ethanol than for water predicts the opposite (Figure 34).[320 p 43]

A good positive correlation (r = +0.96) is demonstrated between the permeability through the inverted frog skin and through the heat-activated, water-loaded cellulose acetate membrane at three different temperatures. But since the ordinate and abscissa of Figure 41 are in the same units, there is actually *one-to-one correspondence* in the permeability over a span of five orders of magnitude. By the method of least squares, the straight line going through or near all the data points are described by the following equation:

$$\log\left(P_{\text{frog skin}}\right) = 0.990 \log\left(P_{\text{cellulose acetate membrane}}\right) + 0.16959, \qquad (3)$$

where the P's are the permeabilities of water and six other solutes of the two systems studied at three different temperatures. Note that the slope is close to one.

The pores of the heat-activated cellulose acetate membrane have been estimated at 44 Å in diameter by Schultz and Asunmaa.[343] This is five times larger than the diameter of sucrose molecules (8.8 Å[311]), to which the membrane is virtually impermeable. All these data are once more in full accord with the notion that water in the wide pores or domains of both systems—as well as in Bigelow and Bartell's copper-ferrocyanide membrane mentioned in [13.1(1)] above—are more-strongly polarized and oriented in accordance with the PM theory .

The same size-dependent q-values for solutes in the polarized and oriented water (Figures 23, 25 and 26) play a major role in selective membrane permeability too, because permeability (P) is the product of the solubility (q-value) and the diffusion coefficient (D).[277 p 43] Only here, according to theory, the differences in permeability are exaggerated as a result of the (moderately) higher intensity of water-to-water interaction within the cellulose acetate membrane and in living cell membrane like the frog skin. It is this (moderately) intensified water polarization and orientation that give us the spread of 5 orders of magnitude in the permeability to water and to sucrose. The same mechanism may also account for the molecular size-dependent permeability reported long ago by Fujita[402] and Michaelis[401] for collodion membranes, by Ruhland and Hoffmann[422] for the sulfur bacteria, *Beggiatoa mirabilis* and by Collander and Bärlund for the algae, *Characeae.*[403]

Summarizing Chapter 13 as a whole, one finds that the concept of a cell membrane containing a continuous barrier of phospholipid bilayer is definitely out. The exceedingly high permeability of the cell membrane to water presents evidence number 1. The much lower interfacial tension (against water) of living cells than that of phospholipid bilayers or lipid layers offers evidence number 2. The failure of valinomycin and other specific K^+ ionophores to increase the K^+ permeability of five types of physiologically active living cells (frog muscle, squid axon, frog egg, mouse liver mitochondria, human lymphocytes), meanwhile enhancing 1000-fold the K^+ permeability through authentic phospholipid bilayers, offers evidence number 3.

In contrast, all three sets of data are in harmony with the new model of cell membrane as simply a variant of the bulk-phase protoplasm found at the cell surface. Like the bulk-phase cytoplasm, the cell membrane contains a matrix of fully-extended proteins, which at once polarize and orient a continuous layer of water. The continuous layer of polarized water then functions as the quasi-impermeable diffusion barrier for large non-electrolytes and hydrated ions (*via* the saltatory route). The membrane proteins also provide fixed β- and γ-carboxyl groups for the adsorption-desorption route of intra-, extra-cellular cation exchange in cells like the frog muscle.

14

The Living State: Electronic Mechanism for its Maintenance and Control

This section begins with a bit of history. It shows how interaction with other scientists and their ideas provided the stimulus as well as conceptual stepping stones toward developing LFCH into a unifying physico-chemical theory of life called the *association-induction hypothesis* (AI Hypothesis, AIH). As such, it is the first in history.

The development of the AI Hypothesis took three steps: LFCH in 1952; the *association-induction hypothesis (proper)* in 1962; the subsidiary *Polarized Multilayer (PM) theory* of cell water in 1965. The late-arriving PM theory was presented *before* the AI Hypothesis proper because, like LFCH presented still earlier, the PM theory also deals with the static *associative* aspects of cell physiology. The AI Hypothesis proper, in contrast, deals also with the dynamic *inductive* aspects of cell physiology. Built upon the association of ions, water and proteins, obviously the AIH proper can only be presented last.

There is a second reason for the inverted order of presentation. The LFCH and the PM theory are focused largely on molecular events, be it ion adsorption or water polarization-orientation. The AI Hypothesis proper, in contrast, ventures out into *the realm of electronic* control mechanisms. Surprising as it may seem, this too is a direction that had not been pursued before—until the advent of the AI Hypothesis.

The presentation of the electronic control is divided into two parts. The first part presented in this chapter (Chapter 14) deals with the electronic control mechanism itself and its role in what is to be described as the *living state*. The second part to be presented in the succeeding chapter (Chapter 15) deals with electronic mechanisms underlying physiological activities of diverse kinds and their control. We are now at the beginning of the first part.

14.1 The launching of the association-induction hypothesis

(1) Prelude

As pointed out earlier, I began my training as a cell physiologist under Prof. Ralph W. Gerard—a better teacher and mentor I could never have found, far-reaching in interests and knowledge, brilliant in thoughts and actions, yet unwaveringly nurturing to his students like myself. As a graduate student, I improved the procedures in making and filling with KCl solution the capillary glass electrode so that one could use it to make accurate and reproducible measurements of the electric potentials of living cells. Thus armed, Prof. Gerard and I continued the study of the resting potentials of frog muscle cells that he and Judith Graham had begun earlier.[88, 441, 442, 443] This line of research soon led me to the subject of selective K^+ accumulation in living cells and the eventual proposal of what was later referred to as Ling's Fixed Charge Hypothesis (LFCH).

In presenting the LFCH in 1952[96 Fig 5] and in a short review written in 1955,[145] I pointed out that in theory the same fixed charge system (i.e.,β- and γ-carboxyl groups)—which selectively adsorb K^+ over Na^+ in the bulk-phase cytoplasm—could also act as the seat for the cellular resting potential when transplanted to the cell surface (Figure 4C).

Hodgkin and Katz made the epoch-making discovery in 1949 that during an *action potential*, the cell membrane switches its preferential permeability to K^+ to a preferential permeability to Na^+.[233] In the light of this exciting new knowledge, I asked myself the following question: If the resting potential arises from the preferential adsorption of K^+ on the fixed β- and γ-carboxyl groups at the resting cell surface—rather than from its preferential membrane permeability—could the same surface β- and γ-carboxyl groups switch their preference for K^+ to a preference for Na^+ during an action potential? If so, how? Clues to possible answers came from studies of the ion exchange resins and of the glass electrodes.

In support of LFCH, I cited in 1952 various inanimate *fixed-charge-systems*. Included are permutits, soils and synthetic ion exchange resins.[96 p 773] They all carry fixed anionic sites and they all selectively accumulate K^+ over Na^+. One recalls that long before, Benjamin Moore and Herbert Roaf had already quoted soils in a similar context[77] (Chapter 7).

Ion exchange resins are better models than soils. First, ion exchange resins are simpler in structure and thus easier to understand. Second, it is the product of a rapidly advancing new technology. Indeed, even as the

LFCH was trying out its new wings, so to speak; more and more was being discovered on the relationships between the chemical makeup of an ion exchange resin and its ion-exchange properties.[15 pp 261–263; 478] Thus the K^+-selecting cation exchange resins I referred to in 1952[96] all carry fixed anionic *sulfonate* groups. When ion exchange resins carrying fixed *carboxyl* groups became available, they were found to select Na^+ over K^+ [(143)]—a new finding first brought to my attention by Prof. E. J. Conway of Dublin in his argument against my thesis that (β- and γ-) carboxyl groups in living cells selectively adsorb K^+ over Na^+ [10.1(3)]. At first, I was dismayed by this "attack." As time wore on, I began to appreciate it more and more for the underlaying truth it called to my attention.

At that time I already strongly believed that β- and γ-carboxyl groups in muscle cells selectively adsorb K^+ over Na^+—for among other reasons, no other fixed *anionic* groups in the living cells are numerous enough to provide enough adsorbing sites for all the cell K^+. Yet carboxyl groups in ion exchange resins do not selectively adsorb K^+ over Na^+ as my theory demands, but do just the opposite. [143] Could one kind of carboxyl group act one way and another kind act another way? If so, how?

J. I. Bregman, who reviewed the subject of cation exchange resins in 1953,[143 p 135] brought attention to the fact that the sulfonate group (which selects K^+ over Na^+) is strongly acidic and has a low pK value, but the carboxyl group (which selects Na^+ over K^+) is weakly acidic and has a high pK value. For explanation of the reversal of ion selectively, however, he cited Teunissen and Bungenberg de Jong,[319] who attributed the difference in the rank order of preference for K^+ and Na^+ in various colloids to the different *polarizability* of the sulfonate and carboxyl groups they carry respectively.

Digging deeper (much later), I discovered that Bungenberg de Jong and his coworkers had done a great deal of important work on the subject of ion selectivity in colloids—published mostly in the 1930's and 1940's. To avoid misrepresenting their ideas, I cite below their opinions mostly *verbatim*.

H.G. Bungenberg de Jong—the same colloid chemist cited earlier—Teunissen and others studied the migration of colloids in water under a DC electric field. They noted that in response to the introduction of different cations in the bathing solution, the direction of migration of the colloids sometimes changed. Bungenberg de Jong (and coworkers) attributed this directional change in migration to a change in the net electric charges of the colloid, and called it "charge reversal."[61 pp 159–334] Bungenberg de Jong further pointed out that "If ...the reversal of charge is generally caused by fixation of a sufficient amount of (positively charged) cations on the ionized (negatively charged) groups (of the colloid)—then

the affinity of cations and ionized groups must depend on *valency, radius and polarizing power of the cation and on the polarizability of the negatively charged ionized groups of the colloid*" (ital. Bungenberg de Jong's).[61 p 287]

He continued: "If we consider for instance the monovalent ions, Li, Na and K, then—exclusively from the point of view of "field strength"—on the surface of these ions, the fixation on a given negatively charged ionized group will be easiest in the case of the smallest ion—Li—and will be increasingly more difficult for the larger Na and K ions. But as a second influence we must consider the energy of polarisation. If the ionized group is more polarisable than water, then the polarisation energy is added to the Coulomb energy. In this case, the above order of cations will not be disturbed, on the contrary, the spread of Li>Na>K will possibly be strengthened.... In the case that the polarisability of the ionized group is smaller than that of water, then the polarisation energy of the water molecules (cation hydration) will oppose the Coulomb energy. Now, the Li ion, the most hydrated ion, will have the greatest tendency to rest in the perfect hydrated state free in the medium." [61pp 287–288] (And the selectivity order will be reversed to K>Na>Li, *added by GL.*)

Bungenberg de Jong and his coworkers also attempted to explain the ion-selectivity-order differences among colloids carrying *similar carboxyl groups* also in terms of their dissimilar *polarizabilities*. The following direct quotation clarifies what they were thinking.

"It seems quite natural to ascribe the lesser polarisabilities of the carboxyl groups of the latter (arabinate, the carboxyl group of which was compared with the carboxyl groups of oleate—*added by GL*) to a certain constitutional influence. They (arabinates) are derived from polymer carbohydrates and thus the presence of hydroxyl groups in the neighbourhood of the carboxyl groups could be the cause of the decreased polarisability..."[61 p 293]

To test the theory that it is the anionic-group polarizability that plays the key role, Teunissen, Rosenthal and Zaayer[144] showed in 1938 that the possession of many hydroxyl groups of arabinate is correlated with a selection sequence of $K^+ > Na^+ > Li^+$ and $Mg^{++} > Ca^{++} > Sr^{++} > Ba^{++}$ while oleate devoid of hydroxyl groups shows inverted sequences.

The ion-exchange-resin models prompted me to seek a more powerful theoretical model capable of explaining not only the ion selectivity of $K^+ > Na^+$ (as in my 1952 LFCH model) but also the inverted order of $Na^+ > K^+$ as well. But my decision to move ahead promptly was triggered by an encounter with George Eisenman, Donald Rudin and Jim Casby and a new idea for ion selectivity changes they later proposed as an extension of the LFCH.

In 1955, I presented my new theory of cellular electric potential at the Federation Meetings at Atlantic City. In the verbal presentation as well as the printed abstract, I pointed out that my new theory of cellular electric potential is "closely related to that of the glass electrode potential."[145]

In my audience was Prof. Harry Grundfest of the Columbia University. Also attending the meetings (but not my talk) was a young MD from Harvard, George Eisenman. Eisenman, Donald Rudin and Jim Casby had just been invited to set up a Basic Research Department in the newly-founded Eastern Pennsylvania Psychiatric Institute (EPPI) in Philadelphia. Among their initial research interests was the electrical activities of the cen-

tral nervous system. In the course of this study, they found it necessary to determine the Na^+ concentration in the (spinal) fluid in the (narrow) central canal of the spinal cord of an experimental animal. At that time, Na^+-selective microelectrodes required to do this job were not commercially available. So they purchased a high-powered electric furnace and set out to make their own Na^+-sensitive electrodes. As Eisenman and coworkers varied the chemical composition of the glass electrodes, electrodes with different selectivity sequences among the five alkali-metal ions, Li^+, Na^+, K^+, Rb^+ and Cs^+ were created.

Grundfest told Eisenman what I had presented earlier at the Federation Meeting and how I was studying the glass-electrode as a model for the electric potentials of living cells. Next thing you know, I was invited to visit the Department of Basic Research at EPPI. And during the visit I described to Eisenman, Rudin and Casby my theory of enhanced counterion association with charge fixation and that of selective K^+ adsorption on the basis of what, in simpler terms, may be described as differences in the the field strength experienced by K^+ and by Na^+ from the fixed carboxyl groups.

Later, over a lunch at a nearby Howard Johnson Restaurant on Henry Avenue, I also thought aloud with my new friends as to how my 1952 model could be modified to fit some of their new findings of varying ion-selectivity order. I recall suggesting a combination of my model of fixed anions with the concept of rigid pores on the glass surface. And how the rigid glass wall of a narrow pore containing a fixed anion at its bottom may force the more hydrated ions to lose some of their water of hydration, thereby creating a different rank order of selectivity. But it was just a passing thought and not further pursued by me. (At that time, I was not aware of Bungenberg de Jong and his coworkers work cited above. I learned of it later.)

Eisenman, Rudin and Casby came up with a new idea to explain the selectivity order differences among the alkali-metal ions. To start, they accepted (implicitly) my theory of full counterion association with charge fixation and also my theory of selective K^+ adsorption as a result of what for simplicity may be called *different electric field strength* K^+ and Na^+ experiences from the fixed anion. As an extension of LFCH, they then postulated that water in the hydration shells of the alkali-metal ion may be squeezed out—not on account of limiting (glass) pore diameters, as I speculated at the restaurant, nor on different anionic group polarizability as suggested by Bungenberg de Jong and his coworkers—but on account of a postulated *change in the field strength* of the fixed anion.

On the basis of these premises, they further postulated that among the five alkali-metal ions, the least hydrated Cs^+ would be the first to shed its hydration shell, Rb^+, the next to do so and so on in an orderly fashion. In consequence, eleven orders of selective ion adsorption were predicted.[146] They then showed that the diverse rank order of ion selectivity they observed in the experimental glass electrodes they made agree with these orders.[148 pp 274–279]

In line with our newly-developing converging interests, Eisenman urged me to leave my (tenured) associate professorship at the Neuropsychiatric Institute of the Medical School of the University of Illinois and join him as a (tenured) Senior Research Scientist at EPPI. He was a persuasive salesman and the advantages offered were substantial. I accepted his offer with no hesitation. On March 1, 1957 my wife Shirley, our baby son Mark and I arrived at Philadelphia, where forsythia was just beginning to bloom.

(2) The c-value *and a quantitative model for the control of the rank order of ionic adsorption*

Brilliant as it was, Eisenman, Rudin, and Casby's idea[146] was an intuitive speculation on a *quantitative issue*. So was Bungenberg de Jong and his coworkers' idea based on anionic polarizability. Thus, there is no *a priori* reason why water of hydration of the ions must be shed in some assumed order. But *to me personally*, the most valuable way Eisenman, Rudin and Casby's extension of LFCH benefited me was a clear "go," further to develop my 1952 *quantitative theoretical model, taking into account* all the prior contributions of Hodgkin and Katz, Conway, Bregman, Bungenberg de Jong and his coworkers. And last but not least, the important contribution of Eisenman, Rudin and Casby.

With the help of Margaret Samuels and Mary Carol Williams, my intense efforts in the months following paid off. The entire finished work was summarized in Chapter 4 of my first book, "A Physical Theory of the Living State: the Association-Induction Hypothesis."[98] Brief summaries of parts of the work follow next.

Earlier, I mentioned the pK values of sulfonate and carboxyl groups. A pK is the negative logarithm of the *acid dissociation constant*; describing the affinity between an ionized acidic group and a *specific* cation, the hydrogen ion, H^+. As such, it is not what I was looking for. What I was looking for is the *independent* quantitative parameter *underlying* the pK. No such parameter existed then or before. To fill in this vacuum, I introduced the *c-value.*[147; 98 pp 57–60; 15 p 155; 107 pp 126–127]

Given in Angstrom units, the c-value measures the displacement of a unit negative charge from the geometric center of the singly-charged oxygen atom of an oxyacid group, so that the net effect of this displacement on the oxyacid group would match the real-world induction-mediated change (for details, see Figure 4.2 in Reference 98 or Figure 6.7 in Reference 107).Thus the carboxyl group of acetic acid has a strong affinity for H^+ (hence a high pK) in consequence of what is now expressed as a high c-value. The carboxyl group of trichloroacetic acid has a weak affinity for H^+

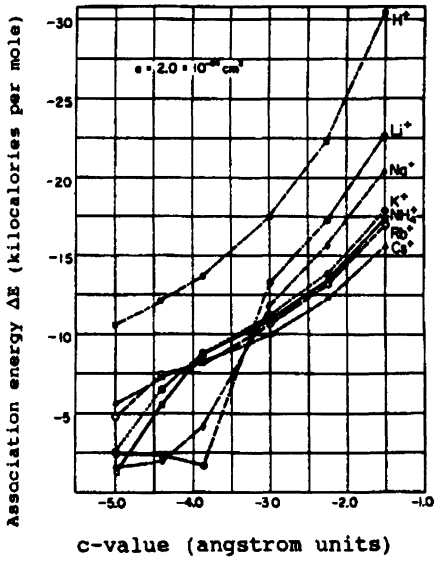

Figure 42. The theoretically computed association-energies in kilogram calories per mole of H^+, Li^+, Na^+, K^+, Rb^+, Cs^+ and NH_4^+ respectively on a singly charged oxyacid group with a polarizability of 2.0×10^{-24} cm^3 and c-value as indicated on the abscissa. An ion, say K^+, which shows a higher negative energy of association of –13 kcal/mole on the fixed oxyacid anion at a c-value of –4.0 Å is preferentially adsorbed over Na^+, which at the same c-value, shows a lower negative association energy of –3.5 Kcal/mole only. However, at higher c-value of –2.5 Å, the preference is reversed since at this point the negative association energy of Na^+ at –16 kcal/mole is higher than that of K^+ at –13 kcal/mole. Preference reversal at different c-values is important in physiological activities according to the AI Hypothesis. (From Ling[98])

(hence a low pK) in consequence of what is now expressed as a low c-value. On first approximation, one may regard the c-value as a measure of the electron density at the charged oxygen atom at an oxyacid group. By analogy, a *c′-value* was also introduced that is a measure of the positive charge density of a fixed cationic group.[98 p 60]

With the c-value (and c′-value) defined, my next task was to determine by energy minimization the most likely mix of the number of hydration water molecules in a linear array (Figure 4.3 in Reference 98; Figure 6 in Reference 173) for each of the five alkali-metal ions (in addition H^+ and NH_4^+) at each c-value and one of the three different values of the polarizability of the fixed anionic group.[98 p 61; 15 p 157; 173 p 15] In this computation, eight types of interaction energies were taken into account. I then used a Born-charging method to determine the average dissociation energy of each of the ions studied and plotted them against the c-value[98 pp 60–78] (see Reference 98 pp. 70–74).

One set of the theoretical results obtained with an anionic polarizability of 2.0×10^{-24} cm^3 is reproduced in Figure 42. *It shows that the high preference of K^+ over Na^+ at a lower c-value indeed switches to a preference of Na^+ over K^+ at a higher c-value*—in harmony with the fact pointed out earlier by Bregman that *sulfonate resin has lower pK and preference of K^+*

over Na^+ while carboxylate resin has higher pK and preference of Na^+ over K^+. This order reversal with c-value change also occurs between other pairs of ions included in the computation.

To put the results in historic context, the following comments may be made.

(1) The quantitative results from this theoretical model confirm the intuitive idea of Eisenman, Rudin and Casby to the extent that the c-value change (equivalent to their field strength change) is the main parameter underlying the changes in the selectivity for the five alkali-metal ions. And by and large, it is the least hydrated ion that is first "dehydrated," also affirming their intuitive idea.

(2) Nonetheless, anionic polarizability, which is not considered at all in the Eisenman-model (for a short and lucid summary of Eisenman's later theory, see Isard[148 pp 70–75]), is in fact an important modifying factor; its impact on the selectivity is complex and involves many operating forces.

As an example, I may mention that throughout the entire spectrum of c-value, the relative preference for H^+ is *lower* than the preference for K^+ and Na^+ when the anionic polarizability is 0.876×10^{-24} cm^3 (Figure 4.9 in Reference 98) (or zero as in the Eisenman model). In contrast, throughout the whole range of c-value studied, the preference for H^+ is *two orders of magnitude higher* than those for K^+ when the anionic polarizability is at 2.0×10^{-24} cm^3 as in the data presented in Figure 42. There is now substantial experimental evidence that the K^+-adsorbing β- and γ-carboxyl groups in the cytoplasm as well as at the surface of frog muscle cells adsorb H^+ with an affinity about two orders of magnitude higher than that for K^+ [107 p 133 n 8, p 248 n 6, p 289] [16.6(3.4)].

It cannot be overemphasized that a *total treatment—as exemplified by the one I undertook—is essential*, where all the eight known types of energies describing the interactions are taken into account and none left out arbitrarily.

Bungenberg de Jong emphasized the importance of the differences in the polarizability of the anionic groups. But polarizability differences are not exclusively found among different anionic groups, they also exist among the cations. Thus the polarizability of a H^+ is zero, that of Li^+ is 0.075×10^{24} cm^3 increasing all the way to Cs^+ in which case it is 2.79×10^{24} cm^3 (see Table 4.1 in Reference 98). To consider only the polarizing force of the cations and the polarizability of the fixed anions is as deficient as considering only the polarizing force of the fixed anions and the polarizability of the cations. *All must be taken into account* as I have done. Even then the results I obtained are no better than a *rough* estimate.

Teunissen, Rosenthal and Zaayer explained the influence of the OH groups introduced on the cation selectivity of the fatty acid oleate as due to changes on the *polarizability* of the carboxyl groups—for which there is no clear-cut supportive evidence. Instead, I suggest that the OH groups introduced could be changing the *pK* values (and hence c-value) of the carboxyl groups for which there is abundant support (see Figure 45).

Figure 42 shows that beside the five alkali-metal ions, H^+ and NH_4^+ were also studied. H^+ is universally present in the cell and its environment. NH_4^+ is also often found in and around the living cells. But it has an additional significance. That is, it may be seen as a prototype of the ever-present *fixed cations*, be it the $\in$-amino group ($R\text{-}NH_3^+$) carried on lysine residue of proteins or the guanidyl group ($R\text{-}C(NH_2)NH_3^+$) carried on arginine residue. By reversibly forming salt-linkages with β- and γ-carbonyl groups, these fixed cations play important roles in physiological and pathological changes of many kinds {see paragraphs in small print in both [10.2(5)] and [15.3(2)]}. In addition, from a historic perspective, salt-linkage formation also explains why past efforts to demonstrate K^+/Na^+ adsorption on the β- and γ-carboxyl groups of native proteins have not succeeded [10.2(2)].

Finally, the reader may ask: "Why is a K^+ over Na^+ preference theoretically predicted in the LFCH model, which did not take into consideration the c-value or polarizability of the oxyacid group?" The answer is, by assuming *a priori* a much larger hydrated diameter of the Na^+ than that of the K^+ as was done in the LFCH model, I have unknowingly chosen a low c-value and a high polarizability—since at low c-value and high polarizability, the more highly hydrated conformation of Na^+ predominate, while a less hydrated conformation predominates in the case of K^+.

(3) The c-value analogue and its control of the protein folding vs water polarization

When I first introduced the association-induction hypothesis in 1962, I put forth the idea that the properties and functions of a protein depend on its amino-acid sequence.[98 p xxx] In Chapter 7 in Reference 98 on the "Theory of Proteins,"[98] I have also shown how and why, through the operation of *inductive effect*, amino-acid sequence determines the pattern of folding or *secondary structure*—including salt-linkage formation, the formation of disulfide bonds as well as the H-bonding of the backbone NH and CO sites. To put these concepts on a quantitative basis, I also introduced the *c′-value analogue* to describe the positive charge density of the backbone NH groups and the *c-value analogue* to measure the electron density of the backbone carbonyl oxygen.[98 p 60; 488; 173]

(3.1) The control of the secondary structure of proteins

Apparently unaware of the AI Hypothesis and my idea on how the primary structure of a protein may determine its secondary structure (see below), Prof. C. B. Anfinson established experimentally that the native protein's secondary structure can indeed be exactly restored from a denatured state when the condition that brought about the denatured state is removed and the normal environment reinstituted.[149] For this important achievement, he won the Nobel Prize for Chemistry of 1972.

Yet, it remained unexplained how and why the primary structure of a protein determines the secondary structure. Except that thermodynamically the native state is seen as the most stable under the condition—which in general principle is important but tells very little more beyond that. Far more important to me at least is the discovery in the 1970's of the dominant role of *short-range influence* of the side chain of each amino-acid residue on its own peptide CONH group's propensity to engage in α-helical, β-pleated sheet or random-coil conformation.[421] For in fact, a possible explanation for the influence of side chains on secondary structure has already been offered by me as early as 1964[488 pp 104–106] and again in 1969.[173 pp 39–42]

Based on the work of Cannon[393] and others[394–396] that the backbone carbonyl oxygen is much more polarizable than the backbone imino group, I suggested that *the strength of H-bonds formed between backbone CO and NH groups in an α-helical structure depends primarily on the electron density (or the c-value analogue) of the backbone carbonyl oxygen.* The higher the electron density of the carbonyl oxygen, the stronger is the hydrogen (H) bond it forms with a peptide NH group. And the stronger the NH-CO hydrogen bond, the greater is the probability of its forming an α-helical H-bond.

At the time this hypothesis was first introduced, there were little quantitative data on the relationship between the nature of the side chain and the protein secondary structure. In years following, the situation had gradually changed. In the mid-80's I had good reason to take up this problem once more.

By this time, the secondary structure of many different proteins had been worked out mostly from X-ray crystallographic analyses. From the data collected, the statistical probability (or *potential*) for forming α-helix, β-pleated sheet, random-coil conformation, etc, for each amino-acid residue has been tabulated[150] (Table 4). With these data on hand, the prospect

Table 4.

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

The α-helical Potential (P_a, w_{hj*}, *j*) of 19 α-amino Acids from Three Sources and the (corrected) Acid-Dissociation Constants (pK_a) of Their Analogous Carboxylic Acids. (Ling[150])

opened up to put my theory of secondary structure to a test. That is, if I can determine the *electron-donating strength* of the side chain of each one of the most common amino-acid residues, their electron-donating strengths should be positively correlated with their respective (empirically-determined) α-*helical potentials.* The key question was: How can I determine the *electron-donating strength* of each of the amino-acid residue's side chain? To understand how I resolved this problem, let us return to the basics.

As mentioned earlier, the pK value is the negative logarithm of the acid dissociation constant. The pK of acetic acid (CH_3 COOH) is 4.76. We now know that this high pK is primarily due to the strong *electron-donating effect* of the methyl group, CH_3[337, 338] (see Figure 45 below). The amino-acid residue derived from the amino-acid, alanine ($CH_3CH_2NH_2COOH$) has a similar methyl group as its side chain. Therefore, 4.76 is a measure of the electron-donating strength of the side-chain of alanine. The pK of formic acid ($HCOOH$) is 3.75, and thus much lower than that of acetic acid. Formic acid is the equivalent of glycine (HCH_2NH_2COOH). So 3.75 is a quantitative measure of the electron-donating power of the side-chain of glycine

residue. And these two cases illustrate how most of the electron donating strengths of all 19 amino acid residues were obtained (Table 4).

Proline and hydroxyproline are left out in this study because their lack of a H atom on the pyrrolidine N atom introduces an absolute bias favoring one conformation and disfavoring the other. The electron density rule, if valid, would not apply in these cases. For reasons that will become clear later, I like to point out that alanine has long been known as a "helix maker" and glycine, as a "helix breaker." In harmony with the theoretical postulation made, the pK's of these two amino acids are respectively at the two extreme ends of the pK spectrum of the 19 amino-acid residues examined (Table 4).

My next task was to carry out a simple linear regression analysis between the electron-donating strengths of the 19 amino acid residues on the one hand, and on the other hand, their α-helical potentials—as given by Chou and Fasman,[151] by Tanaka and Scheraga[152] or by Garnier *et al.* respectively[153] (Table 4). The results confirm the theory.

Analyses of these three sets of comparisons yielded an average *linear correlation coefficient* of +0.75 from the three sets of figures obtained (+0.75, Tanaka and Scheraga; +0.77, Chou and Fasman; +0.72, Garnier *et al.*). Considering that the accuracy of predicting new protein structure based on these α-helical potentials, etc., are themselves in the range of 60% to 80%, the average correlation coefficient of +0.75 must be considered satisfactory.

Efforts were also made to find out if a similar correlation could be demonstrated between the electron-donating strengths of the side chains and the potential for β-pleated sheet conformation. Such a positive correlation, though much weaker, was also found, after excluding all five charged side chains (or glutamate residue only) all known to disrupt β-pleated sheet structure ($r = +0.55, +0.42, +0.39$).[150] (For more details, see original article.)

For clarity I shall briefly reiterate a point made earlier. It concerns the different polarizability of the backbone carbonyl oxygen and the backbone imino group. Each side chain of a protein is attached to a carbon atom flanked on the one side by a carbonyl group (CO) and on the other side by an imino group (NH). Therefore a change in the electron-donating strength of the side chain may not just change the electron density of the carbonyl oxygen. It may also change that of the imino group. How do we know then that only the enhanced or reduced electron density of the carbonyl oxygen determines the strength of the α-helical H-bond?

The answer lies in the fact that *only the carbonyl group is highly polar while the imino group is not*—as C. G. Cannon[393] and Mizushima *et al.*[394] have established long ago. As a result, the same side chain exercising a

strong influence on the electron-donating strength of the nearest carbonyl oxygen exercises much less impact on the proton-accepting strength of the nearest NH group (see [14.3(1)] and Figure 47 below for another demonstration of the same principle in an analogous system).

What has been demonstrated above also offers a clue to solving an apparent paradox.[107 p 120] Since a protein has *only one* primary structure, the dependence of the secondary structure of a protein on its unique primary structure dictates that each protein should also have *only one* secondary structure. Yet it is known that a protein can assume more than one secondary structure—e.g., in response to certain drugs and hormones.[397] On the basis of the hypothesis of protein structure described above, this is now also understandable. The adsorption of a drug or hormone molecule on the collection of amino-acid residues, which together constitutes a *receptor site,* alters the electron-donating strength of the side chains of these amino-acid residues. Accordingly, a new secondary conformation is created. We will return to the subject of drug action in greater detail in [14.3] and [15] to follow.

(3.2) The control of the physical state of the bulk-phase water

In the preceding subsection, I have shown how an *increase* in the electron density or the *c-value analogue* of the carbonyl oxygen favors the formation of α-helical H-bonds—and the β-pleated sheet H-bonds also, though more weakly. This confirms the theory I offered in explanation of the dominant short-range mechanism, which enables the primary structure of a protein to determine its secondary structure. But there is an important *by-product* from this set of computations—if one looks on the converse side of the conclusion.

The finding that *higher* electron density of the backbone carbonyl oxygen favors the formation of α-helical as well as β-pleated sheet conformation implies that a *lowering* of the electron density of the backbone or the c-value analogue of the carbonyl oxygen would enhance the assumption of the alternative conformation. The major alternative conformation is, of course, the "random coil" conformation. Or, in the language of the PM theory, the *fully-extended conformation.*

Now, the extensive data presented in Chapter 11 show that whenever a protein (or a part of a protein) exists in the fully-extended conformation in an aqueous medium, *multilayer polarization and orientation* of the bulk-phase water follows. Thus a decrease in the electron density or c-value analogue of the backbone carbonyl oxygen leads to the assumption of the fully-extended conformation. The assumption of the fully-extended conformation, in turn, leads to multilayer polarization and orientation of the bulk-phase water.

These facts and reasoning allow us to construct a theoretical picture like that shown in Figure 42 for cations: Only instead of c-value of β- and γ-carboxyl groups, we have the *c-value analogue* of the backbone carbonyl groups on the abscissa. Instead of a pair of competing adsorbents like K^+ and Na^+, we have the pair of alternative adsorbents of α-helical H-bonding partners or multilayers of water molecules. At high c-value analogue, α-helical H-bonds would be preferred over multilayers of polarized and oriented water. At a lower c-value analogue, the opposite would be the case.

In summary, we now have a possible theoretical explanation why the primary structure of a protein determines its secondary structure—notwithstanding Prof. Anfinson's well-deserved Nobel Prize for verifying experimentally the same truth. In addition, we also have a theoretical model since 1962, which can explain the selective adsorption of K^+ over Na^+ on β- and γ-carboxyl groups in both the bulk-phase cytoplasm and on the surface of living cells—an ability promptly lost on cell death. Similarly, we now also have a theoretical model, in which water is polarized in multilayers with property permitting size-dependent solute distribution—an ability also promptly lost on cell death. On several occasions above, I have mentioned that death often involves a loss of ATP and early on I had suggested an electrostatic mechanism for this function of ATP. But I also pointed out that this mechanism has been revised. It is this new explanation for the role of ATP and how it may relate to the c-values of the β- and γ-carboxyl groups and the c-value analogue of the backbone carbonyl groups, which will be a major topic for discussion in the following section.

14.2 What distinguishes life from death at the cell and sub-cell level? The new concept of the living state

I do not know what defines life and death of a cell in the membrane-pump theory. In contrast, the AI Hypothesis has given a definition of life and death of a living cell from its inception. Life and death of a cell (and of protoplasm) are defined in the AI Hypothesis with the introduction of the concept of the *living* state. Indeed, this defining concept is part of the title of my first monograph published in 1962: *A Physical Theory of the Living State: the Association-Induction Hypothesis.*

(1) The living state

The term "living state" has been in use sporadically[177] but, as a rule, it is not much more than a substitute for the word, living. However, there are also

important exceptions. Aristotle's concept of life as "form" or organization[309 pp 19–20] offers one example. During the Renaissance (14th to 17th century) scholars noted that under unfavorable conditions, an organism can go into a state of dormancy without losing its life, leading them to the belief once more that the essential part of life is its *organization*.[309 p 20, footnot e2] But even better-focused were what Jean Baptiste Lamarck (1744–1829) referred to in the 18th century as the "*état de chose*"[308, 3 pp 138–139] and what Lepeschkin called "*live state*" another two centuries later.[62 p 269]

Lamarck's "état de choses" and Lepeschkin's "life state" are forerunners of the "living state" of the AI Hypothesis. However, in contrast to these and other earlier concepts, the *living state* of the AI Hypothesis is exactly defined in physico-chemical terms.[98]

Before presenting this definition of the living state according to the AI Hypothesis, we must find out what is the smallest unit of living matter. Many, who are unaware of the true current status of the membrane-pump theory (Chapter 12), often believe that the *whole living cell*, and nothing less than a *whole living cell*, can be alive.[3 p 310] This is not true.

Evidence in support of life at the below-whole cell level are not hard to find. Thus, an isolated piece of nerve axon is severed from the nerve cell's cell body and nucleus and can by no means be regarded as a *whole* living cell. Yet it conducts normal electrical activities on and on and provides the foundation of most of our current knowledge on nerve activities. How can it be considered dead then? Similarly, an isolated droplet of *Nitella* endoplasm (Figure 3) can survive as long as 3 days in a suitable medium.[63] That too is far from being a *whole* living cell.

In the AI Hypothesis, the minimal unit of living matter is not the living cell but its building block, the protoplasm. *By incorporating the concept of the living state defined below, has the concept of protoplasm—introduced a century and half ago by Dujardin, von Mohl and others—become viable for the first time.* Surely, only protoplasm maintained at the living state can serve as " the physical basis of life"[72] as Thomas Huxley eloquently pronounced. Protoplasm not maintained at its living state, and therefore dead, clearly cannot.

That said, I must point out that in the context of the AI Hypothesis, protoplasm comes in all forms. It is not always in the form Dujardin, von Mohl and other microanatomists described it, a viscous but free-flowing "living jelly" (Figure 3), and which colloid chemists call sol or *coacervate*. Cut a frog muscle as we did in making an EMOC preparation (Figure 7) or a

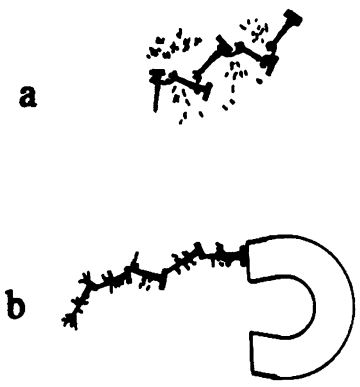

Figure 43. The nail-iron filings-magnet model. A chain of soft-iron nails joined end-to-end with pieces of string assumes a more or less random configuration and does not interact with surrounding iron filings (**a**). A strong horse-shoe magnet brought into contact with the free end of one of the terminal nails causes a propagated magnetic polarization and alignment of the nails and adsorption of the iron filings singly and in multiple layers (**b**). (Ling[173] from the International Review of Cytology by permission of Academic Press)

squid axon;[341 p 36; 342] the firm gel-like consistency of the exposed protoplasm prevents it from flowing out of the cell as the endoplasm of a cut *Nitella* cell does.[63] Diversity in physical consistency notwithstanding, all protoplasms share the capability to exist in the *living state* and to topple over into the *dead state* when the altered environment so dictates.

One way to introduce the physico-chemical definition of the living state is with the aid of a simple model: a chain of soft-iron nails joined end-to-end with bits of string (Figure 43).[173 p 37; 107 pp 31–38] When a strong horseshoe magnet is brought into contact with the end of one terminal nail, it initiates a propagated magnetization. As a result, not only are the assembly of tethered nails brought into a more rigid and ordered conformation, nearby randomly-scattered iron filings also become magnetized and attach themselves singly or in "polarized multiple layers" onto the chain of nails. The assembly of tethered nails, iron filings and horseshoe magnet together exists in a *higher-(negative)-energy and lower-entropy state.* Removal of the horseshoe magnet causes demagnetization of the nails as well as the iron filings—returning them to a *higher-entropy, lower-(negative)-energy state.*

The word, " negative" in parenthesis, was inserted in front of "energy" since the late 80's to forestall misunderstanding. In earlier days, I used to describe the living state simply as a high-energy, low-entropy state. I did that as when I say that a substance has a high adsorption energy on a protein of say, –15 kcal/mole. Note that this *high* adsorption energy is, on an absolute scale, really a very *low* energy or high-(negative)-adsorption energy. Hence the more up-to-date version of the expression, high-(negative)-energy. Parenthetically, I may point out that I am not doing anything outlandish by using the expression "negative en-

ergy." It is commonplace, for example, to use the expression "negative entropy"—as it will be in the discourse presented in [14.3(4)] to follow.

The living protoplasm, whether a viscous fluid or rigid gel, is a closely associated and *cooperatively linked* protein-ion-water system (for definition of *cooperativity*, see subsection (2) following). The protein is the equivalent of the chain of tethered soft-iron nails. K^+ and water molecules are the equivalents of the iron filings. Above all, the equivalent of the horseshoe magnet is the *principal cardinal adsorbent*, ATP, abbreviation for *adenosine triphosphate*. Protoplasm maintained in its living state also has low entropy and high (negative) energy like the magnet-nail-filing model; only in protoplasm, *electronic polarization and depolarization (or induction)* take the place of magnetization and demagnetization.

The living state does not represent a single state. Protoplasm may exist in a *resting living state* or *an active living state*—corresponding respectively to Lepeschkin's *passive,* and *active live state.*[62 p 269] The active living state is lower in its negative energy and higher in its entropy than in the resting living state. Transition from the resting living state to the active living state is the principle mechanism underlying most *physiological activities*, a transition that is as a rule *entropy-driven*. In other words, it is largely the gain in entropy that drives the protoplasm from its resting to its active state (see Lauffer[548] for other examples of entropy-driven processes).

However, the statement that physiological activity is entropy-driven is true only when the activity occurs just *once*—going from the resting living state to the active living state. When repeated activities occur ending with the system returning to its initial resting living state, the entropy as well as the energy of the system remains unchanged. Only work has been performed in the process at the expense of energy derived from the foods consumed. I shall return to this subject in subsection (4) coming.

The ultimate alternative to the living state is the *death state*, in which the protoplasmic assembly attains even lower negative energy and higher entropy. Thus *death too is entropy* driven. This is why life is exquisitely susceptible to a very modest increase of temperature. Frog muscle, for example, is killed by a 41°C temperature,[519 p 19] even though this is the normal body temperature of many birds.[520; 521 p 507]

Each one of these states is separated from the alternative state by an *energy barrier* of varying heights. And it is these energy barriers that prevent the cell or protoplasm from slipping spontaneously from one state to another. In going from one state to another, the change is as a rule abrupt and

all-or-none. In a later section on *cooperative* transition between alternative states [14.3(2)], I shall examine the underlying mechanism for this abruptness and all-or-none trait.

(2) The elemental living machine

In 1939, Engelhardt and Ljubimova[283] electrified the world of cell physiologists with a simple experiment. ATP, the end product of the metabolism of the foods we consume, elicits the lengthening of a loaded artificial muscle fiber made from isolated contractile proteins of muscle cells called "actomyosin." Simultaneously, ATP is hydrolyzed by the ATPase activity of the same contractile protein. This experiment provided a visual demonstration of how the hidden chemical energy stored in the high-energy-phosphate bonds of ATP fuels a dramatic mechanical work performance. I know this subject well; as a graduate student-instructor, it used to be the *piece de resistance* of my lecture series to medical students.

However, as the reader already knows from Chapter 12, the concept of the high-energy-phosphate bond was disproved in the mid-1950's.[133; 134] There is no utilizable free energy stored in any of the phosphate bonds of ATP. Soon after this revolutionary discovery, investigators like Riseman and Kirkwood[109] and Morales and Botts[284] began to look for a different way for ATP to serve its vital role in cell physiology. And earlier in [10.1(2)], I mentioned how once I adopted the idea of Riseman and Kirkwood to explain the role of ATP in maintaining selective K^+ accumulation in living cells.[96 pp 777–778] But I also pointed out that I later replaced that earlier model in the AI Hypothesis with a different one, the inductive model.

The new model adds to the direct electrostatic or *D-effect* transmitted through space (as in the Riseman-Kirkwood model) a better-focused *inductive* or *I-effect* transmitted through the intervening atoms.[98 pp 97–98] Since in most physiological events in the living protoplasm, the inductive component predominates by far, I shall continue to use the word, induction, to represent what, in more rigorous language, should be called the (direct) F-effect as it was in my earlier writing.[98]

Figure 44 presents a diagrammatic illustration of the smallest piece of living protoplasm, which can maintain itself in the *resting living state* and switch reversibly to the alternative *active living state* or irreversibly to the *death state*. In other words, this is a diagram of the *elemental living machine.*

Note that the right-hand side of Figure 44 depicts the elemental machine in its resting living state; the left-hand side of Figure 44 represents the ele-

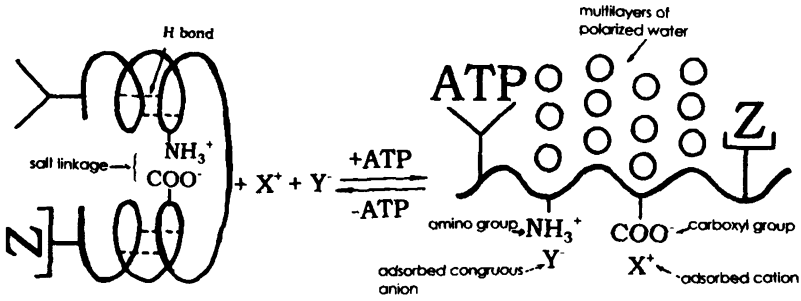

Figure 44. Diagrammatic illustration of how adsorption of the cardinal adsorbent ATP on the ATP-binding *cardinal site* and of "helpers" including the *congruous anions* (shown here as "adsorbed congruous anion") and *Protein-X* (shown as Z) unravels the *introverted* (folded) secondary structure shown on the left-hand side of the figure. As a result, selective K^+ adsorption can now take place on the liberated β- and γ-carboxyl groups (shown on the right side of the figure as carboxyl groups) and multilayer water polarization and orientation can now occur on the exposed backbone NHCO groups and the resting living state is thus achieved and maintained.
(This figure is the latest modified version of an original (simpler) version first published in 1969 in Reference 173.)

mental machine in its active *or* death state. The distinction between the active living state and the death state will be discussed in the subsection immediately following.

Hypothetically, to rejuvenate a moribund elemental living machine and return it to the resting living state, the "introverted" *active* or *dead* protoplasm must be made to open up and reach out to become "extrovert" again. To achieve that, we need (polarized and oriented) water to pry loose the closed-up intra- and inter-macromolecular H-bonds formed among the backbone NH and CO groups. We also need K^+ to pry loose the salt-linkages formed between the β- and γ-carboxyl groups and the fixed cations (∈-amino groups and guanidyl groups). But K^+ cannot do it alone. There has to be certain special or *"congruous" anions* that help K^+ to break open the salt-linkages by displacing at once both the fixed cation and fixed anion from each other.

Congruous anions tend to be different from one cell type to another. It is to a large extent Cl^- in the erythrocyte,[74 p 120] L-glutamate in the guinea pig brain and retina,[285] isethionate in the squid axon,[286] and organic phosphates in the frog muscle.[96 p 758]

Another important "helper" for ATP is what I call *protein-X (or Xs) as* diagrammatically illustrated in Figure 44. Our team at the Pennsylvania

Hospital was in hot pursuit of this illusive agent in red-blood cells. With its identification almost within our grasp,[107 pp 184–186] our laboratory was forcibly closed by the then-reigning establishments of NIH and ONR and their chosen consultants, whose combined wisdom or whatever dictated that our work should be put to an end.[247] Despite the extraordinarily kind and timely support of Dr. Raymond Damadian and his Fonar Company, we lost the essential facilities to continue this pursuit (e.g., radioisotope technology, high speed centrifuge, ion analysis equipments). So protein-X (or Xs) remains unidentified.

However, there are known reasons to suspect that protein-X (or X's) may be, or include *actin,*[107 p 186; 543 p 47] possibly as part of the microfilament system.[544; 545]

(3) What distinguishes the dead state from the active living state?

As pointed out earlier, both (one stroke) physiological activity and death are, according to the AI Model, *entropy-driven*, meaning that it is the large gain of entropy, which drives the system to go from the resting living state to the active living state (as during a single muscle contraction starting from the relaxed state ending in the contracted state) or from the living to the death state. It is therefore not surprising that intense physiological activities often bring on changes in the living cells qualitatively similar to those seen at the dead state. As an example, repeated contractions of a voluntary muscle produce a loss of cell K^+ and gain of cell Na^+ just like those seen in a dying muscle[122] (see Figure 56 below). According to the LFCH, ATP loss plays a key role in both.

For years, muscle physiologists had been baffled by their inability to demonstrate a decrease in ATP concentration after a *single* muscle contraction. This difficulty came from an enzyme called *creatine kinase*, which promptly regenerates the ATP lost during cell activity at the expense of the large reserve of creatine-phosphate (CrP). When Infante and Davies discovered that the poison, fluorodinitrobenzene (FDNB) specifically inhibits creatine kinase, the contraction-induced fall of ATP concentration was readily revealed in muscle treated with this poison.[371]

In normal muscle, both the ATP and CrP are constantly being regenerated through glycolytic and oxidative metabolism. Iodoacetate (IAA), which blocks this regeneration, limits the number of contractions an IAA-poisoned muscle can perform. Indeed with each contraction, the relaxation process of the poisoned muscle becomes a little less complete. Until it

ceases contracting in a sustained shortened *rigor state* of death[372] (see Figure 8.3 in Appendix 1 in Reference 49).

Thus *the main difference between the dead state and the active living state is that one is fully reversible but the other is not, although quantitatively speaking, the extent of changes in the dead state might also be more extensive and profound.* The vital role of metabolism is to make sure that each excursion of functional activity to a low-(negative)-energy and high-entropy state be brought promptly and fully back to the initial high-(negative)-energy, low-entropy resting living state.

(4) What does food provide: energy or negative entropy?

It is at once interesting and enlightening to see what one of the greatest theoretical physicists, Erwin Schrödinger, had to say about *life*. In fact I have already quoted him at the concluding passage of the Introduction section from his booklet "*What is Life? The Physical Aspect of the Living Cell*".[363] In the same volume first published in 1944, Schrödinger poohpoohed the popular belief that we and other living organisms feed on *energy*. Instead, he insisted: "What an organism feeds upon is negative entropy."[363 p 71]

This statement aroused objections from other scientists, to which he responded by admitting that the energy content of food does matter.[363 p 74] Notwithstanding, we are left with no clear-cut answer. Do we feed on energy or do we feed on "negative entropy?" Why has nobody come forth with an answer in the span of over half of a century, during which Schrödinger's little book has been reprinted at least 18 times? The ultimate source of this tardiness, in my view, can only be the membrane (pump) theory, which apparently contradicts Schrödinger's suggestion.

As the reader knows well by now, in the membrane (pump) theory, the living cell contains only *free* solutes and *free* water. And free solutes and free water cannot provide negative entropy. So a living cell, according to the membrane pump theory, contains no more "negative entropy" than a dead one. Indeed, since a dead cell tends to be more rigid (*rigor mortis*), one might expect that the dead cell has more negative entropy than a live one .Yet Schrödinger firmly believed that negative entropy " keeps us from death."[363 p 71]

So either Schrödinger is wrong, or the membrane (pump) theory is wrong. Chapter 12 has shown that the membrane (pump) theory is definitely not right. Does that mean that negative entropy is truly what we consume?

The answer is both No and Yes. And here are the reasons. Extensive evidence presented in [10.2] and Chapter 11 show that the adsorbed state of cell K^+ as well as cell water is part and parcel of the resting living state. Adsorption restricts free motion and creates negative entropy—in full harmony with Schrödinger's emphasis on the requirement of high negative entropy for life to continue. However, to achieve the adsorbed state of cell water and K^+, for example, one cannot eat negative entropy as such and apply it directly onto the water molecules and K^+. But we can achieve this *indirectly*.

Thanks to the monumental achievements of biochemists, we know that through the combined glycolytic and oxidative activities, each glucose molecule metabolized to CO_2 and water generates 36 molecules of ATP.[273 p 517] Adsorption of ATP on the cardinal site of key intracellular proteins (like myosin in muscle cells) leads to the whole-sale adsorption of water and K^+ with a loss of their respective motional freedom and rise of negative entropy. {More detailed discussion on how ATP achieves this vital role will follow in [14.3(4)].}

In summary, we do not consume negative entropy as such. We consume foods with utilizable energy, producing ATP. ATP, in turn, brings about across-the-board adsorption of K^+ but most importantly, across-the-board adsorption of intracellular water as polarized-oriented multilayers. A large rise of negative entropy is thus created and maintained in cells at their resting living state. For repeated physiological activities, when each cycle of activity returns the protoplasm to its initial resting living state, energy in foods is used to resynthesize the ATP split at each cycle of physiological activity.

I have used a horse-shoe magnet to demonstrate the interplay of energy and entropy in a remote, one-on-many control mechanism. However, as also mentioned, the real-world counterpart, according to the AI Hypothesis, is not operated by a magnetic mechanism but by an electronic one and is the main subject of the next subsection.

14.3 Electronic mechanism of remote, one-on-many control

An electric signal starting from one end of a long copper wire—long enough to wind around the equator of the Earth 32 times—will reach the other end in a little less than one second. The extreme speed of electrical transmission along a copper wire, first discovered by Benjamin Franklin, has provided the foundation for great inventions like telegraphy, the tele-

phone and the Internet, which have transformed ordinary people into wizards performing all kinds of magic as part of their daily routine.

Naturally, biologists have wondered if long-distance signal transmission of a similar nature may underlie physiological coordinations also. The answer is No. By virtue of their ubiquity and length, proteins are the only possible candidate for the role of the biological copper wire. Yet proteins cannot serve this role because proteins *per se* are not conductors. Indeed, threads of silk, a *bona fide* protein, have been used to suspend and *insulate* metal balls in classroom demonstrations of electrostatic induction phenomena.[328 p 6]

Of course, we already know that signal transmission can exist as propagated *action potential*s along the surface of nerve and muscle fibers. Less well-known is that at the molecular level, *distant and one-on-many control* underlies the operation of the elemental living machine. Further, the underlying mechanisms for both modes of remote information transmission appear to share a fundamental design demonstrable in a man-made model.

More than two millennia ago, war efforts against the Huns (also known as Hsiung-nu) had led to the building of the Great Wall, which may be seen partly as an apparatus for speedy information transfer over long distances. More than four thousand and five hundred miles long and built over sandy deserts as well as rugged mountains, it is a man-made structure that can be seen from outer space. Paved in such a way that two horses can run abreast on top of the wall, it was also once an elevated and protected superhighway.

There is a watch tower every 200 to 300 yards along the entire length of the Wall. At the first appearance of invaders, sentries manning the watch tower promptly set up fire and smoke signals. Seeing them, the sentinels at the neighboring watch towers immediately set up their fire-smoke signals. And this is swiftly repeated again and again until defenders at long stretches of the Wall are on alert. Mounted soldiers then move swiftly to succor local defenders and engage in battles with the enemies. In its way of relayed smoke signal operation, the Great Wall offers a model of an alternative type of rapid information transmission to that provided by the long copper wire. Here, long-range information transmission is achieved by the repetition of short-range interactions between immediately neighboring watch towers. Both the propagated *action potential* along nerve and muscle fibers and the propagated *induction effect* along proteins chains (according to the AI Hypothesis) share important traits with this model.

(1) Electronic induction in proteins

Proteins are the principle players of the living phenomenon. This is not to deny the importance of DNA and RNA, without which life cannot continue. Nonetheless, since DNA and RNA perform no other function (beside duplicating themselves) than specifying proteins, clearly the roles of DNA and RNA are less direct to life as to those of proteins.

Each protein comprises a linear array of an assortment of amino-acid residues, selected, as a rule, from an assortment of twenty-two. In this, the language of life is not unlike the written (English) language, spelled out from an assortment of twenty-six alphabetic letters. Note that English words like *eat* and *ate* have different meanings though each contains the same three letters. So a protein is also specified by both the kind and number of amino-acid residues and by the order of their arrangement in the protein chain.

The differences between *eat* and *ate* are given and entirely arbitrary. The differences between different arrangements of amino-acid residues are, however, innate. First, their molecular shape or stereochemistry are not the same. Of far greater importance, they differ *electronically* due to the operation of the *inductive effect.*

To illustrate, once more I cite the transformation of a pleasantly-sour vinegar, or acetic acid (CH_3COOH) with a pK value of 4.76 into an insufferably acidic trichloroacetic acid (CCl_3COOH), with a pK value of less than unity.

Now, each chlorine atom in the trichloroacetic acid is more *electronegative* than the hydrogen atom it displaces.[485 Table 3.7] More explicitly, chlorine with 17 positively-charged protons in its nucleus, has a stronger power to attract negatively-charged electrons toward itself than the hydrogen atom (with a single proton as its nucleus) it has displaced. As a result of the chlorine-for-hydrogen substitution, electrons are drawn toward the chlorine atoms. Furthermore, this electron withdrawal extends step-by-step all the way to the negatively-charged oxygen atom of the far-away carboxyl group. Accordingly, the electron density of the carboxyl oxygen atom is reduced and its attraction for the positively charged hydrogen atom or proton (H^+) weakened. Repeated three times, the Cl for H substitutions produce an acid with very little affinity for H^+. A very strong acid is thus created from a weak one through the operation of the inductive effect.

As illustrated in Figure 45, substitution of a hydrogen atom in acetic acid (CH_3COOH) or n-propionic acid (CH_3CH_2COOH) by the chlorine

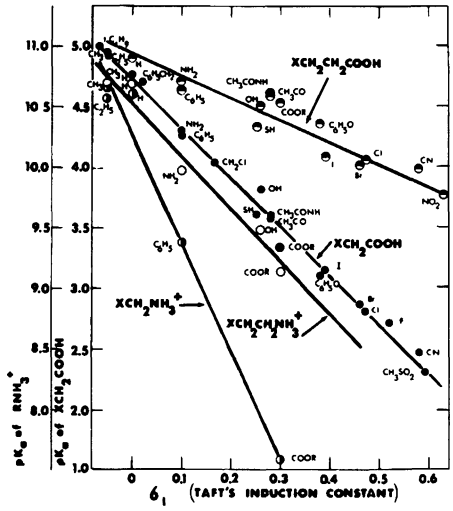

Figure 45. Linear relationships between the acid dissociation constants, pK_a's of two sets of substituted carboxylic acids (acetic and n-propionic acid) and two sets of substituted amines (methylamine and ethylamine) shown on the ordinate and Taft's induction constant, σ_I, of each of the substituent (marked as X in the structural formulae of the acids and amines) shown on the abscissa. (Ling,[453] by permission of Texas Report of Biology and Medicine)

atom and other substituents produces decreases or increases of pK (written as pK_a) in XCH_2COOH and XCH_2CH_2COOH formed, depending on the nature of the subsitituent, X. These changes are governed by sets of empirically-derived *induction constants* for each substituent, including Taft's inductive constants,[506; 338] Hammett's σ constants,[338] and Chiang and Tai's inductive indices.[507; 338; 15 pp 185–188]

Figure 45 shows that the induction constant of a specific substituent remains the same, even though the target groups are different (e.g., carboxyl group vs amino group). But the change in the numeric value of the pK as a result of a specific substitution decreases with an increasing number of saturated hydrocarbons or methylene groups ($-CH_2-$) between the site of substitution and the target group. A simple computation based on the data given in Figure 45 leads to an amazingly high *transmissivity* of 0.48 ± 0.14 through each methylene group ($-CH_2-$).[488 p 100] Thus after passage through *three* successive methylene groups, ($-CH_2CH_2CH_2-$) the induction effect is still a respectable 11% of the original. But there is a much more efficient information highway than chains of saturated methylene groups, namely, the *polypeptide chain.*

Now, proteins are the substance of all protoplasm and are produced in Nature only by living cells—including those living cells making up biochemists who had synthesized proteins. What makes protein unique among molecules is not the collection of amino-acid residues. Conceivably, new

amino acids may be discovered or synthesized, but that would not alter the big picture. *What is truly unique is the polypeptide chain, which is present in all proteins and nowhere else.*[424]

In my opinion, the conventional protein chemistry has not given this *unique structural component* of all life as important a role as it calls for. In contrast, the AI Hypothesis, since its beginning, has laid great emphasis on the polypeptide chain, which is unique in possessing a *partially resonating* structure—as indicated by the 40% double-bond nature of the linkage between the nitrogen atom and its adjacent carbonyl carbon atom in each of its constituent peptide bonds, -CONH-.[485 p 498] This partial resonance makes the long polypeptide chain highly polarizable and thus eminently suited to transmit *induction effect* over distance. In that role, the polypeptide chains share some of the fundamental features of the Great Wall as well as the more humble model of chains of soft-iron nails as the *main information highway* in the elemental structure of life.

How far can each local, or what I call *direct inductive effect*, be transmitted along the polypeptide chain of an isolated protein? There are at least four sets of independent experimental evidence, which provide answers to this question and are diagrammatically illustrated in Figure 46. Together, they have established that the (direct) inductive effect can be effectively transmitted through as many as three peptide linkages plus a small segment of saturated methylene side chain. (More details on this evidence are given in Reference 488 pp. 98–106 and in Reference 107 pp.116–125 and in the legend of Figure 46.). Again by referring to the Great Wall analogy, this distance is the equivalent of the distance between each pair of nearest-neighboring watch towers.

Owing to the rapid attenuation of the inductive effect through saturated methylene groups—though the transmissivity figure cited (0.48) above shows surprisingly how slowly—the functional groups that can effectively respond to the transmitted inductive effect are more likely those carried on *short side chains*. These *proximal functional groups* include the backbone NHCO groups themselves—which are, of course, not side chains but parts of the polypeptide chain itself—and the β- and γ-carboxyl groups we are already very familiar with. Other functional groups on short side chains include those on serine-, threonine-, cysteine-, tyrosine-, phenylalanine-, histidine- and tryptophane-residue.[107 p 148] Since each of these functional groups is separated by no more than two methylene groups from the polypeptide chain, transmission of the inductive effect from the polypep-

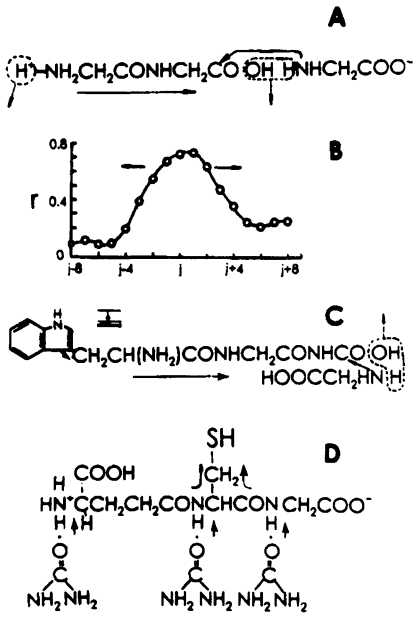

Figure 46. Diagrammatic illustrations of four sets of experimental evidence for the effective transmission of the inductive effect through a length of the polypeptide chain and short segments of saturated hydrocarbon chains. (A) Decrease of the affinity of the terminal amino group for H^+ as indicated by a decrease of its pK_a in response to distant substitution of a carboxyl OH group by a stronger electron-withdrawing glycyl group.[98 p 94] (B) Linear correlation between the α-helical potential of the (19) α-amino-acid residues on the one hand; and the electron-donating power of their respective side chains (expressed as the acid dissociation constants of their α-caroxylic acid analogues) at residue position j and at other immediately neighboring side chains labeled j+m and j–m, where m stands for the number from 0 to 8. j–m represents a residue on the N-terminal side of the jth residue; j+m represents a residue on the C-terminal side of the jth residue. α-helical potentials are the directional information measure given by Garnier *et al.*[153] (For more details, see [14.1(3)] and Reference 150.)(C) Quenching of fluorescence of the indole group of a tryptophane residue in a series of synthetic polypeptides (of tryptophane-$(glycine)_n$) in consequence of the substitution of the hydroxyl group of the most distant carboxyl groups by a stronger electron-withdrawing glycyl group.[107 pp 123–125] (D) Enhancement of SH group reactivity of glutathione by reaction of its backbone sites with urea. (For more details see Reference 185. Note the increasing enhancement with increasing number of peptide linkages shown in Table II of that reference.)

tide chain to these functional groups should present no serious difficulty as the data shown in Figures 45 and 46 have already demonstrated.

Figure 45 also shows that the pK or c-value of the carboxyl groups are the effective targets of the transmitted inductive effect. *Other evidence presented next shows that the strengths of H-bonds—expressed by the c-value analogue—are also effective targets of the transmitted inductive effect.*

The free energy of dimerization of para-substituted acetophenoneoximes (see inset of Figure 47 for its structural formula) replotted from Reiser's data[510 p 446] increases linearly with the rising value of the inductive constant, σ (Figure 47), just as the acid dissociation constants of carboxyl and amino groups increase linearly with the rising value of the inductive constant, σ_I (Figure 45). This is not surprising, because the dimerization

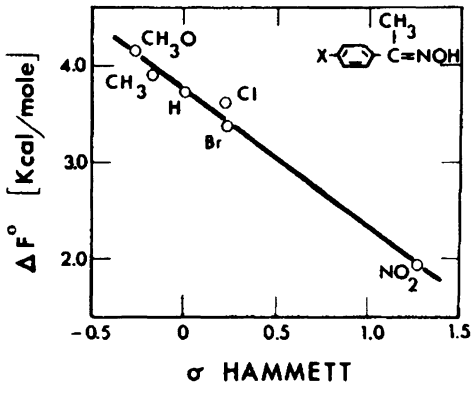

Figure 47. Relationship between Hammett's induction constants, σ's of the substituent X, and the free energy of dimerization of para-substituted acetophenone oximes. The structural formula of the substituted acetophenone oximes is shown in the upper right corner of the figure, where X represents the substituent. The free energies of dimerization of the substituted compounds (ordinate)were calculated from Reiser's data[510] and the assumption that only dimers are formed between the molecules. The fact that powerful electron-donating substituent like the CH_3 strongly enhances the strength of dimerization indicates that it is primarily the electron density of the electron-donating nitrogen atom (rather than the electron-accepting OH group) that determines the strength of the H-bonds formed between a pair of the molecules. (Ling,[453] by permission of Texas Reports on Biology and Medicine)

involves the formation of a pair of H-bonds, which are largely electrostatic[512 pp 339–360] and thus by nature inductive.

Reiser's data has something else to offer. Like the CO and NH groups of a peptide bond, each acetophenone oxime molecule also carries a pair of juxtaposed H-bonding groups: a nitrogen atom (carrying a lone pair of electrons) acting as an electron donor and an OH group acting as electron acceptor. If both groups are equally polarizable, para-substitutions would have no influence on the free energy of dimerization, because the opposing impacts of the substituent on the N atom and on the OH group would cancel out. The fact that the free energy of dimerization increases linearly with increasing electron-donating strength of the substituent shows a higher polarizability of the N atom than the OH group, in parallel with the carbonyl O atom and NH group of the peptide linkage discussed in [14.1(3.1)]. It is the electron density (or its c-value analogue) of the N atom, which is more effectively altered by the para-substitution and which determines the strength of the H-bonds that hold the dimer together.

Evidence that the oxidation-reduction potential of cysteinyl SH groups are inductively affected by H-bonding partner changes at nearby peptide linkages has been reviewed by Ling.[488 pp 106–110] Evidence that the phenolic groups of tyrosine residue and the indole group of tryptophane residue can be similarly affected will be cited in subsection (4) following.

To complete the story, I shall demonstrate that not only are the c-value of the carboxyl groups and the c-value analogue of the electron-donating N

atom (and its analogous electron-donating backbone carbonyl oxygen atom) effective *targets* of the inductive effect; ionic association/dissociation of carboxyl groups and association/dissociation of H-bonds are effective *initiators* of the inductive effect as well, as what follows shows.

Consider a carboxyl group -COOH. It can dissociate into $-COO^-$ and H^+. The Hammett inductive constant, σ, is +0.355 for the (meta-substituted) *undissociated* COOH group but only +0.104 for the (meta-substituted) *dissociated* COO^- group.[522] Thus substituting an undissociated COOH group by a dissociated COO^- group would have produced an inductive effect as large as the substitution of a meta H atom ($\sigma = 0$) by an electronegative substituent with a σ equal to $+0.355 - 0.104 = +0.251$. But substituting a COOH by a COO^- group is just another way of describing the dissociation of a COOH group into a COO^- group. Accordingly, one draws the conclusion that a transmitted inductive effect can be initiated by the breaking or formation of ionic bonds just as it can be initiated by the breaking and formation of covalent bonds (as in the familiar acetic acid/ trichloroacetic acid example). This is not surprising. An ionic bond is also fundamentally electrostatic[511] and thus by nature inductive.

Alterations of the electronic spectrum of phenolic substances can be produced inductively by substitution on the phenol ring involving the formation (and breaking) of new covalent bonds. Burawoy showed that similar alterations can be just as effectively produced by the formation (and breaking) of new H-bonds.[513 p 259] Thus formation of a new H-bond can also *initiate* inductive effects, just as the formation of a new ionic or new covalent bond can.

In summary, examination of model behaviors has established that the inductive effect can be initiated by the formation of new ionic bonds (like those formed between the K^+ and Na^+ with the β- and γ-carboxyl groups), by the formation of new H-bonds (like those formed between the peptide imino group or water with the backbone carbonyl groups) as well as by forming new covalent bonds. The inductive effect thus initiated can be transmitted through as many as three peptide linkages and a short segment of saturated side chains to alter the c-value of β- and γ-carboxyl groups and the c-value analogue of the backbone carbonyl groups, to mention just two. These transmissions are short-range like those between two nearest neighboring watch towers on the Great Wall. Long-range transmissions are achieved by sequential repetition of these short-range transmissions.

(2) Cooperative interaction as the basis for abrupt and coherent transitions between stable states

When I speak of the *protein-ion-water-cardinal adsorbents* assembly being *cooperatively linked* in [14.3(1)] above, I use the word "cooperatively" then and the word, "cooperativity" immediately above in the *statistical-mechanical* sense.[178] In this restricted definition, *cooperativity* means that there is strong *near-neighbor interaction*—which can be both positive and negative—between the individual elements of the assembly. To elaborate on this subject, I begin with the respiratory protein, hemoglobin—which makes up no less than 97% of the total proteins of human red blood cells.[4 p 120, 140]

It has long been known that the uptake of oxygen by hemoglobin is unusual. It does not follow the rule that in a heterogeneous system containing more than one kind of binding sites, the sites with the highest affinity bind first and the sites with the least affinity bind last. Instead, it appears as if the converse is the case. Thus, when the uptake of oxygen of hemoglobin is plotted against increasing oxygen tension, one gets an *S-shaped* or *sigmoid* curve (inset of Figure 55), where steep strong uptake *follows* flat weak uptake.[181–184] This unusual behavior has been expressed in terms of an empirical parameter, n, also known as the Hill's coefficient—introduced by the same A. V. Hill,[183] with whose experimental findings and logical reasoning against the bound water and bound K^+ concept the reader is already familiar. In appearance, n is a measure of "sigmoidity" of the oxygen uptake curve.

According to the once-popular "crevice hypothesis," only heme sites with weak affinity are on the surface of the protein molecule and thus accessible to oxygen in the surrounding medium. Sites with strong affinity are buried inside crevices, which become accessible to oxygen only after prior binding on the weak binding sites has somehow pried open the crevices. However, the total elucidation of the structure of hemoglobin by Perutz and coworkers has revealed that all four heme sites are on the surface of the hemoglobin molecule and thus equally accessible.[329]

Although the sigmoid oxygen uptake of hemoglobin had also been from time to time interpreted in terms of *cooperativity*, to the best of my knowledge it was not until 1964 that theoretical physicist C. N. Yang, Nobel Laureate, and I demonstrated that n equals exp (γ/RT), where R is the gas constant, T is the absolute temperature and $-\gamma/2$ is *the nearest neighbor interaction energy* among the oxygen binding heme sites.[185; 244; 295 p 39; 313; 107]

[pp 136–140] The Yang-Ling cooperative adsorption isotherm introduced in 1964 is given as Equation A4 in Appendix 1.

When n is equal to one, $-\gamma/2$ is equal to zero. In this case, each adsorption is independent and the oxygen uptake curve takes on the familiar "hyperbolic" shape of a Langmuir type of adsorption—as it is the case for the oxygen uptake of myoglobin (left figure in inset of Figure 55). With increasing n, the shape of the curve changes gradually from "hyperbolic" to sigmoid.

A high n value of 3.0 corresponds to a $-\gamma/2$ of +0.649 kcal/mole. A positive $-\gamma/2$ like this signifies that each time a site binds an oxygen molecule, it increases the affinity for oxygen of each of its two flanking (nearest-neighboring) binding sites by this amount. If each of the two nearest-neighboring sites binds oxygen, the middle site will increase its binding energy for oxygen by twice this amount. This sequence of increasing affinity for oxygen exemplifies *ferro-magnetic* cooperative transitions, or as I suggested earlier as an alternative, *autocooperative* transitions (see Section 4 in Appendix 1).

The revealed crystalline structure and autocooperativity of hemoglobin discussed above pose another fundamental question. The four heme sites in a hemoglobin molecule are *far apart*. How can the binding of oxygen on one site affect the affinities for oxygen of other sites a long distance away? Indeed, it is in answer to this basic question that the AI Hypothesis offers a molecular mechanism of long-range information and energy transfer.[107 pp 145–149] The details of this theoretical mechanism will be given in subsection (5) and (6) following. To facilitate presentation, I now add two more simple models in addition to the Great Wall model and nail-filing-magnet model already cited—after ruling out ohmic conduction along some hypothetical biological "copper wires."

A third model for long-range information and energy transfer is a chain of dominos. Toppling one domino sets in motion the toppling of the nearest neighboring domino and this continues *ad infinitum.* But the falling-domino analogy goes only in one direction and is therefore defective, though still useful. A better (fourth) one is a chain of perfectly-balanced, frictionless see-saws, tied end to end with flexible strings.[107 Fig 7.7, p 146] This model can go both ways.

In all four of these models the propagation involves the whole assembly, be it the chain of soft-iron nails, upright dominos, tethered see-saws or vigilant watch-tower sentinels. In agreement, the autocooperative interac-

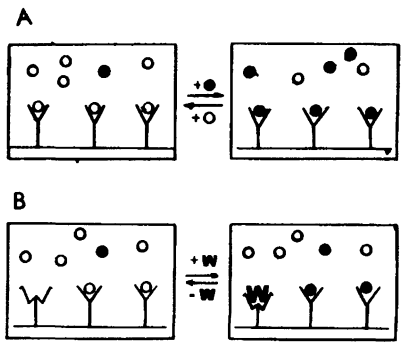

Figure 48. Two types of *cooperative* transitions between i state in which the sites are occupied by solute i (open circles) to the j state in which they are occupied by solute j (solid circles). (**A**) In this type shown under A, called "spontaneous," the transition results from a change in the relative concentration of i and j in the surrounding medium. (**B**) In this type shown under B, called "controlled," a similar transition between the i and j state occurs at constant i and j concentrations, due to interaction of a cardinal site with a cardinal adsorbent, **W.** (This figure is a modification of an original one first published in Reference 313 and shown as Figure 2 in that reference.)

tion we see in oxygen binding of hemoglobin also entails not merely the four oxygen-binding heme sites but *global* changes of the whole protein-ion-water assembly.[107 pp 149–155]

Hemoglobin undergoes such an all-or-none *autocooperative transition* from the relaxed or oxygenated *R-state* to the rigid or tense deoxygenated *T-state* with a small change in the oxygen tension in the environment. This is diagrammatically illustrated in Figure 48A. It can also undergo a similar transition at an unchanging oxygen tension by interacting with a low concentration of a *cardinal adsorbent* like 2,3 diphosphoglycerate (2,3 DPG).[189] This type of controlled autocooperative transition is illustrated in Figure 48B where the cardinal adsorbent is represented by **W**. Experimental data of both kinds of cooperative transition are accurately described by the Yang-Ling cooperative adsorption isotherm (Equation A4 in Appendix 1).[186; 107 Fig 7.3, Fig 7.11]

Among the global changes of hemoglobin beyond oxygenation-deoxygenation and R/T structural transformation, two other concomitant changes may be mentioned: (i) a change in the pK's of anionic groups (Bohr effect);[187] and (ii) a large change in entropy.[188] In terms of the AI Hypothesis, the pK value changes denote a change in the *c-value* of the involved anionic groups; the large entropy gain indicates *c-value analogue* change of the backbone carbonyl groups, leading to adsorption or liberation of water—as only water molecules are numerous enough to bring about the entropy change seen. These two types of changes play central roles in many physiological activities of the living protoplasm as will be made clear again and again as our story unfolds.

Global cooperative change in hemoglobin can also be brought about by ATP.[189] Since hemoglobin is not an ATPase, *the changes in hemoglobin are produced by the adsorption of ATP per se.* In this, ATP (and 2,3-DPG) resemble(s) substances we know as drugs, which, at minute concentration, produce far-reaching responses.

(3) The classification of drugs and other cardinal adsorbents: EWC, EDC and EIC

The specialized part of the cell structure, which takes up drug molecules, are known as *receptor sites*. The concept of the *cardinal sites*, which include the receptor sites, came later with the AI Hypothesis.[98 p 110; 15 p 219; 107 p 32] The function of a cardinal site goes beyond that of a receptor site, which is as a rule found on the cell surface, and *receiving* an externally administered therapeutic agent or drug. Cardinal sites include sites that are part and parcel of the *elemental living machine* found throughout the cell [14.2(2)]. Drugs, hormones, 2,3-DPG, Ca^{++}, ATP and other potent agents, which at very low concentration may interact with cardinal sites, thereby sustaining the resting living state of the protoplasm or bringing about its changes, are collectively called *cardinal adsorbents.*

A receptor site as described in textbooks is distinguished by its steric adaptation to the specific drug molecule it reacts to. A familiar analogy often cited is the *lock and key* (first introduced by Emil Fischer to describe the fitting of substrates on enzymes[444]). Thus only structurally-fitting drugs can produce the desired pharmacological effect. Familiar as this model is, something vital to its function is missing.

In day-to-day experience, a fitting key does not do its job by just sitting there fitting. The fitting key must *do* something. That something, which the ordinary key usually does, is the mechanical act of "turning." There is no equivalent of this "turning" in the conventional cell physiology or pharmacology. It is this basic deficiency, which might underlie Prof. Alfred Burger's complaint about medicinal chemistry cited in the opening page of this document..

In contrast, *the association-induction hypothesis has offered from its very beginning, what is the equivalent of that "turning," i.e., electronic polarization or induction.*

More precisely, a cardinal adsorbent produces its effect by interacting *electronically* with the cardinal site. It may produce an overall effect of donating electrons to the cardinal site. In that case the cardinal adsorbent is

called an *electron-donating cardinal adsorbent*, or EDC. Or it may produce an overall effect of withdrawing electrons from the cardinal site. In that case, the cardinal adsorbent is called an *electron-withdrawing cardinal adsorbent* or EWC. Then there is a third, uncommon type of cardinal adsorbent, which neither donates nor withdraws electrons, but merely occupies the cardinal site. They are called *electron-indifferent-cardinal adsorbents* or EIC.[107 pp 144–145]

Having defined EDC, EWC and EIC, I add some qualifications. That is, a specific drug being an EDC or EWC is limited to a specific type of cardinal site, with which the drug interacts. Since each drug molecule or other form of cardinal adsorbents may possess more than one "center" of activity, it is theoretically conceivable that interacting with a different type of cardinal site, a specific EDC may act as an EWC and *vice versa*. Furthermore, when a weak EDC displaces a stronger EDC, it might give the impression that the weak EDC acts like an EWC. However, in our still very limited study of drug actions on *living cells*, the electron-donating or withdrawing influence of a drug appears to stay unchanging.[398] With model systems carrying poor imitations of the real-world cardinal sites, the situation may be different.

(4) ATP, the Queen of cardinal adsorbents, as an EWC

In [14.2(2)], we have discussed at length how the queen of all cardinal adsorbents, ATP, maintains the resting living state by its adsorption on a specific cardinal site. Indeed, how ATP achieves this function is one of the most important questions in cell physiology. It has also played a key role in mankind's latest attempts to find the energy source of biological work performance. And until the advent of the AI Hypothesis, it had been a long history of repeated failures [16.1].[68 pp 144–148] Has the AI Hypothesis broken this streak of bad luck? I think so and will try to convince you that I am not too far from the mark.

Although ATP does not, as once thought, carry an extra large amount of usable free energy in its two distal phosphate bonds (each equal to –12 Kcal/mole as once thought), the *free energy of adsorption* of ATP on myosin has been firmly established at –14.3 Kcal/mole.[287] Such a favorable adsorption energy assures that virtually all ATP (in muscle cells) is adsorbed on myosin (at least). Note that myosin is not only the the most abundant protein in muscle cells, it also carries from 67% to (more likely) 80% of the K^+-adsorbing β- and γ-carboxyl groups.[122] Strong binding of ATP on

myosin and other intracellular proteins empowers ATP to serve its life-giving role as the *principal cardinal adsorbent.*

In contrast, the hydrolytic product of ATP, ADP—which as a rule does not do what ATP does, does it weakly or does the opposite[289 p 87]—yields a free energy of adsorption on myosin at only half of that of ATP (–6.82 Kcal/mole[288]). Or put differently, the *binding constant* of ATP on myosin is 10^{10} to 10^{11}, which is five to six orders of magnitude higher than that of ADP (10^5). This divergent attribute of ATP and its hydrolytic product, ADP, enables ATP to serve its key role in *switching* between the resting and the active living state (see [14.2(2)] for example).

Having established that ATP binds strongly onto its cardinal site, our next question is, Is ATP an EDC or an EWC? In the following example, I shall provide not only an answer to this important question, but I shall also provide evidence (i) that the inductive effect can be transmitted *through* ionic and/or H-bonds involved in adsorption and (ii) that the attributes of a molecule adsorbed on a protein can be inductively modified by a second molecule adsorbed at a different location on the same protein. But to reach these conclusions, we must first digress a little.

The phenol group of the amino-acid residue, tyrosine, and the indole group of the amino-acid residue, tryptophane, both absorb light in the near-ultraviolet region and give back visible light of a higher wave length—a phenomenon known as *fluorescence.* The ratio of the energy emitted over the energy absorbed is called the *fluorescence yield.* When tyrosine or tryptophane are incorporated into peptides, the fluorescence yield decreases sharply. Additional reduction of fluorescence yield is produced if a proton (H^+) is added to the ionized α-amino group of the peptide formed. Since the *peptide bond*[107 p 121] and the H^+ are both well known for their electron-withdrawing effect, these and many other similar examples have established that *electron withdrawing reduces fluorescence yield (or quenching)*; conversely *electron donating increases fluorescence yield.*[107 pp 123–125] With these facts on hand, let us find out if changes of fluorescence yield may tell us whether ATP is an EWC or EDC.

The antibiotic *aurovertin* exhibits weak fluorescence in an aqueous solution. When aurovertin is added to a solution containing the enzyme protein, *F_1 soluble mitochondrial ATPase*, the antibiotic adsorbs stoichiometrically onto the enzyme and, in consequence, its fluorescence increases 100 fold. Figure 49, taken from Chang and Penefsky,[509] shows that the introduction of ATP or Mg^{2+} produces a marked decrease of the

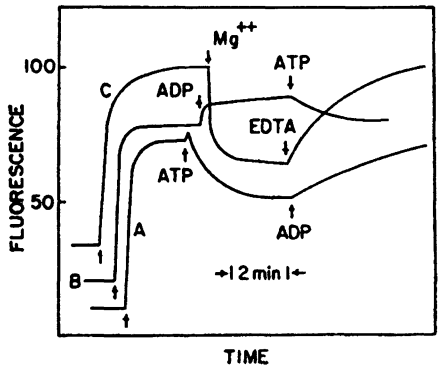

Figure 49. Effects of ATP, ADP, Mg^{2+} and EDTA on the fluorescence intensity of aurovertin-F_1-complex. Reaction mixture contains 3 ml. of buffer and either 1.15 μM (A,B) or 1.50 μM (C) of F_1. At the first set of arrows, 3.3 (A,B) or 2.2 (C) nmoles of aurovertin were added. In all relevant cases, the final concentration of ATP was 1.6 mM: ADP, 0.9 mM and Mg^{++} 3 mM. (Reduction of fluorescence or quenching due to added ATP suggests that ATP acts as an electron-withdrawing cardinal adsorbent or EWC. (GL's addition.)) (Chang and Penefsky,[509] by permission of Journal of Biological Chemistry)

fluorescence yield. This quenching effect shows that both Mg^{2+} and ATP act as *electron-withdrawing cardinal adsorbents* or EWC. In contrast, ADP addition causes an increase of fluorescence yield, signaling that ADP acts as an *electron-donating cardinal adsorbent* or EDC.

Since neither aurovertin nor ATP form covalent bonds with the enzyme, the inductive effect produced by ATP on the fluorescence of aurovertin demonstrates effective transmission of the inductive effect produced by ATP through the ionic bonds, H-bonds and/or other bonds formed in the adsorption of ATP. And this effective transmission occurs twice, once at the ATP binding site on the F_1-ATPase protein, and once at the aurovertin binding site on the same protein.

Having established the important fact that ATP is an EWC (in this and all other cases examined thus far), and before going into details on how this specific attribute of ATP may play a key role in cell functions, I shall examine in the next subsection the broader question: How do drugs and other cardinal adsorbents control cell functions?

(5) What do drugs and other cardinal adsorbents do?

To achieve its goal of physiological control, an EDC or EWC must be able to produce *action over distance* and *one-on-many* impacts. The targets of EDC or EWC action are mostly protein functional groups (singly or in groups), which are located at some distance. And as mentioned above in [14.3(1)], these target groups are, as a rule, close or proximal to the main "highway" of information and energy transfer, i.e., the (partially resonating) polypeptide chain (or backbone). Among these "proximal sites" are the NH and CO groups right on the backbone, and β- and γ-carboxyl groups

carried on short side chains. Earlier we have also demonstrated that the inductive effect can be transmitted through as many as three consecutive peptide linkages in addition to segments of short side chains[107 pp 130–132] (Figure 45).

In the subsection immediately following, I shall present a theoretical model showing how *the adsorption of an EWC on an appropriate cardinal site brings about an across-the-board decrease of the electron density of a succession of proximal sites like the carbonyl groups of the backbone polypeptide and the* β- *and* γ-*carboxyl groups.*[107 pp 145–155] *In contrast, an adsorption of an EDC on an appropriate cardinal site brings about an across-the-board increase of the electron density of similar proximal sites.*

(6) How cardinal adsorbents produce across-the-board uniform electron density changes of many proximal functional groups

The reader is already familiar with the c-value, which expresses the electron density of a β- or γ-carboxyl group, and the equivalent parameter measuring the electron density of a backbone carbonyl group (CO) called *c-value analogue* [14.1(2), (3)]. Similarly, the positive charge carried by a cationic side chain is quantitatively represented by what is known as the *c′-value* and the positive change on a backbone imino group (NH) group as *c′-value analogue.*[173 pp 42–43]

The reader is also familiar with the general pattern of how a change in the c-value of a β- and γ-carboxyl group alters its relative affinity between pairs of monovalent cations like K^+ and Na^+ (Figure 42). Thus, at a low c-value, K^+ is preferred over Na^+. At a higher c-value, Na^+ is preferred over K^+. Analogous changes of preferred partners occur also at the backbone sites[173 pp 42–43] [14.1(3)]. Thus high c-value analogue favors the formation of α-helical H-bonds; low c-value analogue favors the adsorption of water in polarized and oriented multilayers.

The inset of Figure 50 is an illustration of these types of relationships between *c-value analogue* as well as *the postulated c′-value analogue* and the relative affinity for adsorbents a^+, b^+ and a^-, b^- respectively. A rise of the c-value analogue of a backbone carbonyl group (CO) from 1 to 2 in the inset of Figure 50 changes the site from preferring the adsorbent b^+ to preferring the adsorbent a^+. Conversely, a fall of the c-value analogue produces the opposite effect. Similarly an increase of the c′-value analogue of a backbone NH group from 1 to 2 reverses the preference for b^- over a^- to preference for a^- over b^-; a fall of the c′-value analogue has the opposite ef-

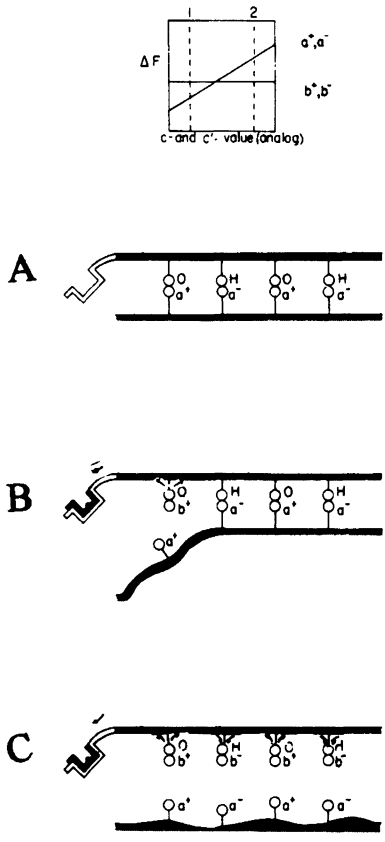

Figure 50. A theoretical model showing how adsorption of a cardinal adsorbent on a controlling cardinal site of a protein brings about an (controlled) autocooperative transition, resulting in an across-the-board uniform change in the electron density of the backbone NH and CO groups. A sterically and electronically specialized region at the left end of the upper protein chain shown as a dark horizontal line makes up a *cardinal site.* Empty circles attached to the protein chains and labeled H and O represent respectively backbone imino (NH) and carbonyl (CO) groups. Inset diagrammatically illustrates how changes in the *c-value analogue* of backbone CO groups alters the relative affinity for adsorbents a^+ and b^+, and how changes of the *c′-value analogue* of backbone NH groups alters their affinity for adsorbents a^- and b^-. See text for a description of how adsorption of the *electron-withdrawing cardinal adsorbent,* **W**, on the cardinal site creates an inductively-propagated, across-the-board and uniform change in all the backbone NH and CO groups. (Modified after Ling.[173] From *International Review of Cytology* by permission of Academic Press)

fect. Armed with these basic postulations—some already established experimentally and others yet to be—we are now ready to tackle the sequence of changes depicted in **A**,**B** and **C** of Figure 50.

To begin, let us suppose that in Figure 50**A**, all the backbone CO sites shown as O and all the backbone NH sites shown as H are respectively at the high c-value analogue and c′-value analogue of 2. In Figure 50**B**, the adsorption of the EWC, **W,** at the cardinal site withdraws electrons from the nearest neighboring O site, decreasing its c-value analogue from 2 to 1. This decrease of c-value analogue reverses its preference for a^+ over b^+ to b^+ over a^+. As a result, the a^+ originally occupying the O site is replaced by a b^+.

Now b^+ is a weaker electron-withdrawing agent than a^+. In consequence, the displacement of a^+ by b^+ releases electrons. Some of the elec-

trons released go back upstream toward the cardinal site-**W** couple enhancing its initial electron-withdrawing effect. Other electrons released go downstream to the nearest H site, causing a decrease of its positive charge and hence a fall of its c′-value analogue from 2 to 1. A reversal of its preference for a^- over b^- to b^- over a^- follows, leading to the displacement of the a^- by b^-. Now b^- is a weaker electron donator than a^-. In consequence, the displacement of b^- for a^- withdraws electrons. Some of the electrons withdrawn come from the O site upstream, further decreasing the c-value analogue of the O site upstream. Some of the electrons withdrawn come from the next O site downstream, lowering its c-value analogue from 2 to 1. A b^+ for a^+ exchange follows. And the cycle repeats itself until all the a^+ and a^- are replaced by b^+ and b^-, respectively, as shown in Figure50**C**.

In Figure 50 the side chains are not represented. Now we are in a position to deal with changes at the functional groups on short side chains also.

In Figure 50 we treated adsorption on the backbone O and H sites individually. In Figure 51, however, each pair of adsorbents a^+ and a^- in Figure 50 (adsorbed respectively on the CO and NH groups of a peptide group belonging to a single amino acid residue) is treated as a single entity and represented by a rectangular box **a**. Figure 51**A** then represents a protein segment before an electron-withdrawing cardinal adsorbent, or EWC (**W**) is introduced. In Figure 51**A** the electron withdrawing power of backbone cationic component (a^+) is represented as a downward arrow in the rectangular box, while the electron donating power of the anionic component (a^-) is represented as an upward arrow.

Figure 51**B** shows the same protein segment after an EWC, **W** occupies the cardinal site. In consequence, the sequence of events described under Figure 50 **A** to **C** takes place. As a result, box **b** (which stands for b^+ and b^- together) displaces box **a** (which stands for a^+ and a^- together). Since the O site or carbonyl group (CO) is highly polarizable but the H site or imino group (NH) is less so [14.1(3)], the electron withdrawing effect at the O site is strong and is represented as a long downward arrow in the **b** box. In contrast, the electron donating effect at the H site is weak and represented as a short upward arrow. The *net* effect of displacing box **a** by box **b** is an electron-withdrawing effect. Since this electron-withdrawing effect is repeated at each CONH peptide group, every functional group on a short side chain experiences an electron withdrawing influence as indicated by the downward arrows along each in Figure 51.

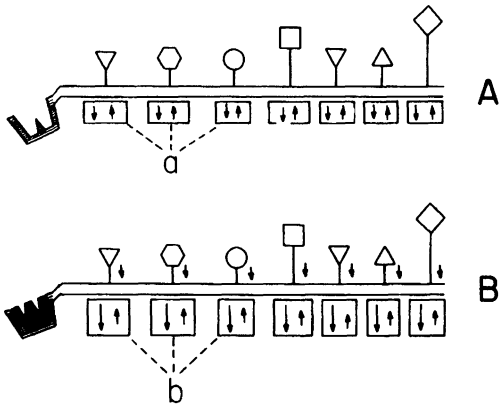

Figure 51. An alternative view of seeing the model of *controlled autocooperative transition* presented in Figure 50 with focus on the impact of the electron-withdrawing cardinal adsorbent (EWC) shown as **W**, on the various functional groups on short side chains (shown as triangles, hexagon, circle and squares). Displacement of the weaker (net-) electron-withdrawing adsorbents **a** (representing Figure 50's a^+ and a^- together here as a single *net-electron-donating* or *net-election-withdrawing* unit) on each peptide NHCO group shown in **A** by the stronger net-electron-withdrawing **b** (representing Figure 50's b^+ and b^- together here as a single net-electron-donating or net-electron-withdrawing unit) shown in **B** is initiated by the adsorption on the cardinal site of the electron-withdrawing cardinal adsorbent (**W**). This adsorption of **W** leads eventually to an across-the-board decrease of the electron density (shown by downward arrows) of all side-chain functional groups on short side chains. Length of the upward arrow in the rectangular boxes indicates strength of *electron-donating* effect; length of downward arrow in the rectangular boxes (and along the side chains) indicates strength of *electron-withdrawing* effect. For details, see text. (This is a new and different figure, though bearing certain similarities to another one first published in 1992 in Reference 107.)

Now, a substantial number of these functional groups on short side chains are β- and γ-carboxyl groups. A lowering of their electron density means a fall of their c-value (from an initial higher value). As a result, a monovalent cation A^+ may be displaced by B^+. This B^+ for A^+ exchange in turn reinforces the **b** for **a** exchanges at the backbone as well as the adsorption of the EWC at the cardinal site. Taken together, the backbone sites and the functional groups on short side chains and their respective adsorption partners (**a**/ A^+/ etc. *versus* **b**/ B^+/ etc.) constitute the alternative basic components of the autocooperative assembly diagrammatically illustrated in Figure 48B and formally in Equation A4 in Appendix 1.

In consequence of the interaction with the EWC, **W**, the backbone CO groups as well as the β- and γ-carboxyl groups (and other functional groups on short side chains) undergo an *across-the-board* electron-density *decrease* from their initial higher values. And exchanges of adsorption partners occur at both sets of sites. Conversely when an EDC adsorbs onto the cardinal site of a protein segment, the backbone CO groups as well as the β- and γ-carboxyl groups (and other functional groups on short side chains)

may undergo an across-the-board electron density *increase* from their initial lower values. And exchange of adsorption partners follow at both sets of sites in the reverse direction. *On account of this coherence, one may say that the whole "gang"* [98 p 97; 15 p 214; 107 p 144] *of cooperatively linked side-chain functional groups on short side chains and the gang of the backbone carbonyl groups behave separately or together as if they were a single site.*

By the same token, *action at a distance and one-on-many impact of a cardinal adsorbent are achieved.*

The physiological roles served by these coherent across-the-board electron density decreases or increases brought on by interaction with *one or more* drug(s) and other cardinal adsorbent(s) will be examined in the immediately-following subsection.

(7) Multiple control of single enzyme site or "gangs" of pharmacological "effector" sites

In this subsection, I shall discuss two specialized areas of cell physiology: enzymology and pharmacology. I shall demonstrate how the molecular mechanism of one-on-many and action at a distance presented above may fill in some critical gaps in our understanding in both fields.

Willy Kühne (1837–1900) introduced the name, enzyme, in 1878.[455] In 1902 V. Henri first suggested that the rate of enzyme activity depends on the concentration of the complex formed between the *substrate* and the *enzyme.*[445] Starting from the same assumption, Michaelis and Menten published in 1913 the essence of what was to be known as the Michaelis theory of enzyme activity.[446 pp 73–77]

The number of enzymes that have been studied is breathtaking. Thus Dixon and Webb, in their classic monograph on enzymes published in 1958, already listed no less than 123 identified, purified and crystallized enzymes.[446] More have been added since then. For each enzyme purified and yet-to-be purified, a routine study almost always includes the determination of a pH profile of the enzyme activity (see Figure 52B). Similar bell-shaped activity curves are also often observed with variation of the concentration of other ions like K^+ (Figure 52 C and E) and Mg^{++} (Figure 52 D). As far as I know, there had been no (electronic) explanation why H^+ as well as the other cations should affect enzyme activities in such a remarkable manner—other than the one offered by the AI Hypothesis.[98 chap 14; 458] I shall return to this subject shortly.

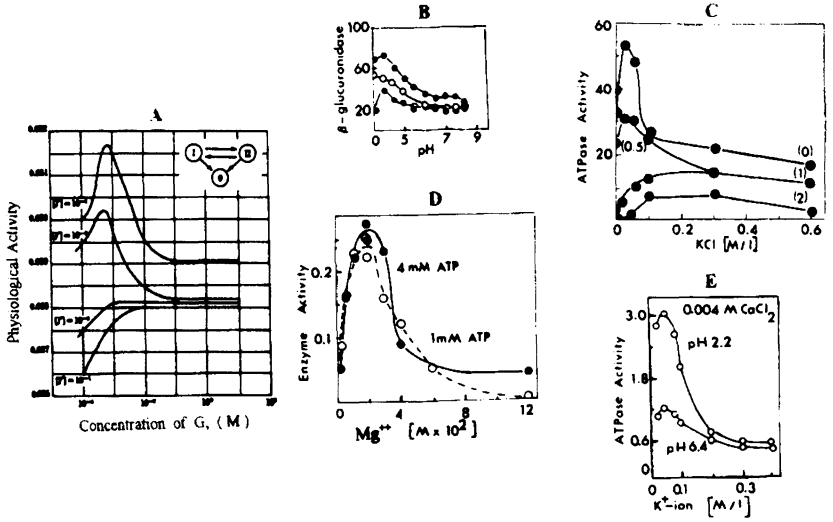

Figure 52. The influence of varying concentrations of two cardinal adsorbents on the activity of various enzymes. Experimental data published by different authors reproduced here to compare with theoretical models that produce similar pattern of interaction. (A) Theoretical model of two mutually-interacting cardinal sites and one enzyme site, interacting with two cardinal adsorbents **J'** and **G** at different concentrations. Concentrations of J' shown near curve (top to bottom): 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} M. (B) Activity of glucuronidase at different pH. Each curve is from a glucuronidase from a different kind of source animal.[451] (C) ATPase activity of myosin B in the presence of varying concentrations of KCl and UO_2Cl.[449] (D) Enzymatic synthesis of deoxycytidinephosphate in the presence of different concentrations of Mg^{2+} and ATP.[452] (E) Effect of pH and KCl concentration on the activity of myosin ATPase.[450] (From Ling[453] by permission of *Texas Reports on Biology and Medicine*)

In 1926, A. J. Clark introduced his *theory of drug action,*[447] adopting a similar principle to that advanced by Henri and Michaelis for enzyme activity. In Clark's theory, the physiological effect brought on by a drug is quantitatively related to the concentration of *drug-receptor complex.* Ariëns, van Rossum and their coworkers took Clark's theory further. They have successfully predicted complex patterns of physiological (or pharmacological) activities produced by one or more drug(s) based on *a priori* assumed changes in the "*affinity*" of the receptor for the drug and in the "*intrinsic activity,*" defined as the physiological activity per unit drug-receptor complex.[448]

Left unexplained are the why's and how's of these assumed changes. Yet the underlying control mechanism is vital to the ultimate understand-

ing of both enzymology and pharmacology as part and parcel of the physiology of the living cells. For after all, most of the enzymes are isolated from living cells and their physiological roles are highly coordinated and interacting events in their truly natural environment. Similarly, drugs do not act alone. Their actions are superposed on the coordinated activities of a team of cardinal adsorbents maintaining the resting living state. On the surface, the control by more than one cardinal adsorbent seems too complicated to contemplate. In fact, it is much simpler because all aspects of cell physiology are reduced to the fundamental properties and behaviors of a common living matter, the protoplasm.

Thus enzyme activity control by H^+, K^+, Mg^{++}, ATP as well as the control of muscle contraction by drugs are basically alike in reflecting the elemental electronic machine, which is the physical basis of life, with the difference that the enzyme site is usually a single isolated site. The pharmacological "effector site," though diagrammatically shown as a single site as in Figures 52 and 53, may in fact comprise a "gang" of sites that acts like a single site.

Figure 52A represents the theoretical influence different concentrations of two substances G and J' may exercise on a protoplasmic system carrying two receptor sites and one enzyme or pharmacological "effector site," each site mutually interacting with the other two sites. Note how the influence of the substance G, is profoundly modified by a second substance (J'), including the complete reversal in the direction of the action—duplicated in essence by real-world enzymes shown in Figure 52 B to E.

Similarly, Figure 53 B and C represent experimental data on the percentage of maximum contraction of frog rectus muscle in response to varying concentrations of two different drugs. In 53A the concentration of one drug, **J**, is given on the abscissa, and that of the second drug, **G**, is shown next to the theoretical dose-response curves, with the symbols a to f representing its increasing concentrations. The pattern of response of the living muscles again mirrors the prediction of the theoretical model based on the AI Hypothesis, containing one receptor site and one "effector site" as shown in Figure 53A.

Alluded to above, the "effector site" in the contraction of frog rectus muscle includes many contractile units (e.g., myofibrils, sarcomere), each of which, as a rule, must be separated from the drug receptor sites by a different distance. Thus, Clark's theory of drug action cannot readily answer the question of how the formation of a drug-effector complex can affect so

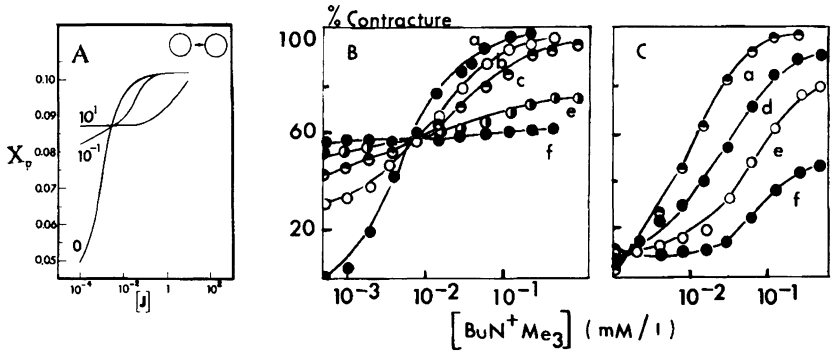

Figure 53. The influence of varying concentrations of two cardinal adsorbents on the intensity of contraction of frog rectus muscle. (A) Theoretical model of one receptor cardinal site and one effector cardinal site interacting with two drugs **J** and **G**. The concentrations of **G** are shown near each curve. The concentrations of **J** are shown on the absissa. (B) Ordinate shows intensity of contraction shown as percentage of maximum contraction of frog rectus muscle in the presence of varying concentrations of butyltrimethylammonium ion shown in the abscissa and the increasing concentrations (a to f) of decapyrrolinium ion shown near each curve. (C) Ordinate and abscissa same as in (B). The increasing concentrations (a to f) of the second drug used, decapiperidinium ion are shown along each curve. Data of van Rossum *et al.*[454] (From Ling[453] by permission of *Texas Reports on Biology and Medicine*)

many effector sites, some close, others far away. By demonstrating that the binding of one or more cardinal adsorbent(s) leads to *across-the-board* uniform change of near and far sites as if they were a single site, the AI Hypothesis offers a possible answer to this major unanswered question.

15

Physiological Activities: Electronic Mechanisms and Their Control by ATP, Drugs, Hormones and Other Cardinal Adsorbents

The membrane theory once gained world-wide acceptance for its success in explaining four major physiological manifestations of the living cells: solute distribution, permeability, swelling and shrinkage and resting potential. The demonstration that the cell membrane is in fact permeable to sucrose and Na^+ made the original membrane theory difficult to defend. In response, the sodium pump hypothesis was installed. In time, this subsidiary hypothesis also met serious contradictions, even harder to defend. And with it, the paradigm of cells as membrane-enclosed dilute solutions is, in my opinion, coming to an end.

In presenting the LFCH and the PM theory, we focused primarily on two basic cell physiological phenomena: selective K^+ adsorption and Na^+ exclusion on one hand; multilayer polarization and orientation of cell water on the other. Both are *static* manifestations of the resting living state. The first part of the present chapter offers new interpretations of the four classic static physiological manifestations mentioned above: solute distribution, permeability, swelling and shrinkage and resting potential in the light of the AI Hypothesis. We then examine how these physiological manifestations are controlled by ATP, drugs, hormones or other cardinal adsorbents through the operation of the electronic control mechanism described in the preceding chapter. In addition, I shall also tackle two *dynamic* cell physiological *activities*: the action potential and true active transport.

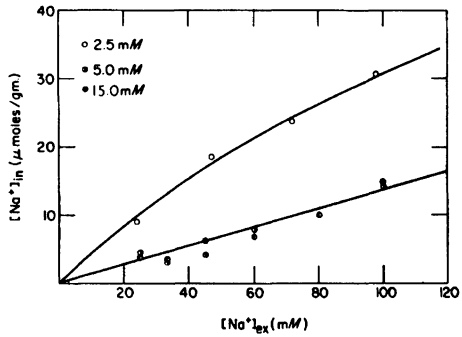

Figure 54. Equilibrium distribution of Na^+ in frog muscle cells at 0°C in the presence of 2.5, 5.0 or 15 mM of K^+ in the bathing medium after a correction was made for Na^+ trapped in the extracellular space of 10%. (Later work revised this figure to 9%, but no attempt was made to revise this graph, which is reproduced as it was published in 1969.) The ordinate is in units of μmoles/gram of fresh muscle cells. The straight line going through or near most experimental points at the two higher K^+ concentrations has a slope of 0.14. A q-value of 0.18 was obtained for Na^+ (mostly as chloride) by dividing 0.14 by the percentage of water in the muscle cells (80%). In this estimate, no correction was made for Na^+ associated with "connective tissue elements" (etc.). Using existing information on hand, I estimated later that the q-value of Na^+ (as chloride) in the water of frog muscle cells is probably between 0.14 and 0.18. 0.15 would be a good estimate. (Ling[173] from the *International Review of Cytology* by permission of Academic Press)

15.1 Selective solute distribution in living cells; cooperativity and control

In this section, we resume our discourse on the distribution of Na^+ and K^+ (and Mg^{++}) in frog muscle cells. More specifically, we focus attention on the influence of ATP on the state of cell water and K^+; and the influence of the cardiac glycoside, ouabain, on the accumulation of this and other alkali-metal ions in frog muscles.

After that, I shall discuss the accumulation in frog muscle cells of D-glucose and the free amino acid, glycine; and their respective control by the hormone, insulin. In addition, I shall also touch upon lactose accumulation in the bacteria, Escherichia coli (E. coli).

(1) K^+, Na^+ and Mg^{2+} accumulation in living cells

In a preceding section [10.2], I have devoted much space establishing the adsorbed state of cell K^+. We now turn our attention to Na^+ and Mg^{2+}, which so far have been kept on a back burner.

Figure 54 shows the equilibrium distribution of Na^+ in frog muscle at 0°C in the presence of a normal external K^+ concentration (2.5 mM) and higher ones.[173] The data show general obedience to the Troshin equation (Equation A1 in Appendix 1), which describes a free fraction of Na^+ in the cell water and another fraction of adsorbed Na^+.

After the adsorbed fraction of Na^+ is chased away by K^+ at the higher concentrations, a *straight-line* plot of the remaining Na^+ in the tissue water against external Na^+ concentration is obtained with a slope of 0.14. After corrections for Na^+ trapped in the extracelluar space and adsorbed on "connective tissue elements," one obtains a q-value of 0.15 for Na^+ (as chloride) in frog muscle cell water. The q-value for sucrose in frog muscle cell water at the same temperature (0°C) is, as seen in Figure 27, 0.13.[156 p 191]

While the distribution of (univalent) Na^+ in frog muscle depends strongly upon the concentration of (univalent) K^+, the distribution of (divalent) Mg^{2+} in both frog muscle and frog ovarian eggs is indifferent to the concentration of (univalent) K^+ as the studies of Ling, Walton and Ling have revealed.[502] Similarly, the distribution of K^+ is also indifferent to the concentration of Mg^{2+} in the bathing medium. This mutual indifference is in conflict with the membrane theory as expounded by Donnan [4.3] where the same Donnan ratio governs the distribution of all permeant ions[98 p 216; 15 p 28; 107 p 17] as well as the resting potential (Figure 4B). By the same token, it is in harmony with the AI Hypothesis, according to which there is no causal relationship between the resting potential and bulk phase ionic distribution, nor obligatory relationship between monovalent and divalent ion distribution as they adsorb in the cells on separate and different types of sites.

In frog muscle there are about 12 mmoles of Mg^{2+}-adsorbing sites with strong affinity for this ion—reaching full saturation at an external Mg^{2+} of 1 mM (or less) (1 mM is the lowest concentration we studied). The q-value of Mg^{2+} (as chloride) is 0.21 at 25°C. To a first approximation, the data can also be described by the Troshin equation.

The Troshin equation is a special case of Ling's general equation for solute distribution in living cells and model systems[156] (presented as Equation A3 in Appendix 1). In the theory, which the Troshin equation stands for, there is no *cooperative* interaction among the adsorption sites. In the AI Hypothesis, on the other hand, *cooperativity* among the β- and γ-carboxyl and other *proximal functional groups* is a universal feature. But it may be undetectable under some conditions.

Autocooperativity becomes detectable when the *nearest neighbor interaction energy* is much larger than zero ($-\gamma/2 >> 0$). $-\gamma/2$ is much larger than zero, when the alternative adsorbents have widely different adsorption energies (for details, see Reference 107 p 139–140), as in the case when frog muscles are in an environment containing a high concentration of Na^+

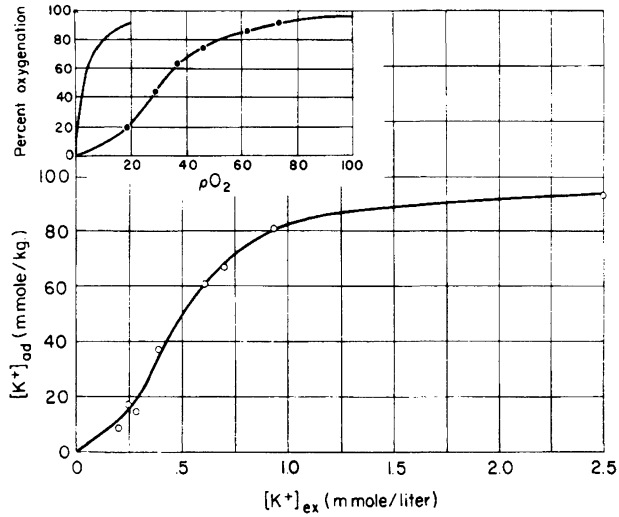

Figure 55. Equilibrium K^+ concentration in frog sartorius muscles at 25°C in Ringer's solution containing an unvarying high concentration of Na^+ concentration (100 mM) but varying (low) K^+ concentration (abscissa). The sigmoid profile resembles that of oxygen uptake by human erythrocytes shown in the inset as the line going through or near the filled circles. The solid line to the left in the inset shows oxygen uptake of isolated myoglobin. (Inset from Eastman *et al.*[440]) (Ling[439] by permission of *Federation Proceedings*)

and *very low* K^+ concentration. Autocooperativity is evident in the (sigmoid) uptake curve of K^+ in Figure 55, which resembles the oxygen uptake curve of human erythrocytes shown in the inset. In contrast, oxygen uptake of myoglobin—shown on the left of the inset— does not exhibit autocooperativity. Instead, it follows a "hyperbolic" uptake curve characteristic of Langmuir adsorption (with no discernible near-neighbor interaction).

Parenthetically, I may add that a *straight-line* distribution curve with a slope significantly lower than unity —like that shown for Na^+ in the presence of higher K^+ concentration in Figure 54, and that shown for Mg^{2+} above an external Mg^{2+} concentration of 1 mM, as well as those of the many larger nonelectrolytes shown in Figures 26 and 27—is *theoretically incompatible with a pump-mechanism.* An outward pumping mechanism needed to maintain a low intracellular concentration of a solute would inevitably predict not a straight line but a *concave-upward* distribution curve as a result of the increasing saturation of pumping sites with rising concentration of the solute being pumped, represented on the abscissa.[174]

(2) Control of solvency of cell water by ATP

Since the introduction of LFC, a major theme of the AI Hypothesis has been a quantitative relationship between the concentraion of ATP and the equilbrium concentration of cell K^+. This postulation has been repeatedly confirmed.

Verification of the postulated relationship between ATP and K^+ concentration has been achieved in a variety of living cells and their derivatives, including resealed red cell ghosts containing residual hemoglobin and other proteins[140] (Figure 33), (blood-bank) human red blood cells,[98 pp 253–254] Escherichia coli[98 p 255] and red blood cells of different mammals.[191] In all these cases, K^+ and Na^+ permeation rates are high so that equilibrium in ionic distribution is readily achieved. (However, the extremely slow permeability to K^+ (and Na^+) in *freshly drawn* human blood cells[465] can make equilibrium time so long that it becomes difficult, if not "impossible," to reach *in vitro* and hence to observe the predicted parallel changes of ATP and K^+ concentration[370].) The postulated relationship between ATP and K^+ concentration has been most extensively tested and confirmed on frog muscle,[107 pp 192–193] including data presented in Figures 17 and 19, as well as Figure 56. However, Figure 56 also contains data on the relationship between ATP and Na^+ concentration in the cells.

According to the polarized multilayer theory of solute distribution, the q-values of large solutes like (hydrated) Na^+ and sucrose increase with a decrease in the degree of water polarization and orientation [11.3(4)]. And that, within limits, the degree of water polarization/orientation varies inversely with the concentration of adsorbed ATP. In short, the theory also predicts that there should be an *inverse* relationship between the equilibrium concentration of Na^+ in the cell water and the concentration of ATP.

Figure 56 shows, once more, how the K^+ concentration in frog muscles declined when the muscles were exposed to a low concentration of the poison, sodium iodoacetate (IAA). As repeatedly noted above, IAA blocks carbohydrate metabolism, reduces the ATP concentration in the cells and eventually kills them. As the cells deteriorated, the change of K^+ concentration paralleled the fall in ATP concentration. In contrast, as the ATP concentration fell, the Na^+ concentration rose.

According to the AI Hypothesis, there are two different ways by which the level of cell Na^+ rises: (i) by taking over the β- and γ-carboxyl groups normally occupied by K^+; (ii) by weakening the polarization/orientation of the cell water, thereby raising the q-value of Na^+ in the cell water. A choice

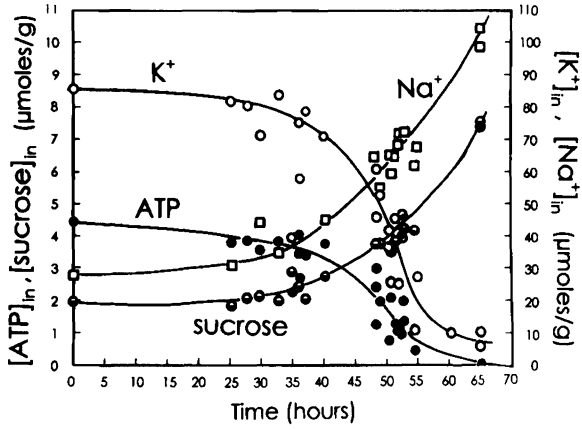

Figure 56. Time course of changes in the concentrations of K^+, Na^+, sucrose and ATP in frog muscles incubated in a Ringer's solution initially containing beside the usual components, 0.1 mM sodium iodoacetate and 10 mM labeled sucrose (0°C). Empty circle, K^+; empty squares, Na^+; solid circle, ATP; half-filled circle, sucrose. All concentrations are given in units of μmoles per gram of fresh muscle tissue weight. (Ling and Ochsenfeld, unpublished)

can be made between the two by introducing a second molecular probe like sucrose.

Figure 56 shows that sucrose, which does not carry a net positive charge and therefore does not compete for the negatively-charged β- and γ-carboxyl groups, nevertheless, follows a time course roughly in parallel with that of Na^+. Therefore, most of the Na^+ taken up in IAA-poisoned muscle must be in the cell water—as its polarization and orientation steadily weakens with progressive ATP depletion.

However, the parallelism, as a rule, is not exact. In work yet to be published, we have evidence that in the rise of Na^+ concentration due to spreading injury in a sartorius muscle EMOC preparation, a part of the rise of cell Na^+ concentration near the cut end is newly adsorbed—as revealed by the fact that this fraction can be chased away by high concentration of K^+ or Rb^+, for example (unpublished).

With the cause for the rise of Na^+ concentration in IAA-poisoned frog muscle roughly known, the *antiparallel* time course of change of the ATP concentration and the Na^+ (and sucrose) together with the *parallel* change of the ATP concentration and K^+ concentration affirm *the fundamental postulate of the AI Hypothesis that ATP controls both the selective accumulation of K^+ and the partial exclusion of Na^+* as depicted in Figure 44. But that is not all.

For we are now in a position to go one step deeper in answering two additional questions of critical relevance: (i) *How* does ATP control the selective K^+ adsorption over Na^+ on some 100 mmoles of β- and γ-carboxyl groups in each kilogram of fresh muscle ? (ii) *How* does ATP bring about a

change of the solvency for Na^+ in 44 moles of water molecules per kilogram of fresh frog muscle? It would seem that the preceding Chapter 14 has already provided the key pieces of information we need to answer these questions. And they are listed under four headings as follows:

(1) Theoretical computations show that K^+ is strongly preferred over Na^+ if the c-value of the β- and γ-carboxyl groups on myosin and other proteins are maintained at a low value [14.1(2)].

(2) Correlation established between the electron-donating strength of side chains and the propensity of the NHCO group of the amino acid residue carrying each of these side chains to engage in α-helical H-bonds leads to the conclusion that low c-value analogue of the backbone carbonyl groups favors multilayer water polarization/orientation. High c-value analogue favors the formation of α-helical H-bonds [14.1(3)].

(3) From fluorescence quenching studies of Chang and Penefsky[509] (Figure 49) and other evidence, we arrived at the conclusion that ATP acts as an electron-withdrawing cardinal adsorbent or EWC [14.3(4)].

(4) Based on evidence for the high *transmissivity* of inductive effect through short length of the polypeptide chains in addition to short segment of methylene groups (Figure 46), a theoretical model has been given showing that occupancy of a suitable cardinal site by an EWC, like ATP, could bring about an across-the-board uniform decrease of the c-value of β- and γ-carboxyl groups and a similar uniform across-the-board decrease of the c-value analogue of the backbone carbonyl groups [14.3(6)].

(1), (2), (3) and (4), when combined, lead to the conclusion that the adsorption of ATP creates an across-the-board uniform decrease of the c-value of β- and γ-carboxyl groups of myosin, and other muscle proteins, leading to the selective uptake of K^+ (over Na^+) on some 100 mmoles of β- and γ-carboxyl groups, which these proteins carry in one kilogram of muscle. The adsorption of ATP also creates an across-the-board uniform decrease of the c-value analogue of the backbone carbonyl oxygen atoms of cell proteins (possibly myosin, actin), resulting in the multilayer polarization and orientation of the 44 moles of bulk phase water found in one kilogram of muscle. A fall in the ATP concentration in response to IAA brings about a corresponding fall in the concentration of selectively adsorbed K^+

pari passu with a fall in the intensity of water polarization/orientation, hence the *exclusion factor*, U_{vp} [11.3(4)]. A corresponding rise of the concentration of Na^+ and sucrose should follow as their respective q-values rise. All these expected changes have been actually demonstrated and illustrated in Figure 56.

I have called ATP the *Queen of cardinal adsorbents* or *principal cardinal adsorbent*, because it holds a unique position in the maintenance of the resting living state (Figure 44). In the resting living state thus maintained, K^+ is selectively adsorbed and Na^+ and sucrose partially excluded in consequence of the polarization and orientation of the cell water—ever ready to shift to another modified living state when a second cardinal adsorbent is brought into the picture, as the next section will illustrate with a specific example, the cardiac glycoside, ouabain.

(3)The control of the rank order in alkali-metal ion adsorption by ouabain

Work described in [10.2] has demonstrated that the great majority of the alkali-metal ions in frog muscle cells is adsorbed on the β- and γ-carboxyl groups belonging mostly to myosin. Exposure of frog muscle cells to a pharmacologically low concentration of the cardiac glycoside, ouabain (3.26×10^{-7}M) changes the β- and γ-carboxyl groups in the muscle cells from preferring the alkali-metal ions in the rank order $Rb^+ > K^+ > Cs^+ > Li^+ > Na^+$ to preferring them in the rank order of $K^+ > Li^+ > Cs^+ > Rb^+ > Na^+$.[280] These observed rank-orders match respectively the ranks orders theoretically computed and shown in Figure 42 at the c-value of approximately –4.20 Å and –3.25 Å. Thus ouabain, which brings about such an across-the-board increase in the c-value of the β- and γ-carboxyl groups, must be an *electron-donating cardinal adsorbent* or EDC. However, in comparison with other changes to be described in sections below e.g., [15.6(2.3)], this c-value rise brought about by ouabain is a relatively modest one.

Nonetheless, the selectivity ratio of Na^+ over K^+ rose nearly 20-fold in response to 3.26×10^{-7} M ouabain, from 0.0037 ± 0.00017 (normal) to 0.48 ± 0.07 (ouabain).[280; 107 Fig 6.9, Fig 8.12] Figure 57 shows that the ouabain-induced changes in the concentration profiles of K^+ and Na^+ follow a sigmoid- or inverted sigmoid- shaped curve.[190] Here the points are experimental and the lines are theoretically calculated according to the Yang-Ling cooperative adsorption isotherm for a pair of competing ions with widely different adsorption energies (Equation A4 in Appendix 1).[190, 279, 280]

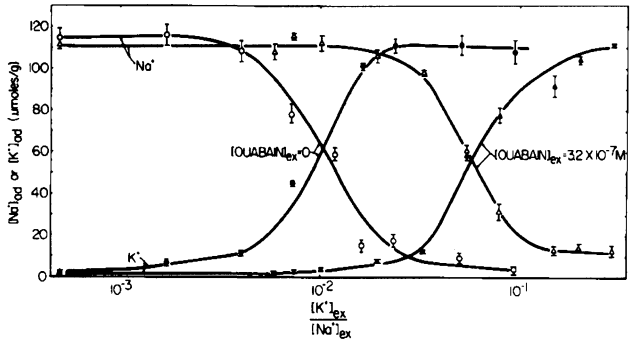

Figure 57. Effects of ouabain (3.26×10^{-7} M) and the relative concentration (or ratio) of external K^+ and Na^+ concentration, $[(K^+)_{ex}/(Na^+)_{ex}]$ (shown on the abscissa) on the equilibrium concentration of *adsorbed* K^+ and *adsorbed* Na^+ in frog sartorius muscle cells at 25°C. Ordinate represents the concentration of adsorbed K^+ or Na^+ in μmoles per one gram of fresh muscle cells. Points are experimental; the lines are theoretical according to Equation A6. In response to the ouabain treatment, the intrinsic equilibrium constant for the Na^+ to K^+ exchange ($K^{\infty}_{Na \to K}$) changed from 100 to 21.7 with little or no change in the nearest-neighbor interaction energy ($-\gamma/2$). In parallel studies reported in the same paper, no detectable change in the concentration of ATP in the muscles was shown for ouabain concentration lower than 10^{-6} M. At ouabain concentration above 10^{-6} M, ATP concentration decline was visible. (Ling and Bohr[190])

The same figure also shows how the abrupt or *all-or-none* change of K^+/Na^+ accumulation in response to ouabain can be duplicated by a *decrease* in the concentration of external K^+ from 2.5 mM to 0.5 mM. And the effect of 3.26×10^{-7} M ouabain can be completely annulled by an adequate *increase* in the concentration ratio of external K^+ over external Na^+.

Ouabain at the low concentration used (3.26×10^{-7} M) has no observable impact on the polarization-orientation of cell water—in agreement with the results of NMR studies of Ling and Cope[276] and of Ling and Bohr,[190] which have revealed that the extra Na^+ gained in response to low external K^+ or to ouabain is all in the adsorbed form and matching the amount of adsorbed K^+ it has displaced[107 p 175; 190 p 44; 15 p 361] (Table 5). What follows in smaller print is a more detailed account of the history and details of this finding. We will resume our main theme after this discursion.

In 1967 Freeman Cope demonstrated that 70% of the Na^+ in isolated frog muscle tissue is not (apparently) detectable by nuclear magnetic resonance (NMR) spectroscopy.[472] Adopting the theory of Jardetsky and Wertz—according to which, the NMR signal of adsorbed Na^+ is so broadened that it is not visible[473]—Cope concluded that 70% of frog muscle Na^+ "is bound." Ling and Cope then showed that frog muscle equilibrated in a low-K^+ Ringer's solution gained roughly equimolar concentrations of NMR-invisible Na^+,[276] just as Ling and Bohr showed later that the Na^+ gained in response to ouabain was also NMR invisible[190]—even though we were puzzled[190 p 444] by a small but significant increase of the *NMR-visible* fraction of Na^+ in both the K^+-depleted and ouabain-treated muscles (Table 5).

Table 5.

	Total K^+ (μmoles/g)	Total Na^+ (μmoles/g)	NMR-visible Na^+ (μmoles/g)	NMR-invisibleNa^+ (μmoles/g)	K^+ remaining + Na^+ gained (μmoles/g)
Control (3)	81.7 ± 2.04	32.2 ± 1.12	12.3 ± 0.72	20.0 ± 0.72	
K-depleted (13)	37.5 ± 3.00	74.1 ± 5.00	26.2 ± 1.53	49.2 ± 3.57	
Change	–44.2	+41.9	+13.7	+29.2	79.4
Center peak intensity			0.33		
Ouabain-treated (3)	43.6 ± 2.61	80.3 ± 0.93	25.8 ± 0.28	55.3 ± 0.98	
Change	–38.1	+48.1	+12.7	+35.3	91.7
Center peak intensity			0.26		

Concentrations of potassium and sodium ions in frog muscles in response to incubation in Ringer's solution containing a low K^+ concentration (0.2 to 0.5 mM) and in response to ouabain (10^{-7} M) at 25°C. Muscles used were mostly sartorius but also included semitendinosus and tibialis anticus longus. Control muscles were incubated sterilely for 3 to 4 days in a normal Ringer's solution containing 100 mM Na^+ and 2.5 mM K^+. Total K^+ and total Na^+ were analyzed by flame photometry from hot 0.1 N HCl extracts of the muscles. NMR-visible Na^+ was determined on 2 milliliter samples of the muscles in a Varian wide-line spectrometer by the method described by Cope.[472] *NMR-invisible* Na^+ concentrations were obtained by subtracting the *NMR-visible* fraction from the total Na^+ concentrations assayed by flame-photometry. More specifically, the visible and invisible fractions of Na^+ in the K^+-depleted muscle and in the ouabain-treated muscles listed were obtained after subtracting from the experimental muscles the corresponding Na^+ concentrations in the control muscles in order to reveal only the *NMR-visible* and *NMR-invisible* Na^+ that had replaced the cell K^+. This subtraction also ensures that the *free* Na^+ within the muscle cells and in the extracellular space does not contribute to the *NMR-visible fraction* of Na^+, which then accurately represents the *center peak* Na^+ concentration (see text). Dividing this concentration by the total (corrected) Na^+ yields the relative intensity of the central peak labeled simply as "center peak intensity." The last column represents the sum of the K^+ remaining (37.5, 43.6) and the Na^+ gained (41.9, 48.1). Number in parentheses in the first column represents the number of individual assays in data given as means ± S.E. (Data taken from Ling and Cope[276] and Ling and Bohr[190])

During a symposium sponsored by the New York Academy of Science and held in January 1972, H. J. C. Berendsen and H. T. Edzes brought forcefully to our attention an alternative theory of vanishing NMR Na^+ signals,[474] a theory which had evolved from the study of NMR spectra of solid crystals since the 1950's.[475 pp 233–248; 476; 477 pp 115–119]

Na^{23} has an atomic nucleus with non-spherical charge distribution. When placed in an asymmetrical electric field, its NMR resonance peak splits into two satellite peaks, which may be too broad to be seen (hence "invisible") and one sharp central peak, which remains visible and is located where it is expected to be located. The relative intensity of the central peak for nuclei with spin quantum number 3/2 like that of Na^{23} is 0.4. The phenomenon is referred to as *first order quadrupole broadening.*[475 p 237]

In solid crystals, imperfections of crystalline structure or impurities produce the kind of asymmetrical field gradients that lead to first order quadrupole broadening. However, when the strength of the quadrupole interactions becomes very strong, the central line may be af-

fected also—in what is described as a *second order quadrupole broadening.*[475 p 246] As a result, the intensity of the central resonance line may become reduced. Thus in the copper alloy containing zinc or silver, the intensity of the central peak of the copper spectrum declines from 0.4 to as low as 0.01, when the zinc or silver concentration approaches 20%.[475 p 248]

With this basic information on hand, we return to Table 5. By subtracting the NMR visible and invisible fraction of the *control* muscle from the respective fraction in the K^+-depleted or ouabain treated muscles, one obtains NMR-invisible and visible spectrum of the Na^+, which has displaced the K^+ in the treated muscles. Since this subtraction eliminates the signal from the free Na^+ in the cell and that in the extracellular space, the NMR-visible Na^+ can only be that representing the central peak of the split signal. Note that the strength of the central peak is not 0.4 as expected from first order quadrupole broadening but are respectively 0.33 and 0.26. This lowering could be the result of more intense quadrupole interaction, which has apparently brought into action the second order quadrupole splitting.

Berendsen and Edzes questioned the validity of our idea that cell Na^+ in normal muscles and in K^+-depleted muscles can be in part adsorbed and in part free. In support of their position, they showed that Na^+ in sulfonate ion exchange resin (Dowex 50), in some rotten and smelly rat muscle as well as in healthy living frog muscle all showed similar NMR Na^+ signals including quadrupole broadening. They then argued that all that is needed are the presence of some fixed anionic sites and a (postulated) diffuse electrical field gradient over a distance of some 100 Å. A homogeneous population of free Na^+ would produce the kind of data Cope and Ling have reported and shown in Table 5. On this, I disagree. (For rebuttal of their additional comments on the T_1 and T_2 of Na^+, see Reference 15 pp 257–269.)

(1) Berendsen and Edzes assumed that their smelly dead muscles do not adsorb Na^+. There is no supporting evidence for this assumption. In fact, existing data point to the opposite, as noted in subsection (ii) immediately above. Thus, days after the cutting of the frog muscle in an EMOC preparation, the (dead or nearly dead) cut end of the muscle cells contained a considerable amount of Na^+ in the adsorbed state (Figures 8 and 17), which can be "chased away" from their adsorption sites by high concentrations of competing K^+ or Rb^+.

(2) Berendsen and Edzes were apparently unaware of the theory and supporting experimental evidence that *site fixation enhances the association of counterions* [10.1(1)]. Instead they assumed that all the Na^+ in the sulfonate exchange resin are parts of a homogeneous population of *free* counterions. Again this was just an off-hand postulation—though one in harmony with the membrane-pump theory and also the theory of ion exchange resin of H. Gregor.[127] Again existing evidence points to the opposite.

Ion exchange resin, Dowex 50, is a cross-linked polystyrene sulfonate containing 40% to 50% of the solid polymer. A simpler model of Dowex 50 is a water solution of the non-cross-linked linear polymer, polystyrene sulfonate. In such a solution, one can easily measure the concentration of free Na^+ with a Na^+-specific glass electrode. By subtracting the free Na^+ concentration thus found from the total Na^+ concentration in the solution, one obtains the adsorbed Na^+. Results of such a study was published by Ling and Zhang in 1983.[478] The fraction of adsorbed Na^+ rises sharply from a little under 30%, when the polymer concentration was only 1%, to 80% when the polymer concentration reaches 5 %. Since increasing cross-linking agents and fixed-ionic-site density reduces the entropy of dissociation of the counterions and thus enhances counterion association, the result from the Na^+ adsorption on

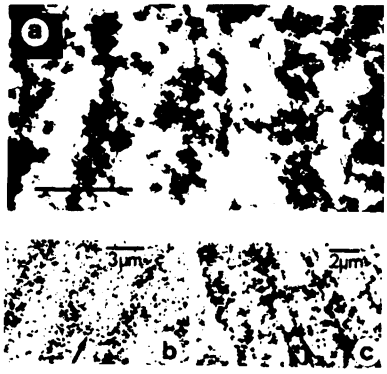

Figure 58. (a) electron microscopic ^{22}Na autoradiogram of a stretched single muscle fiber isolated from a sartorius muscle, which had been equilibrated in a modified Ringer's solution with a low ($[K^+]/[$labeled $Na^+]$) concentration ratio of 5×10^{-3} (see Figure 57). The bulk of the cell K^+ of the muscle fiber has been replaced by ^{22}Na-labeled Na^+. (b) and (c), similar electron microscopic radioautograms of ^{134}Cs- and ^{86}Rb-loaded muscle fibers respectively. Arrow in b indicates line of silver granules from radioactive Cs^+ located in the Z-line. (Edelmann 1980a,[126] from Histochemistry by permission of Springer Verlag; Edelmann 1986[126] by permission of Scanning Microscopy International, Inc.)

non-cross-linked polystyrene sulfonate demonstrates that the greatest part of the Na^+ in the Dowex 50 and other exchange resins must be adsorbed.

(3) Berendsen and Edzes postulated a 100 Å electric gradient within living cells and Dowex 50. Again they gave no supportive evidence. Indeed, existing knowledge argues against the existence of such a 100 Å electric field gradient. In addition, this postulation is superfluous. In presenting the theory of quadrupole line broadening, physicists Brand and Speakman cited the non-uniform electric field created by the simplest of systems, i.e., that of a single point charge.[477 p 115] Lindblom recognized that an anionic binding site for Na^{23} generates the non-uniform electric field around the Na^{23} nucleus for the observed quodrupole splitting of a lecithin-sodium-cholate-water system.[479]

Dowex 50 contains roughly 2 moles of fixed sulfonate groups per liter of resin, yielding an average distance of 7.4 Å between the nearest neighboring anionic charges.[478 p 257] In frog muscle, myosin alone provides some 150 mM of β- and γ-carboxyl groups per liter of cell, yielding an average of 22 Å distance between the nearest neighboring anionic charges. The non-uniform electric field around each of the fixed anion is able to produce the quadrupole broadening as observed.

To leave no doubt that the Na^+ gained by frog muscle cells in response to low external K^+/Na^+ concentration ratio (as well as the Na^+gained in response to ouabain), I reproduce an electron microscopic ^{22}Na autoradiogram of frog muscle fiber (Frame a in Figure 58), in which much of the cell K^+ has been replaced by ^{22}Na-labeled Na^+ during prior incubation in a bathing solution with a K^+/Na^+ concentration ratio equal to 5×10^{-3} (see Figure 57). Note that like the radioautograms of Cs^+-, and Rb^+-loaded muscle fibers shown in frames b and c respectively—as well as in Figures 15, 16 and 18 shown earlier—the silver grains are also lined-up in rows, indicating that the underlying radioactive ^{22}Na are not evenly distributed in the muscle fiber but are also adsorbed onto the A-bands. Berendson and Edzes's claim that Na^+ gained in response to K^+ depletion is free, has thus been proven wrong from another set of independent observations.

In the preceding subsection before the small-print excursion, we have shown how ATP adsorption on cardinal sites brings about an across-the-board decrease of the c-value of the β- and γ-carboxyl groups *pari passu*

with an across-the-board decrease of the c-value analogue of the backbone carbonyl oxygen. Thus, ATP depletion led to the loss of adsorbed K^+ and the depolarization of the bulk-phase cell water. We now find that ouabain also brings about a loss of adsorbed K^+ but here is where the resemblance ends. The Na^+, which displaces the lost K^+ is not free but all adsorbed. Thus, there are no noteworthy changes in the physical state of the cell water, which would be reflected in a change in the concentration of free Na^+.

Now in the theoretical model presented in Figures 50 and 51, the change in the c-value of the β- and γ-carboxyl groups and that in the c-value analogue of the backbone carbonyl groups as a rule go hand-in-hand. The ouabain experiment, on the other hand, shows a c-value change in the β- and γ-carboxyl groups without an accompanying change in the state of the bulk-phase cell water. Nor is this unilateral response limited to ouabain. Other cardinal adsorbents like insulin (see subsection (4) following) and Ca^{++} act in a similar manner.[107 p 172, 177] Indeed, within our admittedly-limited knowledge, only during transient physiological activity {e.g., an action potential [15.6(2)]} or lasting ATP depletion [15.1(2)] have I seen concomitant changes in ion adsorption and water depolarization.

However, there is a difference between the two. The depolarization-disorientation from ATP depletion covers all the cell water; that occurring during an action potential involves only a tiny part of the cell water. As I shall make clear next, size difference may also offer a clue to understanding why ouabain does not cause detectible change in the cell water, while ATP depletion does.

To stay alive, the cell must maintain its bulk-phase water polarized and oriented.[107 pp 177–178] To achieve this aim, I suggested that throughout each living cell, there is a pervasive *matrix-protein system,* which keeps the bulk-phase cell water polarized and oriented. In contrast, during the ouabain-induced K^+ to Na^+ exchange, the accompanying water polarization-depolarization would be confined to the small number of water molecules in direct contact with the protein involved, namely myosin—which carries some 80% of the K^+ (Na^+)-adsorbing β- and γ-carboxyl groups.[122] Two more relevant points are worth mentioning:

(i) Actin, whose ubiquitous and pervasive presence in living cells is well known,[544; 545] may be the major matrix protein in many, if not all cell types. Since there is no free water in the muscle cell [11.2.], yet, as much as 30% to 50% of the muscle cell volume belongs to the I bands,[180 (1990) Figs.2.21CA, 11.17A, 1118A] the responsibility of keeping the water in this part of the

muscle cell polarized and oriented, must fall on the major protein of the I band: actin.

(ii) To maintain the matrix protein(s) at the fully-extended water-polarizing state, ATP is required. One expects that in the slow time scale of the experiments shown in Figure 56 (hours), at any time the ATP concentration throughout the muscle cell is in equilibrium. If true, this would explain why the total K^+ concentration decline in frog muscle shown in Figure 56 goes *pari passu* with water depolarization even though they may occur largely on different proteins, myosin and actin respectively.

(4) Control of D-glucose and glycine distribution by insulin

At 0°C, the metabolism of D-glucose in the voluntary muscle of North American leopard frogs is lowered to beyond detection.[427] The suspension of metabolic change of D-glucose made possible the study of its *equilibrium distribution* without interference from metabolic degradation.

The equilibrium D-glucose concentrations in muscle freed of endogenous insulin (by prior washing at 25°C), when plotted against the external concentrations, yield a straight line (bottom part of Figure 59**A**).[428] This rectilinearness indicates that D-glucose in these washed muscles is virtually all in the cell water (see also Figures 26 and 27).

Exposure to insulin increases D-glucose uptake at 0°C—only if the muscles had been *pre-incubated* at a higher temperature (e.g., 25°C) in a Ringer's solution containing both insulin (e.g., 0.1 unit/ml) and a *primer* (e.g., 24 mM non-labeled D-glucose or D-xylose). Subsequent incubation at 0°C in solutions containing different concentrations of labeled D-glucose (with or without competing non-labeled D-glucose), leads to the accumulation of an *additional* fraction of labeled D-glucose (top three curves of Figure 59 **A**). Figure 59 **B** presents double reciprocal plots of the labeled D-glucose in the cells after the free fraction has been subtracted. The converging double reciprocal plots are, of course, characteristic of Langmuir adsorption isotherms and similar to those shown earlier for adsorbed K^+ and Cs^+ (Figures 12 and 13). And they suggest that the extra D-glucose taken up in response to insulin is adsorbed and thus different from the free D-glucose in insulin-free muscle cells.

We suggested that insulin acts as a *cardinal adsorbent*, bringing about an *autocooperative* conformation change of some intracellular protein(s).[423] As such, it can occur readily only at a higher temperature and in the presence of a high-enough concentration of an effective *primer. With*

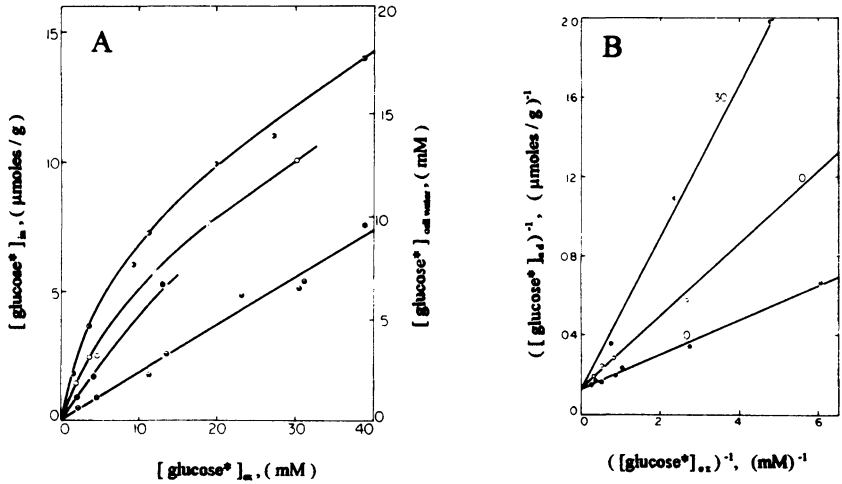

Figure 59. The equilibrium concentrations of labeled D-glucose in insulin-treated frog muscles and muscles washed in insulin-free medium (**A**). Mixed intact small frog muscles were incubated for 6 hours at 25°C with agitation in a Ringer's solution containing no D-glucose and no insulin (bottom curve) or 24 mM D-glucose and 0.1 unit/ml of insulin (top 3 curves). They were then incubated overnight at 0°C in Ringer's solutions containing different concentrations of labeled D-glucose and a fixed concentration of non-labeled D-glucose (topmost curve, no non-labeled D-glucose; 10 mM non-labeled D-glucose for the curve immediately below that one, and 30 mM non-labeled D-glucose for the next lower curve) before they were heated in 0.1 N HCl in a boiling water bath for 20 min. Aliquots of the extracts were then analyzed for radioactivity in a β-scintillation counter. (**B**). The amounts of labeled D-glucose taken up by the insulin-free muscles at each external D-glucose concentration were estimated from the smoothed bottom curve of A. These amounts were then subtracted from each of the D-glucose uptake curves of *insulin-treated* muscle shown in the upper 3 curves of A. The remaining concentrations were plotted in **B** reciprocally against the reciprocals of the equilibrium external concentrations according to Equation A11 in Appendix 1. The convergence of the three curves on a single locus on the ordinate and the divergent slopes in muscles exposed to different concentrations of competing non-labeled D-glucose affirm that the adsorption is of the Langmuir type. The rectilinear bottom curve indicates that in the absence of insulin, all or virtually all D-glucose remains as free D-glucose in the cell water with a q-value of 0.25 ± 0.01 (s.e.) (from 11 sets of data). The total concentration of D-glucose adsorbing sites in the insulin-treated muscles obtained ranged from 7.7 to 12.5 μmoles/g of fresh muscle. The mean and standard error for the D-glucose adsorption constants from 8 sets of data is 124 ± 6.6 (moles/kg).$^{-1}$ (From Ling and Will[428])

insulin at the controlling cardinal site, the primer adsorbs onto hitherto "masked" sites. Subsequent incubation at 0° C merely brings about an exchange of the labeled D-glucose for the adsorbed primer.[15 pp 361–367] Of the 41 sugars and sugar alcohols tested, only 7 are effective primers, including D-galactose, D-fucose and D-xylose. They all share some features of the D-glucose molecule, including an upward oriented OH group on C-3.[429]

Before concluding this section, I shall take two short excursions: one on the accumulation of glycine in frog muscle and another on the accumulation of lactose in a different kind of cell, E. coli.

In 1973, Neville described the accumulation of radioactively labeled glycine in isolated frog muscle at 0°C.[331] Interestingly enough, the accumulation of glycine bears a striking resemblance to the accumulation of D-glucose described above. Thus, exhaustively washed frog muscles accumulate glycine rectilinearly with a q-value of about 0.3. Preincubation at 25°C in the presence of 0.1 unit/ml of insulin and a primer (e.g., non-labeled glycine[542 p 555]) followed by incubation at 0°C in the presence of labeled glycine led to the additional uptake of more (adsorbed) glycine.

When wild type E. coli is incubated in a glucose-free medium containing lactose, the microbes acquire the ability to accumulate and metabolize lactose. Initially, lactose acts as an *inducer,* which activates the *set of three* genes designated as a *lac operon.*[456] Of these, the y-gene specifies the production of a protein named *lactose permease*, which in fact is an alias of a (postulated) outward *lactose pump*. The serious difficulty confronting the membrane pump concept in general for unifacial cells (including E. coli) (Chapter 12) calls for an alternative construct.

In the new interpretation, lactose permease is an insulin-like *cardinal adsorbent* or hormone.[480; 15 p 371] As such, it too can cause the conformation change of some intracellular protein(s), thereby making available hitherto "masked" adsorption sites for lactose and other galactosides. There is some supportive evidence for this model.

If the lactose *permease* is truly a *pump*, it must reside in the cell membrane. Located anywhere else, it cannot transport the sugar from the external medium into the microbial cells. On the other hand, if lactose is an insulin-like hormone, it can serve its role anywhere in the cell. Kolber and Stein could not find lactose permease in the cell membrane fraction from E. coli homogenate. Instead, they found it only in the cytoplasmic fraction.[430 p 694; 15 p 374]

15.2 The control of ion permeability

In [13.2] the *influx* of K^+, Na^+ and other alkali-metal ions into frog muscle cells was analyzed on the basis of a theoretical model of cell surfaces containing fixed anionic sites (β- and γ-carboxyl groups) and two modes of inward permeation: saltatory route and adsorption-desorption route. In the

present section, I shall discuss both ionic influxes and effluxes and their control by ATP and ouabain. However, as a key to understanding the different kind of data presented here and earlier in [13.2], I would like to point out that a major difference exists between influx and efflux studies. In influx studies the inward-migrating ion comes from a uniform population of free ions in the bathing solution. In contrast, in an efflux study the outward migrating ion from the inside the cells may include both free ions in the cell water and adsorbed ions on cell proteins.

(1) Na^+ efflux in normal and IAA-poisoned muscle cells

Not long after the availability of radioactive Na isotopes, Levi and Ussing conducted the first authentic study of the Na^+ efflux from isolated frog sartorius muscles.[268] They washed a muscle loaded with radioactive ^{24}Na in a continual stream of non-radioactive Ringer's solution, while monitoring the remaining radioactivity in the muscle. By plotting the logarithm of the remaining (labeled) Na^+ concentration in the muscle against the time of washing, t, they showed that the efflux curve obtained could be resolved into two straight-line fractions, one fast and one slow (which in appearance resemble those shown in Figure 60 below).

Levi and Ussing then made what seemed to be a highly reasonable assumption. They attributed the slow fraction to labeled Na^+ coming from within the cells, and the fast fraction to labeled Na^+ coming from the extracellular space. They noted, however, that the size of the fast fraction (average 30%) is much larger than the size of the extracellular space widely accepted then (13%)[44] (most up-to-date estimate even lower at 9.2%, see Chapter 9). One way to get rid of the interfering labeled Na^+ in the extracellular space is to study the Na^+ efflux of isolated *single* muscle fibers, which have no extracellular space. (The thin film of isotope-containing surface fluid is washed away instantly.)

Hodgkin and Horowicz were the first to do just that.[489] They showed in 1959 with a Geiger-counter setup that the Na^+ efflux of single muscle fibers, now unencumbered by trapped labeled Na^+ in extracellular space, neatly follows a single exponential time course, apparently confirming Levi and Ussing's original assignment that it is the slow fraction, which represents the intra-, extra-cellular exchange. However, Hodgkin and Horowitz cautiously pointed out that their technique—in which only a portion of the radioactivity emanating from the muscle is "seen" by the sensing element located a distance away—did not permit the first reading to be taken

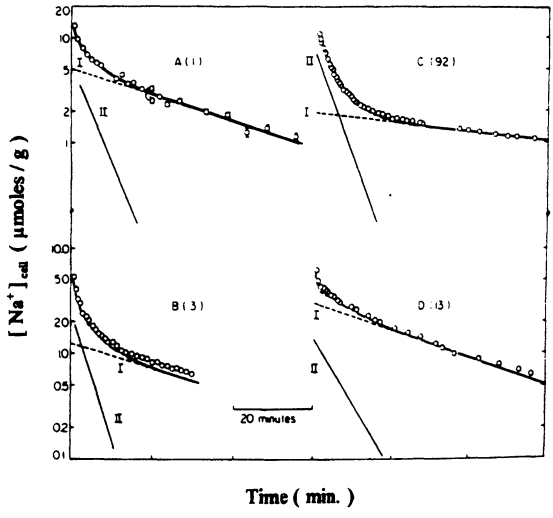

Figure 60. Time course of labeled Na^+ efflux from a single muscle fiber and from 3 small multiple muscle fiber bundles. Number of fiber(s) in each preparation is indicated on the Figure. All fibers were washed in normal Ringer's phosphate at 25°C except B, which was washed at 0°C. Experimental points are in units of μmoles per gram of fresh tissue weight. Correction for a 5% connective tissue element contribution was made on the basis of a composite curve of ^{22}Na efflux from similarly incubated connective tissues from four frogs. (Ling[517])

until 10 minutes after washing began and that afterwards, they could take reading only at 5 minutes intervals.

At that time, the technology of radioactivity measurements was rapidly moving forward. Indeed, three years before Hodgkin and Horowicz's paper appeared in print, Ling and Schmolinske had reported much greater efficiency provided by a well-type γ-scintillation counter[490] (for illustration of setup, see Reference 49 p. 174 or Reference 222 p. 785). With this new technique, I also studied the Na^+ efflux of isolated single muscle fibers (and small muscle fiber bundles), only here the first reading could be taken seconds after washing began, and thereafter at intervals of a minute or less as illustrated in Figure 60.[517]

The efflux curves from single and multifiber bundles thus obtained are just like those from whole sartorius muscles originally reported by Levi and Ussing. As demonstrated in Figure 60, these efflux curves from single and multiple fiber preparation can also be resolved into two fractions (I and II), even though there is no extracellular space to speak of here. To reconcile my findings with those of Hodgkin and Horowicz, I found that if I take away from my curves, like those shown in Figure 60, all experimental points except that taken at 10 minute and thereafter, those at 5 minute intervals, the remaining points can also be nicely fitted with a single straight line (in a semi-logarithmic plot). Therefore, Hodgkin and Horowicz's con-

clusion that the Na^+ efflux follows a single exponential time course is not warranted.[517; 131 p 18]

My study of Na^+ efflux of single and multiple muscle fibers also revealed the reason why Levi and Ussing found the fast fraction from whole sartorius muscles much larger than the size of the extracellular space. What they saw as the fast fraction in fact has two sources: one from the extracellular space, one from within the cells. And it is *the rate of exchange of this fast Na^+ fraction from the cell that reflects the true Na^+ permeability*. Unfortunately, this is the baby that got thrown out with the wash in almost all the work reported in this field except ours.

The rate of the true efflux is one full order of magnitude higher than the literature value obtained (wrongly) from the slope of the slow fraction. The correct *half time of exchange* ($t_{1/2}$) at 25°C is 3.3 ± 0.21 minutes from 21 independent determinations (in contrast to Levi and Ussing's original average $t_{1/2}$ obtained from the slow fraction at 20°C: 34 minutes[268]). Other evidence indicates that the slow fraction represents adsorbed Na^+ and its rate is limited by the rate of desorption.[549]

In the preceding section we have shown how the q-value of Na^+ (Cl^-) rises with decreasing ATP concentration until it reaches unity at zero ATP concentration. Now, a very slow decline of the ATP concentration in frog muscle can be produced by exposing the muscle to a low concentration of sodium iodoacetate (e.g., 0.2 mM IAA). Thus, if the conventional belief that the slow fraction represents intra-, extra-cellular exchange is correct, exposure of frog muscle to 0.2 mM IAA (interspersed by repeated reloading with labeled Na^+) ought to produce a gradual increase in the size of the slow fraction, until at the cell's death, the total concentration of labeled Na^+ in the slow fraction matches that in the loading solution. On the other hand, if the fast fraction represents intra-, extra-cellular exchange, then the fast fraction would become larger and larger until it matches the concentration of labeled Na^+ in the loading solution as the cells die.

Figure 61 shows that it is the fast fraction of labeled Na^+, which increases in size with lengthening exposure to 0.2 mM IAA, until its concentration equals that in the bathing medium, i.e., 100 mM.[49; 307] The two studies described respectively in Figures 60 and 61, in a mutually supportive manner, confirm that it is the fast fraction that represents the true intracellular-extracellular Na^+ exchange.

Earlier I have shown how exposure to 0.2 mM IAA brings about a gradual decline in the concentration of ATP in the poisoned muscles (Figure

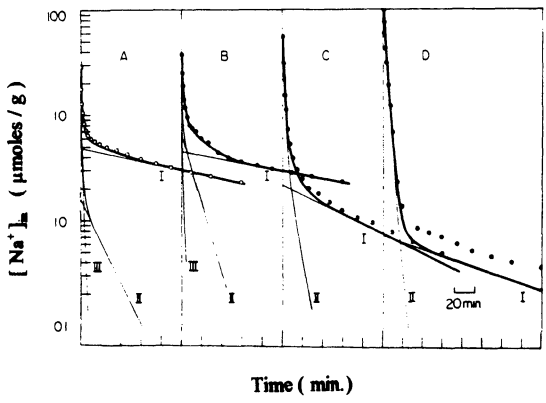

Figure 61. Successive efflux curves of labeled Na^+ in a dying frog muscle. A sartorius muscle was repeatedly loaded with labeled Na^+ and repeatedly washed in a Ringer's solution containing 0.05 mM sodium iodoacetate (IAA). Curve A was the first washing curve, curve B, the next and so on. Heavy solid lines are the best-fitting curve to the experimental points after subtracting the contribution from *connective tissue elements*, similarly loaded with labeled Na^+ and exposed to IAA. These corrected curves were then resolved into a slow fraction (I) and a fast fraction (II) [and occasionally even a third fast fraction (III)]. Note that the slow fraction remained unchanged in magnitude—as indicated by its zero time intercept on the ordinate (A, B)—until it falls to even lower values (C, D). It is the fast fraction (II or II+III), which rose steadily with the duration of exposure to the poison IAA, until it reached the concentration of radioactively labeled Na^+ in the loading solution (100 mM). (Ling *et al.*[307] from *The Journal of Cellular Physiology* by permission of John Wiley & Sons, Inc.)

56). Thus, if the cell surface water, like that in the bulk phase cytoplasm, also depolarizes and desorbs with the fall of ATP concentration as shown in [15.1(2)], then the *rate of efflux* of Na^+ as indicated by the slope of the fast fraction (fraction II) should become steeper with increasing exposure to the poison. The data of Figure 61 show that a gradual steepening in the rate of Na^+ efflux does indeed occur. This observation is also in keeping with the conclusion from influx studies that about half of the exchange goes by the saltatory route through polarized water (Figure 37) and that the intensity of multilayer polarization of the cell surface water, like that in the bulk-phase water (Figure 56), also declines with progressive ATP depletion at the cell surface.

(2) The influence of ouabain on the rank order of the strengths of alkali-metal ion adsorption on cell surface anionic sites

Ling and Fu exposed clusters of a few frog ovarian eggs for exactly 13 minutes (25°C) to radioactive ^{134}Cs-labeled Cs^+ (2 mM) in a Ringer's solution containing in addition (i) either 20 mM of competing NaCl or RbCl; and (ii) either 10^{-6} M ouabain or no ouabain.[398] After the 13-minute exposure, the eggs were washed in successive portions of a non-labeled normal Ringer's solution. The time course of decline of the labeled Cs^+ concentration

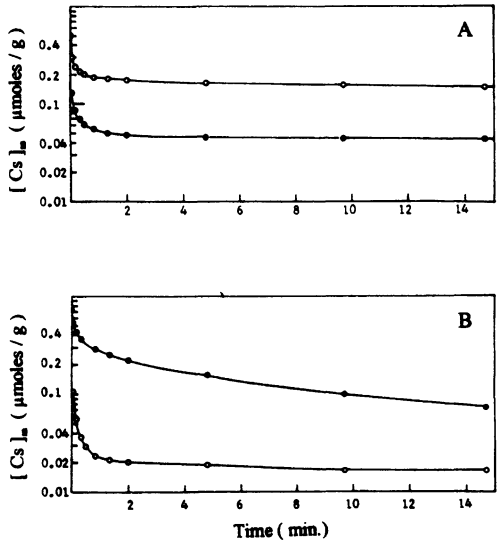

Figure 62. Efflux curves of labeled Cs^+ from small clusters of frog ovarian eggs. Clusters of frog ovarian eggs were incubated at 25°C for 4½ hours in a modified Ringer's solution with or without 10^{-6} M of ouabain. Blotted dry, the egg clusters were incubated at 25°C for exactly 13 minutes in incubation solution containing 2.0 mM tagged Cs^+ and 20 mM of competing non-labeled alkali-metal ions. Again blotted dry, the egg clusters were washed in successive portions of non-labeled Ringer's solution. The radioactivity of labeled Cs^+ detected in the successive portions of washing solution plus the residual labeled Cs^+ in the egg at the end of the experiment, allow the reconstruction of the efflux curves shown. Control eggs (empty circle); ouabain-treated eggs (solid circles). A, competing ion, Na^+. B, competing ion, Rb^+. (Ling and Fu[398])

in the egg clusters was monitored and plotted against the duration of washing in Figure 62. For the limited discussion here, our focus is on the initial labeled Cs^+-ion concentration at washing time 0, which represents the concentration of labeled Cs^+-ion that has entered the cells after the 13-minute-long exposure, i.e., the influx rate.

In Figure 62A, where the competing ion is 20 mM Na^+, the initial concentration of labeled Cs^+ in the control run without ouabain (empty circles) is higher than the initial concentration in the (control) run, where the competing ion is 20 mM Rb^+ (empty circles in B). The stronger blocking of Cs^+ uptake (by the adsorption-desorption route) offered by Rb^+ than Na^+ is in harmony with the rank order of equilibrium distribution of these ions found in normal resting frog muscle $Rb^+ > K^+ > Cs^+ > Li^+ > Na^+$ as pointed out in the preceding subsection. And both are in harmony with the estimate that

the normal muscle cytoplasmic, as well as normal egg-surface β- and γ-carboxyl groups, exhibits a low c-value of about –4.20 Å.

After exposure to ouabain, the picture has changed sharply. The uptake of labeled Cs^+—as indicated by the initial point of the efflux curve of solid circles in A—in eggs exposed to 20 mM of competing Na^+ sharply dropped. This points to a large increase in the effectiveness of Na^+ in blocking the entry of labeled Cs^+ entry into the egg cells. In contrast, exactly the opposite occurred in B, where the competing ion is 20 mM Rb^+. Here, exposure to ouabain sharply *increased* the uptake of labeled Cs^+, indicating that the powerful blocking action offered by Rb^+ in normal eggs is no longer so powerful.

The ouabain-induced increase in effectiveness of Na^+ concomitant with a decrease in the effectiveness of Rb^+ are both in harmony with the ouabain-induced rank order change in the adsorption energy of (frog muscle cytoplasmic) β- and γ-carboxyl groups from the one cited above to that of $K^+ > Li^+ > Cs^+ > Rb^+ > Na^+$. And both sets of data are in agreement with the rough estimate that the c-value of the β- and γ-carboxyl groups in both the muscle cytoplasm and egg surface have risen from about –4.20 Å to about –3.25Å in response to ouabain acting as an EDC.

15.3 Salt ion-induced swelling of normal and injured cells

(1) Cell swelling in isotonic KCl

In reference to the phenomenon of plasmolysis in [4.1(2)], I have mentioned that as the central vacuole of a mature plant cell shrinks in response to a high concentration of electrolytes in the bathing medium, the protoplasm surrounding the central vacuole may do the opposite and swell.[32]

In 0.7% (or 0.120 M) NaCl, frog muscle cells maintain their normal volume indefinitely [4.1(2), (3)]. In contrast, Martin Fischer[78] and von Korösy[196] had long ago reported extensive swelling of frog muscle, when immersed in 0.118 M KCl. Yet the osmotic-pressure- or vapor-pressure-lowering created by 0.118 M KCl and by 0.120 M NaCl are virtually the same. Why do they act so differently on cell volume?

To support his view that the swelling of muscle cells induced by isotonic KCl originates from the protoplasm rather than the (permeability or impermeability of the) cell membrane, Martin Fischer demonstrated similar KCl-induced swelling of fibrin and gelatin, which are not cells and have no membrane covering.[78; 546] Ling & Walton[296] and Ling & Wright[107 p 263]

confirmed and extended Fisher's finding by demonstrating KCl-induced swelling of frog muscle cut into 2- to 4-mm segments, which do not regenerate a new membrane [4.1(4)].

In 1962, Ling offered a theoretical interpretation for KCl-induced swelling of living cells as follows[98 p 249; 107 p 262]:

$$f^- f^+ + X^+ + Y^- \leftrightarrow f^- X^+ + f^+ Y^+, \qquad (4)$$

shrinking swelling

where f^- and f^+ stand for fixed anions (e.g., β- and γ-carboxyl groups) and fixed cations (e.g., ∈-amino groups and guanidyl groups) respectively and X^+ and Y^- stand respectively for free cation (e.g., K^+) and free anion (e.g., Cl^-). The underlying assumption is that the tendency of cells to swell by adsorbing additional (layers of polarized and oriented) water is restrained or prevented by salt linkages, ($f^- f^+$) formed throughout the cell interior among cell proteins, as also cited here earlier in [11.3(7.2)]. When a salt of the right kind and at a high enough concentration is added, it dissociates some of the salt linkages as indicated in Equation 4 above. With the restraining force thus weakened, further swelling occurs until a new and larger equilibrium volume is reached and maintained.

Furthermore, according to the AI Hypothesis and its supportive evidence, when the muscle cells are in their normal resting living state with ATP and its "helpers" in their respective right places and concentrations (Figure 44), the β- and γ-carboxyl groups engaged in salt-linkages are maintained at a proper (relatively low) c-value (e.g., –4.20 Å), where K^+ as well as some fixed cations (as modeled by NH_4^+) are preferably adsorbed (Figure 42) [15.1(3)]. When a high concentration of KCl is introduced, the restraining salt linkages in the muscle cells are torn apart by the K^+ and Cl^- and the cells swell. At the same c-value, the adsorption of Na^+ on the same β- and γ-carboxyl groups is much weaker. Accordingly, a similar concentration of NaCl solution causes little or no swelling. This is true as long as the muscle remains healthy.

[A theoretical equation for cell volume change in concentrated salt solutions was presented by Ling and Peterson in 1973[198] but is not shown here. It incorporates a basic equation derived by Freeman Cope.[141]]

(2) Injury-induced cell swelling in isotonic NaCl

Every child knows that injury can cause tissue swelling. The AI model offers a possible explanation for this pathological manifestation as well.

Alluding to Equation 4 above, the mechanism of injury-induced swelling is similar to that of KCl-induced swelling. Only here, no extraneous electrolyte is needed. Instead, it is the high concentration of NaCl in the patient's tissue fluid that serves the role of the $X^+ Y^-$. One asks, Why should this innocuous, indeed vitally-needed NaCl present in all normal blood plasma and tissue fluids create swelling in an injured tissue but not in a normal one?

The theoretical answer lies in the critical role of ATP in maintaining the proper *low* c-value of the β- and γ-carboxyl groups in healthy cells (Figure 44). Since ATP is an *electron withdrawing cardinal adsorbent* or EWC [14.3(4); 15.1(2)], its depletion following cell injury causes an across-the-board *rise* of the c-value of the β- and γ-carboxyl groups, and with it, a rise in their relative affinity for Na^+ as shown in Figure 42. Accordingly, the normally harmless NaCl in the blood and tissue fluids now becomes as damaging as extraneous isotonic KCl and cell swelling occurs in consequence.

While the increase of the relative affinity for Na^+ with a decline of ATP concentration causes a gain of adsorbed Na^+ in agreement with theory, further loss of ATP leads to (i) additional rise of the c-value of the β- and γ-carboxyl groups and with it, the displacement of most of the adsorbed K^+ and adsorbed Na^+ by some fixed cations to form more salt-linkages[107 pp 127–128] and (ii) the depolarization and disorientation of the cell water {see also [10.1(2)]}. (i) may well be at least partly due to (ii) for the following reason. With water depolarization, the q-values of the (adsorbed) K^+ and Na^+ rise, and with it, the tendency for these ions to stay in the adsorbed state decreases. Since the fixed cations, being fixed in space, are not similarly "favored," they displace the formerly adsorbed K^+ and Na^+, forming more salt-linkages.

In support of this theoretical interpretation of injury-induced cell swelling, Ling and Kwon have shown that to produce the maximum swelling in various injured tissues, both Na^+ and Cl^- in the bathing Ringer's solution are essential.[199] In a modified Ringer's solution, where all the NaCl has been replaced by (isosmotic) sucrose or even $MgSO_4$, no swelling occurs[107 pp 266–271] (Figure 63).

Under otherwise similar conditions, the degree of swelling in the presence of a fixed (high) concentration of NaCl is inversely related to the concentration of ATP. The lower the ATP, the stronger the swelling[199; 107 p 267] (Figure 64). This further confirms that the key to injury-induced cell swelling lies in the decline in ATP concentration.

Earlier in 1969, Saladino *et al.* observed a similar Na^+-dependent swelling in amphotericin-B-poisoned toad bladder cells.[457] These cells too did

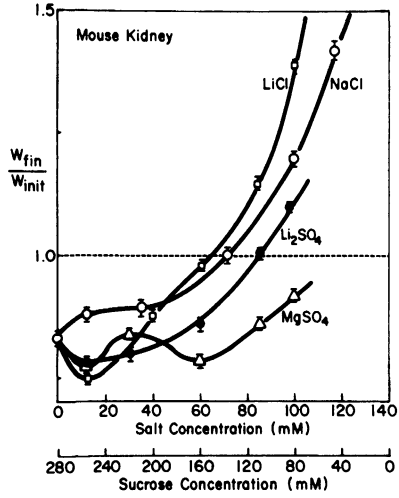

Figure 63. Percentage weight changes as that of initial weights (ordinate) of isolated mouse kidneys in response to chilling (4°C) and anoxia, plotted against the concentrations of salts plus sucrose in the bathing solutions shown on the abscissa. Sucrose is added to maintain a constant isotonic osmolarity. Different salts used are shown against the weight curves of the mouse kidneys incubated in the solution containing that salt-sucrose combination (e.g., 60 mM of NaCl + 160 mM of sucrose). Data show that both Na^+ and Cl^- are essential to produce maximum swelling, but LiCl is even more effective than NaCl. (From Ling and Kwon[199])

not swell in a Ringer's solution, in which NaCl has been replaced by isosmotic concentration of sucrose. Later Nakao and his coworkers made similar observations on human red blood cells kept in physiological saline containing a high concentration of Na^+ and the metabolic poison NaF.[525] As the ATP concentration in the red cells falls to 0.1 mmoles per liter of fresh cells or lower, their normally biconcave-disc shape expands into swollen spheres.

These three sets of independent and mutually-supportive evidence, when viewed together, support the AI theory of injury-induced swelling described above. Just as important, they strengthen the theoretical postulations of the AI Hypothesis that the principal cardinal adsorbent, ATP, is an EWC and that its decline in concentration leads to an across-the-board rise of the c-value of the β- and γ-carboxyl groups. And with that c-value rise, there is an increase in their affinity for Na^+.

15.4 True active transport across bifacial epithelial cell layers and other bifacial systems

In the AI Hypothesis, living cells may be divided into two categories. The more typical category includes muscle, nerve, red blood cells as well as microbes like E. coli. They each possess a single type of cell surface or mem-

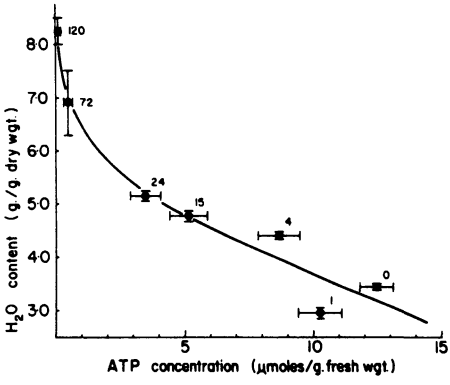

Figure 64. The relationship between the water contents of swelling mouse brains and their ATP contents. Isolated mouse brains were incubated in a cold (4°C) and anoxic mammalian Ringer's solution for varying lengths of time as indicated by the number (of hours) near each set of mean ± S.D. (From Ling and Kwon[199])

brane covering the entire cell. And, accordingly, they are designated as *unifacial cells.*[15 pp 585–586]

The second category of living cells are, as a rule, individual units of a planar sheet of cells marking the boundary between the inside of a multicellular organism or organ and its respective surrounding medium of one sort or another. Examples are the frog skin, the intestinal epithelial cells, the kidney tubule epithelium. The surface membranes of these epithelial cells facing the external medium and that facing the organism's interior are as a rule different. For that reason, these cells are given the name *bifacial cells.*[15 pp 585–586]

Besides epithelial cell layers, there is another kind of bifacial cell, which includes *Nitella* and other giant algal cells. One may recall that from the work of Höfler [4.1], we learned that the outer cell membrane facing the external pond water is permeable to sucrose, but the vesicular membrane (or tonoplast) facing the water in the central vacuole is impermeant or nearly so. Therefore, these cells also have two different cell membranes in each cell and are bifacial.

In the AI Hypothesis, unifacial cells do not possess metabolic pumps in their cell membranes. Results of extensive experimental testings support this view (Chapter 12). In contrast, the entire bifacial cell, membranes and cytoplasm, may be engaged in *true active transport* or pumping of solutes across the bifacial cell layer. Nasonov also suggested that *entire* gland cells and epithelial cells are involved in selective pumping but did not offer a molecular mechanism for this activity.[86 p 168]

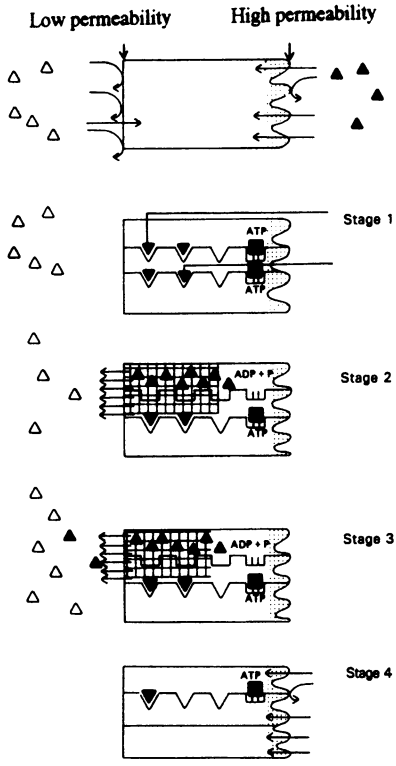

Figure 65. Schematic diagram of the theory of active transport across a bifacial epithelial cell layer. Solute (▲) from the *source* aqueous phase in contact with the mucosal surface at right, is transported actively to the *sink* aqueous phase in contact with the serosal surface at left. Δ represents the same solute in the sink aqueous phase. Straight and curved arrows indicate respectively successful and unsuccessful attempts at entry. ▼ represents the solute adsorbed on sites on the sponge protein in the epithelial cells. Clear area in the cell is filled with polarized-oriented multilayers of cell water. Cross-hatched area represents free water. ■ represents ATP which is mostly adsorbed on the protein that possesses Na,K-activated ATPase activity.

Stage 1. Asymmetrical entry from mucosal surface. Cooperative adsorption on sites under the domination of the cardinal adsorbent, ATP. Stage 2. Last adsorbed Na^+ activates the Na,K-ATPase, triggering ATP splitting. Ensuing cooperative Na^+ desorption and water depolarization at, and including the serosal surface. Stage 3. Diffusion of solute outward through depolarized water in serosal surface affecting transport. Stage 4. Regeneration and reabsorption of ATP onto its cardinal site, completing the cycle. (Ling, 1981[225])

(1) Active Na^+ transport across frog skin

Using Na^+ transport across frog skin as an example, I presented briefly the "cooperative adsorption-desorption pump" for the first time in 1956[136] and in detail later.[225, 15 pp 508–599]

Basically, the two different cell membranes facing the pond water and body fluid act as one-way valves pointing respectively inward and outward. Between these membranes are sponge-like cytoplasmic proteins, which can sop up and thus concentrate Na^+ by autocooperative adsorption and then squeeze it out by autocooperative desorpton. The adsorption-desorption cycle is controlled by ATP, the concentration of which is in turn controlled by the enzyme(s), which splits it and other enzymes, which re-synthesize it (Figure 65).

Stage 1. The mucosal surface has a higher permeability toward Na^+ because both the density and c-value of the β- and γ-carboxyl groups here are

high (Figure 42). In consequence, Na^+ from the external medium is attracted to, becomes selectively adsorbed by and is thus raised to a much higher concentration at the mucosal surface—before entering the cell by the adsorption-desorption route (see Figure 35). Once inside the cell, the Na^+ once more adsorbs, this time onto the β- and γ-carboxyl groups of the "sponge protein(s)," which is (are) under the domination of the cardinal adsorbent, ATP.

In the model presented first in 1981, the concept of molecular switch had not been introduced yet.[225] As of now, we have the option of the two alternatives, either the *sponge protein* and the *switch protein* [14.3(4)] are one and the same, or they may be two discrete proteins joined together by salt-linkages, H-bonds, etc., which can transmit inductive effects across proteins (see [14.3(4)] including Figure 49). In the first case, the Na,K-ATPase will be at once the sponge protein and the switch protein. In the latter case, only the switch protein possesses Na,K-ATPase activity.

Stage 2. As more and more Na^+ becomes adsorbed on the sponge protein, eventually the last-adsorbed Na^+ will trigger into activity the Na,K-activated ATPase, which hydrolyzes the cardinal adsorbent, ATP into ADP and inorganic phosphate. With the key EWC, ATP, gone and the resulting ADP only weakly adsorbed and possibly acting as EDC—for evidence that ADP acts as an EDC, see [14.3(4)] and also Reference 289 pp. 86–87—the sponge protein undergoes an *autocooperative* desorption of the (adsorbed) Na^+ with concomitant water depolarization, which extends into, and includes water in the serosal membrane.

Stage 3. Diffusion of the Na^+ liberated from the sponge protein(s) outward from the depolarized serosal membrane water *via* the wide-open saltatory route.

Stage 4. Regeneration of ATP returns the system to Stage 1 again.

This model has incorporated important discoveries of Koefoed-Johnson and Ussing made in 1958.[226] These investigators found that the electrical potential across the frog skin is sensitive to the concentration of Na^+ in the solution bathing the outer surface of the skin and to the concentration of K^+ in the solution bathing the inner surface of the skin. These findings led them to the conclusion that the outward-facing mucosal surface membrane has a higher Na^+ permeability but the inward-facing serosal surface has higher K^+ permeability.

In the AI Hypothesis, the cellular resting potential is not a membrane potential but a *close-contact surface adsorption potential.* And it is created

by the presence of fixed anionic β- and γ-carboxyl groups on the cell surface (see [15.4(2)] below), which also mediate cation permeation [13.2]. The c-value of the β- and γ-carboxyl groups of the mucosal surface is high, with Na^+ over K^+ preference; that of the serosal surface is low, with K^+ over Na^+ preference (Figure 42).

It was pointed out that in theory the sponge protein with Na,K-ATPase activity could be only a thin layer attached to the serosal membrane. But there are independent evidence, which suggest that the sponge protein(s) exist(s) throughout the epithelial cells:

(i) Maddrell showed that in the Malpighian tubules of the insect, Rhodnius,[227] the rate of transport of K^+ and of Na^+ are strongly correlated to the *total* intracellular concentration of the respective ion. The great majority of these intracellular ions is most probably adsorbed on *cytoplasmic* sites.

(ii) In Necturus kidney cells, the rate of net transport of Na^+ is linearly correlated also to the *total* intracellular Na^+ concentration, which too must be mostly on cytoplasmic sites.[228]

(iii) Following injection of radioactively labeled K^+ into a rabbit, the specific activity of urinary K^+ does not follow that in the arterial plasma. Instead, it quickly attains the specific activity found in the (whole) renal tissues (and thus also mostly due to labeled K^+ on cytoplasmic sites).[229]

These facts raise the question: "Since kidney, salt glands, and other bifacial epithelial tissues offer the richest sources of Na,K-ATPase, could this enzyme be actually found in large quantity in the cytoplasm rather than the cell membranes of these cells?" In reviewing this subject, Skou pointed out that it is not ruled out that the Na,K-ATPase may exist outside the cell membranes.[281 p 604]

(2) Active Rb^+ transport into Nitella cell sap

A central feature of the model of active transport described above is a cyclic change of ion adsorption and desorption on the "sponge protein(s)" of many individual epithelial cells. Unfortunately, verifying this concept in these tiny epithelial cells is by nature difficult and, to the best of my knowledge, has never been attempted. However, marine giant algal cells like *Valonia macrophysa* and fresh-water giant algal cells like *Nitella clavata* have long been known to accumulate K^+ in the vesicular sap to a concentra-

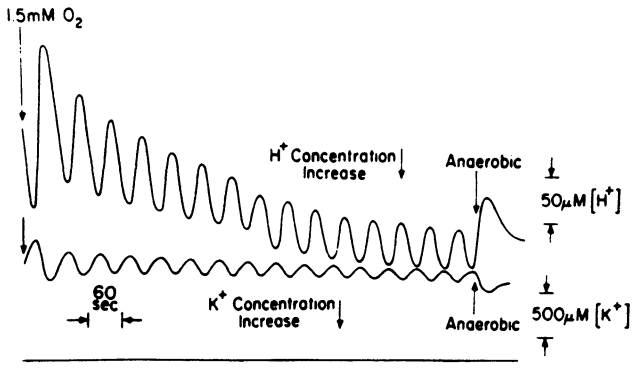

Figure 66. Oscillatory uptake and release of H^+ and K^+ in an aqueous medium containing a suspension of pigeon heart mitochondria. The mitochondria were allowed to become anaerobic before 1.5 mM of oxygen (as H_2O_2) and 17 mg catalase/ml were added. 83 ng valinomycin/ml and 6.7 mM KCl were introduced initially; pH was 0.25 at that time. (Chance and Yoshioka[493] from the *Archives of Biochemistry and Biophysics* by permission of Academic Press)

tion many times higher than in the surrounding sea water or pond water as the case may be.[491 p 125; 15 p 64] In the first brief presentation of what I then referred to as the "cooperative adsorption-desorption pump"—presented in greater detail later and in Figure 65 above—I pointed out that active transport in epithelial cell layers and across the protoplasmic layers of these giant algal cells could share a similar basic mechanism. In support, I cited the work of S.C. Brooks.

But before reviewing Brook's work, I would like to present other evidence demonstrating that a large population of isolated cell components (i.e., mitochondria) can undergo (synchronized) cyclic uptake and release of alkali-metal ions not unlike the kind I suggested above for true active transport.

Discovered in 1965 independently by Lardy and Graven[494] and by Pressman,[495] and illustrated in Figure 66 taken from Chance and Yoshioka,[493] cyclic uptake and release of K^+ and H^+ are demonstrated by a suspension of (pigeon heart or rat liver) mitochondria. Note that the data confirms the *cyclic uptake into and release from* the protoplasm of each individual mitochondrium as theoretically required to explain the ion pumping in individual epithelial and algal cells. The incredible *synchrony* of the uptake and release among a huge population of individual isolated mitochondria is striking and it offers insight toward deeper understanding of the high level of coordination within the living cells as well as among a population of individual cells.

The essential requirements for the type of cyclic changes shown in Figure 66 are: K^+ and H^+ at a correct concentration ratio; a suitable (congruous) anion; ATP or a source of ATP production, like oxygen[15 pp 534–535] and valinomycin. At one time valinomycin was be-

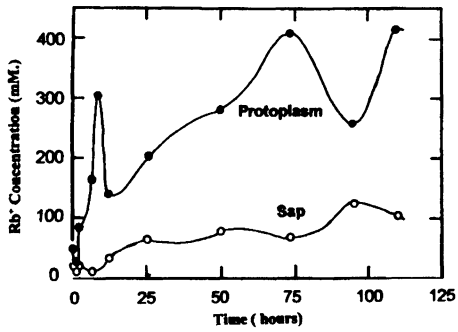

Figure 67. Oscillatory uptake of radioactively labeled Rb^+ by the protoplasm of *Nitella* interstitial cells (top curve) and its delayed release (bottom curve) into the (cell) sap in the central vacuole. Experiment lasted 120 hours in water containing 5 mM labeled RbCl. Each data point represent the mean of 5 determinations in 5 series of studies. pH was 7.3 and the Illumination continuous. (After Brooks[492])

lieved to be a special carrier or ionophore for K^+ through the phospholipid inner membrane of the mitochondria. Later Maloff *et al.*[213] showed that valinomycin does not facilitate K^+ entry into liver mitochondria. Others seriously question the existence of a continuous phospholipid bilayer membrane surrounding mitochondria (and living cells in general)[214, 215] (see Chapter 13).

In 1981, I suggested that valinomycin acts as electron-withdrawing cardinal adsorbent (EWC), posing the c-value of the mitochondrial β- and γ-carboxyl groups at the "razor's edge" to flip autocooperatively between the K^+ adsorption (at low c-value sites) and H^+ adsorption (at high c-value sites).[15 pp 535–536] The fact that EDTA[497] and strontium (Sr^{2+})[498] can substitute for valinomycin is in harmony with this view. Evidence that valinomycin indeed functions as an EWC can be seen in the demonstration that it produces a similar hyperpolarization in giant mitochondria as that produced by the EWC, adrenaline on frog muscle [15.5(2.5.2)].[15 Fig 15.7]

Figure 67 is from Brooks's time course of the uptake of radioactively labeled Rb^+ by the protoplasm and cell sap of *Nitella* cells.[492] Note that the cyclic rise and fall of Rb^+ in the cell sap in the central vacuole follows the cyclic rise and fall of protoplasmic Rb^+ until the level of Rb^+ in the cell sap rises above that in the surrounding medium but always below that in the protoplasm.

15.5 The resting potential

(1) Historic background

(1.1) The membrane potential theory and modifications

In [4.2] I have shown how Bernstein's original membrane theory was founded. And how it ran into difficulty after Na^+ has been shown to traverse the cell membrane with ease. And, as a result, the theory could no

longer predict an inside-negative resting potential of some 90 mV as observed. This debacle prompted A.L. Hodgkin to introduce his ionic theory.

(1.1.1) The ionic theory

In the ionic theory, low *relative* permeability rather than *absolute* impermeability explains why external Na^+ concentration has little influence on the resting potential. Like Bernstein's original membrane theory, the ionic theory is also built upon the tenets of free water and free K^+ in living cells. For some time, discoveries of A. V. Hill and of Hodgkin & Keynes described in Chapter 5 offered what appeared to be definitive proof for this pair of tenets.

Subsequent work described in [10.2(3)], however, reversed the earlier conclusion and demonstrated the adsorbed state of K^+—mostly on β and γ-carboxyl groups in muscle cells. Thus tethered, cell K^+ cannot diffuse freely and generate a membrane potential as suggested in both Bernstein's original membrane theory and in Hodgkin's modified version (Figure 4B).

The adsorbed state of the bulk of intracellular K^+ also explains why the 10 (individuals or groups of) investigators out of 14 could not confirm the predicted logarithmic relationship between intracellular (adsorbed) K^+ concentration and the magnitude of the resting potential[419] (Figure 4B). On the basis of an alternative theory of the cell electric potential called *close-contact surface adsorption potential*—to be fully reviewed in [15.5(2)] below—why four laboratories *did* find the expected relationship between the concentration of intracellular K^+ and the resting potential has also been explained.[15 p 476; 48 p 58; 107 p 315 n5]

The ionic theory also requires a Na pump to maintain the steady low level of this ion in the cell. Findings described in detail in Chapter 12 show that such a membrane pump would consume more energy than the cells could afford—offering yet another reason why the ionic theory of the resting potential is also in grave difficulty. But they are not all as the following shows.

Throughout history, repeated efforts have been made to find an inanimate model displaying a *membrane potential* (Figure 4B). The models investigated included membranes of thin layers of glass,[147; 551] of collodion,[147; 554] of oil[242; 243] and of phospholipids.[499] It turned out that none of the electric potential measured across these artificial membranes is a *membrane potential* (Figure 4B). Instead, in every case, the potential difference registered is the *algebraic sum of two independent potentials, one*

at each surface of the membrane.[241; 243; 147 p 165, Fig 4] Without the solid backup of inanimate models, the membrane potential theory itself and Hodgkins' modification are hard to defend for a third set of contrary evidence.

The equation describing the cellular electric potential in the ionic theory is the familiar Hodgkin-Katz-Goldman equation:[230, 231]

$$\psi = \frac{RT}{F} \ln \frac{P_K[K^+]_{in} + P_{Na}[Na^+]_{in} + P_{Cl}[Cl^-]_{ex}}{P_K[K^+]_{ex} + P_{Na}[Na^+]_{ex} + P_{Cl}[Cl^-]_{in}}, \quad (5)$$

where R, T and F are respectively the gas constant, the absolute temperature, and the Faraday constant respectively. The P's are the permeability constants of each of the designated ions, e.g., P_K as the permeability constant of K^+.

By the time the Hodgkin-Katz-Goldman equation was published, Hodgkin and Katz had already made the important discovery mentioned earlier, i.e., the action potential does not involve just a transient local increase of permeability to all solutes as suggested in Bernstein's theory of the action potential[232] but a transient and local increase in the specific permeability to Na^+.[(233)] Hodgkin and Katz then showed that both the resting potential and the action potential could be described by Equation (5) through manipulating the three permeability constants. That was before the discovery that the steady resting potential is *indifferent to* the concentration of the major external anion, Cl^-.[(234, 235)]

This discovery of the insensitivity of the resting potential to Cl^- posed a fourth set of evidence against the ionic theory. Since the Cl^- permeability (P_{Cl}) is as high as the K^+ permeability (P_K),[45; 46] changes in the external Cl^- concentration should have as much of an impact on the resting potential as changes in the external K^+ concentration—contrary to facts. Unfortunately, the few investigators directly involved either did not see the seriousness of the situation or for other reasons failed to rise to the challenge. Instead, they arbitrarily deleted the Cl^- terms of Equation (5), producing an "equation" of the following form:[236, 237]

$$\psi = (RT/F) \ln\{(P_K[K^+]_{in} + P_{Na}[Na^+]_{in}) / (P_K[K^+]_{ex} + P_{Na}[Na^+]_{ex})\}. \quad (6)$$

This is, of course, no longer a rigorously derived *equation* as Equation 5 was.

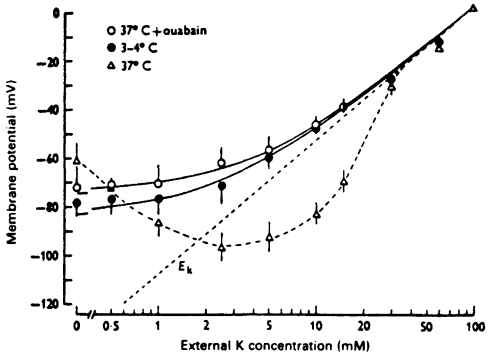

Figure 68. Effects of cooling and of ouabain on the relationship between the logarithm of the external K^+ concentration and the resting potential of "Na-rich" soleus muscle of rats fed a K-free diet for 40 to 49 days. The smooth lines going through or near the experimental points from the chilled (3–4°C) (solid circles, solid line) and ouabain-treated muscles at 37°C (empty circles, solid line) are according to Equation 6. The same equation, however, cannot be made to fit the data of normal muscle at physiological temperature (37°C) and in the absence of ouabain (empty triangles, dashed line). (Akaike,[240] by permission of *The Journal of Physiology* (London))

Since the majority of investigators could not confirm the predicted relationships between ψ and the *intracellular* K^+ and Na^+ concentrations[419] [4.2]—providing a fifth set of contrary evidence—the only experimentally verified part of Equation 6 is that relating ψ to the absolute temperature, T, and to the logarithm of the extracellular concentrations of K^+ and of Na^+. Assembled together, the verified parts look like this:

$$\psi = \text{constant} - (RT/F) \ln\left\{P_K [K^+]_{ex} + P_{Na} [Na^+]_{ex}\right\}. \qquad (7)$$

I shall return to this "equation" shortly.

(1.1.2) Electrogenic pump theory

In the years following the introduction of Equation 5, the resting potentials measured in some living cells did not match those predicted by Equation 5 (or Equation 6). Figure 68, taken from Akaike,[240] offers an illustration. Here the author shows that the resting potential of muscle cells isolated from rats fed a low K^+ diet fits Equation 6 *only* when the muscles had been either chilled to 3–4°C or exposed to ouabain, but not before.

Akaike, like others before him, postulated that the resting potential has two components. One is "passive" and it follows Equation 6. The other is "active" and sustained by a hypothetical *electrogenic pump.*[107 pp 277–279] Low temperature and ouabain suppress the active component, which does not exhibit a predictable quantitative relationship to external K^+ concentration. The authors of this new (electrogenic) pump were apparently unaware of, or for some other reasons(s) ignored, the repeated demonstrations that there is not enough energy to operate the (original) Na^+ pump alone (Chap-

ter 12). So whether this electrogenic pump is the same sodium pump or not, really makes little difference. In either case, it is not easily defensible. Akaike's fine experimental data, however, call for a new interpretation.

(1.2) Phase-boundary potential theories

Under the title: "On the solubility of mixed crystals" (Über die Löslichkeit von Mischkrystallen) Walter Nernst discussed in 1892 the creation of an electrostatic potential difference at the surface of a salt crystal bathed in a solution of the same salt.[360] Nernst first stated that at the inside of each phase there are no free electric charges—which, of course, merely restates the Law of Macroscopic Electric Neutrality.[97 p 330] Nernst then pointed out that only at the contact surface between the solid crystal and the solution is an electrostatic double layer formed. The (dissociated) ion that has the higher *partition coefficient* between the solid phase and liquid phase will endow the solid phase with its kind of electric charge, positive or negative as it may be. And an electrical potential difference is established at the phase boundary, hence the name, *phase boundary potential.*

(1.2.1) Baur's ion adsorption potential theory

In 1917, E. Baur and S. Kronmann visualized a Volta chain as follows:[241]

$$\underset{}{\text{NE / 0.01 N KCl /}} \underset{W_1}{\textbf{ oil }} \underset{}{\text{/ 0.01 N KCl + addition / NE.}}$$
$$W_2$$

The non-polarizable electrodes (NE's) are immersed respectively in the two aqueous phases W_1 and W_2, both of which contain 0.01 N KCl solutions, and are separated from each other by a (rather thick) layer or membrane of "oil" (amyl alcohol). When strychnine sulfate is added to compartment W_2 on the right, the electrode on the left becomes positive. Baur believed that this electric response was due to the adsorption of the positively charged *strychnine ion* in the oil phase, leaving behind in the solution the negatively charged sulfate ion, and a W_2 side-negative electrical potential difference is thus created across the phase boundary. In contrast, if potassium picrate is added to phase W_2, a W_2 side positive electrical potential difference is observed. This was taken to indicate the adsorption of the negatively charged *picrate ion* onto the oil phase, leaving positively charged K^+ behind.

Baur called the electric potential difference thus demonstrated an *ion adsorption potential.* He suggested that the electric organ of electric eels might employ a similar mechanism.[514] Baur's observation on "oil-

potentials" have been confirmed and extended by Ehrensvard and Sillen[242] and by Colacicco.[243] In place of strychnine sulfate and potassium picrate, Colacicco used sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) respectively with comparable results. When water rather than KCl solutions was in both compartments W_1 and W_2, and when SDS was added to one compartment and CTAB to the other, an electrical potential difference as high as 370 mV. was measured across the oil layer.

Baur first published his *ion adsorption potential theory* in 1913.[239] At that time, the prevailing definition of adsorption was different from the one-on-one localized adsorption I have been discussing above {e.g., [10.2(4)]} and to be further discussed in [15.5(2)] below. The definition of adsorption at that time is as follows: "*those substances which decrease interfacial energy tend to concentrate at a liquid-solid or liquid-liquid interface. This phenomenon of concentrating at the interface is called adsorption.*"[64 p 211] It was 1918 when Irving Langmuir (1881–1957) first introduced what he called *localized adsorption.*[117] That Baur's concept of adsorption is probably not of the Langmuir type can also be seen from the nature of the membrane he studied. Amyl alcohol, being a homogeneous liquid, does not contain localized adsorption sites commonly found on the surface of solids and on macromolecules. (For a brief summary of the historical evolution in the meaning of the term *adsorption,* see Reference 107 p. 57, n5.)

Both Baur's and Beutner's, (see below) phase boundary potential theory are in harmony with, and their biological application rests upon, Overton's lipoidal membrane theory[21]—which is, however, no longer tenable in view of contradictory evidence reviewed in [13.1]. Nor is there evidence for the existence of bio-electric potential-generating agents like strychnine sulfate or potassium picrate in Nature, no more so than ion carriers or ionophores [13.2].

Nonetheless, the work of Nernst, Baur, Kronmann, Ehrensvard, Sillen and Colacicco are all highly important because, together, they have established the central role of electric charges at the phase boundary in generating a steady electric potential difference. By the same token, a phase boundary *per se,* like that between the bare surfaces of an oil layer and water, does not generate a steady electric potential difference.

(1.2.2) Beutner's phase boundary potential theory

R. Beutner suggested a different phase-boundary potential,[515] in which the partitioning of whole salt between oil and water phase and their respective dissociation constants in the oil phase are the cause of the potential. For serious criticisms of this theory, see Reference 241 pp. 93–97 and Reference 15 p. 24.

(1.2.3) Horovitz's (and Nicolsky's) theories of glass electrode potentials

In 1923, Horovitz (aka Lark-Horovitz) demonstrated, to the astonishment of many, that a glass electrode, which has been immersed in a silver nitrate ($AgNO_3$) solution, becomes a Ag^+-electrode.[374] Horovitz believed that during the prior immersion in the $AgNO_3$ solution, cations originally present in the glass surface had exchanged for Ag^+ in the bathing solution. And that it is this exchange that endows the glass electrode with its new sensitivity to external Ag^+ ion concentration.

At first, Horovitz called the glass electrode potential an *ion adsorption potential.*[374] That was 1923. He changed it to an *ion exchange potential* two years later in 1925.[375] His still later version presented in 1931 is given in an equation form:[362]

$$\psi = \text{constant} - (RT/F)\ln(C_1 + AC_2), \tag{8}$$

where R, T and F are the gas constant, the absolute temperature and the Faraday constant respectively. C_1 and C_2 are respectively the concentrations of the two species of ions in the adjoining solution. Note that A is not a simple adsorption constant but an *exchange constant* equal to $\mu_2 K_1/\mu_1 K_2$, where μ_1 and μ_2 are the *mobilities* of the two species of ions in the solid phase. K_1 and K_2 are what he referred to as the "integration constants in the expression for the thermodynamic potential of the ions in the solid phase-solution tension."

In 1937, B. P. Nicolsky also wrote an equation for the electric potential of a glass electrode exposed to two species of free ions:[373]

$$\psi = \psi^\circ + (RT/F)\ln(a_1 + K a_2), \tag{9}$$

where a_1 and a_2 are the *activities* of the two species of ions 1 and 2 in the bathing solution. ψ° is a constant. K is the *exchange constant* of the two species of ions. The great emphasis Nicolsky placed upon this *exchange constant* is made clear in these words:

"It is clear that one also cannot regard as satisfactory the conception of exchange adsorption of cations advanced by Horovitz" (citing Horovitz's 1923 article[374]). Nicolsky went on to say ".... Horovitz in his later publications,[375; 362] considered that between the glass and the solution, a cation exchange (not adsorption) (*Nicolsky's interpolation*) takes place.... Such a point of view seems to the author of the present paper (i.e., Nicolsky, *GL's insertion*) to be the most correct one.... ."[373 p 600]

Horovitz was not the first to study the glass electrode potentials. Nor did he, to my knowledge, make the (published) suggestion that the glass electrode potential shares a basic mechanism with bioelectric potentials. It was Cremer who first cited in 1906 the glass membrane as a model of the cell membrane and the H^+ diffusion potential (or membrane potential, see Figure 4B) across such a glass membrane, as a model of the cellular electric potential[466] (see also Haber and Klemensiewicz[468; 15 p 123]).

Based on his studies of electrical potential difference across collodion membranes, L. Michaelis[401] came to the support of Cremer's diffusion potential theory for his collodion model and bioelectric potentials. Michaelis and Perlzweig rejected Horovitz's ion exchange theory for glass-electrode potential as inapplicable to the collodion model on the ground that collodion, unlike glass, is electrically neutral and does not carry electric charges required in Horovitz's theory.[518] Michaelis and Perlzweig could not have foreseen the surprising twist and turn of history that was to happen soon. And at a place no other than Michaelis's own laboratory.

World War II brought an end to the supply of Schering brand of collodion, which Michaelis's laboratory had been using. As a result, his student Sollner and others had to prepare collodion themselves from scratch. To their disbelief, the purer the collodion they made, the worse electrode it made. Until at last, they discovered that it is an *impurity* in the original Schering brand that was responsible for the high electric potentials they had previously measured.[330] That impurity, which can be deliberately added by oxidizing the collodion with HBr or NaOH, is negatively-charged *carboxyl groups.*[541] In other words, the collodion electrode that works is similar to glass electrode potentials, after all. Both depend on electric charges on the surface for the ion-sensitive electric potential difference observed.

But neither Michaelis, nor his students, nor anyone else to my knowledge had taken full cognizance of this important discovery. Or re-thought the logical process that led to their earlier choice of the membrane potential model for bioelectric potentials. That is, not until 1955 when I first published the brief note mentioned in passing in [14.1(1)].

(2) The close-contact surface adsorption (CSA) theory of cellular electrical potentials

The AI version of the cellular electric potential was briefly presented first in 1955[145] [14.1(1)], even though the idea had begun earlier as illustrated in

Figure 4C taken from a 1952 publication.[96] In this theory, *it is the* β- *and* γ-*carboxyl groups in a microscopically thin layer of the cell surface*[377] *and the* K^+ *(and* Na^+*) they adsorb in a close-contact, one-on-one fashion, which give rise to the resting potential.*

Up to now, this theory has been known as the *surface adsorption (SA) theory.*[107 p 280] However, to forestall confusion with other names like Baur's *ion adsorption theory* (of oil layer potential) and Horovitz's early (and later abandoned) *ion adsorption theory* and his later (and Nicolsky's) *ion exchange theory* (of glass electrode potentials), I have decided to change the name of the SA theory henceforth to *close-contact surface adsorption (CSA) theory* of cellular electric potentials.

(2.1) The importance of close-contact once more

The *close-contact surface adsorption (CSA) theory* differs from all other phase boundary potential theories and the glass electrode potential theories in a key feature. Only the CSA theory can provide a *quantitative* mechanism for the kind of overwhelming preference for one ion over another between a pair of ions bearing the same number of electric charges (e.g., K^+ vs Na^+)—seen often in living systems as well as their models like the glass electrode. The reason is similar to the one already discussed at length in [10.2(4.1)] on equilibrium ion distribution: The *only* differences between pairs of ions like K^+ and Na^+ are in their *short-range attributes*—which cannot be "perceived" unless they are *in close contact* with the adsorbing sites. And not until 1952 has (one of) a plausible set of two explicit reasons been offered for the mandatory close-contact adsorption on fixed β- and γ-carboxyl groups, i.e., *those given in the theory of enhanced association with site fixation* summarized in [10.1(1)].

The theory of *enhanced association* with site fixation provides a new basis for understanding some of the most outstanding features of life—reflected in its diverse interaction with ions and water. For, in its absence, one could only assume that counterions like K^+ and Na^+ are fully dissociated from, and thus not in close contact with, anionic sites—as dominant theories like that of Arrhenius and of Debye-Hückel on dilute ionic solutions, of Linderstrom-Lang on protein solutions,[467] and of H. Gregor on ion exchange resin[127] would have prescribed.

Close-contact association of counterions are important for yet another reason. It permits important attributes of the *adsorbing sites* to be perceived. They include the c-value of the β- and γ-carboxyl groups and the c-

value analogues of backbone carbonyl groups. Without close-contact, changes in the c-value and c-value analogues would have no meaning as none would be "seen," recognized and responded to.

(2.2) Out of the union of two failed models for the membrane potential, a super-model for the close-contact surface adsorption (CSA) potential

In 1955, I first introduced the idea that the resting potential of living cells and the electric potential of glass electrodes share a similar basic mechanism. Comparative studies of the cellular resting potentials and inanimate models have repeatedly and consistently affirmed this postulation. As an example, an electrode made of Corning 015 glass shows high sensitivity to H^+ but no sensitivity to K^+. But coating this glass electrode with a microscopically thin layer of (oxidized) colodion endows the electrode with a new sensitivity to K^+—indistinguishable from that of an oridinary oxidized collodion electrode.[107 p 284; 376] This finding shows that it is indeed the nature of charged groups on the suface (rather than the overall membrane permeability to ions), which determines the polarity and magnitude of the electric potential measured in agreement with the close-contact surface adsorption (CSA) theory. Parallel studies on the resting potential of frog muscle led to a similar conclusion that it is the nature of surface anionic sites rather than membrane permeability which determines the potential.[106 p 166; 314 Figs 5 to 6; 377 Fig 2]

Historically, the glass membrane and the collodion membranes were introduced respectively by Cremer and by Michaelis as models for the membrane potential.[401; 466] Both failed [15.5(1.1.1)]. Yet, when combined, they turned into a super-model for the close-contact surface adsorption potential—as witnessed by the following:

(2.2.1) Both model and living cell show similar rank order of sensitivity to alkali-metal ions

The effectiveness of the alkali-metal ions in depolarizing the surface potential of the oxidized-collodion-coated glass (CG) electrode follows the rank order: $Rb^+>K^+$, $Cs^+>Na^+>Li^+$,[(376)] mimicking the rank order of their adsorption constants on frog-muscle-cell-surface β- and γ-carboxyl groups: $Rb^+>Cs^+>K^+>Na^+$ [(222)] and the rank order of effectiveness in depolarizing the guinea pig heart muscle: $Rb^+>K^+>Cs^+$.[(246)] All three sets of data indicate a low c-value of the respective carboxyl groups involved.

The reader may ask: "According to the AI Hypothesis, the low-c-value of the β- and γ-carboxyl groups involved can be traced to the presence of the EWC, ATP and its helpers

on controlling cardinal sites. The carboxyl groups of the oxidized collodion are not under the control of ATP. Why do they exhibit a rank order indicative of a similar low c-value also?" In answer, I point out that even though the carboxyl groups on nitrocellulose are not under the control of ATP, they are under the "control" of other electron-withdrawing groups right on the oxidized nitrocellulose molecules. They include the OH groups (see Figure 45) on the D-glucose units of the cellulose (see fine print in [14.1(2)]) and the nitro group on the nitrocellulose molecules. Both groups are electron-withdrawing (see Figures 45 and 47).

(2.2.2) Both model and living cell are indifferent to external Cl^-

Since fixed (negatively-charged) anions like the β- and γ-carboxyl groups repel, rather than attract and adsorb other (negatively-charged) free anions like Cl^-, the resting potential of muscle and nerve is insensitive to the concentration of external Cl^- as observed (see subsection (1.1.1) above). Similarly, the oxidized collodion-coated glass electrode or CG electrode shows no sensitivity to Cl^-. On the other hand, exposure of an (unoxidized) collodion-coated glass electrode to a solution of positively-charged *polylysine* followed by drying at 40% humidity, endows the electrode a sensitivity to external anions like Cl^-.[15 Fig 14–23] The acquisition of anion sensitivity of these *polylysine-treated, collodion-coated glass* (PCG) electrodes demonstrates once more that it is electrically charged sites on the surface that determine the nature and polarity of the electric potential measured.

(2.2.3) Both model and living cell demonstrate approximately 150 times greater sensitivity to H^+ than to K+

The resting potential of frog muscle shows a sensitivity to H^+ 170 times greater than to K^+.[107 pp 288–289] The surface potential of the CG electrode also shows a sensitivity to H^+ 130 times greater than to K^+.[107 p 287]

(2.2.4) Both model and living cell are insensitive to external Mg^{++}

The resting poential of frog muscle shows no sensitivity to the concentration of external Mg^{2+}[503]—even though the muscle cell membrane is highly permeable to this ion[503] and should have a strong depolarizing action on the resting potential according to the membrane (pump) theory. The CG electrode also shows no sensitivity to external Mg^{2+}.[376] Now, pairs of closely-placed carboxyl groups found in chelators like EDTA (ethylenediaminetetraacetic acid) and clusters of closely-placed carboxyl groups found in carboxyl ion exchange resins both show strong affinity for divalent ions like Mg^{++}.[501] The insensitivity to Mg^{2+} of the β- and γ-carboxyl groups on the cell surface, and of the carboxyl groups on the CG electrode, suggest that neither exists in closely-placed pairs or clusters. By exclusion, they must both exist as spatially-isolated sites.

(2.3) The original equation for the resting potential

The equation of the resting potential according to the AI Hypothesis was first introduced in 1959;[245] simplified, it reads as follows:

$$\psi = \text{constant} - (RT/F)\ln\left\{K_K[K^+]_{ex} + K_{Na}[Na^+]_{ex}\right\}, \qquad (10)$$

where R, T, and F are respectively the gas constant, the absolute temperature and the Faraday constant. K_K and K_{Na} represent respectively the (close contact) *adsorption constant* of K^+ and of Na^+ on the cell surface β- and γ-carboxyl groups.

There are three independent variables in this equation: the absolute temperature T, the extracellular concentration of K^+, $[K^+]_{ex}$, and the external Na^+ concentration, $[Na^+]_{ex}$. By now, the predicted relationship of the resting potential to each of these three variables has been experimentally confirmed.[290 to 294]

Since I announced in 1955 that the resting potential of living cells and the glass electrode potential share a similar basic mechanism [14.1], I have reached the conclusion that Equation 10 (rather than either Equation 8 of Horovitz or Equation 9 of Nicolsky) more accurately describes the glass-electrode potential—for the reason cited earlier. Equation 10 differs from both Equation 8 and Equation 9 in the meaning of the coefficients. In Equation 10, the coefficients are the (*close-contact*) *adsorption constants*; in contrast, the coefficients in Equation 8 and 9 are *exchange constants*, which, as emphatically pointed out by Nicolsky, are not adsorption constants, certainly not close-contact adsorption constants. If the cations are not engaged in *close contact* adsorption, it would be virtually impossible to explain (some) glass-electrodes's strong preferential selectivity between a pair of monovalent cations like K^+ and Na^+, which differ from each other only in short-range attributes.

Note also that Equation 10 is the *entirety* of a rigorously derived equation,[219 pp 160–161] which, however, also bears formal resemblance to (the mangled and thus incomplete) Equation 6. The difference between Equation 6 and Equation 10 include differences in the meanings of the coefficients. The coefficients in Equation 6 are used to represent ion *permeability constants* (P_K, P_{Na}). In contrast, the coefficients in Equation 10 are, as just pointed out above, *close-contact ion adsorption constants* (K_K and K_{Na}).

A statement of A.S. Troshin that the (close contact) surface adsorption (CSA) theory I introduced is not different from the *phase theory of biopotentials* of Nasonov and Aleksandrov[92 p 396] is mistaken and startling. The CSA theory is founded on the *existence* of a resting potential measured across an uninjured resting cell surface. In sharp contrast, Nasonov and his coworkers, like L.Hermann long ago in his well known alteration theory,[87] *denies the existence* of such a potential difference across the surface of an *uninjured* cell *at rest* (see

statement of Nasonov quoted *verbatim* on pages 41 and 42). This denial of the existence of the resting potential is almost incomprehensible now in view of the vast amount of highly quantitative data on the resting potentials recorded world-wide as illustrated, for example, in Figures 68, 69 and 70. In my opinion, this denial of the existence of the resting potential is simply the consequence of the unavailability of newer tools like the Gerard-Graham-Ling microelectrodes[88] and the giant squid axons,[47] which together revolutionized the measurement of cellular electric potentials.[441 to 443] On the other hand, there is some resemblance at the qualitative level between Nasonov's phase theory of the action potential and the CSA theory of action potential to be reviewed in [15.6].

(2.4) Edelmann's deciding experiment

Ludwig Edelmann, then a young German biophysicist, recognized the significance of knowing what the coefficients in the two outwardly similar equations (Equation 6 and Equation 10) *truly* stand for, and set out to find the answer.[246] He first measured in the guinea pig papillary muscle both the (real-life) permeability constants, P's of K^+, Rb^+ and Cs^+ and their respective (real-life) close-contact surface adsorption constants, K's. He then recorded the effect of each of these three cations on the resting potential of the papillary muscle.[88] And compared the results with (i) that predicted by Equation 6, fitted with the permeability constants he had just measured, and (ii) that predicted by Equation 10, fitted with the close-contact adsorption constants he had also measured. The result was without ambiguity. The coefficients are *the (close-contact) adsorption constants (K's)* of the cations on the surface anionic sites.

For making this important discovery and standing by it, Ludwig Edelmann lost his job and had to start a new career as an electron microscopist.[547] For the details of this shameful story and how, as an electron microscopist, he proved to be just as brilliant and as true to his profession as the work described in [10.2(5)] illustrates, see also Reference 348.

(2.5) Control of the resting potential

In the AI Hypothesis, the K^+ and Na^+-adsorbing β- and γ-carboxyl groups at the cell surface and throughout the protoplasm are fundamentally alike. Thus, it is reasonable to introduce the same Yang-Ling cooperative adsorption isotherm (Equation A4), which has been successfully applied to ion distribution in the bulk-phase cytoplasm [14.1],[279; 190; 15 pp 208–224; pp 345–361] into the equation for cellular electrical potential as well—as I did in 1979.[248] Hopefully this more advanced equation (Equation A19 in Appendix 1) may provide a more powerful tool (than Equation 10, for example) to achieve a deeper understanding of the nature of the cellular electric potential and its control by drugs. And it did.

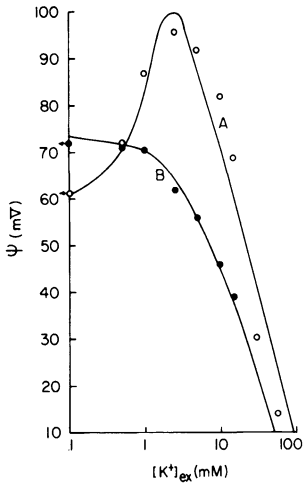

Figure 69. Effect of ouabain (10^{-4} M) on the resting potential of "Na-rich" rat soleus muscle. (A) Control Na^+-loaded muscle; (B) ouabain-treated Na^+ loaded muscle. Na^+-loaded soleus muscles were isolated from rats fed a low K^+ diet. Experimental points are the same as shown in Figure 68 and are taken from Akaike.[240] Their resting potentials were measured in the presence of varying concentrations of external K^+ with or without ouabain. Both solid lines going through or near most data points are theoretical based on Equation A19. In (A), $-\gamma/2 = 0.67$ Kcal/mole, $K^{oo}_{Na\to K} = 96.3$, constant$_1$ = 116 mV. In (B), $-\gamma/2 = 0$ Kcal/mole, $K^{oo}_{Na\to K} = 35$, constant$_1$ = 76.4 mV. (From Ling, Baxter and Leitmann[314])

(2.5.1) Control by ouabain and other EDC's by raising the c-value of surface β- and γ-carboxyl groups

In order to explain the normal resting potential, Akaike[240] and others[238] invoked yet another (electrogenic) pump, apparently unaware that there is not enough energy to operate just one sodium pump alone [Chapter 12]. To seek an alternative explanation, I re-analyzed Akaike's data shown in Figure 68 with the aid of Equation A19.

Figure 69 represents a replot of the two sets of Akaike's data, one obtained in the presence of ouabain and the other one without.[314] The solid lines are both theoretical according to Equation A19. The agreement between the experimental data and theory in both the control and ouabain-treated muscles confirms the CSA theory. In addition, the agreement suggests specifically that ouabain at 10^{-4} M reduces the *(close contact) adsorption exchange constant*, $K^{oo}_{Na\to K}$ of the muscle cell surface β- and γ-carboxyl groups from 96 to 35, and the *nearest neighbor interaction energy* ($-\gamma/2$) from 0.67 Kcal/mole to 0. A consultation of the theoretical curves of Figure 42 shows that a drop of $K^{oo}_{Na\to K}$ could only occur if there is an *increase* of the c-value in response to ouabain. This confirms the earlier conclusion of Ling and Bohr,[190] from its impact on the bulk-phase adsorption of the alkali-metal ions in frog muscle, that ouabain acts as an EDC [15.1(3)].

However, at a much lower concentration (3.26×10^{-7} M), ouabain produced on the bulk-phase cytoplasmic β- and γ-carboxyl groups of frog muscle a lowering of $K^{oo}_{Na \to K}$ from 100 to 21.7, but no change in $-\gamma/2$ (Figure 57).[190] So the possibility exists that the observed zero $-\gamma/2$ here in Akaike's data was due to a concomitant fall in the ATP concentration as a result of the much higher ouabain concentration Akaike used (10^{-4} M). (For suggestive evidence for this interpretation, see Figure 9 in Reference 190, describing the relationship between ouabain concentration and ATP concentration.)

In 1949, Ling and Gerard reported a rapid depolarizing effect of sodium azide (1 mM) on the resting potential of frog muscle.[95, 1949c] In 5 to 10 minutes, the potential fell from 80 mV to a new steady level at around 40 to 60 mV. In 1961 Abood *et al.* reported a similar depolarizing effect of 2,4-dinitrophenol (DNP).[504]

In a plot of the resting potential against the logarithm of $[K^+]_{ex}$ similar to Figure 69, Ling *et al.* demonstrated that azide, like ouabain, acts also as an EDC. As such, both ouabain and azide generate an across-the-board rise of the c-value of the surface β- and γ-carboxyl groups . In consequence, the relative preference for Na^+ versus K^+ increases, thereby lowering $K^{oo}_{Na \to K}$ in Equation A19, and the resting potential.[15 Fig 14.18] The data of Abood *et al.* on DNP are not detailed enough for a similar analysis. However, other evidence from the studies of isolated rat liver mitochondria suggests that DNP indeed acts as an EDC[496 p 84]—in harmony with its depolarizing effect on the resting-potential.

(2.5.2) Control by adrenaline as an EWC by lowering the c-value of surface β- and γ-carboxyl groups

I demonstrated in 1952 a rise of the resting potential of frog sartorius muscles after a 3–5 hour exposure to 2.73×10^{-5} M adrenaline from 82.3 ± 4.4 (S.D.) mV. to 92.9 ± 3.8 mV.[96] In 1958 Burnstock confirmed my observation on the effect of epinephrine on the resting potential in smooth muscle.[500]

Figure 70 plots the resting potential of the control frog muscle and its pair exposed to adrenaline. Note that adrenaline increased the resting potential at all external K^+ concentrations. Comparing the two theoretical curves, which go through or near most of the data points, one finds that the main impact of adrenaline was to raise $K^{oo}_{Na \to K}$ of the surface β- and γ-carboxyl groups from 40 to 66.7. $-\gamma/2$, on the other hand, remains unchanged at 0.75 Kcal/mole. In the context of the AI Hypothesis, this ability of raising the relative preference of K^+ in comparison to Na^+ can only occur in response to an across-the-board *decrease* of the c-value of the surface β-

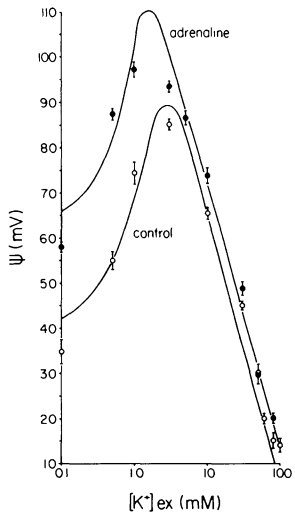

Figure 70. Resting potentials of frog sartorius muscles exposed to adrenaline (2.73×10^{-5} M) (25°C) and their controls. Both solid lines going through or near the data points were theoretical, calculated according to Equation A19 in Appendix 1. For the control muscle, $-\gamma/2$ is 0.75 kcal/mole, $K^{\infty}_{Na \to K}$ is 40 and constant$_1$ equals 105 mV. For the epinephrine-treated muscle, $-\gamma/2$ is 0.75 kcal/mole. $K^{\infty}_{Na \to K} = 66.7$ and constant$_1$ equals 125 mV. (Ling, Baxter and Leitmann[314])

and γ-carboxyl groups (Figure 42). A drug or cardinal adsorbent capable of lowering the c-value, as epinephrine does, is an EWC

In summary, Equation 19 can quantitatively describe: (i) the resting potential of frog muscle in the presence of varying external K^+ concentration and a fixed Na^+ concentration; (ii) the resting potential in the presence of different external K^+ and a fixed concentration of Na^+ and of an EDC like ouabain and sodium azide, producing an across-the-board *increase* in the c-value of the cell surface β- and γ-carboxyl groups, causing a decrease of their preference for K^+ over Na^+ and the depolarization of the resting potential; and (iii) the resting potential in the presence of varying K^+ concentration and a fixed concentration of Na^+ and of an EWC, like epinephrine, producing an across-the-board decrease of the c-value of the surface β- and γ-carboxyl groups, and an increase of their preference for K^+ over Na^+ and hyperpolarization of the resting potential.

15.6 The action potential

The action potential, the physical basis of the nerve and muscle impulse, represents a propagated local depolarization of the resting potential. As such, it too brings to mind the workings of the model of the falling domino, of the tethered see-saws and of the Great Wall of China.

(1) Hodgkin and Huxley's theory of the action potential

In the textbook version of cell physiology, all living cells are covered with a continuous phospholipid bilayer, in which scattered proteins are fully or partially submerged. Across the phospholipid bilayer membranes are also (postulated) wider "Na channels" (3×5 Å[249]) and narrower "K channels" (3 Å in diameter[250]). Both the Na channels and K channels are closed when the (excitable) cell is at rest. This is, as mentioned earlier, in essence the fluid-mosaic theory of Singer and Nicolson[200]—against which I have summarized a list of evidence for its rejection (Chapter 13).

In 1952, Hodgkin and Huxley introduced a new theory of the action potential.[251; 252] In this theory, the front end of an arriving *action potential* triggers a momentary opening of the Na channel.[251] Since there is a *standing* "Na potential," from the much higher Na^+ concentration in the external medium than in (what these authors thought to be free) cell water, Na^+ rushes into the cell through the opened Na channels, momentarily and locally annulling the inside-negative resting potential and creating, in addition, an inside-positive "overshoot."[252, 92 p 378] This is promptly followed by the closing of the Na channel and opening of the K channel. Since there is also a standing K potential from the much higher concentration of (what they thought to be) free K^+ in the nerve or muscle fiber than in the surrounding medium, opening of the K channel precipitates an outward flow of cell K^+, creating the delayed inside-negative "after potential."[251]

As pointed out repeatedly above, both Bernstein's membrane potential theory as originally proposed and the Hodgkin's ionic theory introduced later, are in serious difficulty for a host of reasons including the following: the adsorbed state of intracellular K^+ [10.2(4)]; not enough energy to run the postulated Na pump (Chapter 12); no inanimate model to be found [4.2(3)]; indifference of the resting potential to highly permeable Cl^- [12.9(1.1)]; indifference of the resting potential[503] to highly permeable Mg^{2+} [357; 503] [15.5(2.2)]; indifference to intracellular K^+ concentrations [4.2(1)]. But there is also strong evidence contradicting specifically the Hodgkin-Huxley theory of *action potential.*[251, 252] The most important two will be discussed next.

(1.1) There cannot be a *standing* Na potential

The concept of a standing Na potential would have been more plausible before the extensive and unanimous demonstration[36] that the resting cell membrane is quite permeable to Na^+ {Figure 60; [15.2(1)]}, and that cell

water is not normal liquid water but one with much-reduced solvency for this ion [11.3(5)]. This is not to mention that—if we assume, for argument's sake alone, that such a "standing" Na potential exists—it would be promptly dissipated while the cell is still at rest.

The number of positive charges needed to change the voltage of a one-microfarad condenser (like a cell membrane) by 120 millivolts is 0.12×10^{-6} coulomb, equivalent to the electric charges, which 1.2×10^{-12} moles/cm^2 of a monovalent cation as Na^+ carries.[237 p 81] The most up-to-date and accurate Na^+ influx rate of a resting frog muscle cell at room temperature is 6.87×10^{-11} cm^2 sec (see Reference 49, Table IVB). Dividing 1.2×10^{-12} moles/cm^2 by 6.87×10^{-11} cm^2 sec, one finds that it would take only *17 milliseconds* to dissipate such a hypothetical standing Na potential, while the postulated Na channels remain closed all the time. Therefore, the central postulated mechanism for the action potential rests upon information no longer valid and therefore cannot work.

(1.2) The allegedly ion-specific Na channel is *not specific to* Na^+

To serve the purposes intended, both the Na and the K channels must be strictly ion-specific. Otherwise, when the Na channel opens, the inward movement of Na^+ (driven by the alleged "standing" Na potential) would be annulled by the outward movement of K^+ *at the same time* through the open Na channels (driven by the alleged oppositely-oriented K potential). In consequence, the anticipated sequential impacts of the Na^+ and K^+ migrations on the electric potential would not happen and there would be no action potential. In 1965, Chandler and Meves[255] demonstrated in squid axon that the postulated Na channel, when open, does indeed admit K^+ also. This finding has once more made the ionic theory of action potential difficult, if not impossible, to defend.We now turn our attention to an alternative theory.

(2) The close-contact surface adsorption (CSA) theory of action potential

The theory of resting and action potential according to the AI Hypothesis is based on the concept that the resting potential is a *close-contact surface adsorption potential.* As such, the CSA theory does not suffer from the difficulties upon difficulties confronting the membrane-pump theory.[15 p 462; 107 p 273] And the CSA theory is supported by all the inanimate models that were engineered to verify the membrane model (but failed).

As the reader knows by now, the high concentration of K^+ in resting cells results from preferential adsorption; the low concentration of Na^+ from weak adsorption and low solvency in (polarized-oriented) cell water. This is true in the bulk-phase cytoplasm as it is at the cell surface. There is

no *standing* Na potential, nor *standing* K potential, because opening a (hypothetical) Na gate would not in a resting cell cause an inrush of Na, nor the opening of a (hypothetical) K gate cause an out-rush of K. Instead, it is only during activation that *a local Na potential, and shortly after a local K potential, are momentarily and sequentially created* and just as rapidly and sequentially canceled.

A local *transient* Na-potential is created when, in response to an above-threshold stimulus of one kind or another, a key cardinal adsorbent essential for the maintenance of the resting living state at the cell surface is suddenly removed or destroyed. In consequence, the c-value analogue of the backbone carbonyl groups rise sharply and the cell surface water becomes suddenly depolarized and disoriented locally. With the force keeping the external Na^+ from rushing in *en masse* removed, a local Na potential is thus momentarily created. But this is only one facet of the story.

As the backbone undergoes its transition from the fully-extended, water-polarizing conformation to a folded conformation, there is also a parallel across-the-board rise of the c-value of the β- and γ-carboxyl groups to a higher value more favorable for Na^+ adsorption and less favorable for K^+ adsorption. Accordingly, some of the K^+ adsorbed on anionic sites at, and near the cell surface are set free. A transient K-potential is thus locally created shortly in the wake of the creation of the Na potential. A displacement of the adsorbed K^+ by external Na^+ follows, adding a new component beyond that caused by the rising c-value analogue of the backbone carbonyl oxygen atoms and liberation of cell surface water.

In Figure 71 one finds a diagrammatic illustration of a submicroscopic portion of the surface of an excitable cell at rest (top) and during activity (bottom). The adsorption exchange of Na^+ for K^+ goes *pari passu* with the depolarization of water.[257; 317] Here the liberation of water from the backbone in one portion of a cell surface protein is partially instrumental in causing the c-value increase of the β- or γ-carboxyl group downstream. The resulting exchange of the adsorbed K^+ for Na^+ is in turn helping to bring about the liberation of water polarized and adsorbed by the backbone NHCO sites downstream [14.3(6)]. And this continues until the recovery phase begins and the process goes in reverse, ultimately to restore the cell surface to its resting living state.

Note that the transient depolarization of cell surface water with concomitant c-value rise of the β- and γ-carboxyl groups creates the transient preference for Na^+ over K^+. However, this Na^+ preference is *relative* and

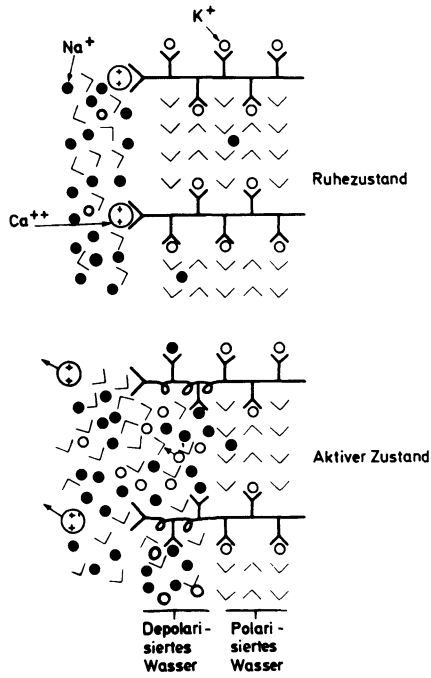

Figure 71. Diagram of a region of the cell surface of a resting excitable cell labeled "resting state" ("Ruhezustande") and a partially activated region labeled "active state" (Aktiver Zustand). At the resting cell surface, the controlling cardinal sites are occupied by the cardinal adsorbent Ca^{++}, K^+ is selectively adsorbed over Na^+ on the cell surface β- and γ-carboxyl groups and the water molecules are adsorbed in polarized-oriented multilayers on the backbone NH and CO groups of fully-extended protein chains. Detachment of Ca^{++} from the cardinal sites leads to an autocooperative change of the system to the *active state*. In this active state, the *c-value* of the β- and γ-carboxyl groups rises to a higher value, at which Na^+ is preferred over K^+ (rather the other way around during the resting state at a lower c-value). Concomitantly, the *c-value analogue* of the backbone carbonyl oxygen also rises to a higher value resulting in an enhanced propensity of the backbone CO groups to form α-helical H-bonds with fixed backbone NH groups. In consequence, cell surface water becomes depolarized and desorbed and external Na^+ rushes into the cell surface through the depolarized cell surface water, displacing the K^+ from the β- and γ-carboxyl groups. See text for observation of concomitant swelling not shown in this figure. ● = K^+. O = Na^+. V = water molecules. (Ling,[317] by permission of Wissenschaftliche Verlag)

not absolute, in the same way that the K^+ preference of the β- and γ-carboxyl groups in the bulk-phase protoplasm of the resting cells is relative and not absolute. Thus when K^+ is removed from the bathing medium, the same β- and γ-carboxyl groups normally occupied by K^+ will readily take up Na^+ as experimentally demonstrated in Figure 57. Similarly when Na^+ was removed from both the inside and outside of the squid axon and replaced by K^+, K^+ took the place of Na^+ and moved into the cell *via* the open "Na channel" as observed by Chandler and Meves[255] [15.6(1.2)].

While the Chandler-Meves finding seriously contradicts the Hodgkin-Huxley theory of action potential, it presents no problem to, indeed it distinctively affirms, the AI Hypothesis. Since the bulk of cell K^+ in a resting cell is adsorbed [10.2(3)], cell K^+ would not move out of the cell instantly at the time the cell surface water is depolarized—as the *free* K^+ in the (arti-

ficial intracellular) perfusion fluid of Chandler-Meves did. Instead, only after Na^+ has rushed into the cell surface through the depolarized water—*pari passu* with the transient rise of Na^+ preference of the surface β- and γ-carboxyl groups brought about by the c-value rise[256]—will adsorbed K^+ be displaced by the Na^+ that has passed through the cell surface. A part of the displaced K^+ will then move out of the cell, creating the *after potential*. But by this time the cell surface has returned to the normal resting state with resting c-value at its β- and γ-carboxyl groups and cell surface water once again polarized and oriented.

The following are summaries of independent experimental confirmations of the major predictions of the AI theory of the action potential.

(2.1) The identification of the anionic groups mediating ionic permeation and generating the resting potential as β- and γ-carboxyl groups carried on cell surface proteins

Long before the AI Hypothesis was formally presented in 1962, I assigned two different roles for the cell surface β- and γ-carboxyl groups. As sites mediating selective ionic permeation and as sites generating the resting potential.[217, 219, 244] In 1965, Ling and Ochsenfeld demonstrated that the anionic groups mediating the entry of K^+ into resting frog muscle cells are indeed β- and γ-carboxyl groups —on the basis of the pK value of 4.6 to 4.7 estimated.[222] From earlier rough data, I also estimated that the anionic group generating the resting potential of frog muscle also has a pK of similar value (*ca.* 4).[107 pp 288 –289]

From his studies of the guinea pig Purkinje muscle fibers, Edelmann provided further evidence that the surface β- and γ-carboxyl groups generating the resting potential and those mediating ionic permeation are the same.[246] He made this identification by correctly predicting the magnitude of the depolarizing action of Rb^+, K^+ and Cs^+ on the resting potential on the basis of their (close-contact) adsorption constants obtained from permeation studies [15.5 (2.4)].

(2.2) The selective preference for ions of the cell surface β- and γ-carboxyl groups is mutable, rather than fixed as in the ionic theory

Evidence that the ionic selectivity of the nerve surface is indeed mutable has been provided by Khodorov.[261; 107, Fig 11.19]

(2.3) The anionic groups mediating the entry of Na^+ into squid axons during an action potential are the same β- and γ-carboxyl groups but with a much higher c-value

In the AI Hypothesis, the c-value of an anionic oxyacid group can be estimated from its pK value, and also from the rank order of preference for the five alkali-metal ions. The pK value of the β- and γ-carboxyl groups mediating the entry of K^+ into *resting* frog muscle is, as just mentioned, 4.6–4.7.[222] In contrast, the pK value of the acidic group mediating the entry of Na^+ during an action potential is higher—as predicted by the AI Hypothesis. Thus, the value obtained from squid axon is given as 5.2 by Hille[258, 259] and 6.5 by Stillman *et al.*[260] All three values (4.6–4.7; 5.2; 6.5) fall within the range of known pK values of β- and γ-carboxyl groups from 3.65 to as high as 7.3.[120; 121] This and the ready reversibility of the pK value change observed[258; 259] are also in harmony with the theory that upward shift of the c-value of the same β- and γ-carboxyl groups confers the Na^+ preference to sites, which at rest prefer K^+. The study of the rank order of ion selectivity further affirms this view.

Thus, the rank order of selectivity of the surface β- and γ-carboxyl groups for alkali-metal ions at 25°C is $Rb^+ > Cs^+ > K^+ > Na^+$ in *resting* frog muscle[222] and $Rb^+ > K^+ > Cs^+$ in resting guinea pig heart muscle.[246] Both correspond to a relatively low c-value (ca –4.0 Å, Figure 42). In contrast, the rank order of the *active* surface of giant squid axon according to Hille is $Li^+ = Na^+ > K^+ > Rb^+ > Cs^+$,[(259)] corresponding to a high c-value (ca –3.0Å, Figure 42) and, according to Chandler and Meves:[255] $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$ with permeability ratios relative to Na^+ of 1.1 (Li^+): 1.0 (Na^+): 0.083 (K^+): 0.025 (Rb^+): 0.016 (Cs^+) corresponding to that of an even higher c-value of about –2.65 Å.

(To recognize the relevance of the relative *permeabilities* of the Chandler-Meves data, one must take into consideration that the ratio of the adsorption constants of a pair of cations on surface sites—which underlies their rank order we want to know—can be, to a first approximation, equated to the ratio of their respective permeabilities—as can be seen from a comparison of Equations A16 and A10, and the known preference for the adsorption-desorption route in the permeation of most cations, see Figure 37).

(2.4) Swelling of nerve fiber accompanying an action potential

In [15.3(1)] above, I have shown how isotonic KCl, but not isotonic NaCl, causes swelling of healthy muscle cells because in healthy and resting cells, K^+ but not Na^+ is preferred by the β- and γ-carboxyl groups—includ-

ing those normally engaged in salt-linkages, which maintains the normal cell-volume. Cell injury, however, leads to a loss of the principle EWC, ATP. An across-the-board rise of the c-value of these carboxyl groups follows in consequence. At the higher c-value, Na^+ present in tissue fluids becomes an effective competitor for these β- and γ-carboxyl groups causing salt-linkage dissociation and cell swelling (Figure 63).

Now, we have presented an hypothesis of the action potential, which too invokes an across-the-board increase of the c-value of the (cell surface) β- and γ-carboxyl groups and their enhanced preference for Na^+ albeit only briefly during the passage of an action potential. Since the nerve axon and muscle fiber are also constantly exposed to the high concentration of Na^+ in the tissue fluid or Ringer's solution, and since some of these surface β- and γ-carboxyl groups are also engaged in volume-preserving salt-linkages, consistency demands that a transient swelling accompanies a normal action potential in a nerve axon, for example.

Such a transient swelling during the passage of an action potential has indeed been reported by Tasaki, Isawa and coworkers in a set of highly ingenious, carefully controlled experimental studies on isolated crab nerves.[523; 524]

(2.5) The propagated c-value increase of the surface β- and γ-carboxyl groups goes *pari passu* with the depolarization of cell surface water molecules

The AI model predicts that concomitant with the abrupt increase in the inward Na^+ permeability, there is an increase in the permeability to nonelectrolyte molecules of large size, which have low q-values and low permeability in the resting cell (surface) water ([13.7], Figure 41).

Villegas, Blei and Villegas demonstrated in 1965 just what the AI Hypothesis has predicted.[262] Thus, they showed that in activated squid axon, there is an abrupt and transient increase in the permeability toward erythritol, mannitol and sucrose (Table 6). Furthermore, this increase of permeability to these large non-electrolytes occurs only in the presence of external Na^+—also in harmony with theory. It would be hard to overestimate the importance of this piece of work in winding up the evidence for the key role of water depolarization in the creation of an action potential.

Table 6.

	Permeability in 10^{-2} cm/sec[a]			
Molecule	Resting axon (axon a)	Stimulated at 25/sec (axon b)	Net increase 25 stimulations/sec (paired data) (b – a)	Calculated permeability during activity[b]
Erythritol	3.6 ± 0.4	6.1 ± 1.0	2.5 ± 0.8	110
Mannitol	2.3 ± 0.4	4.0 ± 0.5	1.7 ± 0.3	75
Sucrose	0.9 ± 0.1	1.8 ± 0.3	0.9 ± 0.3	40

Permeation of C^{14}-erythritol, -mannitol and -sucrose into resting and stimulated squid axons. [a]The values are mean ± SE of ten nerve fiber pairs. [b]Calculated permeability during activity obtained by considering that the permeability change per impulse lasts 1 msec.
(Villegas *et al.*,[262] from *The Journal of General Physiology* by copyright permission of the Rockefeller University Press)

16

Summary Plus

This section is not a standard summary, reiterating what has been said earlier. It does that. But in addition, it also does something not possible until the whole story has been told—overviews. Then there are also bits and pieces of information, which are presented here for the first time, because their significance becomes evident only after the overviews have been drawn.

16.1 Early history

Chapter 1 begins with the discovery of the living cell. The inauguration of cell physiology soon followed. However, *life* in a broader context had captivated the minds of ancient philosophers long before the discovery of the living cell.

Thales (ca. 640–548 B.C.), reputedly the first natural philosopher of the West, chose *water* as the first principle of the cosmos.[309 p 22; 526] Hippo of Samos (450 B.C.) perceived life as water.[309 p 19] Heraclitus (500 B.C.) and Democritus (420 B.C.) considered the human body to be made up of not just water but earth and fire as well.[309 p 34 p56]

Both Paracelsus (1493–1541) and Francis Bacon (1561–1626) saw life as fire. Paracelsus further alleged that something in the air is essential for both ordinary fire and Flama vitalis *or living fire*. In 1774, Joseph Priestley (1733–1804) captured that "something in the air" but did not know it at first. Instead, he thought it was a purer form of air, or dephlogisticated air.[529 p 144] After a vigorous debate, Priestley gracefully and enthusiastically accepted the idea of his arch opponent, Antoine Lavoisier (1743–1794).[527; 346]

According to Lavoisier, what Paracelsus called living fire is *respiration.*[527] Like ordinary fire, respiration entails not a loss of *phlogiston* from the combustible material—an idea which Georg Stahl (1659–1734) championed[352 p 122] and was widely adopted at the time—but a chemical combi-

nation of the combustible material with what Priestley had discovered and, to which Lavoisier gave a different name, oxygen. Both ordinary fire and living fire consume oxygen and produce carbon dioxide.

Louis Pasteur (1822–1895) showed that life can also go on without oxygen-containing air.[528] In an air-free or anaerobic environment, fermentation of plant cells produces alcohol, but glycolysis of animal tissues produces lactic acid.[128] Extensive investigations by many talented biochemists paved the way for Lohmann's discovery of ATP in 1929[272]—as the end product of all energy metabolism, aerobic as well as anaerobic.

In 1941 Lipmann introduced his highly popular, though ill-fated, *high-energy phosphate bond* hypothesis.[132] A mere 15 years later, Podolsky and Morales with the aid of more sophisticated methods of measurements than ones used earlier, found no extra energy stored in the phosphate bonds of ATP.[133]

The high-energy phosphate bond concept is thus the last of a succession of failed attempts to explain the energization of living activities. Engelmann's heat-engine theory for muscle contraction[347] was disproved by Fick on thermodynamic grounds.[383; 530 p 144] A.V. Hill's lactic acid theory for muscle contraction[357] did not survive Lundsgaard's demonstration that iodoacetate-poisoned muscle can contract normally without producing lactic acid.[372; 530 p 145] The disproof of the high-energy-phosphate bond concept left life sciences clueless on the pivotal issue of energization of physiological activities—altogether or only for a few years, depending on the inquirer's awareness of the AI Hypothesis.

Starting out as a part of Ling's fixed charge hypothesis (LFCH), the AI Hypothesis has offered a new theory of how *ATP* may energize life activities without violating the Law of Conservation of Energy—a law first discovered by physiologist-physicist Hermann von Helmholtz when he was barely 26 years old.[352 p 213]

But beyond the ideas of *life as water* and *as fire*, there were still other philosophical thoughts on life from ancient Greeks. Both Anaximenes (ca. 550 B.C.) and Aristotle (384–322 B.C.), for example, equated life with *organization* or *form*.[309] Lamarck's *Ètat de chose* mentioned in [14.2(1)] may be regarded as a sequel. So is Lepeschkin's *live state*.

However, *motion*, the most conspicuous expression of life, was not recognized as characterizing life at first. According to historian Thomas Hall, this neglect arose from the confusion from attributing *all* motions to life (*psyche*).[309 p 267] Not until the 17th century did Descartes (1596–1650) dis-

miss psyche as the cause of animal and plant movements and begin the search for their true causes.[309 p 268] In one direction this search merged with the trail begun with *life as fire* just discussed. In another direction, Dutrochet (1776–1847)—possibly the first recorded experimental cell physiologist in history—equated life with movement.

16.2 The membrane (pump) theory

The low resolving power of early microscopes might have misled Theodor Schwann, the accepted founder of the Cell Theory, into the belief that cells are typically membrane-enclosed bodies of dilute solutions. Schwann's error was quickly corrected from the microanatomists's perspective. Less fortunate were the cell physiologists, who were misled into the same erroneous generalization. But this mistake has stayed on.

The membrane theory, often attributed to Wilhelm Pfeffer, was the first general theory of cell physiology. (For doubts about the validity of this assignment, see Chapter 3). On the assumptions—as Schwann had assumed before—that all living cells are membrane-enclosed dilute solutions and that the enclosing cell membrane is permeable to water but *permanently* impermeable to substances like sucrose and NaCl, proponents of this theory could explain, or so it seemed at the time, four classic cell physiological manifestations of the resting living cell: (i) selective permeability, (ii) selective solute accumulation and exclusion, (iii) cell volume control and (iv) electric potentials.

The membrane theory's apparent strength in simplicity is also its fatal weakness. When Nasonov, Aizenberg and Kamnev demonstrated that the cell membrane is in fact permeable to sucrose and galactose, the paradigm of cells as membrane-enclosed dilute solution faced grave difficulties.

In the crisis thus created, the sodium pump was installed. However, one (sodium) pump is not enough. To keep the cell afloat, an ever-lengthening list of pumps has been introduced. Yet the sodium pump at the cell membrane alone would require at least *15 to 30 times* the total energy available to the cell—under rigorously controlled conditions and with the assumption that the cell needs no energy at all beyond pumping sodium—the membrane theory was in serious trouble again, this time with no easily-discernable escape route in sight.

The energy shortage, however, is far from being the only unfavorable evidence against the membrane-pump-theory. It may be the most persua-

sive one, because the issue is simple and the inconsistency demonstrated between theory and facts staggering. However, a part of the original membrane theory has survived, namely, the existence of some sort of a covering protoplasmic skin or cell membrane, as Pfeffer advocated. But even this surviving part requires drastic modifications from the one widely accepted. Nor is the new cell membrane *semipermeable* (according to the original van't Hoff definition) and the seat of osmotic activities. A summary of these modifications required will be presented in [16.6(3.2)] to come.

16.3 Early protoplasm-oriented cell physiologists and their contributions

At the turn of the 20th century, a number of adventuresome investigators came forth with alternative concepts on the living cell. Fischer, Moore, Roaf, Lepeschkin, Nasonov, Ernst all the way down to Troshin agreed on one point. What the advocates of the membrane (pump) theory had considered as reflecting the attributes of the cell membrane originates (often) from the bulk-phase protoplasm.

More specifically, the efforts of these early protoplasm-oriented cell physiologists were focussed on *two* of the four subjects of cell physiology mentioned above: *solute distribution* and *volume control*. Left largely untackled by these investigators are cell *permeability* and *electric potentials*. Nasonov went so far as to deny the existence of a cell membrane (hence selective *membrane permeability*) or a *resting potential*[86 p 164, 178](Chapter 8).

These early protoplasm-oriented cell physiologists often chose gelatin as an inanimate model for protoplasm. They believed that protoplasm and gelatin are both colloids. Lepeschkin and Troshin argued that living cells represent the special form of colloids known as *coacervates*. Overton presented evidence that at least a part of cell water exists as *imbibition water* ("Quellungswasser").

That adsorption could provide a mechanism for the accumulation in cells of solutes like K^+ at a level *higher* than in the surrounding medium was first suggested in 1908 independently by Benjamin Moore & Herbert Roaf together[77] and by Martin Fischer alone.[78] In addition, Fischer invoked the "partition law" to account for solutes found at concentration *lower* than that found in the external medium. This was an important new insight in cell physiology at that time; but Fischer did not exploit it beyond the short comment he made in a lengthy document on edema.[78] Later, Troshin

welded these twin concepts—adsorption and partial exclusion—into the two-term Troshin equation (Equation A1 in Appendix 1). And he demonstrated that the distribution of various nonelectrolytes in a *complex coacervate* (of gelatin and gum arabic) and in living cells obeys this equation.

The protoplasm-oriented cell physiologists suffered a severe setback from Hill's reputedly conclusive evidence—bolstered by the later demonstration of free K^+ by Hodgkin and Keynes—that cell water is normal and cell K^+ is free. The inability of early colloid chemists to refute Hill's claim, to provide a more cogent definition of colloids (and coacervates) beyond molecular size, and to explain the underlying difference between gelatin and other proteins, might have also weakened the cause for the colloid-oriented cell physiologists in the eyes of some fellow-scientists at least—as seen in the following *verbatim* quotation:

> "Aside from technical difficulties, protein chemistry has also suffered from the abortive effects of an ill-founded idea, which could be called the *colloid concept* (ital. original)."[538 p 4]

16.4 Ling's fixed charge hypothesis (LFCH)

Before its collapse, the membrane theory was bolstered by the eminent theories of dilute solutions including those of Arrhenius, Debye, Hückel, van't Hoff and others. In contrast, the early protoplasm-oriented cell physiologists had no intellectual pillars like these on which to lean. Both Carl Ludwig and Martin Fischer openly lamented the poverty of relevant physico-chemical knowledge. However, by the time I began my career as a cell physiologist, things had improved considerably. Statistical mechanics, protein chemistry, colloid chemistry, polymer chemistry had either already entered a stage of maturity or were in the process of doing so. It was these new advances in knowledge, which made possible what had been denied my forbears—the option of dreaming up possible physico-chemical mechanisms for cell physiology, and eventually of putting forth a general physicochemical theory of life itself, the *association-induction hypothesis*.

In addition, useful experimental techniques also came into existence at just the right time. Examples are the radioactive tracer technology and the Gerard-Graham-Ling capillary microelectrode. Thus, the disproof of the sodium pump hypothesis on the basis of energy considerations (Chapter 12) relied on the employment of both of these tools.

After the introduction of LFCH (Ling's fixed charge hypothesis) in 1952, it took me ten more years to develop and publish the association-induction (AI) hypothesis. For the convenience of summarizing, I sort the theory into two parts: *association* and *induction*. I shall begin with association.

The most abundant components of the living cell in terms of mass are water first and proteins next. The most abundant components in terms of number are water first and K^+ second . *Association of* K^+ with proteins is the central theme of LFCH presented in Chapter 10; a*ssociation of water* with proteins is that of the PM theory presented in Chapter 11.

The first theoretical question the LFCH raised and attempted to answer concerns the act of *association* itself. Why should K^+, for example, engage in (close-contact) association with fixed anionic groups, when we know that at the same ionic strength K^+ *does not associate* with similar anionic groups in a free aqueous solution? To the best of my knowledge, the LFCH has published (possible) answers to this question for the first time in history.

Two mechanisms have been offered for the enhanced close-contact association with *site fixation,* respectively *electrostatic* and *kinetic*. (i) Overlapping electric field reduces the entropy of dissociation and increases association. (ii) Fixation of the adsorption site nullifies half or more of the potentially-effective bombardments by solvent molecules, which would have torn apart an associated pair of free ions or non-ionic particles in a solution. The electrostatic mechanism enhances the association of only particles bearing opposite electric charges. The kinetic mechanism suffers no such restriction; it applies to all associated molecules, bearing net electric charges or not. By the time the LFCH was published, Kern's striking *experimental* demonstration of the enhanced counterion association with charge fixation had been around for four years.[469]

The LFCH also offered: (1) another historical first in the form of a *quantitative mechanism* for the selective adsorption (hence accumulation in cells) of K^+ over Na^+, (2) the salt-linkage hypothesis to account for prior repeated failures to demonstrate selective K^+ adsorption on isolated proteins and (3) an answer to the question why K^+ is lost on cell death—built on the *still-to-be-fully-evolved* theory of ATP function as the *principal cardinal adsorbent*.

The essence of the LFCH was presented within an hour's time at a Symposium held in Baltimore in 1952. It has taken me and others more than 40 years to complete its experimental confirmations to be summarized next.

With the then-newly-introduced EMOC setup, I demonstrated that the seat of selective K^+ accumulation (and Na^+ exclusion) in frog muscle cells is the bulk-phase cytoplasm rather than the cell membrane (and postulated pumps located in the cell membrane). The adsorbed state of cell K^+ as well as the nature and locations of the adsorption sites have been established by six sets of mutually supportive evidence: (i) the low intracellular electrical conductance, (ii) the strongly reduced mobility of cell K^+, (iii) the altered X-ray absorption fine edge structure of cell K^+, (iv) the widely different activity coefficients of K^+ measured in different cell types with an intracellular K^+-specific microelectrode, (v) the obedience of intracellular K^+ to the prediction of a Langmuir isotherm, demonstrating close-contact, one-on-one adsorption, (vi) the identification of (most of) the K^+-adsorbing sites as β- and γ-carboxyl groups carried respectively on aspartic and glutamic residues on myosin in frog muscle cells.

Next, we move on to the theory and facts on the *association* of intracellular water.

16.5 The polarized multilayer (PM) theory of cell water

In the PM theory, virtually all cell water assumes a dynamic structure different from that in normal liquid water. This departure originates from interaction of cell water with a matrix of fully-extended protein chains. The carbonyl (CO) and imino (NH) groups of these fully-extended protein(s) offer properly-spaced, alternatingly negatively-charged N sites and positively-charged P sites. Together, these N and P sites adsorb a layer of (oppositely-oriented) water molecules or dipoles. The water molecules thus oriented and polarized, in turn, polarize and orient a second layer of water molecules and this continues until all the cell water is oriented and polarized.

A prediction of the PM theory is that proteins, which for structural reasons (e.g., gelatin) or in response to certain denaturants (e.g., urea, guanidine HCl, NaOH), exist at least in part in a fully-extended conformation, behave like the postulated fully-extended protein in living cells. In contrast, proteins in what is conventionally called native conformation,[107 p 37, n5] will not behave this way, or do so weakly. This theoretical perception led

to the classification and deployment of two kinds of models. *Extrovert* models like gelatin imitate the (postulated) fully-extended proteins in living cells; *introvert* models, including most of the so-called native proteins, do not or do so weakly.

Another prediction of the PM theory is that water in living cells and in the presence of extrovert models suffers *motional restrictions.* (In contrast, the presence of introvert models has less or no impact.) This prediction has been put to world-wide testing. Thus (i) NMR studies of the *correlation times* of water protons; (ii) ultra-high frequency dielectric studies of *(Debye) reorientation time* of (whole) water molecules and (iii) quasi-elastic neutron scattering studies of the *rotational diffusion coefficient* of water molecules *unanimously* confirmed the predictions of greater motional restriction of water molecules (and their protons) in both living cells and in aqueous solutions of extrovert models than in plain liquid water.

In addition, more detailed confirmation of the predicted *similar* behaviors of living cells and extrovert models and *dissimilar* behaviors of introvert models extend to a host of other attributes of cell water as well. They include *osmotic activity*, *freezing point depression*, *vapor sorption at near saturation vapor pressure* but above all, *(partial) solute exclusion.*

Based on a quantitative theory of solute distribution introduced in 1993, I demonstrated how a small difference of only 126 cal/mole—over and above the enormous normal water-to-water interaction energy (heat of vaporization) of 9.7171 kcal/mole—is able to explain quantitatively the phenomena of sucrose-, and Na^+-exclusion from living cells, which have interested cell physiologists from the very beginning.

The PM theory of solute distribution, and in particular, the *size-rule*, also explain why and how the historic mistake was committed in regarding the equal distribution of urea and ethylene glycol as definitive proof that cell water and K^+ are both free in living cells.

In harmony with the concept that what the bulk-phase protoplasm is and does, protoplasm elsewhere in the cell can also be and do, the PM theory also offers a new theory of the physico-chemical makeup of the *cell membrane* (a name accepted not without reservation because this structure may not have a sharp delineating boundary on its inner side as in all true membranes). In the new model, the continuous phase of the cell membrane—like the continuous phase of the bulk-phase cytoplasm—is not lipids or phospholipids but multilayers of polarized and oriented water. This new model of the cell membrane, in agreement with facts, is virtually

permeable to everything but with vastly different rates of permeation. Nor does this membrane have much to do with the osmotic activities of the cell, which are primarily expressions of the bulk-phase protoplasm (as witnessed by the normal osmotic behaviors of cells without an intact membrane.)

A series of mutually-supportive findings confirms this theory of (polarized-oriented) water membrane. They include the demonstration that the diffusion of water in frog ovarian egg and giant barnacle muscle fiber is *bulk-phase limited.* That is, the rate of diffusion of water through the cell membrane is the *same as that through the bulk-phase cytoplasm,* both *orders of magnitude* faster than water diffusion through a phospholipid bilayer. Valinomycin (10^{-7} M), which increases 1000-fold the K^+ permeability of bilayers of (authentic) phospholipids bilayers, has *no* detectable influence on the K^+ permeability of frog voluntary muscle, frog ovarian egg, giant squid axon, human lymphocytes and mouse-liver mitochondrial inner membrane.

Finally, the permeation of water and six non-electrolytes through a water-loaded cellulose-acetate membrane quantitatively matches the permeation rates through an (inverted) living frog skin of the same substances at the same temperatures (0°, 4° or 25°C). The cellulose acetate membrane, which, like the frog skin, is virtually impermeable to sucrose, has (polarized and oriented) water-filled pores with average diameter *five* times that of the (virtually impermeant) sucrose molecule. This disproportion in size has once again eliminated a *sieve* mechanism for sucrose's near-impermeability but agrees with the polarized-water model following the *size-rule.*

Before closing this summary of the *associative aspect* of the AI Hypothesis on cell water, I shall fill in on some additional gains provided by the PM theory.

First, the PM theory has at long last offered the first (possible) explanation of why gelatin is different from most native proteins (by being, for structural reasons, permanently in the fully-extended conformation), and of what a colloid is (macromolecules or big aggregates of smaller entities polarizing water or other polar solvents in multilayers) or a coacervate (i.e., the stand-alone cooperative phase containing parallel-oriented colloids).

Second, based on results of early NMR investigations testing the PM theory, Raymond Damadian invented what was to be known as *magnetic resonance imaging* or MRI.

16.6 The association-induction hypothesis (proper)

The inductive arm of the AI Hypothesis concerns itself with the maintenance and control of the resting *living state*, with reversible physiological activities and with irreversible death. Not all of these life-and-death problems have been of broad interest in the context of the membrane theory or to the early protoplasm-oriented cell physiologists—even though some of these life-death problems had been of great interest to natural philosophers of ancient times.

(1) The resting living state

According to the AI Hypothesis, the foundation of life rests upon the existence of cells and protoplasm in the resting living state. In this state, the three major components of the living cell are at equilibrium in an associated state. Chapters 10 and 11 summarize evidence for this concept.

(1.1) The resting living state as a (metastable) equilibrium state

The equilibrium AI model stands in sharp contrast to the notion that life represents a *steady state.* Like a flame, whose unchanging shape belies ceaseless unseen chemical reactions, life in the steady-state model is also regarded as the overall expression of biochemical reactions, steadily producing and splitting ATP for the pumping of Na^+ and other work performances. It is true that in my opinion the membrane pump hypothesis has passed its productive days (Chapter 12). Nonetheless, it would be only prudent to re-examine it from time to time in the light of new findings and new knowledge, if only to remind us where we had been before and why.

Like all other chemical reactions, biochemical reactions come to a halt when the temperature falls to the absolute zero, or close to it, say at the temperature of liquid nitrogen. If life truly represents the summation of ceaseless chemical reactions like that described in the steady state model, it would inevitably come to an end or death at such a low temperature. In contrast, an equilibrium state does not necessarily undergo cataclysmic changes in being cooled to a very low temperature. It is now common knowledge that living cells, even whole human embryos, can be kept alive but dormant in liquid nitrogen.[176] And at any time, normal life can start again in response to simple thawing. Cryopreservation of the living cell confirms the existence of living protoplasm and cell in a (metastable) equilibrium state.

(1.2) The resting living state as a low-entropy state

In the AI Hypothesis, the living cell or protoplasm has low entropy due mostly to the adsorbed state of the bulk-phase cell water, cell K^+, congruous anions, ATP etc.—as has been experimentally verified in Chapters 10 and 11. This hypothesis also predicts that the cell or protoplasm has the propensity to topple spontaneously and lose the motional restriction of its adsorbed water and adsorbed K^+ with a modest rise of temperature—a prediction, which has also been affirmed by the demonstration that frog muscles die on being heated to a mere 41°C.[519]

Thermodynamics tells us that the free energy change (ΔF) in a reaction is related to the enthalpy or heat-content change (ΔH) and entropy change (ΔS) by the relation: $\Delta F = \Delta H - T\Delta S$. The *spontaneity* of the transition of the frog muscle from its normal resting living state to the dead state at 41°C indicates that ΔF of this reaction is negative. The initiation of this transition by such a small increment of temperature demonstrates that the entropy change (ΔS) associated with the spontaneous transformation must be positive and large so that $T\Delta S$ can exceed the heat-absorbing, hence positive enthalpy change (ΔH), and thus yield a negative free energy change (ΔF) for the reaction to proceed *spontaneously*: A positive change of entropy in going from the living to the dead state shows that the entropy of living cells must be lower than that in the dead cells in agreement with theory.

(1.3) Do the major components of the living cell exist in a different physical state or conformation?

The three major components of protoplasm and living cells are water, proteins and K^+. The adsorbed state of K^+ and of water in living cells has been established (Chapters 10 and 11) and the evidence already summarized above. It is the conformation of intracellular proteins that calls for special attention. Indeed, to find out the truly *native* conformation of a protein inside a resting living cell carries the same risk as that in trying to find out the life or death of a cat inside a black box, where opening the door of the box inevitably triggers the release of a poison instantly killing the cat (if it is alive). This parable tells us that we cannot determine the true conformation of intracellular proteins within the resting living cell by grinding up the cell, extracting the protein with solvent and other steps, by which means what are commonly called native proteins are usually prepared. A less invasive approach is a must.

(1.3.1) Conformation of hemoglobin in red blood cells from K^+, Na^+ distribution

Red blood cells, like most other living cells, selectively accumulate K^+ and partially exclude Na^+. Hemoglobin makes up 97% of the intracellular pro-

teins of red blood cells.[74 p 117, 119, 120] From the established adsorbed state of both cell K^+ and cell water (Chapters 10 and 11), one would expect hemoglobin to play a major role in the adsorption of K^+ and exclusion of Na^+. Yet dissolved in water at the same concentration as that found in normal living red blood cells (36%), the so-called *native* hemoglobin adsorbs little or no K^+ or Na^+.[(484; 114 p 224)] Nor does water in a 36% solution of native hemoglobin exclude Na^+.[(306 Table 1A)]

According to the AI Hypothesis, hemoglobin in the living red blood cells can do what isolated native hemoglobin cannot do, because in the resting cell, hemoglobin assumes a different conformation. The following two sets of evidence support this view.

Exposure to 0.4 M NaOH not only liberates all the β- and γ-carboxyl groups for the stoichiometric and selective adsorption of Na^+ or K^+ [10.2(2)], it also liberates the backbone NHCO groups to polarize-orient the surrounding water and to reduce its solvency for sucrose (and Na^+) [11.3(4)]. These experiments show that hemoglobin has the innate capability of adsorbing K^+ and reducing solvency of water for Na^+. And apparently it will do both, if the conditions are right. And the following experiment suggests what these conditions are in the hemoglobin's natural environment—within the red blood cells.

When freshly drawn red blood cells are lysed in a *hypotonic* solution, they lose (all or part of) their hemoglobin, their K^+ as well as their ability partially to exclude Na^+. If sucrose is added to the suspension of red cell "ghosts" thus obtained so that isotonicity is restored, continued incubation at 37°C will lead to the re-accumulation of K^+ and exclusion of Na^+—*only if* (i) ATP at suitable concentration is present in the lysing solution and (ii) some tightly bound, lysing-resistant protein(s) [protein X(s)] as well as some hemoglobin are allowed to remain in the ghosts [14.2(2)].

In fact, the concentration of K^+ reached at equilibrium and the extent of Na^+ exclusion are both *quantitatively* related to the amount of proteins (mostly hemoglobin) left in the ghosts (Figure 33). Resealed "white" ghosts containing no hemoglobin neither reaccumulate K^+ nor extrude Na^+. By the method of least squares, the data given in the top half of Figure 33 can be represented as a straight line. Extrapolation of this straight line to a protein concentration found in normal red blood cells yields an intracellular K^+ concentration close to the K^+ concentration found in normal red cells.[140 Fig 5] Furthermore, this hemoglobin required for K^+ reaccumulation and Na^+ extrusion does not have to be the hemoglobin originally present in

the cells. Foreign hemoglobin introduced into the lysing solution and subsequently captured in the resealed ghosts will do also.

Together, these findings suggest that (i) in living red blood cells hemoglobin exists in a conformation different from that of isolated native hemoglobin but with both the backbone NHCO and β- and γ-carboxyl groups free to adsorb water and K^+ respectively and (ii) hemoglobin originally present or introduced from a foreign source assumes this conformation in response to ATP and its helpers, protein X (X's) (and congruous anions, Cl^-) much as that illustrated in Figure 44.

(1.3.2) Conformation of myosin in frog muscle cells from nonelectrolyte distribution

Gelatin, PEO, NaOH-denatured hemoglobin, like all the other extrovert models, exist in solution in the fully-extended conformation. As such, they demonstrate size-dependent exclusion of nonelectrolytes. In a plot of the q-values of nonelectrolytes against the logarithm of their respective molecular volumes (or molecular weights), a Z-shaped curve is found (Figures 25 and 28). In contrast, a flat line of unchanging q-value at unity or near unity with variation of nonelectrolytes size (up to a molecular volume of about 500 cc.) is shown by *native* proteins (Figure 25). Just like nonelectrolytes in solutions of extrovert models and not at all like solutions containing native hemoglobin [11.3(4)], (Figures 28 and 29), the q-values of 21 nonelectrolytes in frog muscle cells, when plotted against their respective molecular volumes, also show a Z-shaped curve. We conclude that the major muscle proteins, myosin (and actin [15.1(3)]) exist at least in part in the fully-extended conformation in resting frog muscle cells.

This conclusion is strongly boosted by the long list of other attributes of cell water, which mimic those in the presence of extrovert models but not that of introvert models in Section [11.2].

(1.3.3) Conformation of myosin and actin in frog muscle cells from vapor sorption

Frog muscle contains some 80% water and 20% proteins. According to the PM theory, each gram of muscle cell protein must sorb on the average 4 grams of water. Using the newly introduced *null-point method*, Ling and Hu studied vapor sorption of both introvert native proteins and extrovert gelatin, PEO, PVP and PVME—at the rarely-studied near-saturation relative vapor pressures.

The results show that only extrovert models in the fully extended conformation sorb enough water to approach that of the living frog muscle at the same (physiological) vapor pressure. In contrast, all native proteins studied sorb from a quarter to one half as much water as that of frog muscle cells [11.3.(7.1)]. Once more, the data show that at least a major part of myosin and actin must be in the fully-extended conformation to sorb enough water to match that seen in intact living muscle cells.

Parenthetically, the free intracellular solutes (i.e., Na^+ and Cl^-) are too low in concentration to influence significantly the water uptake. The more abundant intracellular solutes like K^+, ATP, creatine phosphate are adsorbed and thus osmotically inactive or nearly so.

(1.4) In maintaining the resting living state, ATP is indispensable

To produce the drama of life, many cardinal adsorbents must each play a designated role. None of them could play its designated role if ATP were absent. For ATP adsorption on key cardinal sites is indispensable in preserving the resting living state. As illustrated in Figure 44, each adsorbed ATP keeps an *elemental living machine* in the living state.

Two outstanding expressions of the resting living state are the selective adsorption of K^+ (over Na^+) and the polarization-orientation of the bulk-phase water, from which Na^+ and sucrose are partially excluded. The AI model predicts that there should be a *positive* linear relationship between the equilibrium concentration of K^+ and the concentration of ATP in the living cell. And that there should be a *negative* relationship between the equilibrium concentration of Na^+ and sucrose and the concentration of ATP.

The data presented in Figure 19 and part of the data in Figure 56 affirm the positive *temporal* relationship between K^+ and ATP among a population of muscle cells. The data presented in Figure 17 affirms the positive *spacial* relationship between cell K^+ and cell ATP within the same cells. Another part of Figure 56 affirms the negative relationship between Na^+ (sucrose) and ATP.

(1.5) Where does the excess (cell) water-to-(cell) water interaction energy in the resting living cell originate?

I have shown in [11.3(4)] that the (negative) water-to-water interaction energy in living cells is higher than in normal liquid water by an amount represented by what is called an *exclusion intensity*, or U_{vp}. From solute exclusion data, we estimated an U_{vp} of –126 cal/mole among frog muscle

cell water. If, for simplicity, we assume all this –126 cal/mole to represent exclusively interaction energy—rather than a mix of (mostly) interaction energy and (some) entropy as it truly is[168]—then the total excess interaction energy of water in 1 kilogram of fresh muscle cells would add up to 44 × (–0.126) = –5.56 Kcal, where 44 represents the number of moles of water in 1 kilogram of resting muscle cells. Where does this amount of energy come from? Could it all come from ATP adsorption? The answer is No.

ATP exists in resting frog muscle at a concentration of 5 mM. Multiplying this figure by the adsorption energy of –14.3 Kcal/mole[287][14.3(4)], the total adsorption energy of ATP is only –14.3 × 0.005 = –0.072 Kcal., far below the excess water-to-water interaction energy of –5.56 Kcal.

The source(s) of the excess energy is easier to explain with the help of a few simple models.

(i) *A pair of water dipoles.* As pointed out in [11.2], each water molecule can be considered as an electric dipole. When two such dipoles are placed in *parallel*, head-to-head and tail-to-tail, their mutual interaction energy is repulsive. On the other hand, if they are in an *antiparallel* orientation, head-to-tail, tail-to-head, the interaction is attractive. The basic source of the excess water-to-water interaction energy in living cells is from similar antiparallel orientations of neighboring water molecules.

(ii) *A solution of gelatin.* In normal liquid water, thermal agitation keeps most water molecules from maintaining an antiparallel orientation for long periods of time. This situation changes when gelatin at a suitable concentration is introduced. Because at least half of the gelatin backbone exists in the fully-extended conformation [11.3(2)], and their CO and NH groups are fixed in space, the *principle of enhanced association with site fixation* [10.1(1)] drives the water molecules to adsorb and stay adsorbed onto these CO and NH groups. This adsorption not only polarizes and orients the water molecules in immediate contact with the protein, it also forces the assumption and maintenance of antiparallel orientation of water molecules farther removed. Thus the CO and NH groups of the fully-extended gelatin chain act as "nucleation centers" for the more ordered arrangement of the bulk-phase cell water with enhanced water-to-water interaction energy. Note that before and after the introduction of gelatin, the system is in a stable dynamic structure at equilibrium. The high

entropy-low (negative) energy state of the normal liquid water shifts to a lower entropy-higher (negative) energy state of water in polarized-oriented multilayers in consequence of the interaction with gelatin. The energy component of the enhanced U_{vp} represents this higher-(negative) energy from such a trade-off.

(iv) *The living cell.* Intracellular proteins like myosin and actin in muscle cells and hemoglobin in red blood cells act in a similar manner, like gelatin as "nucleation centers," promoting the dynamic structure of lasting antiparallel orientation of the cell water molecules. And with it, the excess water-to-water interaction observed.

(2) Global coherence and internal connectedness in protoplasm

Frog muscle contains 44 M of water, 100 mM K^+-adsorbing β- and γ-carboxyl groups and 5 mM of ATP. Since all the cell water and K^+ are under the control of the cell ATP, each ATP molecule controls on the average 20 K^+ and 8800 water molecules.

In the AI Hypothesis, this type of far-reaching control rests upon a combination of two basic mechanisms: a short-range inductively-propagated transmission in one dimension along the length of the polypeptide chains and short side chains like those carrying β- and γ-carboxyl groups [14.3(6)]; and a three-dimensional propagated polarization-orientation through the bulk-phase water. I shall review the one-dimensional transmission first.

(2.1) Native hemoglobin *in vitro*

The highly accurate data on the oxygenation/deoxygenation of human hemoglobin by Lyster[557] are rigorously described by a Yang-Ling adsorption isotherm (Equation A4 in Appendix 1)[107 Fig 7] deploying a single intrinsic equilibrium constant ($K^{oo}_{i\to j}$) of 0.1 $(\text{mm Hg})^{-1}$ and a single *nearest-neighbor-interaction energy* or $-\gamma/2$ of +0.67 Kcal/mole[107 p 139]—even though the nearest neighboring heme sites are separated by long stretches of polypeptide chains derived from an assortment of different amino-acid residues.

(2.2) NaOH-denatured hemoglobin *in vitro*

A look at the amino-acid sequence in the hemoglobin chains[531, 536] reveals that the distance between each pair of nearest neighboring β- and γ-carboxyl groups in hemoglobin varies widely—ranging from 1 (e.g.,

α74asp–α75asp) to as many as 23 (β50asp–β13asp). Nonetheless, a single $-\gamma/2$ equal to +0.824 Kcal/mole describes the Na^+ adsorption on all the β- and γ-carboxyl groups of the entire NaOH-denatured hemoglobin molecule.[114]

(2.3) Muscle proteins *in vivo*

In frog muscle, as much as 80% of the K^+-adsorbing β- and γ-carboxyl groups belong to myosin.[122] This protein is even larger than hemoglobin[361] and has a diversity of the distance between nearest-neighboring β- and γ-carboxyl groups like that in hemoglobin. Nonetheless, the K^+ (and Na^+) uptake curve of *whole* frog muscle cells is also rigorously described by a *single* $-\gamma/2$ equal to +0.62 Kcal/mole.

That (i) the oxygen uptake on heme sites of native hemoglobin, (ii) the Na^+ adsorption on the β- and γ-carboxyl groups of NaOH-denatured hemoglobin and (iii) the K^+ adsorption on the β- and γ-carboxyl groups of whole frog muscle cells should in each case be described by a single $-\gamma/2$ is at once astonishing and informative. For this singleness of $-\gamma/2$ tells us that the transmitted interaction along the polypeptide chains between any pair of nearest-neighboring heme groups in hemoglobin, between any pair of nearest neighboring β- and γ-carboxyl groups in NaOH-denatured hemoglobin and between any pair of nearest neighboring β- and γ-carboxyl groups in (diverse) frog muscle proteins must all be *independent of the distance separating the nearest neighboring sites.*

The independence of $-\gamma/2$ from the distance between the nearest neighboring sites is in full harmony with the theoretical model of long-distance transmission—built on repeated short-range propagations as depicted in [14.3(6)] and diagrammatically illustrated in Figures 50 and 51. As in an even simpler model, the impact on each falling domino is the same wherever that domino may be in the domino chain. (See [16.6(4.4)] for more evidence on global connectedness.)

We now proceed to the three-dimensional propagation of the inductive effect among the bulk-phase water molecules—as already pointed out in small print in the later part of [11.2]. That is, under ideal conditions of strongly polar NP surface and absolute zero temperature, the extra-energy between the nearest neighboring water molecules within the dynamic structure of polarized and oriented multilayers is a *constant* and stays undiminished as the polarization-orientation proceeds from the N and P sites to water molecules farther and farther away. Of course, in the real world, the

ambient temperature is not absolute zero, nor is the NP surface perfect. Accordingly, the propagation of the inductive effect is limited. Yet all the data cited above show how far-reaching even the real-world propagation can be.

(3) Interpretation of the four classic physiological manifestations

During its heyday, the membrane theory appeared able to explain the four major physiological manifestations on the basis of one simple assumption—cells as membrane-enclosed dilute solution. All that is now history. It is the turn of the AI Hypothesis to explain the same set of physiological manifestations —based on the almost equally simple assumption that living cells are made of protoplasm maintained in the resting living state by ATP and its helpers.

(3.1) Solute distribution

At 0°C and in the absence of insulin, D-glucose and glycine (and 20-some additional nonelectrolytes studied [11.3(4)]) are found virtually all in the muscle cell water. As such, their equilibrium concentrations in cell water are rectilinearly related to their external concentrations with slopes equal to the solute's respective q-value, which in turn obeys the *size-rule* (Figures 26, 27 and 59). Similar rectilinear distributions with low q-values were also shown for Na^+ in frog muscle in the presence of high concentrations of competing K^+ (Figure 54) and for Mg^{2+} at external concentration higher than 1 mM [15.1(1)].

Exposure to insulin and a primer at 25°C creates specific adsorption sites for D-glucose in frog muscle cells (and for glycine) (Figure 59). Exposure of E. coli to lactose in a glucose-free medium leads to the production of a trio of proteins, one of which has been hitherto regarded as a lactose pump and given the name of lactose permease. Severe difficulty of the pump concept for all unifacial cells (including E. coli) calls for a new interpretation. The fact that this permease promotes the accumulation of lactose in E. coli much as insulin promotes the accumulation of D-glucose in frog muscle and that it is not found in the membrane fraction of E. coli homogenate but only in the cytoplasmic fraction supports the notion that the lactose permease is in fact an insulin-like *intracellular hormone*.

For the quantitative explanations of the equilibrium distribution of sugars and other nonelectrolytes, the simple two-term Troshin equation (Equation A1) is often adequate. However, the Troshin equation cannot explain the accumulation of oxygen in red blood cells (right curve in the Inset of

Figure 55) nor the accumulation of low concentration of K^+ in the presence of high concentration of Na^+ (Figure 57). In contrast, *all solute distribution thus far encountered can be quantitatively described by Equation A7 for solute distribution on just one type of adsorption sites (and of course, also by the general equation for more than one type of adsorption sites, A6).* Both Equation A6 and A7 differ from the Troshin equation in that only Equations A6 and A7 incorporate *cooperative interaction* among adsorption sites.

And I have shown in subsection (2) above how it is that in *autocooperativity* one finds clues on what distinguishes living protoplasm from a piece of dead ion exchange resin, which, like living protoplasm, also selectively accumulates K^+ over Na^+ and which, like the living protoplasm, also contains polarized and oriented water demonstrating size-dependent solute exclusion.

As mentioned above, the adequacy of Equation A7 with a single adsorption isotherm to describe *all* the known solute distribution data is truly amazing. Especially so, if we take into account that to describe adsorption on a *single purified isolated native protein* like bovine serum albumin[439] [Fig 6] requires a composite of several adsorption isotherms. The pervasive physiological uniformity in adsorption is in harmony with the concept of a common elemental living machine and the kind of synchrony among different elemental living machines that the population of isolated mitochondria exemplify in Figure 67.

(3.2) Solute permeability

In the AI Hypothesis, the cell surface, conventionally referred to as the cell membrane, is largely a two-dimensional replica of the three-dimensional cytoplasm. In harmony with this view, the general equation for solute permeability (A13 in Appendix 1) as well as its various simplified versions shown as equations A14 to A16 find counterparts in the equations for solute distribution.

Based on the location in the body, cells can be divided into two categories: exposed *bifacial* cells like the epithelial cells of the frog skin and protected *unifacial* cells like frog muscle and ovarian eggs. Since the bifacial epithelial cells of frog skin, like all other cells, must also receive nutrients from, and dump toxic wastes into, the blood stream, some part of its covering membrane must be like the protected unifacial cells. Therefore, the task of preserving valuable components and keeping out noxious elements from

the outside must be delegated to the *outer surface* of the frog skin epithelial cells. In agreement, the rate of permeation of L-glucose *across* frog skin (including the outer surface of the epithelial cells) is more than 3000 times slower than that of water[217] (Figure 41).

The demonstrated bulk-phase limited diffusion of radioactively labeled water in frog eggs and barnacle muscle cells tells us that the diffusion coefficient of water through the cell membrane of these and other similar protected cells is equal to that through the cytoplasm. And both differ from that in normal liquid water by a factor of only 2 to 3 (see legend of Figure 39).These data suggest that in protected unifacial calls, permeation rates in general are much higher than those across the outer membrane of bifacial cells.

In work yet to be published, Diane Graham and I studied labeled D-glucose efflux from one kind of protected cell, frog muscle. From 20 independent assays, we obtained a half-time of exchange ($t_{1/2}$) at 0°C of 12.9 ± 1.86 min. (s.e.). I estimate that the $t_{1/2}$ at 25°C for D-glucose would not be far from that of Na^+ at 25°C, i.e., 3.34 min.[49 p 150] All these studies on frog muscle suggest that the permeability of protected cells to essential nutrients like D-glucose are indeed orders of magnitudes faster than the extremely slow permeation of other nonelectrolytes of similar or even smaller size through the (exposed) frog skin as shown in Figure 41, or the membranes of the (exposed) *Nitella* and *Chara* internodal cells as shown in Figure 34.[358]

The divergent needs of exposed and sheltered cells are once more evidence against the conventional membrane theory, in which the continuous phase of the cell membrane is invariably a phospholipid bilayer. This inadequacy was brought into sharp focus by R. E. Wood *et al.*,[435] when they showed that the permeability of phospholipid bilayers to D-glucose is 1,000,000 times slower than that of the cell membrane of another protected cell, the red blood cell.

In contrast, the AI model of cell membrane composition and structure is distinguished by its great flexibility, giving rise to highly different permeability to ions, nonelectrolytes as well as ampholytes like free amino acids and even giant protein molecules—to cells according to their specific needs.

(3.3) Cell volume control

The volume of a living cell is essentially the sum of the volume of its proteins and that of its water. The ratio of cell water to cell proteins is not con-

stant but characteristic of each cell type. This diversity of water/protein ratio reflect the interplay of three factors—each attributable ultimately to the differences in the nature and amounts of cell proteins.

The primary driving force for the accumulation of water in living cells, called factor (1), is the multilayer polarization-orientation of water on some fully-extended intracellular proteins. The equilibrium volume of a cell is determined by the balance of this factor for expansion and two factors opposing it: (2) salt-linkages distributed throughout the cell restrict water uptake and expansion; (3), low q-value solutes bring about cell shrinkage [11.3(7.2)].

That isolated frog muscle cell maintains its normal water content in a vapor phase with partial vapor pressure equal to that of a frog Ringer's solution, that 95% of frog muscle cell water follows the Bradley adsorption isotherm [11.2], and that only fully-extended or extrovert models like gelatin, PEO and PVME, sorb enough water to match that of the living cell [11.3(7.1)] all affirm (1). That healthy living cells swell strongly in an isotonic solution of (salt-linkage-disrupting) KCl but not in an isotonic solution of (non-salt-linkage disrupting) NaCl [15.3] affirms (2). That cells shrink in high concentration of NaCl with low q-value but swell in isomolar solutions of solutes like ethylene glycol, which have high q-values [11.3(7.2)] affirms (3).

The demonstration that injury-induced swelling shares a basic mechanism with KCl-induced swelling and that this swelling is directly dependent on the concentration of NaCl in the bathing medium and inversely dependent on the concentration of ATP in the cells [15.3] confirm at once the AI Hypothesis of cell volume control and the critical role of ATP in maintaining the resting living state as an EWC.

(3.4) The resting potential

For over 60 years, since the publication of Bernstein's membrane theory of cellular resting potential, efforts had been made again and again to find an inanimate model for the postulated membrane potential. All have failed [Section 15.5(1.1.1)].

In my opinion, the fault lies in the theory itself. A real-world membrane of any measurable thickness cannot give rise to a membrane potential as the membrane theory suggests (Figure 4B). For as soon as the permeant ion species enters the membrane, it will be stopped by the attraction of the oppositely-charged impermeant ion species left behind. As a result, an

electric double layer is formed at the interface, establishing a (lasting) potential difference not across the whole membrane but across one (or both) phase boundary—much as the theory of Nernst has made clear [15.5(1.2)].

While the membrane theory could not find an inanimate model to confirm its validity, every one of the failed models turns out to be a cogent model for the close-contact surface adsorption (CSA) theory. Two of these models are of special interest: the glass membrane (especially in the light of the Horovitz's discovery of the role of surface cations in generating the electric potential); the collodion membrane (especially in the light of Sollner *et al.*'s discovery of the role of surface carboxyl groups in generating the electric potential). But the best inanimate model of all, is a combination of the two—in the form of oxidized collodion-coated glass (CG) electrode, as well as its variant, the poly-lysine-treated collodion-coated glass (PCG) electrode.[376 p 286]

While both the CG and the PCG models clearly demonstrate that the potential measured has nothing to do with membrane permeability to ions, each underscores how surface fixed ionic sites determine the magnitude and polarity of the potential. But it is the extreme cogency of the CG model that is nothing less than amazing: (i) both the resting potential of frog muscle and the CG electrode potential show no sensitivity to Cl^-; (ii) both the resting potential of frog muscle and the CG electrode potential show no sensitivity to divalent cations like Mg^{2+}; (iii) both the resting potential of frog muscle and the CG electrode potential show sensitivity to alkali-metal ions following a rank order corresponding to a relative low c-value; (iv) both the resting potential of frog muscle and the CG electrode potential show a sensitivity to H^+ roughly two orders of magnitude higher than for K^+.

So far, all or almost all the accurately measured resting potentials of one kind of cell or another can be described quantitatively by Equation A19 or A21 in Appendix 1 (or their simpler variants), all of which are derived from the general equation for solute distribution with cooperative interaction, Equation A3 also in Appendix 1 (or its simpler variants).

(4) Physiological activities as reversible cooperative transitions mediated by inductive effects

In Chapters 10 and 11, we have established that in resting frog muscle cells at say 30°C, K^+ is adsorbed singly on β- and γ-carboxyl groups and water molecules are adsorbed as polarized-oriented multilayers on the CO-NH

groups of the fully-extended protein chains. Yet with a modest rise of some 10°C, all the muscle cells are dead. In consequence, K^+ and cell water are both freed. In explanation, I have pointed out why this high sensitivity to a small temperature rise bespeaks a low entropy state of the living cells. And how, according to the AI Hypothesis, the underlying mechanisms for the observed changes in the state of cell K^+ and cell water are electronic in nature; they can be expressed in terms of changes in parameters called the c-values and c-value analogues.

(4.1) c-value and c-value analogue: the key board of life

In 1902, E. Overton showed[359] that muscle contraction requires Na^+ (or Li^+). Almost half a century later, Hodgkin and Katz found that nerve and muscle excitation involves a local increase in the permeability to Na^+. The ensuing inrush of Na^+ into the cell gives rise to the action potential. If there is no Na^+ in the surrounding medium, there can be no inrush of Na^+, nor action potential, nor excitability. These findings provided the foundation for the Hodgkin and Huxley theory of the action potential published in 1952 [15.6(1)]. Even though both authors have made fantastic contributions to this field and even though their theory of action potential is truly brilliant, this theory as such was not to last [15.5(1)].The resting potential is not a membrane potential. Nor can the action potential be a modified version of a membrane potential. There was a need for a new theoretical interpretation.

In 1955, I suggested that the cellular resting potential is closely related to the glass-electrode potential. Both originate from the presence of fixed anionic sites on the surface. On nerve and muscle cell surfaces at rest, these sites are in the form of K^+-preferring β- and γ-carboxyl groups [10.1(3)]. Thus the resting cell surface acts like a potassium-sensitive glass electrode. Yet on activation, it somehow metamorphoses into a sodium-sensitive electrode.

Hints of the feasibility of such a metamorphosis came from one of the inanimate models I had cited in support of the LFCH in 1952, the ion exchange resin. The (earlier) sulfonate type of ion exchange resins I cited prefer K^+ over Na^+, but the converse is true for the late-arriving carboxylic exchange resins. Bregman linked K^+ preference to strong acidic groups and Na^+ preference to weak ones. However, he interpreted the ion-preference difference among different ion exchange resins in terms of the different polarizabilities of their acidic groups, a concept introduced and developed earlier by Bungenberg de Jong and his coworkers.

However, it was Eisenman, Rudin and Casby's extension of the LFCH that changes in the field strength of the anionic sites in glass could lead to reversal of ion preference that prompted me to further develop the LFCH. The result is the association-induction (AI) hypothesis. Included in this general physico-chemical theory of the living cell is the basic concept that physiological activities may entail across-the-board changes in the electron density of the singly charged oxygen atom of the β- and γ-carboxyl groups and that these changes can be represented as changes in a parameter called the c-value.

The c-value (in units of Angstroms) is the independent parameter underlying the acid dissociation constants, or pK values, of oxyacid groups. Thus acetic acid, CH_3COOH, has a high pK of 4.76. Accordingly, it has a high c-value. Substituting one of the methyl H atoms with a Cl atom produces monochloroacetic acid, $ClCH_2COOH$. Since the chlorine atom has a stronger power to draw electrons toward itself than the H atom it has displaced, a propagated inductive effect is set in motion. In the end, a reduction in the electron density of the singly charged carboxyl oxygen occurs. With this charge reduction, a decrease in the affinity of the negatively-charged carboxyl group for the positively charged protons or H^+ results, producing a fall of the pK to 2.8 and a lower c-value. Substitution of two H by two Cl produces dichloroacetic acid with a pK of 1.3 and a still lower c-value. Finally, substituting all three H's with three Cl produces trichloroacetic acid with a pK of less then unity and a still lower c-value. All these instances illustrate how atomic substitutions can inductively alter the attributes of *distant* functional groups in a step-by-step quantitative manner.

With the c-value defined and a linear model adopted, I computed the association energies of 5 alkali-metal ions plus H^+ and NH_4^+ (with NH_4^+ seen also as a possible model for the fixed cations). The results show that a rise in the c-value can indeed produce a reversal of preference from K^+ over Na^+ to that of Na^+ over K^+. Just as important, the computed results offer a possible explanation of why c-value change may also play a role in the engagement or disengagement of salt linkages.

In contrast to the *c-value* of the β- and γ-carboxyl groups, which are carried on short side chains, the *c-value analogue* describes the electron density of the carbonyl oxygen sitting right on the backbone. The relationship between the c-value analogue and its preferred partners was derived from the following facts and postulations: (1) the propensity of a peptide NHCO

to engage in an α-helical (or β-pleated sheet) conformation is postulated to vary directly with the *electron-donating strength* of the side chain of that specific amino acid residue type; (2) the electron donating strength of the side chain of an amino acid residue could be derived from the pK value of the corresponding carboxylic acid (e.g., acetic acid for alanine; formic acid for glycine); and (3) the propensity or potential of the peptide of each type of amino acid residues in forming α-helical, β-pleated sheet and random coil conformation (equivalent to our fully-extended conformation) could be, and were, established empirically.

A positive linear correlation coefficient averaging + 0.75 was demonstrated between three sets of empirically derived α-helical potentials of the 19 amino acid residues and the pK values of their corresponding carboxylic acid. This good correlation in turn, led to the discovery of the rank order of preference for the two major competing partners for the backbone CONH groups. At high c-value analogue, the backbone peptides prefer to engage in α-helical conformation. At low c-value analogue, they prefer to adsorb, polarize and orient multiple layers of water molecules.

(4.2) Changes in the partners of ionic and hydrogen bonds as initiators as well as targets of transmitted inductive effect

The acetic-acid-to-trichloroacetic-acid transformation is a classic example of the inductive effect. The initiator here is a substitution involving the breaking of an old *covalent bond* and formation of a new one. The target of the inductive effect in this case is what one calls an *ionic bond* between the carboxyl group and H^+. However, in the AI Hypothesis, a broader application of the inductive theory has been introduced. In this broader application, H-bonds can also be the *targets* of transmitted inductive effect. Changes in the partners of both ionic and H-bond can also *initiate* a transmitted inductive effect [14.3(1)]. Supportive experimental evidence were all taken from the literature, indicating that these concepts *per se* are not new, only their applications to cell physiology are.

These extensions of the inductive effect are vital to cell physiology, because the activation energy involved in the breaking and formation of the H- and ionic bonds are low enough to occur "spontaneously" at ambient temperature, but the activation energy involved in breaking and formation of covalent bond are as a rule far too high to occur without the participation of specialized enzymes, for example. The c-value and c-value analogues are respectively quantitative measurements of the underlying cause for the

different strengths of ionic and hydrogen bonds formed. And cardinal adsorbents exercise their long range impact on the c-values and c-value analogues leading to across-the-board formation or dissociation of new ionic or H-bonds at near and distant locations.

(4.3) Classification of drugs and other cardinal adsorbents

In conventional pharmacology, there is no explanation for what drugs really do beyond their mechanical fitting on stereospecific receptor sites. In contrast, the AI Hypothesis, from its very inception, sees the living cell as an *electronic machine*. In that context, all chemical agents that at low concentrations bind onto cardinal sites and produce changes (or no change) of cells and protoplasm fall into one of the three categories: electron-donating cardinal adsorbents or EDC's, electron-withdrawing cardinal adsorbents or EWC's and finally electron-indifferent cardinal adsorbents or EIC's.

In the subsection immediately following, I shall review how EDC's and EWC's act on different facets of cell physiology.

(4.4) Maintenance and modulation of the resting living state by various cardinal adsorbents

With ATP and all the other helpers secure in their places, other cardinal adsorbents like ouabain, insulin and adrenaline can then fine-tune the diverse protoplasm to serve their respective specific function for a particular moment or more lastingly.

(4.4.1) Maintenance of the living state by ATP as EWC

ATP is different from the other cardinal adsorbents. As the *principal cardinal adsorbent*, ATP alone (with the assistance of "helpers") can sustain the living state. Other cardinal adsorbents can only modulate the living state. (For the rare condition under which the living state was transiently maintained in the absence or virtual absence of ATP, see Reference 107 p. 196.)

On solute distribution in cytoplasm: As an EWC, ATP controls the specific ionic adsorption on β- and γ-carboxyl groups by creating and maintaining an across-the-board lowering of their c-value (Figure 42) so that K^+ is strongly preferred over Na^+. As an EWC, ATP also keeps the key intracellular proteins in their fully-extended conformation by maintaining an across-the-board low c-value analogue of the backbone carbonyl groups so that the backbone CONH groups can polarize and orient virtually all the cell water. The q-value of (hydrated) Na^+ and sucrose in the water thus polarized and oriented are far below unity, in accordance with the *size-rule*.

On solute permeability of the cell membrane: Like the bulk-phase cytoplasm, the cell membrane proteins are controlled by ATP in an entirely parallel manner. Depletion of ATP depolarizes and disorients the cell surface water and accelerates the rate of nonelectrolyte and electrolyte permeation rate *via* the less-restrictive or no-longer-restrictive saltatory route.

On cell volume control: In normal cells equipped with its full complement of ATP, the c-value of intracellular β- and γ-carboxyl groups are low. And accordingly they strongly prefer K^+ over Na^+. In consequence, isotonic KCl causes extensive cell swelling by dissociating volume-maintaining salt linkages, but isotonic NaCl—present in the normal blood plasma—cannot. During cell injury, however, ATP level falls. With loss of this major EWC, the c-value of the β- and γ-carboxyl groups rises; and with it the preference for Na^+ also rises. Now the normally harmless isotonic NaCl in the plasma becomes as effective as isotonic KCl in bringing about (pathological) cell swelling.

On resting potential: ATP controls the resting potential in a way entirely analogous to its control of ionic permeability. To maintain the normal resting potential in frog muscle also requires the major congruous anion, which is largely creatine phosphate (CrP) in this tissue. Metabolic poison alone or in conjunction with bursts of electric stimuli can produce stepwise fall in the concentration of CrP and the resting potential follows the level of CrP step-by-step.[107 pp 294–299] With complete ATP depletion, the resting potential falls to zero.

(4.4.2) Modulation of the resting living state by ouabain as an EDC

Like all the other non-ATP cardinal adsorbents, the pharmacological function of cardiac glycoside is to modulate the resting living state maintained by ATP and its helpers. While the impact of a single drug ouabain is reviewed here, Figures 52, 53 and related text describe interactions among two or even more drugs and/or other cardinal adsorbents acting concomitantly.

On solute distribution: In a typical experiment reported by Ling and Bohr in 1971[190] on the impact of ouabain on the K^+ and Na^+ distribution, we incubated sterilely at 25°C four small intact frog muscles weighing some 400 mg. together. Of this about 60 mg. belong to extracellular space and connective tissue elements. The remainder of 340 mg. are "pure" muscle cells. The volume of the Ringer's solution is 110 ml or 0.11 liter and it contains initially ouabain at the low concentration of 3.26×10^{-7} M. After

72 hours of incubation, a new distribution equilibrium was established, at which *virtually all the 0.11 moles/kg. of K^+ has been mole-for-mole replaced by (0.11 moles/kg. of) Na^+.*[190 p 442]

These details are given to provide the factual basis in answer to the question: How can a minute amount of ouabain bring about such a wholesale displacement of a vastly larger amount of K^+ in the cell by an equally large amount of Na^+. To leave no stones unturned, I shall resurrect both the original membrane theory and its pump modification as viable alternatives to the AI Hypothesis. And on this much broadened canvas, begin our search for a reply.

(1) The old membrane theory: In this theory both the K^+ originally present in the cells and the Na^+, which replaces it, are free. And the only mechanism that keeps the asymmetrical distribution is a dam-like cell membrane. Within these limits, ouabain can bring about the wholesale replacement of cell K^+ by Na^+ only by "punching holes" in the membranes in one way or another, so that K^+ would leak out and Na^+ would leak in. And in the end the cell will contain little K^+ but a lot of Na^+, exactly as they are in the bathing Ringer's solution.

(2) The membrane pump theory. In this theory the low concentration of (free) Na^+ and high concentration of (free) K^+ in the cell result from the continued activity of a pump located in the cell membrane, ceaselessly pumping Na^+ out and K^+ in. Ouabain acts as an inhibitor of this pump and that is why exposure to ouabain leads to the displacement of cell K^+ by Na^+. In fact, this model is not just my conjecture but part of real-life history (for details, see References 52 and 539).

(3) The AI Hypothesis. In this hypothesis, the bulk of cell K^+ in normal resting cells is adsorbed singly on β- and γ-carboxyl groups and the cell water exists in the dynamic structure of polarized multilayers with reduced solvency for Na^+ and other large solutes like sucrose. On the basis of these (verified) postulations, one can offer three alternative interpretations for the action of ouabain.

In Model 3A, the action of ouabain is like that produced by the depletion of the EWC, ATP. Salt-linkage formation displaces cell K^+; depolarization-disorientation of cell water raises the q-value, and hence intracellular concentration of free Na^+.

In Model 3B, ouabain as an EDC, raises both the c-value of the β- and γ-carboxyl groups and the c-value analogue of the backbone carbonyl

groups. As a result, adsorbed Na^+ displaces adsorbed K^+. Depolarization-disorientation of the bulk of cell water raises the level of free Na^+ also.

In Model 3C, ouabain raises the c-value of the β- and γ-carboxyl groups but not the c-value analogue of the backbone carbonyl oxygen atoms. As a result, adsorbed Na^+ displaces adsorbed K^+ mole for mole. The level of free cell Na^+ remains unchanged.

Model 1 can be rejected forthwith because, by disrupting the cell membrane in one way of another, it predicts a sudden increase of the efflux rate of labeled Na^+ from no efflux at all, contrary to facts. There is a steady Na^+ efflux in the resting cell to begin with, and ouabain either does not change this normal efflux rate of labeled Na^+ or slows it down, depending on whether ouabain is added after or before the exposure of the cells to radioactive Na^+.[540]

Model 2 can also be rejected for a variety of reasons (see below). Only one needs to be cited here. A frog sartorius muscle mounted in an Effectively Membrane-pump-less Open-ended Cell (EMOC) preparation is, as the name indicates, effectively without a functional cell membrane and its postulated pumps. Yet as shown in Figure 8B, such a muscle gains Na^+ and loses K^+ in response to ouabain just as a normal muscle suspended in an ouabain-containing normal Ringer's solution does. Therefore, the ouabain induced changes in K^+ and Na^+ concentration has nothing to do with the activities of the cell membrane or its (postulated) pumps.

Model 3B can also be rejected because it would predict a doubling or at least a large increase in the total cell Na^+ concentration beyond the sum of original cell K^+ and Na^+ concentrations, contrary to facts: the sum of cell K^+ and cell Na^+ remain unchanged in response to ouabain (Figure 57).

The key difference between Model 3A and Model 3C lies in the physical state of the Na^+ taken up by the muscle cells in response to ouabain. If all the extra Na^+ taken up in response to ouabain is in the adsorbed state, Model 3A, which predicts gain of only free Na^+, would be ruled out also. The evidence presented in [15.1(3)], including both NMR data shown in Table 5 and autoradio-electron micrographs shown in Figure 58a demonstrate that the extra Na^+ gained in response to ouabain is adsorbed. (The adsorbed state of the extra Na^+ gained would rule out Model 1 and Model 2 also.)

Having eliminated all the other alternative interpretations that I could find or dream up, I now proceed to analyze the data on the basis of the (only) surviving Model 3C, which, of course, reiterates our earlier conclu-

sion [15.1(3)] that ouabain causes a wholesale displacement of adsorbed K^+ by adsorbed Na^+ mole for mole but no change in cell water.

I now return to my objective mentioned above: to determine how many β- and γ-carboxyl groups swap their (adsorbed) K^+ for (adsorbed) Na^+ in consequence of the adsorption on an appropriate cardinal site of *one single* ouabain molecule.

I assume that *all* the ouabain molecules initially present in the bathing solution (in quantity equal to $0.11 \times 3.26 \times 10^{-7}$ or 3.59×10^{-8} mole) have entered the muscle cells and each ouabain molecule has successfully occupied a cardinal site of the right kind. The total number of β- and γ-carboxyl groups, which had exchanged their K^+ for Na^+ in response to ouabain is $0.110 \times 3.40 \times 10^{-4}$ equal to 3.74×10^{-5} mole. Dividing the number of ouabain molecules in the muscle (3.59×10^{-8} mole) into the number of β- and γ-carboxyl groups affected (3.74×10^{-5} mole), we obtain the number of β- and γ-carboxyl groups controlled by each ouabain molecule. It equals 1042.

That one thousand and forty-two (1042) β- and γ-carboxyl groups were made to give up their K^+ for Na^+ by the adsorption of a single ouabain molecule is astonishing. Furthermore, each of these 1042 K^+ or Na^+ that have replaced them is not part of a random population of migrating ions like those depicted in Model 1 and 2, but adsorbed one-on-one in close contact on an individual β- and γ-carboxyl group, each of which has a specific *identity* and *location* in the cell. Since the only difference between a K^+ and a Na^+ lies in their respective short-range attributes, the change in each of the β- and γ-carboxyl group to effect the switch from a K^+ to a Na^+ can only reflect changes in the carboxyl group's own short-range attribute such as the local electric charge density, i.e., its c-value. Or one can look on it in another way. One may say metaphorically that a minute amount of ouabain has somehow transformed our protoplasmic "ion exchange resin" of the strongly acidic sulfonate type to that of a weakly acidic carboxylate type. Such a change could not be due to pore size limitation, an idea I toyed with in a Howard Johnson's Restaurant in Philadelphia in 1957 but abandoned later, nor due to changes in the polarizability of the functional groups as suggested by Bungenberg de Jong in the 1940's, but can be accounted for by the idea first suggested by Eisenman, Rudin and Casby intuitively as an extension of the LFCH, a change in their field strength, aka the c-value (Figure 42).

Then, of course, the changes in the c-value of these β- and γ-carboxyl groups brought about by ouabain are *all-or-none*. Physicists, in studying the inanimate world, discovered that this type of all-or-none change occurs in what they refer to as ferromagnetic (or auto)cooperative phenomena, distinguished by a large positive *nearest neighbor interaction energy* (e.g., $-\gamma/2$). The striking and different feature of biological autocooperative phenomena lies in the fact that the *nearest neighbor* is not so near at all—as I have discussed at some length in [16.6(2)] above and offered a molecular mechanism to account for it in the theoretical model presented in [14.3(6)].

After all, in theory at least, there is no limit on how long a chain of dominos can continue falling, nor how distant the smoke-and-fire signals on the Great Wall can continue advancing, nor how far the near-neighbor interaction of the theoretical model portrayed in Figures 50 and 51 can continue spreading. Just like the copper wire has made the world a coherent one, this extraordinary efficiency in long-range propagation may well be a *unique* feature of the coherent living protoplasm. Unquestionably, it is built upon the distinguishing traits of the only *unique* chemical structure of life, the *partially resonating polypeptide chain.*

Ouabain may give rise to its effect by stages, producing an impact on other cardinal adsorbents, which in turn relay the message to still other cardinal adsorbents until the effect finally reaches the β- and γ-carboxyl groups involved. But one thing is certain. At the concentration employed (3.26×10^{-7} M) ouabain does not change the normal ATP level.[190 Fig 9] And even if a relay is involved, each one of the participant cardinal adsorbents must still be able to act in an one-on-many manner in controlling distant sites, not fundamentally different from the case when there is no relay operation.

On permeability: In studies on the effect of Rb^+ and Na^+ in blocking the entry of labeled Cs^+ into frog eggs, Ling and Fu[398] have shown that the presence of ouabain raises the affinity of surface β- and γ-carboxyl groups for Na^+ and lowers that of Rb^+ (Figure 62). As such, ouabain acts as an EDC producing an across-the-board rise of the c-value of these carboxyl groups much as it does on the equilibrium distribution of these ions in the cytoplasm of frog muscle.[280]

On cell volume: Depletion of ATP causes cell swelling that in magnitude quantitatively depends on the concentration of Na^+ in the medium (Figure 63). In theory, *depletion of* a pre-existing EWC (like ATP) and

gaining of a previously non-existent EDC (like ouabain) should produce similar effects. In work to be published, Ling and Bohr have demonstrated precisely such an effect: ouabain—at a concentration (3.26×10^{-7} M) far too low to affect the ATP level—produced muscle swelling in magnitude quantitatively dependent on the Na^+ concentration in the medium.

On the resting potential: The data of Akaike first shown in Figure 68, when plotted in Figure 69 according to Equation A19 in Appendix 1 also show that ouabain acts as an EDC. As such, it causes an across-the-board rise of the c-value of the β- and γ-carboxyl groups of rat muscle cell surface, increasing their preference for Na^+ and decreasing their preference for K^+ in an autocooperative manner and quantitatively obeying the theoretical Equation A19.

(4.5) Cyclic reversible physiological activities

(4.5.1) True active transport

100 mM of Na^+ is found in the blood plasma of an animal that spends most of its time in fresh pond water containing only some 0.2 mM Na^+.[15 Table 3.3] Yet, instead of losing Na^+ to the pound water, frogs actually take up Na^+ from the pond water.

Figure 41 demonstrates the extremely low permeability of (inverted) frog skin toward sucrose. Since the q-values of sucrose and NaCl are often not far apart, one may expect the Na^+ permeation rate to be also slow—due most likely to the more-intensely polarized-oriented water in the outer membrane of the epithelial cells. However, to move Na^+ from the pond water to inside the frog against a 500 to 1 concentration gradient requires a more complicated setup. In the AI model, this setup consists of a pair of membranes working like one-way valves and with a sponge protein placed between them, which cyclically sops up and releases Na^+ (Figure 65).

In more detail, the outer surface of the frog skin carries a high density of β- and γ-carboxyl groups of high c-value. At such a high c-value, these anionic groups adsorb Na^+ strongly, raising the local Na^+ concentration far above the concentration in the pond water, completing the first step of Na^+ transport.

The Na^+ accumulated at the cell surface then enters the cell by the doublet and/or triplet adsorption-desorption route. Once inside the cell, it adsorbs onto the sponge protein's β- and γ-carboxyl groups, which also are under the control of ATP but have high c-value. The last Na^+ adsorbed acti-

vates the Na,K-ATPase (of the sponge protein) and the ATP is dephosphorylated.

With ATP gone, across-the-board desorption of Na^+ follows. Concomitantly depolarization and disorientation of the bulk-phase water take place in the cytoplasm, extending to, and including the serosal cell membrane. The desorbed Na^+ finds its way out of the epithelial cells into the blood stream *via* the depolarized water of the cytoplasm and the serosal membrane. Regeneration of ATP starts the next cycle.

The reader may ask, How can ATP, an EWC, make the β- and γ-carboxyl groups preferentially adsorb Na^+, which according to the AI Hypothesis is preferred at *high* c-value sites? A possible answer is that ATP may not be acting alone here. Indeed, with an auxiliary ouabain-like EDC acting in conjunction with ATP, we will have a set of uniformly Na-adsorbing β- and γ-carboxyl groups like those in the bulk-phase cytoplasm of frog muscle under (the combined action of ATP and) ouabain (Figure 57). {For control by more than one cardinal adsorbent, see also [14.3(7)].}

The model for true active transport across frog skin demands that the epithelial cells are engaged in cyclic activities, which are difficult to monitor in view of the small size of the individual epithelial cells. We are thus indebted to S. C. Brooks for his demonstration of cyclic Rb^+ uptake of *Nitella* protoplasm from the external medium and its subsequent (cyclic) release into the central vacuole (Figure 67).

(4.5.2) The action potential

Understanding the physiology of the action potential is the pinnacle of achievement in cell physiology of the 20th century. Unfortunately, the membrane theory, on which the conventional interpretations are based, is, in my opinion, in essence wrong. The insides of nerve and muscle cells are not dilute solutions containing free ions and free water, as once believed. A membrane potential does not seem to exist in the real world, living or nonliving. The majority of researchers could not find the predicted relationship between the intracellular K^+ concentration and the resting potential. The resting potential is indifferent to the concentration of (highly permeant) Cl^- and (highly permeant) Mg^{2+} in the bathing solution. In addition, there are also more specific arguments directed against two key concepts of the Hodgkin-Huxley theory of action potential. There cannot be a standing Na potential; nor is the alleged Na channel specific to Na^+.

According to the AI Hypothesis, the action potential, like the resting potential on which it is built, is an electric phenomena generated by

(spatially-isolated) β- and γ-carboxyl groups on the cell surface. The action potential represents a propagated autocooperative change sweeping across the surface protoplasm of nerve, muscle and other excitable cells. The process entails a transient across-the-board increase of the c-value of the surface β- and γ-carboxyl groups leading to a rise of Na^+ preference—*pari passu* with a transient across-the-board increase of the c-value analogue of the backbone carbonyl groups, leading to the depolarization of the surface water and a rise of the q-values for Na^+ (and sucrose).

Experimental confirmation came from the demonstrated transient rise of the pK values of the surface β- and γ-carboxyl groups mediating the Na^+ entry, and by the demonstrated transient shift of their reference from the resting order of $Rb^+ > K^+ > Na^+$ corresponding to a lower c-value, to an inverted order corresponding to a much higher c-value.

Confirmation of the AI model of the action potential continued with the demonstration by Tasaki and his coworkers[524] that during the passage of an action potential in a crab nerve, there is a transient and local swelling of the nerve axon, which in time parallels the action potential.

Confirmation of the AI model of the action potential reached a climax with the demonstration by Villegas, Blei and Villegas[262] that during an action potential the squid axon surface becomes momentarily more permeable to erythritol, mannitol and sucrose, if Na^+ is present in the external medium. This Na^+-dependent increase of non-specific permeability to nonelectrolytes of high molecular volumes again fits hand-and-glove with the AI model of the action potential. It demonstrates a coordinated across-the-board increase of the affinity for Na^+ adsorption with a rise of c-value of β- and γ-carboxyl groups *pari passu* with water depolarization and disorientation following a concomitant rise of the c-value analogues of the backbone carbonyl groups. The cogency of these confirmatory experimental findings for the AI Hypothesis of the true active transport and, especially, of the action potential are beyond one's fondest hopes. After all, the original authors of these first-rate experimental efforts as a rule did not know much, if anything, of the AI Hypothesis—a shortcoming, which I hope that the present writing may remedy in time to come.

(5) The death state

A physicochemical theory of life is incomplete without an accompanying physicochemical theory of death; because life and death are, like the two sides of a coin, inseparable.

(5.1) Life and death of protoplasm

After Thomas Huxley delivered in 1869 his famous Sunday evening lecture on "The Physical Basis of Life,"[72] he was passionately applauded; and just as passionately attacked. Thus the Scottish philosopher, James Hutchinson Stirling made light of the concept of a single substance that is at once lobster, mutton and roast beef as well as the man who eats them.[3 p 308] Stirling pointedly ignored the obvious difference between roast beef, which is cooked, and the feasting man, who is not. But to defend against this specific attack, Huxley needed to define both life and death at the below-cell level. For only then could he separate without amibiguiity roast beef from the man who eats it. But to define life and death at below-cell level was not easy to do then, as others also discovered.

August Weismann (1834–1915), who separated living matter into *germ plasm* and *soma*, encountered a similar difficulty when he tried to unite the two under the broader notion of protoplasm. Thus, he conceded that since at the time nobody knew what kind of chemical difference exists between dead "proteid" and the living, one could only confess that it is merely an assumption that the only difference between the two are chemical-physical in nature.[3 p 344] On that historical state of affairs, historian Thomas Hall offered his own opinion in 1969 that what was needed but not yet available was a chemicophysical theory of life in general.[3 p 344] Notwithstanding, Hall went on to describe contemporary opinions held by many professional biologists as essentially similar to that expressed by Stirling—against the concept of protoplasm and for the concept that life occurs only in cells considered as wholes.[3 p 310]

Did Hall know that seven years before he made the statement above, just such a general chemicophysical theory of life had been offered? It bears the title, familiar to the reader by now: "A Physical Theory of the Living State: the Association-Induction Hypothesis."[98]

Reasons are also given in [14.2(1)] why the popular belief that life can only occur in whole cells is wrong and why protoplasm, existing in the living state, is the more fundamental unit of life.

(5.2) How protoplasm dies

According to the AI Hypothesis, the resting living state is a metastable equilibrium state. Like a coin standing on its edge, a system in a metastable state can be easily toppled by all kinds of perturbations. However, I shall limit myself to just two: by heat and by poisons.

In discussing the death of frog muscle at an elevated temperature, I pointed out that the driving force is the entropy gain. Now, frog muscle dies at a temperature at which the muscle and other body parts of birds remain quite normal. Clearly, not all protoplasm dies at the same elevated temperature. Can the entropy gain on death be so different among the protoplasms of frogs and birds? Not likely. The q-values of Na^+ (and of sucrose), which are quantitative expressions of the physical state of cell water, tend to be close to one another in the same type of cells from different animals and even plants.

More likely, the divergent killing temperatures originate from varying strengths of the stabilizing forces in maintaining the structure of the living protoplasm—such as that provided by salt linkages [15.3]. This interpretation is in harmony with Perutz's theory of *thermostability* of living organisms[322] and its supportive evidence: the same enzyme isolated from the more thermostable bacteria contain more salt-linkages. Although the end is essentially similar, poisons bring about death *via* a different route.

When frog muscles are subjected to ten poisons of widely different toxicological properties, their lethal outcome is quantitatively measured by their respective impact in reducing cell ATP (Figure 19). This rule observed is in harmony with the theory of the critical role of ATP in maintaining the living state [16.6(1.4)]. Since ATP is an EWC, its removal leads to an across-the-board rise of both the c-value of the β- and γ-carboxyl groups and the c-value analogue of the backbone carbonyl groups. Desorption of K^+ and water follow as the protoplasm dies. And the proteins in the dead assume conformations, ironically, close to what is conventionally called "native."

(5.3) The anatomy of dead protoplasm or cell

In the AI Hypothesis, the resting living state represents a state of high (negative) energy and low entropy. Heat destroys this state by liberating the adsorbed K^+ and water with a gain of entropy; poisons achieved the same end by disrupting the regeneration of ATP.

The increase of c-value analogue following ATP depletion favors the desorption of the cell water, leaving the backbone CO and NH groups free to engage in α-helical (and β-pleated sheet) folding. The consequence of the c-value rise of the β- and γ-carboxyl groups is the displacement of adsorbed K^+ by either Na^+ or fixed cations (in forming salt linkages). But adsorption of Na^+ is *only partial and transient, to be followed ultimately by*

the displacement of the adsorbed Na^+ by fixed cations. Thus the formation of salt linkages, both within and between protein molecules, explains why dead muscle protoplasm tends to be stiffer than live ones, and as such bears a closer resemblance to nail or horn rather than carboxyl exchange resin loaded with Na^+. Yet the surrounding medium, be it sea water, blood, tissue fluid or a Ringer's solution, is as a rule rich in Na^+.

In a general way, all these changes in dying can be traced to the *auto-cooperative* transition of the (protoplasmic) protein-water-ion assembly. In one state, the living state, ATP, K^+ are adsorbed on β- and γ-carboxyl groups and water is polarized and oriented on the fully-extended protein chains; in the other state, the death state, ATP is gone, and adsorbed K^+ and congruous anions as well as water are set free—as illustrated with varying details in Figures 44 and 48—*via* a step-by-step process depicted in Figure 51. However, there is also a specific question that needs to be addressed. Why should fixed cations be the final partners of all the β- and γ-carboxyl groups rather than Na^+?

The theoretically computed results shown in Figure 42 (and its supportive evidence) give us grounds for recognizing a K^+ over Na^+ preference at low c-value, and a Na^+ over K^+ preference at higher c-value. But the relative positions of the fixed cations (e.g., ∈-amino groups and guanidyl groups) are not known beyond that based on the assumption that they would resemble the position of NH_4^+ (which is known) [14.1(2)]. However, the NH_4^+ curve does not give us any indication that NH_4^+ and hence fixed cations would be preferred over Na^+ at still higher c-value —as ATP concentration approaches zero. Is there a complementary mechanism that may account for the ultimate salt-linkage takeover? There is and it has been briefly mentioned in [15.3(2)].

Equation 4 in [15.3(1)] can be borrowed to illustrate this additional mechanism, if we assume X^+ and Y^- in this Equation to stand for K^+ and the congruous anion respectively. In a healthy cell at rest, the surrounding medium is polarized-oriented cell water and the equilibrium stays to the right side of Equation 4 (with both K^+ and congruous anions adsorbed). Favoring this trend is the low q-values of K^+ (and of the congruous anion) in the polarized-oriented cell water. Thus the entropy gained on dissociation of either K^+ or the congruous anion is relatively small due to the restricted motional freedom of these ions in the polarized-oriented cell water.

When the cell begins to deteriorate and the concentration of ATP starts to decline, the c-value of the β- and γ-carboxyl groups rises, leading to the

displacement of K^+ by Na^+ at a time before a critical change of the c-value analogues takes place. Up to this critical juncture, low q-value, which favors K^+ adsorption also favored Na^+ adsorption.

The situation changes drastically when the cell dies with total ATP depletion and cell water turns into normal liquid water. As a result, the q-values of Na^+ and congruous anion rise to approach unity and the desorption of these ions is now awarded with full motional freedom. Thus for K^+, Na^+ and the congruous anion, cell or protoplasm death sharply enhances their tendency to desorb. A different fate awaits the fixed cations.

Being fixed, neither the ϵ-amino group of lysine residues nor the guanidyl group of arginine residues (nor the terminal α-amino groups) can gain much motional freedom *under any condition*. Therefore, cell death and water depolarization, which favor the desorpton of K^+, Na^+ and congruous anions, offer no comparable gain of motional freedom to the fixed cations. As a result, the reaction depicted in Equation 4 goes fully to the left in cell death. And the dead protoplasm is locked from end to end in salt linkages of *rigor mortis*.

16.7 A sketch of the history of Mankind's search for understanding of life

I conclude the "Summary Plus" with a pictorial diagram shown in Figure 72. It is an admittedly incomplete and somewhat arbitrary attempt to put into a coherent framework the key concepts in the evolving science of life throughout history. The main reason that prompted me to make this effort is that I think that it could with a minimum of words portray scientific research for what it really is: An open-ended, continuing cooperative enterprise of representatives of the entire human race, living, dead and yet to come. As such, scientific research is unique among all human enterprises. It gives our race an unending continuity and a measure of immortality even as we individuals come and go.

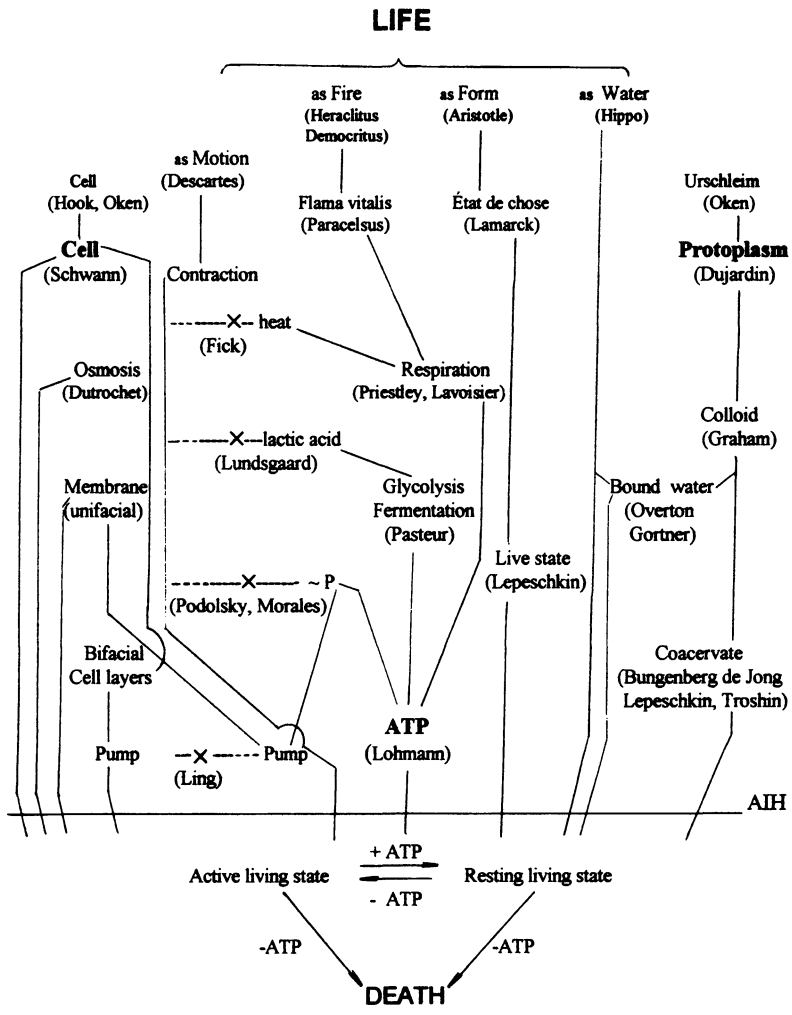

Figure 72. A historical sketch of Mankind's search for understanding of life at the cell and protoplasmic levels. For the historical role (and portrait) of Lorenz Oken (1779–1851) mentioned only briefly in the text of this volume, see Reference 15 p. 6.

17

Epilogue

Crossword puzzle and fox hunt: two models for scientific research

Science resembles the solving of a crossword puzzle. An ordinary crossword puzzle can be done easily by one person. In solving the scientific puzzle of *Nature*, an inherent difficulty lies in its immense size. Inevitably, a division of labor is inaugurated and uncoordinated multiple starts are launched. Thus, with perfectly good intentions, fragmentation, the deadliest disease of science, was set in motion. And, for a science like cell physiology, there is a second pitfall.

In solving the cell-physiological puzzle, correct basic physico-chemical concepts rather than words are used to fill the blank spaces. Since these physico-chemical concepts were being discovered *at the same time* the cell-physiological research was on-going, the early cell physiologists were bound to make wrong entries because the right ones were not yet known. By the time the right physico-chemical ideas came along, wrong entries had already been written into textbooks and taught worldwide year after year to generation after generation of young people at their most impressionable age.

The crossword puzzle analogy emphasizes that there is only *one unique solution* for each man-made puzzle as well as for the puzzle of Nature. A fox hunt is a cogent model in another aspect. It underscores the *critical need of a correct guiding theory.* A guess (theory) must be made early on the general direction the fleeing fox has taken, before the chase can begin. Once that guess is correctly made, the rest of the hunt has a much greater chance to proceed fruitfully.

If not, the leader of the hunt must have the courage and wisdom to make changes promptly. Nonetheless, if the discovery of a wrong theory is made late in the game, changing direction is difficult.

The secret of past success in major directional changes

These great inborn difficulties notwithstanding, science has made major directional changes in the past. These changes are referred to as *scientific revolutions*. What was the secret of their success in the past? Before attempting to answer, let us not forget, successful or not, a scientific revolution has never been easy. For their roles in a scientific revolution, Bruno lost his life at the stake, Galileo was imprisoned for life and Semmelweis died in an insane asylum. Revolutionaries, who did live to see the success of their work, were found more often at the time of, or after, the Enlightenment movement of Western Europe.

Replacing a broadly accepted but wrong guiding theory in science with one closer to truth has been as a rule initiated by one (or a few) individual(s). This is what I call Step 1 of a scientific revoluton.[107 p 319] However, if science is to survive as a continuing cooperative effort, the new and closer-to-truth revolutionary theory must be accepted by the scientific community as a whole. This conversion of the scientific majority is what science historians call a scientific revolution, but which I believe is Step 2 of that process. (In an earlier writing, I called Step 1 a scientist's scientific revolution, and Step 2 an historian's scientific revolution.[107 p 319]) Step 2 is as a rule more difficult to accomplish than Step 1. Thus, the great physiologist-physicist, Hermann von Helmholtz expressed a similar view in his 1881 Faraday lecture: "...it is often less difficult for a man of original thought to discover new truth than to discover why other people do not understand and do not follow him."[537 p 1]

To illustrate how Step 2 of past scientific revolutions actually did get accomplished, I cite two specific examples.

(i) Joseph Priestley (1733–1804) was the Unitarian minister-scientist who, as mentioned earlier, discovered oxygen but believed it to be "dephlogisticated air." When Antoine Lavoisier argued that it was really oxygen, Priestley fought him tooth and nail—until he realized at last that Lavoisier was right. Then Priestley made a 180-degree turn and praised Lavoisier's "chemical revolution" with overflowing admiration and enthusiasm: "There have been few, if any, revolutions in science so great, so sudden and so general....of what is now named the new system of chemistry."[346] Rapid and broad acceptance of Lavoisier's new theory soon followed.

(ii) Michael Faraday (1791–1867) came from a poor family in London. He had no formal education. Yet he revolutionized the field of physics with his iconoclastic concepts of curved electric and magnetic lines of force and his field theory. He was almost entirely rejected by his peers. Some even suggested that "he ought to return to sixth form mathematics before venturing into the deep ocean of Laplacian physics."[366 p 507] But there were also striking exceptions.

William Thompson (Lord Kelvin) and especially James Clerk Maxwell both recognized the importance of Faraday's revolutionary concepts. Later Maxwell was to introduce his own famous theory of light as electromagnetic waves, which marked one of the great forward leaps in physics. Yet through it all, Maxwell always insisted that the core of Maxwell's advanced field theory were the ideas Faraday expressed in his life's work.[54 p 509, p 513] Thus Step 2 of another major scientific revolution was once more successfully carried out.

Eighty-nine years after Faraday's death, Albert Einstein—possibly the greatest scientist of all time—wrote: "For us, who took in Faraday's ideas so to speak with our mother's milk, it is hard to appreciate their greatness and audacity."[365 p 101] And Faraday did all this "without the help of a single mathematical formula."[537 p 1]

The two sets of historical events cited above tell us that the secret of the successful scientific revolutions of the past lies in the deep belief in *fair play* or *sportsmanship* among the key participants in particular and, lesser though still importantly, the scientific community in general. One asks, "Is this secret formula so lofty that one cannot expect it from ordinary people?"

Not so at all. Indeed, every participant in a competitive sport, accepts and lives by it without a second thought. It is true that occasionally one hears of someone in sports who used illegal drugs or even attempted to beat up a referee, but that is a rare exception. Almost all participants of competitive sports know how to win and how to lose. In this obedience to a widely accepted ethical rule of fair play, the very spirit of sports resides. This spirit is not inborn. *It is taught from the day the child learns to play the game*—by fathers, amateur coaches, etc., who love the game, understand it and teach its rules of fairplay and sportsmanship, which make the game possible. But another factor in favor of fair play and sportsmanship in sports is that everyone can see and fully understand what is going on. After all, they are exhibitions.

I am sad to say that the same spirit, which is as vital to the health and survival of science as it is to competitive sport, has not done well in the field of cell physiology in the later half of the 20th century. The decay began slowly and almost imperceptibly after the introduction in the 1940's of large-scale government funding of scientific research. To determine who gets support and who is refused, the *peer review system* was born and universally adopted.[348; 349]

Public funding of research *per se* is a *great blessing* to science and scientists, including myself. Unfortunately, those chosen to serve on peer-review panels are—unlike most referees and umpires in competitive sports—themselves competitors for the money they control. All too frequently, they forget the vital role of fair play and sportsmanship in science and see an impending major scientific progress as a threat to their personal advantage and prestige and use the entrusted power to suppress it.[247; 350] Unlike sports, these activities are as a rule not open to public view.

(Creating and putting into practive something better than the widely-practiced "peer review" system—so that truly innovative ideas are encouraged rather than suppressed—is a matter of great urgency. Until this reform is successfully carried out, the peer review system will remain a blemish on the wisdom and integrity of a great leading Nation like the United States, which rightfully would not tolerate infringement of freedom in far less important issues.)

Fragmentation and its impact on the future of science

In his "History of Physiology," Karl E. Rothschuh pointed out that with the increase in practicing physiologists, the number of scientific journals have risen to such an extent that "Physiology has even ceased to be one whole and distinct teaching subject, a fact which virtually spells the end of the discipline as a certified field of scientific endeavor."[352 p 349]

This comment was made by Rothschuh years ago on what is known as organ physiology, e.g., renal physiology, digestive physiology, etc. But cell physiology has fared no better. It too has split into biochemistry, biophysics, pharmacology, cell biology, molecular biology, mathematic biology, etc., etc. Indeed, things have gotten much worse with the universal adoption of the peer review system, which further exacerbates fragmentation.

A verified unifying theory to put Humpty-Dumpty together again

"The eternal mystery of the world is its comprehensibility" (Immanuel Kant). To begin with, simple and coherent things are easier to understand (and to remember.) Thus the comprehensibility of Nature may be tied to its underlying simplicity and coherence, which are couched in such admonitions as that of Occam's razor, "What can be done with fewer is done in vain with more."

However, a fragmented approach is like "viewing the sky from the bottom of a well" (Chinese proverb). As such, it hides from view Nature's innate simplicity, coherence and comprehensibility. The question is: How can we heal this fragmentation? My answer is: *Begin with a unifying* theory.

Early on, I pointed out why the membrane theory at one time appeared to be a unifying theory (Chapter 4). Unfortunately, as more and more new facts came to light, they left no doubt that the membrane theory is not headed in the right direction. Then alternative theories based on the concept that cells are solid and made of protoplasm were introduced. Unfortunately, the protoplasm-oriented cell physiologists did not produce a unifying theory. The time was not yet right.

Not only were the essential basic physico-chemical sciences themselves still in their early development or not yet in existence (Chapter 7), powerful new scientific tools like radioactive tracer technology, which have played key roles in critically testing the alternative theories, were still to come. Nor did these investigators enjoy the benefits of public financial support, which was not in place until the end of World War II in the United States, for example.

All these had undergone profound changes when I came on the scene as I have pointed out repeatedly in the text of this volume. Whether or not the AI Hypothesis will remain the one and only unifying theory, as I believe it will, is a judgement that can be made only in the future. Notwithstanding, there is no denial that the AI Hypothesis is the first-in-history physico-chemical theory of life at the cell and below-cell level. And this whole volume testifies to how it agrees with the experimental studies designed to test its validity.

How a dedicated biology teacher holds the key to a better future for basic life science

Let us begin with what a teacher does not want. No teacher worthy of that title wants knowingly to teach or present a wrong theory as truth—nor teach only (beautifully-illustrated) trivia.

However, a great teacher does more than just not doing wrong or meaningless things. He or she can expose the students to the right underlying rules of fair play and sportsmanship in the same way that loving fathers and volunteer coaches teach youthful sand-lot baseball players. And in the process, inspire in a few students an abiding love for the subject taught, and prepare him or her for a career in the service of all mankind that is always interesting and unswervingly relevant. To discover what specific tool may help our teachers to fulfill this critical role, let us return to the life of Michael Faraday once again.

At the age of fourteen, Michael Faraday was an apprentice to a small bookbinding business. He could easily have continued with what he had learned as an apprentice and spent the rest of his life as a journeyman bookbinder. But that was not to be. What then inspired this young bookbinder's apprentice to dream of a career of a modern Galileo or Isaac Newton? From what I could gather, his dream—where it all began—started with two articles on the *history of science.*

As young Faraday was glueing together the pages of a set of the *Encyclopedia Britannica*, something in the printed pages caught his eyes. It was an article entitled, "History of Electricity" written by a Mr. James Tytler. Tytler, in turn, took most of his material from Joseph Priestley's book, "The History and Current Status of Electricity."[379] Faraday was so excited by what he read that he began to conduct experiments on the mantle-piece of his employer's shop. His scientific equipments were fashioned out of two glass bottles, which he bought from an old rag shop for six pence and one penny respectively. Soon he was defending his own theory of electricity among a gathering of young friends intent on "improving their minds." Unfortunately, as his love for science grew more and more fervent, the prospect of becoming a professional scientist, or even just continuing as an amateur scientist, became less and less bright. His apprenticeship was drawing to a close. He felt hopeless and depressed.

Then suddenly and unexpectedly came a lucky break. The janitor of the Royal Institution—home of such illustrious scientists like Sir Humphrey

Davy—was fired for engaging in a brawl. And young Michael Faraday was hired to replace him. This was how Faraday began his life career as one of the greatest scientists in history.

At the beginning, he was mostly engaged in helping Davy and others. But before long, he was on his own. Despite its lofty-sounding name, the Royal Institution had no regular income. Faraday, like the other members, had to earn his expenses. One way on which he relied was giving public lectures on scientific subjects. He took great pains to learn how to be an effective speaker and in the end, became good at it. On one occasion, his lecture was so engaging that school children gave up their Christmas parties to hear his talk, which bore the title: "The Chemical History of the Candle."

So it seems that in the early intellectual environment of Michael Faraday, narratives on *history* of one sort or another kept on popping up. Is there something special about *history* that reaches out to the young mind? If so, it would not be surprising. The English word, *history,* is synonymous with the word, *story*. When one reads to a child, be it Winnie the Pooh or Peter Rabbit, it is always a story or history. A story or history is always "moving" and coherent. It tells of experiences with which the listener is familiar, including a reassuring happy ending.

With these examples in the background, one can see why cell physiology has been losing ground steadily (to such a degree that knowledgeable scientists began to believe that (all) science is approaching an end). Being fragmented and, in my view, wrongly-headed, the conventional cell physiology has no story to tell and, of course, is truly at an end. With no story to tell, students and teachers would be hard put to develop a genuine interest in it—as Faraday himself and his young audience did on a Chrstmas Day long long ago. But all this is changed now.

The AI Hypothesis has made cell physiology truly coherent for the first time. Convinced that the best way to bring future generations of the likes of Michael Faraday into the field of cell physiology is to tell them its story or history, I gradually convinced myself of the need for a book like this one, which too is a story and bears that magic word in its subtitle.

While teachers and their students are an important segment of the audience I am trying to reach, they are not the exclusive audience I am looking for—as I have briefly mentioned in the Preface. Others I am trying to reach include all kinds of scientists, especially those close to biology and medicine, who want to update their basic knowledge; molecular biologists seeking the link between genetics and cell and subcellular physiology;

physicists looking for new fertile terrains to apply their talents and knowledge; researchers in medicine and in pharmacological companies searching for new ways to cure diseases and invent drugs; school-board members eager to offer the right and up-to-date guidelines for school curriculum they supervise; science reporters and editors who may open the eyes and minds of an even larger body of intellectually adventurous book readers. True, teachers and their wards hold the key to reverse the senseless unending teaching of meaningless long-ago-disproved ideas. But we need the help of everyone to accomplish the momentous task lying ahead.

"Science, The Endless Frontier" can be as shining and as promising as ever

Fifty-five years have gone by since Vannevar Bush wrote his report to President Franklin D. Roosevelt, bearing the title: "Science, The Endless Frontier."[400] Those, who have read only Horgan's "End of Science" (see Preface), may be misled into thinking that Bush was wrong and that science does not offer an endless frontier. Those who have gone through the present volume, may realize how right Vannevar Bush was and still is. And in all probability will continue to be.

As an illustration, the invention of MRI shows not only that cell physiological science is burgeoning, it also shows how physics is as alive as ever, because, among other reasons, advanced cell physiology is physics. And then, advanced physics is also advanced cell physiology. For after all, it is the cell physiological activities of the trillions of brain cells in men and women who call themselves physicists that have created physics.

It would be wonderful if I could present a list of all the exciting avenues open to cell physiological research in the future. But as space is limited, I must satisfy myself with just two.

First is a reminder that many of the seemingly dead-end scientific accomplishments now gathering dust in some unreachable storage libraries—and sooner or later in danger of becoming parts of some garbage dump—be they biochemistry, or biophysics or molecular biology etc., etc. will become alive again, when viewed in the new light of the unifying AI Hypothesis. Better still, in them will be found truths that show conflicts, real or apparent, with the predictions of the AI Hypothesis. They will be the spring-board for the continued growth in future cell physiological science.

Second is one specific direction that answers the question with which I began this story: How to develop an inexhaustible arsenal of weapons

against cancer, AIDS and other deadly diseases, which are the true enemies of our species? These weapons are in the form of drugs. Unlike the kind we have, they will be *rationally designed* and thus on target and without untoward side effects. And they can be cheap.

It is truly astonishing to listen to political debates on how to stave off the impending bankruptcy of the Medicare/Medicaid programs as the US population ages and more and more people consume larger and larger quantities of prescription drugs. For this to happen in as wealthy a country as the US tells just how extremely inefficient and expensive it is to obtain useful and safe drugs through *random trial and error*—as it is being done now, a process which also leaves patients of AIDS in poor countries around the world to die like abandoned cats and dogs.

What chance is there for improvements if we just do more of the same—along the line of the membrane-pump theory? Indeed, the theoretical mechanism of drug action now being taught in textbooks remains not much more than the same old lock-and-key model. The conventional concept of drug action begins and ends with receptor-site fitting. The fitting drug does not do anything. Because there is nothing proposed to respond to drugs other than site-fitting, one cannot go very far—indeed anywhere at all—on such a dead-end road.

In contrast, the AI Hypothesis in its very name, association and induction, has already spelled out the basic mechanism of drug action. It is an *electronic* one, a direction more specifically explained in Chapters 14 and 15. Thus, based on the theoretically derived relationship between the c-value and the rank order of selectivity of K^+, Na^+ and other alkali metal ions (and other criteria) and the c-value analogue and the rank order of selectivity between helical structure and water polarization-orientation, one can already determine experimentally whether a specific drug or other cardinal adsorbent functions as an *electron-withdrawing cardinal adsorbent* or EWC or an *electron-donating cardinal adsorbent* or EDC. This is a big step forward. Nothing like this has ever occurred before.

In addition, this type of experimentation carried out has already begun to produce new, valuable information from a variety of intact living cells, normal as well as cancerous. Among the cardinal adsorbents—which include all drugs—the most important is ATP. And from our studies of its influence on different physiological manifestations in living cells, we reached the conclusion that ATP is an EWC. Ouabain, in contrast, is an EDC. Why ATP is an EWC and why ouabain is an EDC are questions that

will challenge the best of the coming generations of new biologists, chemists and physicists, but especially those who have mastered the essence of all three fields of science. And hopefully this volume may stimulate the training of scientists of this kind. But all that is for the future. For the moment, we return once more to something at our current rather primitive level of progress.

As pointed out repeatedly, a crucial step in verifying a cell physiological theory is the invention or discovery of an *inanimate model.* An inanimate model shares major attributes with living cells, but one upon which it is much simpler and easier to test the theory. In almost all the subsidiary theories of the AI Hypothesis, one or more suitable inanimate model(s) was found by 1992—with one important exception.

That exception is an inanimate model showing how drugs and other cardinal adsorbents at minute concentrations can induce an across-the-board change of the c-value of a large number of β- *and* γ-*carboxyl groups, and that with their c-value change, the theoretically predicted change in the relative adsorptive preferences for* K^+, Na^+, *for example, can follow.*

It is thus with great pride and joy that I, together with my associate, Dr. Zhen-dong Chen (as well as Margaret Ochsenfeld), can now make the preliminary announcement that a few of these inanimate models have been found and the essence of the changes predicted by the AI Hypothesis tentatively confirmed. Having said that, I must add that the demonstrated changes are very small though statistically significant. They are, after all, models and not the real thing.

Not the least earnest message, which we want to share with the next generation of cell physiologists and their teachers, is about our experience as what I shall call less-than-popular scientists—though even now we have more facilities than Faraday had to make do with in his time. All these experiences suggest that if you always do your best with whatever you may have, you will be at a better starting position for a scientific career than Michael Faraday was when he learned with unspeakable joy that he was appointed the "fag and scrub" man of the Royal Institution.

Appendix 1

1. *The Troshin equation*[92]

$$C_c = C_s K\left(1+\frac{A}{C_s K+a}\right), \qquad \text{(A1)}$$

where C_c and C_s are the concentration of the solute under investigation in the cell water (or coacervate models) and in the external medium respectively. K is the "coefficient of proportionality" characterizing the aqueous phase in which the cell or coacervate is found. A is what Troshin calls the limit of adsorption and a is a constant characterizing the curvature of the rise in the adsorption isotherm.

2. *The Bradley adsorption isotherm*[278]

Bradley developed an isotherm for the adsorption of polarized multilayers of gaseous molecules possessing a permanent dipole moment:

$$\ln\left(\frac{p_o}{p}\right) = K_2 K_1^{\ a} + K_4, \qquad \text{(A2)}$$

where p_o /p is the reciprocal of the relative vapor pressure. a is the amount of gas sorbed. Under specified conditions, K_1, K_2 and K_4 are all constants.

3. *Ling's equation for solute distribution in cell water*[168]

$$q = \exp\left\{\frac{1.23 v \Delta E_s\left[1-(1-b)\frac{(kv)^n}{1+(kv)^n}\right]-(\Delta E_v + 1.23\Delta e^*)v}{RT}\right\}, \qquad \text{(A3)}$$

where q is the equilibrium distribution coefficient of the solute in question—equivalent to K in the Troshin equation (A1). v is the molecular volume (molar volume) of the solute and it is in cm^3. b is a small fractional number describing the probability of (very large) molecules in finding adsorbing sites on the water lattice. k and n are parameters describing the steepness of the declining probability of finding adsorbing sites with increase of molecular volume. ΔE_s is the *specific surface (or solute) polarization energy change* per cm^2 in units of $cal.mol^{-1}\ (cm^2)^{-1}$, when the solute is moved from normal liquid water to the polarized cell

water. ΔE_v is the *specific solvent polarization energy*, equal to the difference between the energy spent in excavating a hole 1 cm^3 in size in the polarized (cell) water and the energy recovered in filling up a 1 cm^3 hole left behind in the surrounding normal liquid water; it is in units of $cal.mole^{-1}(cm^3)^{-1}$. Δe^* is the *increment of the activation energy* for overcoming the greater rotational restrictions per unit surface area in units of $cal.mole^{-1}(cm^2)^{-1}$, when a solute is transferred from normal liquid water phase to the polarized water phase. R and T are the gas constant and absolute temperature respectively.

4. *The Yang-Ling adsorption isotherm*[185]

We begin with a one-dimensional chain of uniformly spaced adsorption sites of similar nature. Each adsorption site can adsorb two species, p_i and p_j, which are also found in a free solution of infinite volume with which the one-dimensional chain is in equilibrium. (Either p_i or p_j can also represent a vacant site). Only the nearest neighbor interactions are taken into account. Then for a chain of N sites, the average number of adsorbed p_i's per chain is

$$[p_i]_{ad} = \frac{[f]}{2}\left[1 + \frac{\xi - 1}{\sqrt{(\xi-1)^2 + 4\xi\exp(\gamma/RT)}}\right]. \tag{A4}$$

Now,

$$\xi = \frac{[p_i]_{ex}}{[p_j]_{ex}} \cdot K^{\infty}_{j\to i}, \tag{A5}$$

where $[p_i]_{ex}$ and $[p_j]_{ex}$ are the concentrations of the ith and jth solute in the bathing solution respectively. $K^{\infty}_{j\to i}$ is the intrinsic equilibrium constant for the jth to ith adsorption exchange during which no change in the number of i-j pairs is created as it is in the case when the middle site of a triad of sites originally occupied by a jth solute is exchanged for an ith solute when the two flanking sites are occupied respectively by one ith solute and one jth solute. $-\gamma/2$ is the nearest neighbor interaction energy, defined as the energy change of the system each time a new i-j pair is created on two immediately neighboring sites. [f] is the concentration of adsorption sites.

When $-\gamma/2$ is larger than zero, the adsorption of an ith solute favors the adsorption of more ith solute. This type of cooperative interaction is usually referred to as *ferromagnetic*. When $-\gamma/2$ is less than zero, the adsorption of an ith solute favors the adsorption of the alternative jth solute. This type of cooperative interaction is usually referred to as *anti-ferromagnetic*. However, since in cell physiology we are not dealing with magnetism most of the time, to avoid confusion, I have suggested the use of *autocooperative* and *heterocooperative* interaction in place of ferromagnetic and anti-ferromagnetic interaction respectively.[244; 313; 295; 107 p 138]

Equation A4 was derived on the basis of one-dimensional Ising model.[353] There is a "theorem" that "There can be no phase transition in one dimension."[354] However, this theorem may not be true always (see Reference 355, 356).

5. Ling's general equation for solute distribution in living cells and simplified versions[136]

$$[p_i]_{in} = \alpha q_i [p_i]_{ex} + \sum_{L=1}^{N} \frac{[f]_L}{2}\left(1 + \frac{\xi_L - 1}{\sqrt{(\xi_L - 1)^2 + 4\xi_L \exp(\gamma_L / RT)}}\right), \qquad \text{(A6)}$$

where $[p_i]_{in}$ represents the intracellular concentration of the ith solute in units of μmoles per gram of fresh cell weight. $[p_i]_{ex}$ represents the concentration of the ith solute in the external medium and is in units of mmoles per liter or millimolarity. $[f]_L$ is the concentration of the Lth type of fixed anions—usually β- and γ-carboxyl groups—among a total of N types and it is in units of μmoles per gram of fresh cell weight. α is a fractional number equal to the weight percentage of water in the cells. q_i is the (average) equilibrium distribution coefficient of the ith solute in the cell water (see Equation A3 above). ξ_L is as defined in Equation A5 above but refers to the Lth type of adsorption sites only among a total of N types of adsorption sites.

A simplified version of Equation A6 is the case where there is only one type of adsorption site. In this case, ξ_L reduces to ξ and

$$[p_i]_{in} = \alpha q_i [p_i]_{ex} + \frac{[f]}{2}\left(1 + \frac{\xi - 1}{\sqrt{(\xi - 1)^2 + 4\xi \exp(\gamma / RT)}}\right). \qquad \text{(A7)}$$

As pointed out in 1992,[107 pp 161–162] almost all the experimental data examined up to that time can be described by the simplified version A7 or other simplifications of A6 including

$$[p_i]_{in} = \alpha q_i [p_i]_{ex} + \sum_{L=1}^{N} \frac{[f_L][p_i]_{ex} K_i^L}{1 + \sum_{i=1}^{n} [p_i]_{ex} K_i^L}, \qquad \text{(A8)}$$

where a total of n types of competing solutes are present, and K_i is the adsorption constant of the ith solute on the Lth type of adsorption sites among N types, and is in units of $(M)^{-1}$. When only two types of solutes, i and j, are present, Equation A8 further simplifies to

$$[p_i]_{in} = \alpha q_i [p_i]_{ex} + \frac{[f][p_i]_{ex} K_i}{1 + K_i [p_i]_{ex} + K_j [p_j]_{ex}}. \qquad \text{(A9)}$$

When only one type of solute, the ith, is alone present, Equation A9 further reduces to its simplest form

$$[p_i]_{in} = \alpha q_i [p_i]_{ex} + \frac{[f][p_i]_{ex} K_i}{1 + K_i [p_i]_{ex}}, \qquad \text{(A10)}$$

and in this form, it is identical to the Troshin equation presented with Troshin's original symbols as Equation A1 above.

Equation A9 can be written in a reciprocal form, when $[f]K_i >> \alpha q_i$:

$$\frac{1}{[p_i]_{in}} = \frac{K_i}{[f]}\left(1+\frac{[p_j]_{ex}}{K_j}\right)\frac{1}{[p_i]_{ex}}+\frac{1}{[f]} \quad . \tag{A11}$$

When the experimentally determined values of $1/[p_i]_{in}$ are plotted against $1/[p_i]_{ex}$ in the presence of varying concentrations of competing jth solute, they yield a family of straight lines converging on the same locus on the ordinate. The values of adsorption constants K_i and K_j , as well as the concentration of the adsorption sites, [f], can be determined from the slopes and the intercepts of the straight lines obtained.

6. *Ling's equation for cell permeability*[107 Eq. 26]

In the AI Hypothesis, the protoplasm making up the cell surface and the cytoplasm are fundamentally alike. As a result, an equation for (surface-limited) permeation can be obtained by extending Equation A6 for solute distribution. For cells having N types of adsorption sites, the initial rate of permeation of the ith solute (v_i) in units of moles/cm^2/sec can be described by the following equation:

$$v_i = A_i[p_i]_{ex} + \sum_{L=1}^{N}\frac{v_i^{max(L)}}{2}\left[1+\frac{\xi_L - 1}{\sqrt{(\xi_L-1)^2+4\xi_L\exp(\gamma_L/RT)}}\right], \tag{A12}$$

where $A_i[p_i]$ represents the rate of entry of the ith solute *via* the saltatory route. A_i is a constant in units of cm/sec. $v_i^{max(L)}$ is the maximum rate of permeation of the ith solute *via* the Lth type of sites by the adsorption-desorption route. ξ_L and γ_L have the same meanings as described under Equation A4 and A5 but refer only to the Lth type of surface sites.

A simpler version of this equation described cell surface with only one type of adsorption sites:

$$v_i = A_i[p_i]_{ex} + \frac{v_i^{max}}{2}\left[1+\frac{\xi-1}{\sqrt{(\xi-1)^2+4\xi\exp(\gamma/RT)}}\right]. \tag{A13}$$

Other simplified versions A14, A15 and A16 can be derived from Equation A8, A9 and A10 respectively:

$$v_i = A_i[p_i]_{ex} + \sum_{L=1}^{N}\frac{v_{max}K_i^L[p_i]_{ex}}{1+\sum_{i=1}^{n}K_i^L[p_i]_{ex}}, \tag{A14}$$

$$v_i = A_i[p_i]_{ex} + \frac{v_{max}K_i[p_i]_{ex}}{1+K_i[p_i]_{ex}+K_j[p_j]_{ex}}, \tag{A15}$$

and

$$v_i = A_i[p_i]_{ex} + \frac{v_{max}K_i[p_i]_{ex}}{1+K_i[p_i]_{ex}} \quad . \tag{A16}$$

It should be pointed out here that in all these equations, the adsorption-desorption route is *via* the doublet mechanism. For the triplet variety, a more elaborate equation was presented by Ling and Ochsenfeld in 1965.[222]

7. *Ling's equation for cell volume*[195]

The following equation describes the water content of living cells in equilibrium with varying concentrations of external solutes, which have different q-values in the cell water:

$$a = \frac{1}{\log K_3} \cdot [\log [\log \left(1 + \frac{(1-q)n_2}{n_1} \right) - K_4] - \log K_1] , \qquad \text{(A17)}$$

where a is the number of grams of water taken up per 100 grams of dry protein (initial dry weight). n_2 is the molal concentration of the solute and n_1 is equal to 55.51, the number of moles of water in which n_2 moles of the solute are dissolved. K_1, K_3 and K_4 are constants under the same physical condition.

The cell volume (V_{cell}) in cm^3 of cells, which before the experiment begins weighs 1 gram in weight, is described by the following equation:

$$v_{cell} = W_{prot}(\%) \left\{ \frac{a}{100} + V + \frac{aqM[C]_{ex}}{100d} \right\} , \qquad \text{(A18)}$$

where the three terms in the larger bracket on the right are respectively the volume of cell water, the volume of cell proteins and the volume of the dissolved solute S in question. W_{prot} (%) is the weight of proteins in the cells in percentage (w/w) of 1 gram of fresh cells as they are before the experiment begins and is to all intent and purposes equal to the cells's initial dry weight. V is the averaged partial molar volume of the proteins and equals about 0.73. q is the equilibrium distribution coefficient in the cell water of the solute S under study and M is its molecular weight. $[C]_{ex}$ is the molal concentration of the solute S in the external bathing solution and d is the density of the solute S.

8. *Ling's equation for the resting potential*[248]

In 1979 Ling introduced his general equation for the resting potential in which the cooperative interaction between the nearest neighbor adsorption sites is taken into consideration,

$$\psi = \text{Constant} + \frac{RT}{F} \ln \frac{1}{[K^+]_{ex}} \left(1 + \frac{\xi - 1}{\sqrt{(\xi-1) + 4\xi \exp(\gamma / RT)}} \right) , \qquad \text{(A19)}$$

where

$$\xi = \frac{[K^+]_{ex}}{[Na^+]_{ex}} \cdot K^{\infty}_{Na \to K} \cdot \qquad \text{(A20)}$$

ξ, $K^{\infty}_{Na\to K}$ and $-\gamma/2$ have the same meanings defined under Equation A5 except they refer only to the surface β- and γ-carboxyl sites, rather than bulk-phase sites and that the adsorbents refer specifically to Na^+ and K^+ rather than i and j.

Equation A14 can also be written in the following form:

$$\psi = \frac{RT}{F}\ln[f^-] + \frac{RT}{F}\ln\frac{1}{2[K^+]_{ex}K_K}\left[1+\frac{\xi-1}{\sqrt{(\xi-1)^2+4\xi\exp(\gamma/RT)}}\right], \quad \text{(A21)}$$

where K_K is the adsorption constant of K^+ on the surface anionic sites. In the special case, where the nearest neighbor interaction energy $-\gamma/2$ is equal to zero, Equation A21 reduces to

$$\psi = \frac{RT}{F}\ln[f^-] - \frac{RT}{F}\ln\left\{K_K[K^+]_{ex} + K_{Na}[Na^+]_{ex}\right\}, \quad \text{(A22)}$$

where K_{Na} is the adsorption constant of Na^+. Equation A22 is a simpler version of the more general equation for the resting potential of non-interacting sites as follows:

$$\psi = \frac{RT}{F}\ln[f^-] - \frac{RT}{F}\ln\left\{1+K_K[K^+]_{ex} + \sum_{i=1}^{n}K_i[p_i]_{ex}\right\}, \quad \text{(A23)}$$

where p_i is the ith among a total of n competing monovalent cations and K_i is its adsorption constant on the surface anionic sites.

9. *Ling's equation for the action potential*[15 p 494]

$$\psi = \text{constant} + \frac{RT}{F}\ln\frac{1}{2(K^+)_{ex}K_K}\left[1+\frac{\xi-1}{\sqrt{(\xi-1)^2+4\xi\exp(\gamma/RT)}}\right] - \frac{RT}{F}\ln\left[\frac{[Na^+]^{free}_{in}}{q_{Na}} + \frac{[K^+]^{free}_{in}}{q_K}\right], \quad \text{(A24)}$$

where ψ represents the magnitude of the action potential. $[Na^+]^{free}_{in}$ and $[K^+]^{free}_{in}$ are respectively the concentrations of free Na^+ and K^+ in the surface cell water and q_{Na} and q_K their equilibrium distribution coefficients in the cell surface water. Other symbols are as in Equation A21.

A Super-Glossary

for Words, Terms and Basic Concepts Used in this Book

Å: See Angstrom unit.

a_K: As used in this volume, a_K represents the activity of the potassium ion, K^+. As such, it is equal to the product of the concentration of the ion (C_K) and its activity coefficient (γ_K).

A-band: optically dense band in a striated muscle fiber (see Figs. 15, 16)

absorb: to take up (like a sponge)

α-amino group: an amino group (NH_2) when attached to an α-carbon atom (immediately adjacent to a functional group) Example: the amino group of the amino acid glycine (NH_2CH_2COOH) is an α-amino group.

α-helical conformation: the principal secondary structure of most globular proteins. In this conformation each amide group is attached by a H-bond to the third one from it in either direction along the polypeptide chain.

α-helical potential: the empirically determined propensity or potential of a specific type of amino acid residue to engage in an α-helical structure

α74asp-α75asp: a nearest neighboring pair of aspartic-acid residues located respectively at position 74 and 75 on the α-chains of a hemoglobin molecule

abscissa: the horizontal axis. See ordinate.

absolute temperature: the temperature of the Kelvin scale, which has its zero at –273.16°C It is a temperature at which a gas would show no pressure, if the general gas law held for all temperatures. It has degree units of the same magnitude as the centigrade scale and represented as °K.

acetic acid: CH_3COOH, a weak acid found in vinegar

acetylphenoneoxime: an organic chemical having a structure shown in the inset of Fig. 47

acid dissociation constant: the equilibrium constant for the dissociation of a specific kind of acid Thus for acetic acid, the acid dissociation constant (represented as K) is equal to the product of the concentration of the H^+ ion multiplied by the concentration of the acetate ion and divided by the concentration of the unionized acetic acid at the equilibrium condition. K for acetic acid is 1.76×10^{-5}. The negative logarithm of K is called pK. The pK of acetic acid is thus 4.76.

acronym: a word formed from the initial letter(s) of successive parts of a compound term

actin: the major protein component of the thin filaments of striated muscle and of microfilaments in many cell types

action potential: the transient local electrical potential variation, which causes an active locus to be electrically negative to the quiescent region of the nerve or muscle surface. It is the physical basis of the nerve or muscle impulse.

active living state: the state of a living cell or protoplasm when it is "in activity"

active transport: movement of ions or molecules against a concentration and/or electrical gradient by a mechanism that requires the expenditure of energy

activity: The attributes of ions or molecules as a component in a solution may be weakened due to interaction with other components of the system so that they appear as if they are lower in concentration than they actually are—by a fractional number, f, called the activity coefficient. The *activity* of the component is the product of the concentration (C) and the activity coefficient and often represented by the letter a.

activity coefficient: A factor introduced by G.N. Lewis to correct for other-than-ideal behavior of substances in solution. An activity coefficient of 0.8 for a sodium ion, for example, means that in that specific environment, the sodium ion is or does only 80% of what a sodium ion in an ideal environment is or does.

actomyosin gel: a gel formed from a complex of the two muscle proteins, actin and myosin

ad infinitum: (Latin) without end

adenosine diphosphate (ADP): the major hydrolytic product of ATP when acted upon by, for example, an enzyme called ATPase

adenosine triphosphate (ATP): a critically important nucleotide compound found in virtu-

ally all living cells It is the end-product of the living cell's energy metabolism.

ADP: See adenosine diphosphate.

adrenaline: also known as epinephrine It is a hormone produced by the adrenal glands and has wide-spread physiological effects on tissues innervated by the sympathetic nerves.

adsorb: to take up on the surface of a solid or on specific sites of a solid or macromolecule

adsorbent: the substance that is adsorbed

adsorption: the occurrence of high concentration of any substance at the surface of a solid or liquid (For more details and history, see Ref. 107, p37n5.) (See also close-contact adsorption and localized adsorption.)

adsorption constant: an equilibrium constant for the (close contact) adsorption of a substance on a fixed site and usually given in units of reciprocal moles. Example: the adsorption constant of ATP on the major muscle protein myosin is 10^{10} to 10^{11} M^{-1}.

adsorption isotherm: a line on a chart or an equation describing the relationship between the pressure (concentration) of a gas (solute) and the amount of gas (solute) adsorbed at a constant temperature

adsorption site: a specific locus on a solid surface or a macromolecule, which has the potential of adsorbing an ion or molecule

adsorption-desorption route: one of the two major modes of entry or exit of an ion or molecule into or from a fixed charge-carrying system like the surface of a muscle cell This mode of entry or exit involves the adsorption of the ion or molecule from one side of a fixed site at the cell surface and its subsequent emergence on the other side of the cell surface.

"adsorption-staining method": a special technique introduced in 1991 by Ludwig Edelmann,[282] which makes visible and thus identifiable special anionic loci in ultra-thin electron microscopic sections by exposing the thin sections of the frozen tissue to electron-dense ions like Cs^+ in the presence of Li^+

"affinity": attraction often in a highly specific manner

$AgNO_3$: silver nitrate. water-soluble, colorless crystals

AIH, AI Hypothesis: acronyms of the association-induction hypothesis

alanine: one of the common α-amino acids found in most proteins

alkali-metal ions: members of the group of univalent basic metals belonging to the first group of the periodic table Highly reactive, they readily give off one electron becoming univalent alkali-metal ions. Examples: Li^+, Na^+, K^+, Rb^+, Cs^+

alkalinity: having properties of an alkali, exhibiting a pH higher than 7.0

all-or-none: marked by either exhibiting full effect or none at all

alteration theory: a theory of the origin of what was then measured between the cut end and intact surface of a nerve or muscle and known as *injury potential* (or demarcation potential) According to this alteration theory, the electric potential difference measured as injury potential exists only at the site of injury (or activity). Its chief advocate was Ludimar Hermann (1838–1914), Emil DuBois-Reymond's student.

amino acid: any one of a group of some twenty organic compounds containing an amino group and a carboxyl group Amino acids make up proteins.

amino-acid sequence: the linear sequential order of arrangement of various amino acid residues in a polypeptide or protein

amino group: $-NH_2$ or in its ionized form, $-NH_3^+$

ammonium sulfide: $(NH_4)_2S$, water-soluble crystals at below 0°C but decomposing at higher temperatures

amphiuma: an amphibian inhabiting Southern United States It has a snake-like body and four small limbs.

Amphotericin B: also known as Fungizone, an antifungal antibiotic produced by Streptomycetes culture

amyl alcohol: one or a mixture of various saturated aliphatic alcohols containing 5 carbon atoms

anaerobic: living, be active or occurs in the absence of free oxygen

Angstrom unit: a unit of length equal to 10^{-8} centimeter, named after 19th century Swedish physicist, A. J. Ångstrom

animalcules: antiquated name for tiny animals

anion: a negatively-charged ion The name (as well as those of ion, cations and other related subjects) was introduced by philologist, Rev. William Whewell of Trinity College in

response to the request of Michael Faraday to designate the charge-bearing particles (in an electrolyte solution) that migrate to the positively-charged electrode or anode.

antiferromagnetic cooperative transition: a cooperative transition, which shows unfavorable or negative near-neighbor interaction energy, i.e., $-\gamma/2 << 0$, in the terminology used in this volume

antiferromagnetism: A number of ferromagnetic substances contain two interlocking types of atoms or atom groupings, referred to as sublattices, which tend to magnetize oppositely. Such substances tend to show a cancellation of the total magnetization. This phenomenon is referred to as antiferromagnetisim.

antiparallel orientation: orientation of individual polar elements that is opposite to that of parallel orientation

apparent equilibrium distribution coefficient (ρ-value): After diffusion equilibrium has been reached, the ratio of the concentration of a substance in the water within a living cell or model system over that in the surrounding medium is called the (true) equilibrium distribution coefficient (or q-value), if the substance present in the cell and in the surrounding medium are all in a free state. In the case where an adsorbed or otherwise non-free form of the substance also coexists with the substance in the cell water in a free state, the (unresolved) equilibrium distribution coefficient is then referred to as an apparent equilibrium distribution coefficient or ρ-value. Note that the ρ-value may be equal to, or larger than the true equilibrium distribution coefficient or q-value but never smaller than the q-value.

arginine: a common α-amino acid found in most proteins It carries an additional positively-charged guanidyl group beyond the α-amino group (and α-carboxyl group) shared by all α-amino acids. Like lysine, its presence in a protein endows the protein with a positively charged guanidyl side chain.

arginine residue: when an arginine participates in the formation of a polypeptide or protein, it becomes an arginine residue in the polypeptide or protein.

Arrhenius's theory of ionic dissociation: In 1887, S. A. Arrhenius, Swedish physicochemist (1859–1927) proposed the theory that in a water solution, a substantial portion of acids, salts and bases dissociate spontaneously into positive and negative ions, which are then free to move around independently.

association-induction hypothesis: In 1962 G. N. Ling introduced the first physico-chemical theory of life at the molecular and electronic level. In this hypothesis the three major components of the living cells, proteins, water and ions are largely associated and electronic polarization or induction provides the basic mechanism for the coordination and coherence of functional activities.

atomic weight: the relative weight of the atom on the basis of oxygen as 16 rounded off to the nearest integer. The atomic weight of a pure isotope gives the number of neutrons and protons in the atom's nucleus.

ATP: See adenosine triphosphate.

ATPase: an enzyme, which hydrolyzes ATP into ADP and inorganic phosphate

aurovertin: an antibiotic, which strongly inhibits mitochondrial oxidative phosphorylation and first introduced by H. Lardy to investigate mitochondrial functions

autocooperative adsorption: A type of adsorption, in which the adsorption of an ith adsorbent on one site enhances the affinity of the two nearest-neighboring sites for the ith adsorbent. In other words, a type of adsorption, which shows positive nearest-neighbor interaction, i.e., $-\gamma/2 >> 0$.

autocooperative interaction: interaction in which a primary event favors subsequent events of similar nature

autocooperative transition: a cooperative transition that is abrupt and "all or none" as a result of the strongly positive nearest-neighbor-interaction energy in the assembly

autocooperativity: being, or in possession of the trait exhibited by an assembly showing autocooperative attributes in consequence of the possession of strongly positive nearest-neighbor-interaction energy

autoradiography: the science and technique of localizing a chemical substance, X, in living cells and tissues by introducing into the cell or tissue a radioactively labeled substance X

and after air-drying, freeze-drying or other technique to remove the water in the sample, covering the sample with a thin film of photo emulsion in the dark After due exposure and development, the silver granules visualized point to the locations of substance X in the living cell or tissue.

auxiliary cardinal adsorbent: a cardinal adsorbent, which functions as a modifier of the living state Its action is, as a rule, superposed on that of the principal cardinal adsorbent, ATP, which plays the primary role of maintaining the living state.

Avogadro number: The number of individual atoms in a gram atom, of ions in a gram ion, or of molecules in a mole is called the Avogadro number and equal to 2.02×10^{23}.

Avogadro's Law: Equal volumes of all gases, under the same conditions of temperature and pressure, contain equal numbers of molecules.

axon: a nerve fiber, the long process extending from and continuous with the cell body of a nerve

axoplasm: the gel-like protoplasm making up the bulk of nerve axons

backbone: the polypeptide chain of a protein minus the side chains

Baur's ion adsorption potential theory: the theory of steady phase-boundary electric potential proposed by E. Baur In Baur's concept of adsorption—as was virtually universal at his time— the adsorbed entities are not stuck on localized sites but are mobile at the (oil-water) interface. (See Langmuir adsorption isotherm and close-contact adsorption.)

β- and γ-carboxyl groups: When a pair of dicarboxylic amino acids, aspartic and glutamic acids are incorporated into the polypeptide chain of a protein, they endow the protein with two negatively charged side chains, one in the form of the β-carboxyl group of the aspartic acid residue and the other in the form of the γ-carboxyl group of the glutamic acid residue.

β50asp-β73asp: a symbol used in this volume to denote a specific pair of nearest-neighboring aspartic residues at position 50 and 73 respectively on the β-chains of human hemoglobin

β-galactoside permease: a protein isolated from E. coli and seen by some investigators as an inward lactose pump transporting lactose and other β-galactosides into E. coli cells against concentration gradients (See lac operon.)

β-pleated sheet conformation: An extended polypeptide may form H-bonds with its neighboring polypeptide chains and together form a flat sheet. There are two ways how this can be done: with the NH-CHR-CO all in the same direction (parallel chains) or in opposite direction (antiparallel chains). The antiparallel chain is that ascribed to the β-form of the keratin-myosin groups of fibrous protein, and also of silk and is known as the β-pleated-sheet conformation.

Beggiatoa mirabilis: the name of a sulfur bacteria

Berthelot-Nernst distribution law: If to a system of two immiscible liquid phases I and II in direct contact, a third substance, S, soluble in both liquid phases, is introduced and the equilibrium concentrations of S in the two phases are represented respectively as I_S and II_S, then the equilibrium concentration ratio, (I_S/II_S) (the distribution coefficient or partition coefficient), is a constant regardless of the concentration of S. In the special case where S is a gas, the distribution law (also referred to as partition law) is known as Henry's law.

BET theory: the theory of multilayer water sorption proposed by Brunauer, Emmett and Teller, bearing the initials of their respective last names

bifacial cells: cells with two different kinds of surfaces or membranes, usually making up sheets of single cell layers separating two different media Examples: epithelial cells of frog skin and of kidney tubules

billiard type of triplet route: a mode of entry of an external ion into a living cell or model system where beside the fixed ion and the entrant ion, a third ion of the same polarity as the entrant ion is involved If this third ion comes from the opposite side as the entrant ion, the triplet route belongs to the billiard type. (occurs also in exit)

binding constant: a constant that measures the strength of binding of ligand to a binding site

bioelectrical potential: electric potentials transient or lasting, measured across different loci in a living organism The most widely studied bioelectric potentials are the resting potential and the action potential of nerves and muscle cells.

blood plasma: the cell-free liquid of normal blood

Bohr effect: change of the oxygen affinity of hemoglobin with a change in the pH of the medium

Born-charging method: the method of evaluating the association energy between a fixed ion and its free counterion by evaluating the work performed when the counterion is brought from infinity to its location of closest association—as used by physicist Max Born

bound water: water bound to colloids by unspecified mechanisms, producing attributes different from that of normal liquid water (For history and details, see Ref. 64 pp. 278–306.)

bovine serum albumin: a protein isolated from cow's blood It has a molecular weight of 64,000 and is made of 582 amino acid residues all in a single chain.

Boyle's Law: At constant temperature the volume (V) of a definite mass of gas is inversely proportional to the pressure (P). PV = constant. Law due to English physicist-chemist, Robert Boyle (1627–1691).

Br^{86}-efflux analysis method: one of the methods used to determine the extracellular space of frog sartorius muscles

Bradley multilayer adsorption isotherm: a theoretical equation published by R. S. Bradley in 1936, relating the adsorptive uptake of multiple layers of gaseous molecules (with a permanent dipole moment, e.g., water vapor) on polar surfaces at various partial vapor pressures at an unchanging temperature (Equation A2 in Appendix 1)

butyltrimethylammonium ion: a quaternary ammonium ion

Bryopsis plumosa: a marine plant

bulk-phase limited diffusion: In contrast to a surface-limited diffusion in which the rate-limiting step of the diffusion is at the surface, in a bulk-phase limited diffusion the rate-limiting step is not confined to a specific location but exists throughout the bulk-phase of the diffusion medium.

c-value: a quantitative parameter introduced as part of the AI Hypothesis Rigorously defined elsewhere (see text), it is to a first approximation the electron density of the singly charged oxygen atom of an oxyacid group. The c-value of an oxyacid group represents the electronic variable underlying the different acid dissociation constants or pK values. Weak acid with high pK has a higher c-value; strong acid with low pK has a lower c-value.

c′-value: counterpart for the c-value but applicable to cationic sites High c′-value denotes strong positive charge; low c′-value denotes low positive charge.

c-value analogue: a parameter which measures the electron density of the carbonyl oxygen atom of a polypeptide chain or protein

c′-value analogue: a parameter which measures the positive charge density of the imino group of a polypeptide chain or protein

Ca^{2+}, Ca^{++}: calcium ion

capillary condensation: condensation of gaseous molecules as normal liquid in narrow pores

carbodiimide: a carboxyl reagent, which combines and destroys carboxyl groups

carbohydrate: any of the neutral compounds of carbon, hydrogen and oxygen like sugars and starch, much of which are formed by green plants and make up a major class of animal foods

carbonyl group: a bivalent radical CO occurring in the peptide group (CONH) of protein backbones and in simpler molecules including aldehydes, ketone, carboxylic acids and amides

carbonyl oxygen: the oxygen atom belonging to a carbonyl group (CO)

carboxyl group: a univalent radical (COOH) typical of organic acids (See also β- and γ-carboxyl groups.)

carboxyl group specific reagent: a chemical reagent, which has a specific affinity for, and hence focussed destructive power for the carboxyl group

cardiac glycosides: Cardiac glycosides are complex steroids with one or more carbohydrate residues attached through the oxygen atom at position 3 of the steroid nucleus. Cardiac glycosides like digitalis increases the force

of myocardial contraction without concomitant increase in the heart's oxygen consumption. The highly water-soluble ouabain is a cardiac glycosides much studied by cell physiologists for its control of the K^+ and Na^+ accumulation in living cells.

cardinal adsorbent: an adsorbent on a cell protein that critically control the metastable cooperative state of a gang of protein sites

cardinal site: a locus on a protein composed of one or more closely placed side chain and/or backbone functional groups that critically control the metastable cooperative state of a gang of interacting neighboring sites

carrier theory: a theory that traffic of a substance between the inside of a cell and its surrounding medium is mediated by a complex molecule, which shuttles back and forth transporting its molecular passengers

catalyze: to bring about the acceleration of a chemical reaction by a substance (catalyst), which itself remains unchanged at the end of the reaction

cause vitae: the underlying cause of life As used by believers in vitalism, *cause vitae* is not only unknown but also unknowable.

cell membrane: In the textbook version of the membrane theory or its variant the membrane-pump theory, each living cell is covered with a bilayer of phospholipids, in which islets of fully submerged or partially submerged proteins molecules are imbedded. (For an up-dated version based on the AIH, see Chapter 13.)

cell physiology: the scientific study of the functions of living cells

cell theory: the theory, formally introduced by Theodor Schwann in 1839, that all living organisms are made of (as a rule) tiny units called cells

cellular electrical potentials: See resting potential and action potential.

cellulose acetate membrane: a thin water-loaded translucent membrane manufactured from cellulose acetate and marketed by Eastman Kodak Co., Rochester Before use, the membrane is heated at 90°C for half an hour to "activate" and make it more selective in its differential permeability toward solute molecules.

center(s) of activity of a drug molecule: the idea that a drug molecule may possess more than one center or point of (effective and functional) attachment to a receptor site

central canal: a narrow passage going through the center of the spinal cord

central vacuole: the large sap-filled space inside mature plant cells (see Fig. 1A)

centrifugation method, Ling-Walton centrifugation method: a simple and rapid method introduced by G. N. Ling and C. Walton in 1975 to remove quantitatively the extracellular fluid of the sartorius or isolated muscle-fiber bundles by spinning the muscle in a hermetically sealed packet at 1000 g. for 4 minutes

cetyltrimethylammonium bromide: a cationic detergent containing a long chain quaternary ammonium ion

CG electrode: (oxidized) collodion-coated glass electrode

Characea: the family of giant algae found in fresh water ponds including *Chara* and *Nitella*

"charge reversal": a phenomenon extensively studied by Bungenberg de Jong If in a steady DC electric field, the migration of, say, a negatively-charged colloid particle toward the positively charged electrode reverses its direction of migration when a salt (X^+Y^-) is introduced, it would suggest that an excess of X^+ has been taken up by the colloid.

chelate: (**chela**, a pincer-like organ or claw from Grk. chele, claw) of, or relating to, or having a ring-like structure that usually contains a metal ion held by coordination bonds

Chiang-Tai's inductive index: On the surface, Chiang-Tai inductive index resembles other inductive constants introduced earlier (Hammett's inductive constant, σ; Taft's inductive constants, σ_I) in that it too offers a list of numerical values expressing the relative inductive power of various atomic and group substituent. However, here is where the similarity ends. All prior inductive constants were entirely empirical in origin and therefore limited to substituents on which published data are available. In contrast, M. C. Chiang and T.C. Tai have offered theoretical formulae, which enable the computation of inductive indices for new substituents never published before. Without Chiang and Tai's work, the study summarized in Table 4, for example, would be

difficult if not impossible to complete. (For details of this important work, see Ref. 509.)

chloroform: $CHCl_3$, a colorless volatile heavy liquid used as a solvent or general anesthetic

chloroacetic acid (monochloroacetic acid): $ClCH_2COOH$, colorless, white, deliquescent crystals See dichloroacetic acid.

Clark's theory of drug action: proposed by A.J. Clark in 1927, in which the intensity of drug action is directly proportional to the concentration of the drug-receptor complex

close-contact adsorption: adsorption in which the adsorbed entity and the adsorption site are in close direct physical contact

close-contact surface adsorption (CSA) potential: a theory of resting (and action) potential of living cells and model systems (e.g., glass electrode, or CG electrode) in which the electric potential difference originates from the presence of fixed anionic sites at the cell or model surface and countercations adsorbed on these sites in a close-contact manner First introduced by G. N. Ling in 1955 and 1959, it had been until recently described simply as a surface adsorption (SA) potential.

CO group: usually refers to carbonyl groups on the polypeptide chain $(\text{-}\mathbf{CO}CHRNH\text{-})_n$

coacervate: Under the right condition (e.g., elevated temperature, addition of salts, gum arabic) a solution of a certain colloid by itself or in conjunction with other polar macromolecules separates out from a colloid-poor phase. This colloid-rich phase was named coacervate and the phenomenon as coacervation by H. G. Bungenberg de Jong and H. R. Kruyt in 1929.

coacervation: the process that leads to the formation of coacervate (see coacervate) It involves the separation of a single homogeneous aqueous solution into two phases with a clear boundary between them.

coefficient of proportionality: A.S. Troshin's name for what is called *distribution ratio* in the Berthelot-Nernst distribution law or the *equilibrium distribution coefficient* or *q-value* in the AI Hypothesis See Berthelot-Nernst distribution law, and/or q-value.

Collander and Bärlund's lipoidal filtration theory: a theory of the makeup of cell membranes proposed in 1933 by R. Collander and H. Bärlund, in which the continuous phase of the cell membranes is lipoidal, punctured by small pores that act as molecular sieves allowing the passage of small solutes but not that of larger ones

collodion membrane: Collodion is the name of a solution of 4 g. of pyroxylin (largely nitrocellulose) in a mixture of one part ethyl alcohol and one part ether. By casting collodion on the surface of a glass test tube, soaking it in water, a thimble of collodion membrane is formed.

collodion membrane bearing carboxyl groups: Pure collodion does not carry carboxyl groups. However, either as an impurity or added deliberately by oxidation, collodion membranes bearing carboxyl groups can be obtained.

colloid: a term introduced by Thomas Graham in 1861 from the Greek word for glue or gelatin as representing substances like gelatin that diffuse slowly and do not form crystal In years following colloid became entangled with the word macromolecule and lost its identity until a new and simple definition was given by G. N. Ling {see [11.3(2)]}.

colloid chemistry: scientific study aimed at the elucidation of the properties and behaviors of colloids and the colloidal condition

competitive inhibition: a term introduced by enzyme chemists to describe the lowering of the rate of enzyme activity by a substance, which competes against the substrate for the enzyme site In recent years, the terms has been broadened to describe similar competitive inhibition in the effectiveness of drug action, the rate of permeation of ions and in the selective adsorption of solutes, for example.

complex coacervate: coacervate the formation of which involves the formation of salt-linkages among its macromolecular components

complexions, Ω: the number of equally probable micromolecular states of the assembly The number of equally probable micromolecular states is determined by the number of particles in the assembly, the total energy they share and the quantum-mechanically allowed energy levels. (For a simple additional exposition, see Ref. 98 pp 4–12.)

compound microscope: a microscope consisting of an objective lens and an eye piece mounted in a drawtube

concentration gradient: Gradient means slope; a concentration gradient means a slope in the concentration of a substance starting out high in one phase and ending low in an adjoining phase.

condenser: For storing electric charge, a device consisting of two metal plates separated by air as an insulator is called a condenser.

conductance: the reciprocal of the resistance of a substance, a solution or circuit to the passage of a current

congruous anion: an anion which for steric, electronic or other reason(s) is preferentially adsorbed by the fixed cations in the protoplasm of a specific type of living cell at rest

CONH group: the group known as the "peptide linkage"

contractile proteins: A protein which under specified conditions, contracts reversibly is often referred to as contractile protein, usually referring to the actin-myosin system.

converging double reciprocal plot: See reciprocal plot.

cooperative phenomenon: Ralph Fowler first used the term "cooperative transitions" to describe changes of state of a system, in which the interaction among individual atoms or molecules tends to increase in importance with the progress of the change under consideration. This type of cooperative phenomenon cannot be understood in terms of the properties of individual constituent atoms or molecules alone, but is the result of, and therefore can be explained in terms of their interaction.

cooperative adsorption-desorption pump: a theoretical model proposed by G. N. Ling first in 1965, in which all-or-none type of autocooperative adsorption and desorption play central roles in the active transport or pumping of ions and solutes across bifacial cell layers

cooperative assembly: A collection of atoms and molecules being in close contact with one another are engaged in cooperative interaction among them so that the entire assembly acquires properties beyond the summation of the properties of isolated individual components.

cooperatively linked: Entities that are cooperatively linked are engaged in near-neighbor interaction among themselves.

cooperatively linked protein-ion-water system: a system of proteins, ions and water in close association and linked to one another in a cooperative manner

copper ferrocyanide: red-brown water-insoluble crystals formed when copper sulfate is mixed with potassium ferrocyanide

copper sulfate: usually refers to the cupric salt, either anhydrous or with 5 water of hydration. It is readily soluble in water, forming a blue solution.

Corning 015 glass: a glass manufactured by Corning Co. for making good pH electrodes. It shows high specificity for the H^+ and little or no sensitivity to interfering ions like K^+ or Na^+ for example.

Coulombic energy: electrostatic interaction energy between charged entities, equal in magnitude to the product of the charges of each entity divided by the product of the distance between them and the dielectric constant

counter-cation, counter-ion: As a result of the Law of Macroscopic Neutrality, fixed (negatively–charged) anions are as a rule accompanied by an equal number of ions of the opposite electric charge. These counterions are in the form of counter-*cations*, if the fixed ions are negatively charged.

counter-ion: See counter-cation.

covalent bond: a type of chemical bond resulting from the sharing of electrons between two atoms

creatine kinase: an enzyme that catalyzes the reversible transfer of a phosphate group from a creatine phosphate molecules to an ADP molecule, forming creatine and ATP or the converse

creatine phosphate (CrP), phosphocreatine: Found in high concentration in muscle cells, it is also the major congruous anion of this cell type. Through the action of the enzyme, creatine kinase, CrP acts as a reservoir of the terminal phosphate for the resynthesis of the principal cardinal adsorbent, ATP, when it is hydrolyzed.

crevice hypothesis: an once-popular hypothesis to explain the peculiar S-shaped oxygen

binding curves of hemoglobin In this hypothesis, heme sites with strong binding energy for oxygen are buried inside "crevices" inaccessible to oxygen until the "crevices" are somehow pried open by the binding of oxygen onto weaker oxygen-binding heme sites on the surface of the hemoglobin molecules. Complete elucidation of the hemoglobin molecule has revealed the equal accessibility of all four heme sites, disproving the hypothesis.

cross-link: a cross-wise connecting part (as an atom or group) that connects parallel chains in a complex molecule (as a polymer)

cross-word-puzzle: a puzzle in which words are filled into a pattern of numbered squares in answer to correspondingly numbered clues and in such a way that the words read across and down

CrP: short for creatine phosphate

cryoprotectant: certain organic chemicals including glycerol and dimethyl sulfoxide (DMSO) Added to the incubating medium, a cryoprotectant shields living cells and even whole embryos from freezing and thawing injuries when they are frozen and preserved for long periods of time in liquid nitrogen and later brought back to active life by thawing.

crystalloid: a collective name introduced by Thomas Graham in 1861 for sugars, salts and other molecules, which diffuse rapidly as solutes in a water solution and which form crystals under suitable conditions

Cs^+: cesium ion. one of the heavier alkali-metal ions

Cs^{134}: one of the radioactive isotopes of the cesium ion

cuttlefish: (Sepia) a ten-armed marine cephalopod mollusc differing from the related squids in having a calcified internal shell

cysteine: a common amino acid found in many protein hydrolysates A cysteine residue in a protein contributes an sulfhydryl group on a short side chain.

cysteinyl SH group: the SH or sulfhydryl group of a cysteine residue in a protein or peptide

cytoplasm: the protoplasm of a cell outside the nuclear membrane

D_K: a symbol used in this volume to represent the diffusion coefficient of K^+

decapiperidinum ion: a long-chain organic cation

decapyrrolinium ion: a long-chain organic cation

D-effect: direct electrostatic effect mediated through space

D-fucose: the D-isomer of one of the hexoses; also known as 6-desoxygalactose

D-galactose: a hexose, one of the few sugars other than D-glucose, which are found in animals (In combination with D-glucose, it forms lactose in mammalian milk.)

D-glucose: the D-isomer of the hexose also known as dextrose or grape sugar. It is the main energy source of many living organisms including humans.

D-sorbitol: See sorbitol.

D-xylose: a naturally occurring pentose, often isolated from agricultural wastes (e.g., corn cobs). As a rule, not metabolized by animals or yeasts.

Danielli-Harvey paucimolecular theory: a theory proposed in 1939 by E. N. Harvey and J. Danielli for the chemico-physical make-up of the cell membrane, in which a core of lipid bilayers are covered on both side by layers of globular proteins

DC electric field: direct current electric field

dead state, death state: the counterpart of the concept of the living state introduced in the AI Hypothesis The dead state or death state is the state of the cell or protoplasm when it has reached a low (negative) energy and high entropy state but unable to revert back to the high (negative) energy-low entropy living state

de Boer-Zwikker polarization theory: See polarization theory.

Debye dielectric reorientation time, (Debye dielectric relaxation time), (τ_D): the rate constant for the decay of the macroscopic polarization when the applied field is abruptly removed

debye: unit of electric polarizability equal to 10^{24} cm^3, named after Peter J. W. Debye, Dutch-born American physicist (1884–1966)

Debye-Hückel theory of dilute ionic solution: a theory of dilute ionic solution proposed by P. Debye and W. Hückel in 1923, in which ions of one electric charge in a dilute solution are surrounded by a cloud of oppositely charged ions In this theory there is no, or lit-

tle close-contact association of oppositely charged ion pairs.

***de facto* multiatomic ion**: By itself a Na^+ comprises a single atom. However, being surrounded by a more or less permanent shell of water molecules (of hydration), Na^+ is in reality or *de facto* multiatomic.

denature, denaturation: See denatured protein.

denatured proteins: In 1931 H. Wu offered the first definition of protein denaturation thusly: **denaturation** is a change in the natural protein molecules whereby it become insoluble in solvents in which is was previously soluble."[107 p 37 n4] Since then, the meanings of native and denatured protein have changed a great deal and the word "native" is used often to designate the crystalline folded form of a (globular) protein. In this context, denaturation refers to the change from the folded introvert conformation to the fully-extended extrovert conformation. Since there are extensive evidence now that major proteins in their genuinely *native* environment inside living cells exist not in the so-called native (introvert) comformation but in the extrovert fully-extended conformation [16.6(1.3)], the word denaturation has not only lost its original intended meaning, it has actually acquired willy-nilly the inverted meaning.

deoxygenated state: The protein hemoglobin can exist in two states. In one state, the heme sites are occupied by oxygen molecules; in the other they are not occupied by oxygen. The state without oxygen on its heme sites is called the deoxygenated state.

deoxyribonucleic acid, (DNA): a nucleic acid especially abundant in the nuclei of animal and plant cells Upon hydrolysis it yields adenine, guanine, cytosine, thymine, phosphoric acid and deoxyribose

depolarization: causing to become wholly or partially unpolarized

dialysis: the separation of substances on the basis of their different rates of diffusion through selectively permeable membranes, especially the separation of proteins from crystalloids

dichloroacetic acid: Cl_2HCOOH, a derivative of acetic acid, in which two of its methyl hydrogen atoms have been replaced by chlorine atoms

dielectric: a nonconductor of direct electric current

dielectric constant: also known as specific induction capacity Represented as ϵ, it is defined by the equation: $F = QQ'/(\epsilon r^2)$, where F is the force of attraction between two charges Q and Q' separated by a distance of r in a uniform medium with a dielectric constant ϵ.

dielectric saturation: In the intense field near an ion, particles of the polar solvent become strongly oriented so that the effective dielectric constant can no longer have the value usually given but is greatly reduced. This phenomenon is known as dielectric saturation.

differentiation: the act or process of becoming different

diffusion: a process, which leads to the equalization of concentration in a single phase Diffusion of particles in liquids, gases or solids results from their spontaneous movement caused by thermal agitation.

diffusion barrier: a barrier, which prevents or slows down the equalization of concentration in two contiguous spaces

diffusion coefficient: the weight of material passing in unit time across a plane of 1 sq. cm. when the concentration gradient is unity Diffusion coefficient measures the rate of diffusion; its magnitude can often tell us about the environment of the diffusing substance.

diffusion equilibrium: A diffusing substance has reached diffusion equilibrium when the amount of the substance entering any region in the space where diffusion occurs equals the amount of the substance leaving that region.

diffusion head: The word, head, means the difference in elevation of two points in a body of fluid and the resulting pressure of this fluid at the lower point expressible as the height. Diffusion head denotes a similar head in molecular diffusion of a substance dissolved in a fluid rather than *en masse* movement of a fluid.

dimerization: the formation of a compound from two similar molecules or entities

2,4-dinitrophenol: a highly toxic derivative of phenol with two nitro groups introduced respectively at the 2 (ortho) and 4 (para) po-

sitions It is a powerful inhibitor of oxidative phosphorylation, a key step in energy metabolism.

2,3-diphosphoglycerate (2,3-DPG; DPG): a diphosphate ester of glycerol It is an intermediate in glycolysis of animal tissues; found in significant concentration in erythrocytes.

dipole: a pair of equal and opposite electric charges or magnetic poles of opposite sign separated by a small distance

dipole moment: the product of one of the charges of a dipole unit by the distance separating the two dipolar charges

direct current: electric current, which does not change its direction with time

direct inductive effect (D-effect): inductive effect mediated through intervening space

disaccharide: a sugar that upon hydrolysis produces two monosaccharides Example: sucrose, lactose

dispersive X-ray microanalysis: A setup, which by combining an electron microscope, an energy-dispersive X-ray detector and a multichannel analyzer, makes possible the analysis of minute quantities of elements in a microscopic specimen.

displacement, D: a vector defined by the relationship: $\mathbf{D} \equiv \mathbf{E} + 4\pi\mathbf{D}$, where **E**, another vector is the electric field intensity and **P**, also a vector, is the electrical polarization

disulfide bond: -S-S-, as in the example of the linkage between two cysteine groups in forming cystine

DNA: See deoxyribonucleic acid.

(40%) double bond: Two atoms can be joined by a single bond or a double bond. But under certain conditions, the actual bond involves both single and double bond by resonating between the two structures, or one may say, it is a resonance hybrid between the two. An empirical curve relates the distance measured between the two atoms and the amount of single bond, double bond character of a specific bond. Based on this empirical relationship and the actually measured bond length, one may arrive at the conclusion that a specific bond is 40% double bond and 60% single bond.

double reciprocal plot: a way of presenting the experimental data in which one plots the reciprocal of the dependent variable against the reciprocal of the independent variable A converging double reciprocal plot means that the (straight-line) theoretical plots with different slopes converge toward the same spot on the ordinate. (For examples, see Fig. 12 and Fig. 59B.)

doublet adsorption-desorption route: one mode of ion entry into (or exit from) a living cell or model It involves the adsorption of the entrant ion on an oppositely-charged fixed ion, a libration step around the fixed ion and desorpton from the fixed ion and entry into (or exit from) the living cell or model.

Dowex-50: a sulfonate type of cation exchange resin marketed by Dow Chemical Co.

drug: a substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease

dynamic: of or relating to activity

dynamic structure: a structure marked by continual change

E. coli: short for Escherichia coli

ϵ-amino group: the amino group carried on the side chain of the lysine molecule (or residue in a protein) attached to the fifth or ϵ-carbon atom counting from the carboxyl group

Ebola: a new deadly virus Named after a little river in Africa flowing through the region where the disease caused by this virus was first discovered As much as 80% to 90% of the infected victims died from intense bleeding. The disease is highly infectious and apparently transmiited through direct contact with the victim's body fluids.

ectoplasm: the outer relatively rigid and granule-free part of the cytoplasm

EDC: short for electron-donating cardinal adsorbent

edema: an abnormal accumulation of serous fluid in connective tissue or serous cavity

EDTA: short for ethylenediaminetetraacetic acid

effective collision: at room temperature, water (and other) molecules in an aqueous solution, for example, are moving about constantly due to thermal agitation and they collide among themselves and against other particles. If the particles were in an associated state and if the collision tears them apart, one may speak of that specific collision as an effective collision

effectively membrane-pump-less open-ended cell (EMOC): a multicellular preparation

usually derived from a frog sartorius muscle In this preparation part of the cell membrane is amputated whereas the intact part of the cell membrane is made effectively non-functional by being suspended in moist air, which can neither act as a source of the hypothetical inward K^+ pumping, nor a sink for the hypothetical outward Na^+ pumping (For illustration, see Fig. 7).

effector site: one of a cluster or gang of reactive sites, which produces a physiological activity in response to an agent adsorbed at another spatially separate site known as receptor site

efflux: a continual outward flow or migration

EIC: short for electron-indifferent-cardinal adsorbent

eight interaction energies: the eight interaction energies we considered in computing the data shown, for example, in Fig. 42, including: (i) charge-charge; (ii) charge-permanent dipole; (iii) charge-induced dipole; (iv) permanent dipole-permanent dipole; (v); permanent dipole-induced dipole; (vi) induced dipole-induced dipole; (vii) London dispersion energy; (viii) Born repulsion energy

electric field: a region, in which electric forces are acting

electric field intensity, electric field strength: the force exerted on a unit charge A unit field intensity (or strength) is the field, which exerts the force of 1 dyne on unit positive charge.

electric line of force: A line of force in an electric field is a curve so drawn as to have the same direction of the electric intensity.

electric potential: The electric potential at any one position is the work necessary to bring a unit positive charge from an infinite distance to this point.

electric potential difference: The electric potential difference between two points is measured by the work necessary to carry a unit positive charge from one point to the other.

electrogenic pump: a hypothetical concept in which the cell membrane or its constituent by transporting charged particles (ions) steadily in an asymmetrical way creates a standing electric potential difference between the inside of a cell and its surrounding medium

electrolyte: a nonmetallic conductor, in which current is carried by the movement of ions Examples are acids, bases and salts.

electrolytic conductor: a conductor in which the current is carried by ions

electromagnetic waves: waves propagated by simultaneous periodic variation of electric and magnetic field intensity They include radio waves, visible light, X-ray.

electron density: The smallest of all atoms is hydrogen. Its nucleus contains just one proton carrying a single positive charge. In quantum mechanical terms, the normal hydrogen atom may be seen as a positively charged nucleus surrounded by a spherically symmetrical *ball of negative electricity*—the electron blurred by a time exposure of its rapid motion. When 3 H atoms on the methyl group of acetic acid are replaced by 3 strongly electronegative Cl atoms, its pK value drops from 4.76 to below unity—due to the inductively transmitted effect reducing the affinity of the carboxyl group for H^+.There are two (arbitrary but convenient) ways of looking at this event. In one way, one visualizes a movement of the single excess electron charge of the carboxyl oxygen away from the H^+ it attracts—this is the essence of the concept of c-value measured in unit of distance (Å). Another way is to see the change as a result of a reduction of the "ball of negative electricity" i.e., the *electron density* surrounding the singly charged oxygen atom.

electron-donating cardinal adsorbent, (EDC): a cardinal adsorbent which donates electrons to the (cardinal) site on which it is adsorbed

electron-donating power: See electron-donating strength.

electron-donating strength: the relative strength, with which a substituent donates electron to the molecular entity it combines with The methyl group, CH_3, has a high electron-donating strength when compared to that of a hydrogen atom, H. For this reason, acetic acid, CH_3COOH, has a stronger affinity for its H^+ and is accordingly less willing to give off its H^+ and thus falls into the category of a *weaker* acid (pK, 4.76) than formic acid, HCOOH (pK, 3.75), which

has less affinity for its H^+, is more willing to give off its H^+ and is thus a *stronger* acid.

electron indifferent cardinal adsorbent (EIC): a cardinal adsorbent which neither donates to, nor withdraws electron from a cardinal site

electron microscope (EM): an electron-optical instrument, in which a beam of electrons focused by an electron lens is used to produce an enlarged view of a minute subject on a fluorescent screen or photographic plate

electron microscopy: the science and technique of the electron microscope

electron-withdrawing cardinal adsorbent (EWC): a cardinal adsorbent, which withdraws electrons from the cardinal site

electronegative: An atom is more electronegative if its nucleus carries more positively-charged protons and thus has greater power to draw (negatively-charged) electrons toward itself.

electronic polarization-depolarization: the cycle, usually repeated, of separating electronic charges followed by their being united to a more neutral arrangement again

electrostatic field strength: See electric field strength.

electrostatic forces: of or relating to forces produced by static electricity

electrostatic repulsion: repulsion produced by static electricity

elemental living machine: the smallest unit of life according to the AI Hypothesis

EM section: very thin sections of imbedded tissue for electron microscopic viewing

embedding: to prepare (a microscopic specimen) for sectioning by infiltrating with and enclosing in a supportive substance

EMOC preparation: See effectively membrane-pump-less open-ended cell (preparation).

Encyclopedia Britannica: The first edition of this great encyclopedia was published in Great Britain in 100 installments between 1768 and 1771.

endocrine: secreting internally especially in the body by way of the blood stream

endocrine gland: a gland (as the pituitary) that produces a hormone, also known as ductless gland

endogenous: being or developing within the cells

endoplasm: inner relatively fluid part of cytoplasm

endosmosis: name introduced by Dutrochet describing the inward movement of water into living cells and model systems

energy barrier: a continuous barrier of electrostatic repulsion, which impedes the passage of ionic particles

energy metabolism: the collection of enzyme-catalyzed biochemical reactions, which degrade energy-rich chemicals like D-glucose in order to synthesize and thus replenish hydrolyzed (principal cardinal adsorbent) ATP during cyclic activity and in maintaining the resting living state

energy minimization: a method of evaluating the most stable configuration of a group of interacting molecules or ions by determining in what configuration is the total interaction energy at its minimum

enhancement of ionic association through charge fixation: a basic theory offered in LFCH to account for the high state of ionic and molecular association in living and non-living fixed charge system (e.g., protoplasm) It has two components, one electrostatic and the other kinetic. {For details, see [10.1(1)].}

enthalpy: also known as heat content (H), a thermodynamic quantity given by $H = E + PV$, where E is the internal energy of the system; P, the pressure and V, the volume

entropic cause for enhanced counterion association due to charge fixation: The Law of Macroscopic Neutrality forbids the departure of any significant quantity of counterions from leaving the fixed charge system. As a result, virtually all counterions stay within the much smaller volume inside the fixed charge system rather than the much larger volume of the free bathing solution. Since entropy, especially transational entropy, is directly related to the volume accessible to the counterion, and low entropy favors association, charge fixation enhances counterion-association.

entropy: that portion of the energy of a substance, which is unavailable for the performance of useful work It is due to the various modes of motion of the molecules and may be considered a measure of the degree of randomness of the molecules. Entropy S is directly proportional to the heat content H (or H_{rev}) and inversely proportional to the absolute tem-

perature, T. Thus S = H_{rev}/T. In statistical mechanics, entropy is related to the number of complexions Ω by the relation, S = kT lnΩ, where k is the Boltzmann constant.

entropy of dissociation: entropy gain accompanying the dissociation of an adsorbed or associated entity

entropy-driven: that which proceeds spontaneously in a direction made probable by the gain of entropy

enzyme: any of the numerous proteins produced by the living cells, which catalyze biochemical reactions at room temperature

enzyme kinetics: the study of the rate of enzyme-catalyzed biochemical reactions

enzymology: the science of the study of enzymes and the reactions they catalyze

epithelial cells: cells of the epithelium

epithelium: a membranous cellular tissue that covers a free surface or lines a tube or cavity of an animal body and serves especially to enclose and protect the other parts of the body, to produce secretions and excretions and to function in assimilation

equilibrium: a state of balance between opposing forces or reactions. For water in contact with an air phase containing water vapor in a closed vessel, equilibrium is reached when the number of water molecules leaving the liquid phase exactly matches the number of molecules entering the liquid water phase.

equilibrium distribution coefficient: See true equilibrium distribution coefficient and apparent equilibrium distribution coefficient.

equilibrium phenomena: phenomena that describe systems at equilibrium

erythritol: one of the four-carbon polyols or tetritols Found in lichen, grass etc., it is twice as sweet as cane sugar or sucrose.

erythrocytes: red blood cells

erythrocyte ghosts: During hemolysis of red blood cells by hypotonic solutions or lytic agents, dim colorless outlines of the cells may be seen and are known as ghosts. They represent incompletely-destroyed framework or the stroma of the cells.

Escherichia coli: gram-negative colon bacteria, the most abundant bacteria of fecal matter and studied intensively in 1886 by T. Escherich

état de choses: things as they are

ether (ethyl ether; diethyl ether): highly volatile and inflammable liquid, used as inhalation anesthetic with a high degree of safety

ethylenediaminetetraacetic acid (EDTA): an important chelating agent with strong affinity for divalent cations

ethylene glycol: $HOCH_2CH_2OH$, a viscous, hygroscopic, sweet-tasting but poisonous liquid, used often as an antifreeze agent

EWC: short for electron-withdrawing cardinal adsorbent

exclusion intensity, (U_{vp}): an overall expression of the effectiveness of the polarized-oriented water's ability of excluding solutes. It is equal to the sum of the volume component of an energy term and an entropy term (See Ref. 168 pp.152–157.)

exosmosis: term introduced by Dutrochet, meaning outward movement of water from living cells and model systems

extended conformation: the conformation in which the protein chains are extended Often used to denote the β-pleated-sheet conformation in solid or crystalline proteins. To be distinguished from *fully-extended conformation* used in the PM theory, which as a rule refers to proteins in the fully extended conformation and interacting with bulk-phase water.

extracellular space: the space in between living cells in a tissue like the muscle It is usually filled with tissue fluid or Ringer's solution.

extrovert model: proteins or linear polymers with properly-spaced exposed oxygen or nitrogen atoms, each carrying lone-pair electrons, which interact directly with the bulk-phase water

f^-, f^+: fixed anion and fixed cation respectively

FDNB: short for fluorodinitrobenzene

F-effect: the combined direct electrostatic effect transmitted through space (D-effect) and electrostatic inductive effect transmitted through intervening atoms (I-effect), sometimes also called direct F-effect

F_1 soluble mitochondrial ATPase: a specific water-soluble ATPase isolated from mitochondria

Federation Meetings: yearly meetings of the Federation of American Societies of Experimental Biology of the United States

fermentation: an enzymatically controlled, anaerobic breakdown of energy-rich compounds like glucose into alcohol as that occurring in yeasts

ferromagnetic cooperative transition: Iron, nickel and cobalt are described as ferromagnetic since they exhibit the properties of a magnet. This property originates from the existence of each atom of these metals as a magnetic dipole. And for that reason, the orientation of a particular atom depends on the orientation of its neighboring atoms—a fundamental characteristic of cooperative phenomenon. A ferromagnetic cooperative transition tends to be all-or-none, either all in the ordered magnetized state or the disordered random state.

fibrin: a white insoluble fibrous protein obtained from fibrinogen found in normal blood by the action of the proteolytic enzyme, thrombin in the clotting of blood

fibrous protein: In contrast to globular proteins, fibrous proteins assume at least in part the fully-extended and other extended conformations. Examples include collagen and gelatin.

field overlapping enhances counterion association: A cation exchange resin bead, as a typical fixed charge system, contains only fixed anionic sites. Here each cation experiences not only the electrostatic attraction of a closely located fixed anion but also that of other fixed anions as well. A resting living cell or protoplasm, on the other hand, is as a rule an amphoteric fixed change system containing roughly equal number of fixed cations and fixed anions. Here the field experienced by a counter-cation is weakened by the fixed cations nearby. But even in a perfectly structured fixed system resembling the NaCl crystal where each Na^+ is surrounded by six Cl^- and each Cl^- is surrounded by six Na^+, the field experienced by each ion is more than that of one fixed ion of the opposite sign—as precisely described by the Madelung constant. Furthermore, the distribution of fixed anions and fixed cations in living cells are as a rule not uniform. Charge distribution along individual protein molecules[180 Fig. 3.5] and in different regions of the cell[15 Fig. 15.5] tend to be segregated, thereby producing regions of fixed charges of one sign predominantly and in which the influence of field overlapping can be more pronounced in enhancing counterion association.

field theory: first introduced by Michael Faraday on the electric field produced by changing magnetic flux

field strength: See electric field strength.

first order quadrupole broadening: When atoms whose nuclear charge distribution are not spherically symmetric are placed in an asymmetrical electric field, the NMR signal of these atoms may split into one center peak and two satellite peaks. For atoms with nuclear spin equal to 3/2, like Na,[23] the relative intensity of the center peak is 0.4. For cases in which, the size of the center peak does not differ significantly from the expected intensity (0.4), the quadrupole broadening is described as that of the first order.

fixed anion: a negatively-charged functional group that is a part of, and thus anchored on a protein or other macromolecule or a fixed charge system

fixed cation: a positively-charged functional group that is a part of, and thus anchored on a protein, another macromolecule or a fixed charge system

fixed charge: a chemical group that is spatially fixed and carries a net positive or negative charge

fixed charge system; a three-dimensional system consisting of a fixed framework onto which are fixed a large number of charge-bearing groups

fluid-mosaic model of cell membrane: a model offered by Singer and Nicolson for the cell membrane, consisting of a continuous phospholipid bilayer penetrated fully or partially by islets of protein molecules

fluorescence: When a molecule absorbs a quantum of radiation, the molecule enters an excited state. If the molecule is stable enough, the excited molecule may return to its initial ground state by emitting radiation usually of a longer wave length. This type of emission is called fluorescence.

fluorescence yield: the ratio of the emitted radiation energy over that of the exciting radiation in fluorescence is called the fluorescence yield

fluorodinitrobenzene, FDNB: a reagent which reacts with terminal amino groups of proteins It is also a specific inhibitor of the enzyme, adenylic kinase.

formic acid: HCOOH, a rather strong acid, first observed from distillates of ants (colonial insects belonging the family, Formicidae)

free energy, Gibbs free energy, F: also called *useful energy* by Guggenheim It represents the work, which a system is able to perform in a given isothermal process at constant pressure. The free energy F is related to the enthalpy or heat content H and the entropy S and absolute temperature T by the thermodynamic relation: $F = H - TS$. Now $H = E + PV$, where E is the energy (also called total energy, or internal energy), P and V are the pressure and volume respectively. Therefore, $F = E + PV - TS$. (See also Helmholtz free energy.)

free energy of adsorption: See standard free energy of adsorption.

free energy of dimerization: the free energy change entailed in the association of two identical molecules (or monomers) into a double molecule or dimer

free energy of solute distribution: See standard free energy of distribution.

freeze-drying: a procedure used in preparing microscopic sections for viewing under an electron microscope It involves the freezing of the specimen in liquid nitrogen followed by sublimation of the water in the frozen specimen before infiltrating it with an imbedding material.

freeze-substitution: a procedure used in electron microscopy, during which water in the frozen biological specimen is replaced at a very low temperature by acetone, which facilitates the infiltration of embedding material and minimize distortions of the original fine structure of the specimen

freezing point depression: the lowering of the freezing point of a solvent like water

frozen hydrated preparation: A biological specimen as a rule contains large amount of water. In the standard procedure in preparing sections for viewing under an electron microscope, this water is first removed by drying under vacuum and replaced directly or indirectly with an imbedding medium, which then can be polymerized into a solid form. In a frozen hydrated preparation, all these intermediary steps are eliminated and the specimen is viewed in its original frozen, hydrated state.

fucose: See D-fucose.

fully-extended conformation: According to the PM theory, the backbone NH and CO groups of a fully-extended protein molecule are not engaged in intra- or inter-macromolecular H-bonds but are directly exposed to and interact with the bulk-phase water.

"fully-satisfied state": a state of water molecules when all its H-bonding groups are fully engaged in forming H-bonds with H-bonding sites on macromolecules or with other polarized and oriented water molecules

"fusing": joining as if by melting together

g: unit of force equal to that exerted by gravity at the earth's surface

γ_K: the activity coefficient of K^+

$-\gamma/2$: the nearest neighbor interaction energy in a cooperative adsorption, equal to the additional energy gain with each new neighboring ij pair formed {See [14.3(2)] and/or section 4 in Appendix 1.}

γ-scintillation counter: a devise for detecting γ-radiation where the γ-emitting radioactive sample is placed within a cavity or "well" inside a solid scintillator which emits light each time it encounters a γ-radiation and the light pulses are then amplified by a photomultiplier and then registered on a counter

galactose: See D-galactose.

"gang": a chain of similar H-bonding, ionic or other types of sites, which function autocooperatively and coherently as a unit

Gay-Lussac's Law: At constant pressure, the volume of any gas expands by the same fraction of its volume at 0°C. for every 1° rise of the temperature. Due to French physicist-chemist, Joseph Louis Gay-Lussac (1778–1850). Also known as Charles's Law due to J.A.C. Charles (1787).

Geiger counter: an instrument introduced by Hans Geiger for detecting and measuring the intensity of radiation

gel state: a more rigid state of a colloid than a sol

gelatin: denatured collagen usually obtained from animal skin, hoofs, etc., by boiling

gene: Historically a gene is an element of the germ plasm, which controls the transmission of a

hereditary character. A gene is now known to be a stretch along a chromosome that codes for a functional product, i.e., either RNA or its translation product, a polypeptide or protein.

Gerard-Graham-Ling microelectrode: for some time also known as Ling-Gerard microelectrode, glass- capillary microelectrode In 1942 Judith Graham, A. J. Carlson and R. W. Gerard first reported the use of fine-tipped glass capillary electrode for the measurement of the resting potential of frog muscle cells. War slowed down the progress, so that the electric potential as reported in 1946 in Graham's Ph.D. thesis (and elsewhere) varied over the range of 41 to 80 mV, too scattered for quantitative determinations. The task of improving the technique of making the microelectrode and of filling the electrode with salt solutions fell on G. N. Ling, who as a graduate student under Prof. R.W. Gerard, inherited the project. The efforts paid off so that by 1947, the difficulties in obtaining reproducible quantitative data were overcome. Thus, the resting potentials reported by Ling and Gerard in 1949 obtained with the improved technique was 78.4 ± 5.3 mV from 1350 measurements with Ringer's-solution- filled electrodes, and 97.6 ± 5.7 mV. from 207 measurements with a 3 M KCl-filled electrodes. A tool was thus ready to enable the exact measurements of the electrical potentials activities of a vast variety of living cells as well as subcellular organelles. (For more details, see Refs. 88, 441–443.)

germ plasm: The living organism can be seen to consist of two parts: soma and germ plasm. Soma represents all except the germ plasm, which is devoted to inherence. A chief advocate of this concept was August Weismann (1834–1915).

giant barnacle muscle cells: muscle fibers (diameters varying from 0.4 mm to over 1.0 mm) isolated from the depressor muscle of the giant barnacle, *Balanus nubilis*

giant squid axon: See squid axon.

Gibbs free energy: See free energy.

gland cells: cells belonging to a variety of glands (e.g., salivary gland, adrenal gland)

glass electrode potential: the electric potential difference measured between the inside surface of a glass electrode and an outside solution bathing the electrode To be noted is the fact that the inside electric lead requires no mediation of a solution (direct metal plating would do) but an external solution is essential.

globular protein: In contrast to fibrous proteins, which are much longer than wide, globular proteins are roughly speaking globular in shape. Most so-called native proteins exist as globular proteins. Under proper conditions, globular proteins can form crystals.

glucose: See D-glucose, L-glucose.

glucuronidase: an enzyme that hydrolyzes a glucuronide Example is β-glucuronidase, which hydrolyzes the β-glucoside of D-glucuronic acid. (D-glucuronic acid is one of the carbohydrate derivatives possessing both aldehyde and carboxyl groups, which are known as uronic acids.)

glutamate: See L-glutamate.

glutamic acid: See L-glutamic acid.

glycine: CH_2NH_2COOH, a common α-amino acid found in almost all protein hydrolysates

glycine-NaOH buffer: a solution containing a mixture of glycine and NaOH at a specific ratio so that the pH of the solution is maintained at a stable specific value Useful range of pH maintained: 8.2–10.1

glycolysis: Animal tissues like the skeletal muscle utilize glucose anaerobically by way of what is called glycolysis. The overall reaction of glycolysis is that one glucose molecule is converted to two molecules of lactate, two molecules of ADP and inorganic phosphates are converted to two ATP molecules and four electrons are transferred to pyruvate.

glycolytic activity: metabolic activity involved in glycolysis

gram ion: the quantity of an ion that has a weight in grams numerically equal to its molecular or atomic weight

gram molecule: the quantity of a compound or element that has a weight in grams numerically equal to its molecular weight

gravity: the gravitational attraction of the mass of the earth, the moon or a planet for bodies at its surface

Great Wall: the Great Wall of China built on the northern boundary of China mainly to protect herself against northern invaders, who

called themselves Huns, Tartars, Mongols and still others names

guanidine HCl: $HN=C(NH_2)_2$, HCl, well-known protein denaturant freely soluble in water

guanidyl group: $NH_2C=NH(NH)–$, At neutral pH the guanidyl group picks up a H^+ becoming a cationic group.

gum arabic: a highly water soluble gum from the acacia tree

H^+: the hydrogen ion, proton

H_2SO_4-Na_2SO_4 buffer: a system of buffer solution containing varying ratios of sulfuric acid and its salt, sodium sulfate, maintains stable pH between neutral to as low as 1.3

half-life: the time required for half of something to undergo a process, like that in the decay of radioactivity of an isotope

Hammett's σ constant: an empirically determined set of constants, which describes the relative effectiveness of specific substituent on aromatic molecules in producing an inductive effect on a target functional group a distance away

H-bond: hydrogen bond, a linkage consisting of a hydrogen atom bridging two electronegative atoms such as oxygen and nitrogen

H-bonding partner: The imino group and the carbonyl group of a protein, for example, are H-bonding partners to one another in the H-bond formed between their respective nitrogen and oxygen atom.

HBr: hydrogen bromide, a colorless corrosive and non-inflammable gas

half time of exchange: the time needed for one half of the ions or molecules in one phase to exchange with similar ions and molecules in an adjoining space, usually determined with the use of radioactively labeled isotopes

HDO: deuteriated water, heavy water Water in which one of the hydrogen atoms has been replaced by its isotope, deuterium.

heat of dephosphorylation: the amount of heat evolved when 1 gram mole of a phosphate-containing compound such as ATP is dephosphorylated

heat of neutralization: the amount of heat evolved when one gram mole of an acid or base is neutralized at constant temperature and pressure

heat content change, enthalpy change, ΔH: the change in the heat content or enthalpy, which equals the internal energy of a body plus the product of its volume and pressure

heat-activated cellulose acetate membrane: See cellulose acetate membrane.

Helmholtz free energy, work function, A: a thermodynamic function defined by A = E –TS, where E is the energy or internal energy, T and S are the absolute temperature and entropy respectively It represents the maximum work that can be made available in a given isothermal process. Note that free energy, or Gibbs free energy represented as F and work function or Helmholtz free energy represented as A are different by the relation F = A + PV. Since most cell physiological activities occur in solution where there is little change in volume or pressure, changes in Gibbs free energy and Helmholtz free energy are to all intents and purposes the same. (See also free energy.)

heme: the deep red, iron-containing prosthetic group of hemoglobin

heme site: Each hemoglobin molecule contains 4 heme groups. Since each heme can bind one oxygen molecule during the oxygenated state, each heme on a hemoglobin molecule offers a binding site for oxygen and is sometimes referred to as a heme site.

hemoglobin: the pigment-protein complex of red blood cells by means of which these cells are able to transport oxygen from the lungs to the body tissues Each hemoglobin molecule comprises 4 subunits: two called α-chains and two called β-chains

hemolysis: lysis of red blood cells with liberation of hemoglobin

Henry's Law: At constant temperature, the mass of gas dissolved by a given volume of solvent is proportional to the pressure of the gas with which it is in equilibrium. This law was introduced by W. Henry in 1803 and is a special case of the Berthelot-Nernst partition law.

heterocooperative interaction: cooperative interaction with a negative nearest-neighbor interaction energy or $-\gamma/2$ For a system with two alternative adsorbents, i and j, a heterocooperative interaction means that the adsorption of one species, i, enhances the adsorption of the alternative adsorbent, j. Heterocooperative interaction, a term introduced in the AI Hypothesis, is equivalent to

what is called antiferromagnetic cooperative inter-action.

hexose: a monosaccharide like D-glucose that contains six carbon atoms in the molecule

H^3HO: tritiated water, in which one of the hydrogen atoms of a water molecule has been replaced by the radioactive tritium atom. Tritiated water is of great value in the study of movement of molecular water in living systems.

high energy phosphate bond: the concept that certain organic phosphate compounds like ATP contain extra amount of utilizable free energy (–12 to –16 Kcal /mole) in its phosphate bonds that can be tapped to perform biological work In 1956 Podolsky and Morales demonstrated that the enthalpy of the ATP phosphate bond is only –4.7 Kcal/mole and thus not different from what has been called low energy phosphate bonds like that in AMP.

high negative energy: See negative energy.

high(negative)energy-low entropy state: In the AI Hypothesis, the living cell and protoplasm may exist in a resting living state, in which the total negative energy is high and the total entropy is low. This state is thus referred to as high (negative) energy-low entropy state.

Hill coefficient, n: an empirically determined constant represented by the letter, n, introduced by A.V. Hill to measure the "sigmoidity" of the oxygen binding curve of hemoglobin In 1964, Ling demonstrated[488] that n in fact equals – exp (γ/ 2kT), where $-\gamma/2$ is the nearest neighbor interaction energy of an autocooperative phenomenon, k is the Boltzmann constant and T, the absolute temperature.

histidine: a basic α-amino acid found in most protein hydrolysates

Hodgkin-Huxley theory of action potential: a theory of the nerve and muscle action potential introduced by British physiologists A. L. Hodgkin and A. Huxley. Based on the membrane-pump theory, this theory postulates the sequential opening and closing of specific Na^+ and K^+ channels as the physical basis for the nerve or muscle impulse.

homogenate: a ground up paste

hormone: a chemical substance secreted into the body fluids by an endocrine gland, which has a specific effect on the activity of other organs

Hsiung-nu: The word nu means slaves. Hsiung nu is the deprecatory name used by ancient Chinese against northern tribes. Hsiung-nu thus really means Hun-slaves, since the word Hsiung is also pronounced Hun. Witness the Chinese word chosen to represent the first syllable of the word, Hungary, is also Hsiung (pronounced as Hun).

Huns: same as Hsiung-nu, a collective name for northern Mongolian tribes. After being defeated by Han soldiers, part of these tribes moved west. As time went by, they grew stronger. Around 450 AD they took control of large part of central Asia and Europe under their able leader, Attila.

hydrated ion : In water, alkali-metal ions like K^+ are surrounded by a more or less permanent layer(s) of hydration water and are called hydrated ions.

hydrated K^+, hydrated Na^+: See hydrated ion.

hydration water: water molecules attached more-or-less permanently to ions, nonelectrolytes, macromolecules including proteins, etc. See hydrated ion.

hydrogen bond: See H-bond.

hyperbola: a plane curve generated by a point so moving that the difference of the distances from two fixed points is a constant

hyperbolic: of, or relating to a hyperbola

"hyperbolic" curve: It is an age-honored tradition to describe a plot of the rate of enzyme reaction (y) as ordinate against the substrate concentration (x) as abscissa where y rises sharply initially at low x, slowing down steadily thereafter at increasing x [and other similar plots like the oxygen uptake curve of myoglobin (See left curve in Inset of Fig. 55.)] as representing part of a rectangular hyperbola and thus hyperbolic. This is an approximate but inexact description. In a rectangular Cartesian x–y plot, the equation describing a rectangular hyperbola is $x^2 - y^2 = a^2$, where a is a constant. The equations of the asymptotes are $y = x$ and $y = -x$. In other words, y rises steadily with x as x approaches infinity. This is at odds with both the Henri-Michaelis-Menten equation of enzyme kinetics or Langmuir adsorption isotherm, in both of which y approaches a constant value as x approaches infinity. For

this reason and in due respect for tradition, I have used the word hyperbolic from time to time but under quotation marks.

hyperpolarization: to produce an increase in the potential difference across (a cell surface)

hypertonic: at a concentration higher than that which would maintain living cells at their normal sizes

hypotonic: at a concentration lower than that which would maintain living cells at their normal sizes

hydroxyproline: a common α-amino acid found in some protein hydrolysate Like proline but unlike most other α-amino acids, when a hydroxyproline is incorporated into a polypeptide chain or protein, its pyrrolidine nitrogen no longer carries a hydrogen atom and is consequently unable to form an α-helical or other inter- or intra-macromolecular H bond.

IAA: abbreviation of iodoacetic acid

I-bands: the optically less dense bands of muscle striations It corresponds to the region of the sarcomere between the ends of the thick-filaments.

iceberg theory: a theory according to which crystalline ice surrounds protein molecules

ideal gas: See perfect gas.

I-effect: short for inductive effect transmitted through intervening atoms

imbibition water: water taken up by colloidal materials often causing it to expand

imino group: relating to or containing the NH group or a substituted group NR united to a radical other than an acid radical

impermeant: that which cannot permeate

impulse: a wave of excitation propagating along the surface of living cells like nerve and muscle that results in physiological activity or inhibition

in vitro: Latin in glass (test tube)

in vivo: Latin in living (matter)

inanimate model: a non-living model

indole group: 2,3-benzopyrrole, leaflets with intensely fecal odor Paradoxically, at very low concentration, it offers a pleasant odor and is used in perfumery.

inducer: molecules that cause the production of larger amounts of the enzymes involved in their uptake and metabolism, compared to the amounts bound in cells growing in the absence of an inducer Thus lactose acts as an inducer in the activation of the *lac operon* in E. coli.

inductive effect: When the hydrogen atoms on the methyl group of an acetic acid are replaced by the more electronegative (possessing greater power to draw electrons toward itself due to larger nuclear positive charges) chlorine atoms, it cause unequal sharing of electron pairs between neighboring carbon atoms and this unequal sharing propagates to reach the singly-charged carboxyl oxygen atom leading to a weakening of the affinity of the negatively charged oxygen for H^+ and a marked lowering of the pK. The long range impact produced by the Cl for H substitution exemplifies the inductive effect.

inflection point: a change of curvature with respect to a fixed line from convex to concave or vice versa. In a standard titration curve of a (pure) acid, a change of curvature from convex upward to concave downward occurs at the mid-titration point, at which the pH of the solution is equal to the pK of the acid being titrated.

influx: inward flow

influx profile: By plotting the fractional uptake of a substance into a living cell or model system at time t against the square root of t, one obtains a distinct profile, which can be used to determine the rate-limiting step in the diffusion process (Fig. 38).

Infusoria: early name given to minute living organisms including protozoa and bacteria

initiator: that which initiates

integration constant: In calculus, integration of differentials or diffential equations produces a function plus a constant, which is called an integration constant.

interaction energy: energy produced as a result of an interaction between two entities

intercellular space, extracellular space: space between cells making up a tissue

intercept: the distance from the origin where a curve crosses a coordinate axis

interfacial tension: The work required to enlarge the surface of separation between two immiscible or partially miscible liquid (in dynes per cm) is the interfacial tension.

intestinal epithelial cells: epithelial cells forming the inner lining of the intestine

intramacromolecular H-bonds: H-bonds formed within the same macromolecule, e.g., α-helical H-bonds

"intrinsic activity": physiological activity produced by a unit receptor-pharmacon complex

intrinsic equilibrium constant ($K^{oo}_{i\to j}$): in a system containing two alternative adsorbents i and j, the equilibrium constant for an i to j exchange on a site flanked on one side by an i adsorbent and on the other side by a j adsorbent, so that the i to j exchange in the midsite does not alter the number of ij neighboring pairs in the system

introvert model: A globular protein with all or almost all of its backbone carbonyl groups engaged in α-helical or other intra- and/or intermacromolecular H-bonds, constitutes an introvert model. Introvert models as a rule do not interact or interact weakly with the bulk-phase water.

inulin: a tasteless water-soluble polysaccharide from tubers and rhizomes of various plants

inulin probe method: a common method using inulin as probe to determine the volume occupied by the extracellular space of living tissues

iodoacetate: ICH_2COOH, a metabolic poison inhibiting glycolysis and the regeneration of ATP

ion: an electrically charged atom, radical or molecule formed by the loss or gain of one or more electrons (For historical origin of this work, see anion.)

ion exchange resin: synthetic resin containing fixed ionic groups used to purify ions or (ion-containing) water

ion exchange resin sheet: flat sheet of ion exchange resin cast on an inert nylon fabric

ion permeation: diffusion of an ion through or into a barrier

ionic bond, electrovalent bond: The bond formed between a positively charged ion or group and a negatively charged ion or group is electrostatic in nature and sometimes called an ionic bond.

ionophores: molecules which can combine selectively with certain ions and ferry them across water-insoluble phospholipid bilayers. The antibiotic valinomycin, for example, is a K^+ specific ionophore.

ionic dissociation: dissociation of salts, acids or bases producing charged particles or ions

ionic strength: G. N. Lewis and M. Randall introduced in 1921 the concept of the ionic strength, I, defined as one half of the *sum* of the terms obtained by multiplying the molality (m or concentration) of each ionic species present in the solution by the square of its valence, z: $I = \frac{1}{2} \sum_i z_i^2 m_i$.

ionic theory: the theory proposed by A. L. Hodgkin in 1951 for the electrical potential of living cells

isethionic acid, isethionate ion: hydroxyethylsulfonic acid ($HOCH_2CH_2SO_3H$); hydroxyethyl-sulfonate ion ($HOCH_2CH_2SO_3^-$)

Ising model: See one-dimensional Ising model.

isobutyric acid: $(CH_3)_2CHCOOH$, liquid with pungent odor

isotherm: an equation or law describing the quantitative relationship between different reactants at equilibrium at the same temperature

isotonic: a solution possessing the same osmotic concentration as the plasma of a given animal

K^+: potassium cation, (positively-charged) potassium ion

K^{42}, ^{42}K: one of the radioactive potassium isotopes with a half-life of 12.4 hours, emitting two β-particles of 3.6 and 2.4 MEV respectively and also γ-radiation of 1.5 MEV

$K^{oo}_{i\to j}$: See intrinsic equilibrium constant. also known as close-contact adsorption exchange constant when i and j refer respectively to Na^+ and K^+ specifically

$K^{oo}_{Na\to K}$: close-contact adsorption exchange constant of Na^+ and K^+

K-channel: hypothetical narrow pores in the nerve and muscle cell membranes that are selectively permeable to the smaller (hydrated) K^+ but not the larger (hydrated) Na^+ Both the narrow K-channels and wider Na-channels (permeable only to the larger hydrated Na^+) are closed when the cells are at rest but open momentarily and sequentially during activity.

K^{42}-labeled K^+: If there are many grey-colored carps in two adjoining ponds, it is not easy to tell accurately how fast they are traveling between the two ponds. The task would be made easier if at a certain time we introduce a number of gold-colored carps into one

pond and count the number of gold carps in the other pond at different times after the initial release. K^{42}-labeled K^+ are employed just like the gold-colored carps to monitor the rate of exchange of K^+ in two pools of this ion, one inside a living cell and another outside.

K^+-sensitive microelectrode: a device with a sharp tip which possesses specific sensitivity to K^+, and can penetrate into the interior of a living cell to monitor the activity of this ion in the thin layer of fluid that collects around the sensitive tip of the microelectrode (See Fig. 11 and text for more details.)

K^+ surrogate: a cation which can serve as a substitute for K^+

Kelzan: a commercially available bacterial polysaccharide of large molecular weight

KI: potassium iodide, colorless or white cubic crystals soluble in water

kidney epithelial cells: epithelial cells lining the renal tubules in kidneys

kinetic: of or relating to the motion of material bodies and the forces and energy associated therewith

kinetic energy: the energy possessed by a body due to its motion, given by the formula: K.E. = $\frac{1}{2}$ mv^2, where m is the mass of the body and v its velocity

L-glucose: the enantiomorph (mirror image) of D-glucose Unlike the ubiquitous D-glucose, L-glucose is rarely if ever found in Nature.

L-glutamate: the anion of dissociated L-glutamic acid The sodium salt has long been used in China as a food additive and is marketed in more recent times under the name "Accent" in the US.

L-glutamic acid: $HOOCCH_2CH_2CH(NH_2)COOH$, one of the common α-amino acids found in most protein hydrolysates

lac-operon: a segment of the ring-like DNA of E. coli, which contains the base sequence coded for the synthesis of three proteins: β-galactosidase coded by the Z-gene, β-galactoside permease coded by the Y-gene and β-galactoside acetylase coded by the AC gene

lactose: milk sugar Upon hydrolysis each mole of lactose yields one mole of D-galactose and one mole D-glucose.

lactose permease: See β-galactoside permease.

Langmuir adsorption isotherm: an equation describing the "hyperbolic" (See "hyperbolic" curve.) relationship between the concentration of free solute and that of its adsorbed form on localized sites, introduced in 1918 by Irving Langmuir (1881–1957) (For an illustrative example, see second term on the right-hand side of Equation A10 in Appendix 1.)

Laplacian physics: French astronomer and mathematician, Marquis Pierre Simon de Laplace (1749–1827) was the last of the leading 18th century European mathematicians. His two great works one on the theory of probability and the other on celestial mechanics together showed how the study of astronomy and the development of mathematics were closely interwoven. Laplacian physics is just an expression for mathematically oriented physics.

latent heat of vaporization, heat of vaporization: the difference in the heat content or enthalpy of one mole of a substance in liquid and vapor forms at the same temperature and pressure

Law of conservation of energy: Although energy may be converted from one form to another, it cannot be created or destroyed. This law was first introduced by H. von Helmholtz in1847 when he was a military surgeon barely 26 years old. But *Annalen*, the major journal for physics, rejected his manuscript entitled "Über die Erlhaltung der Kraft" ("On the Conservation of Energy"). However, he received warm praises from the military boss, who mistook his "Kraft"for military power.

Law of macroscopic neutrality: This law states that for any subject of macroscopic dimensions, the total number of positive charges it carries must be at all time essentially equal to the total number of negative charges it carries. A departure from this neutrality corresponding to a quantity of charged particles or ions far too small to be detected chemically would create an electrostatic potential far above what is compatible with known facts. The following example, modified after one given by E.A.Guggenheim,[97 pp 330–331] shows why. Consider a spherical living cell 0.1 micron (10^{-4} cm or 10^{-6} m) in radius. Let us imagine that, against the Law of

Macroscopic Neutrality, we removed a (minute) quantity of K^+ equal to 10^{-18} mole from the cell. This removal leaves the cell with an excess of negative charges of 10^{-18} faradays or $0.965 \times 10^5 \times 10^{-18} = 9.65 \times 10^{-14}$ coulombs. Now, the electric potential ψ of a charged sphere of radius r in an aqueous medium is determined by $\psi = Q/\epsilon r$, where Q is the electric charge and ϵ, the *permittivity* of the aqueous medium (equal to $78.5 \times 1.11 \times 10^{-10}$ coul./volt m $= 8.72 \times 10^{-9}$ coul./volt m. at 25°C). Substituting the values, we have $\psi = (9.65 \times 10^{-14})/(8.72 \times 10^{-9} \times 10^{-6}) = 11$ volt. This is far above the electrical potential difference observed across normal living cells, which are in the range of 0.1 volt and lower. Assuming that this spherical cell contains the usual 100 mmoles/kg. of K^+, the normal K^+ content of the spherical cell is then $(4/3)\pi r^3 \times 10^{-4} = 4.19 \times (10^{-4})^3 \times 10^{-4} = 4.19 \times 10^{-16}$ mole. The hypothetical quantity of K^+ removed (10^{-18} mole) is thus only $10^{-18}/(4.19 \times 10^{-16}) = 2.39 \times 10^{-3}$ or 0.239% of the total cell K^+. Nonetheless, removal of even this small a quantity of free cation by itself (unaccompanied by an equivalent amount of free anion or in exchange of an equivalent amount of free cation) is not permitted.

Law of parsimony: See Occam's razor.

Law of partition, Law of distribution: See Berthelot-Nernst distribution law.

Leucocyte, leukocyte: See white blood cell.

LFCH: See Ling's fixed charge hypothesis.

Li^+: lithium ion, carrying a single positive electric charge

libration: revolving around a primary object

ligand: a group, ion or molecule coordinated to a central atom in a complex Example, oxygen in hemoglobin

light microscopy: the use of, or investigations with microscopes magnifying and making visible small objects illuminated with ordinary light

linear conformation: straight-line or fully-extended conformation usually referring to macromolecules like proteins, which may assume more than one conformation

linear correlation coefficient: Usually represented by the letter, r, a linear correlation coefficient is a number varying from 0 to 1.0, which measures the closeness of the linear relationship between two variable Y and X. r^2, called *coefficient of determination*, is a rough estimate of the proportion of the variation of Y that can be attributed to its linear regression on X.

linear regression analysis: a functional relationship between two variables often empirically determined is called regression When that relationship can be represented by a straight line, that regression is called linear. The statistical examination for a possible linear regression between two variables is called linear regression analysis.

linear model: a theoretical model (in the AI Hypothesis) of a singly charged oxygen atom of a fixed oxyacid anion interacting with a monovalent cation in which the free cation, the fixed oxyacid anion as well as a varying number of water molecules are arranged in a linear array

linear polymer: a nonbranching long molecule built from many similar smaller units (called monomers) joined end-to-end

linear protein: a protein existing in the fully-extended conformation

Ling's fixed charge hypothesis, LFCH: a quantitative (statistical) theory of selective accumulation of K^+ over Na^+ in living cells and models, based on the theory of enhanced counterion association with a fixed ion carrying an opposite electric charge and preferential (one-on-one) selective adsorption due to stronger electrostatic interaction with the smaller hydrated K^+ over the larger hydrated Na^+ and associated concepts

Ling's theory of enhancement of association through site fixation: See theory of enhancement of association through site fixation.

Ling-Walton centrifugation method: See centrifugation method.

living state: according to the AI Hypothesis, the high (negative) energy, low entropy state assumed by protoplasm and cells of all plants, animals and microbes between their birth and death

localized adsorption: a concept first introduced in 1916–1918 by Irving Langmuir, in which adsorption takes place at specific localized sites on solid surfaces

lock and key analogy: first introduced by Emil Fischer to illustrate his concept that a sub-

strate binds onto a site on an enzyme because the substrate fits the steric and geometric characteristics This concept was later extended to include drug action where drugs are like keys that fit onto stereospecific receptor site.

lone pair electrons: Each atom contains a nucleus carrying a certain number of positively charged protons and a matching number of negatively-charged electrons orbiting around the nucleus in groups of increasing number—except the outermost shell which cannot exceed eight. This is the well-known octet rule. When the outermost shell has 8 electrons, the maximum stability is reached as in the case of the noble gas, neon, for atoms of the first row of the periodic table, and argon for the second row. Thus of the eight electrons of the oxygen atom in H_2O, two single covalent bonds each with two electrons are formed, leaving four of the valence electrons unshared. Quantum mechanical treatment of valence shows that to form a covalent or electron pair bond, a stable orbital for each atom and two electrons with opposing spins are required. The most stable electronic structure of an atom is reached when all its stable orbitals are used either in electron pair bond formation or in occupancy by an unshared pair of electrons with opposing spins. In a water molecule, the two unshared pairs of electrons with opposing spins are examples of lone pair electrons.

long-range attributes: inherent characteristics that can be perceived from a distance away

lymphocyte: a colorless weakly-motile cells produced in the lymph tissues and making up about 20–30% of the leukocytes of human blood

lysine: a basic α-amino acid found in most protein hydrolysates When present in a protein as a lysine residue, it endows the protein with a fixed cationic ε-amino group.

lysine residue: Like almost all other α-amino acids, lysine possesses an α-amino group and an α-carboxyl group each attached to the first carbon or α-carbon atom. When lysine is incorporated into a polypeptide or protein, its α-amino group reacts with the α-carboxyl group of the immediately neighboring amino acid on one side to form a peptide bond (after losing a molecule of water) while its α-carboxyl group also forms a peptide bond (after losing a water molecule) with α-amino group of its neighboring amino acid residue on the other side. The reminder of the lysine residue forms the lysine residue's side chain. The lysine side chain carries a positively charged ε-amino group which is one of the important fixed cations of all proteins.

lysis: a process of disintegrating or dissolution (as of red blood cells)

μ: Greek alphabet for m and pronounced mu like in mule. It is used as the symbol for the distance represented by one micrometer , i.e., 10^{-4} cm.

macromolecule: a large molecule usually containing many smaller units called monomers joined together end-to-end by covalent bonds

Madelung constant: In ionic crystals, the mutual potential energy of two ions (and electrostatic attraction) can be expressed by the sum of two terms, one attractive—which extends to far distances—and one repulsive—which falls off rapidly. The attractive term is expressed by $(Ae^2z^2)/R$, where e is the electronic charge, z, the valency and R, the smallest interionic distance. A is a constant called the Madelung constant. It is always higher then unity, indicating that each cation (or anion) is interacting not just with one single anion (or cation) but other anions (or cations) further away also. Thus A for NaCl crystal is 1.74756. A for Fluorite, $M^{++}X_2^-$, is 5.03738.[485 pp 507–508]

magnetic resonance imaging, MRI: a way of visualizing the interior of the human patient without surgery or exposure to harmful X-ray based on the different NMR relaxation times or T_1 and T_2 of water protons in normal living cells and in cancer and other diseased cells. Invented by Raymond Damadian using his original field focussing technique, it was improved on by technical advances made by Paul Lauterbur and others.

magnetization-demagnetization: The act of producing a cooperative transition of ferromagnetic metals to the magnetized state by the imposition of a strong magnetic field from

outside is called magnetization. Demagnetization means the opposite action of removing the state of magnetization of the ferromagnetic materials.

magnetoelectric: characterized by electromotive forces developed by magnetic means

malignant tumor: cancer, a life-threatening abnormal growth, which grows in an uncontrolled manner, and may proliferate and invade normal tissues

Malpighian tubule: any of a group of long blind vessels opening into the posterior end of the alimentary canal of most insects, functioning primarily as an excretory organ.

masked sites: Chemical groupings inherently capable of interacting with ions or other solutes are not able to do so because they are already occupied in some way and thus appear masked.

matrix protein, matrix protein system: a hypothetical system of one or more protein(s) pervasively present throughout the living cell One function of the matrix protein system is to maintain the bulk-phase cell water in its normal state of polarization and orientation in spite of local perturbations from time to time.

membrane fraction of crab-nerve homogenate: a suspension of isolated crab nerves is first ground up in a (tight-fitting glass pestle-glass tube) homogenizer, until a more or less homogeneous suspension is obtained. By centrifuging the suspension at different speed in an ultracentrifuge, different fractions of the homogenate can be obtained. The fraction containing bits and pieces of materials identified as the torn cell membranes is what is called the membrane fraction of crab nerve homogenate.

membrane potential: a lasting electrical potential difference theoretically postulated to exist across cell membranes or their models on account of different permeability to ions of different electric changes in the environment. {For evidence and other reasons, which deny its existence in Nature, see [15.5(1.1.1)] and [16.6 (3.4)].}

membrane pump theory: In a later version of the membrane theory after the demonstration that resting cell membranes are permeable to sucrose, hydrated Na^+ and other large solutes, pumps were installed in the cell membrane to keep their intracellular concentrations low. Among them, the most prominent pump is the sodium pump.

membrane theory: a theory of the living cells as membrane-enclosed dilute solutions introduced in the 19th century to explain the osmotic swelling and shrinkage and other related cell physiological manifestations

metabolism: the sum total of chemical reactions occurring in a living cell or organism by which energy is provided for vital processes and activities, and new materials are assimilated to repair worn structures and build new ones

metastable equilibrium state : Like a coin standing on its edge, a system in a metastable equilibrium state is in an equilibrium state but not the most stable one. With relatively small perturbation, a system in a metastable equilibrium state may topple into an alternative more stable state.

meta-substitution: See para-substitution.

method of least squares: a statistical method of finding the best fitting straight line or other theoretically-derived curve for a group of experimental data points The method relies on finding the theoretical curve which gives the smallest sum of the squares of the departure (both positive and negative) of individual data points from the theoretically predicted values.

methyl alcohol: methanol, wood alcohol, CH_3OH

methylene group: $-CH_2-$, a fundamental structural unit of straight-chain or aliphatic compounds

Michaelis-Menten kinetics: study of the rate of enzymatic reactions with variation in the substrate concentrations, in the concentration of competing agents, etc. Double reciprocal plots of the rate of enzyme activity against that of the substrate concentration is a key feature of these studies to obtain the quantitative data sought after.

Michaelis' theory of enzyme activity: This theory is based on the assumption that the rate of enzyme activity is directly proportional to the concentration of the enzyme-substrate complex, which in turn is related to the substrate concentration according to the Langmuir adsorption isotherm.

microelectrode: a small electrode See Gerard-Graham-Ling microelectrode, ion-specific microelectrode.

microinjection: injection of fluid materials into the interior of a living cell with the aid of a glass capillary micropipette.

micrometer, μ: a distance equal to 10^{-4} cm

millimole, mmole: name for a specific number of molecules or ion equal to one one-thousandth of the Avogadro number (2.02×10^{23}), or a mole

mitochondria: granules and filaments in the cytoplasm which may be separated from the remainder of the cytoplasm by ultracentrifugation. Mitochondria are the seats of many metabolic reactions. A major function of mitochondria is to produce ATP. That is why in living cells they tend to congregate near work-performing, energy-demanding cytological structures like myofibrils.

mitochondrial inner membrane: Each mitochondrion as a rule contains an outer membrane and an inner membrane. The inner membrane has many inward directed folds or cristae. Inside the inner membrane is the matrix in which much proteins as well as DNA and RNA are found.

mobility: rate of movement usually at molecular scale

molal: of, or pertaining to, the concentration of a solute in gram molecules dissolved in 1000 grams of the solvent

molar: of, or pertaining to, the concentration of a solute in gram molecules dissolved in 1000 ml of the solution

molar volume, molecular volume: volume in cubic centimeters (cc) of one mole of a chemical substance

mole: the expression for a certain number of molecules or ions equal to that of the Avogadro number, 2.02×10^{23}

molecular switch: a molecular mechanism, which can turn on and off certain physiological activity at the cell or below cell level

molecular volume: See molar volume.

monactin: an antibiotic which combines reversibly and preferentially with K^+ It is known as a K^+-specific ionophore since its presence can dramatically increase the rate of transport of K^+ across phospholipid bilayers.

monochloroacetic acid: $ClCH_2COOH$, acetic acid in which one of the methyl hydrogen atom has been replaced by a chlorine atom

monochromatic: having or consisting of one color, or in other words having or consisting of electromagnetic wave of one specified wave length

monomers: the individual chemical units that forms a polymer when joined together

monosaccharide: a sugar that cannot be decomposed into simpler sugars. They are polyhydroxy aldehydes and ketones. Examples: D-glucose, D-galactose

motional restriction: hampering and limiting the movement of some substances

MRI: short for magnetic resonance imaging

mucosal surface: The surface of the epithelial cell layer in contact with the intestinal content is called the mucosal surface, whereas the other surface is called the serosal surface.

multilayers of polarized-oriented water: the dynamic structure of multilayers of water molecules polarized and oriented by a checkerboard of alternating negatively and positively charged sites or a matrix of linear chains carrying alternatingly negatively charged and positively charged sites

muscle fibers: muscle cells

mutation theory: the theory that in genetics, the attributes of the offsprings are sometime altered by changes of the genes by a process called mutation as a result of which, the DNA molecules involved are altered chemically

myocardium: the middle muscular layer of the heart wall

myofibrils: one of the fine, longitudinal filaments making up a muscle fiber Each myofibril consists of a linear sequence of individual units called sarcomeres.

myoglobin: a red iron-containing protein pigment in muscle

myoplasm: muscle cytoplasm

myosin: the most abundant protein of muscle

myosin B: a name once used to describe actomyosin

n: See Hill coefficient.

Na^+: sodium ion, a sodium atom which has lost one electron and thus becomes a monovalent cation carrying a single positive charge

Na channel: In the theory of action potential of Hodgkin and Huxley, the cell membrane is normally impermeable to Na^+. However, during activity, pore-like structures in the phospholipid bilayer cell membrane called the sodium channel suddenly swing open allowing a momentary inrush of Na^+ creating the spike of the action potential before its closure.

Na potential: In the theory of action potential of Hodgkin and Huxley, the tendency or potential of Na^+ to rush into the cell when the Na channel is opened is called the Na potential. It is supposed to be present at all times due to the much higher concentration of this ion in the external medium than in the cell. In contrast, the sodium potential according to the AI Hypothesis is created momentarily and reversibly with the depolarization of the cell surface water and the increase of the c-value of the surface β- and γ-carboxyl groups.

Na-selective microelectrode: a glass microelectrode which can penetrate and record the Na^+ activity in a living cell

NaCl: sodium chloride. table salt

NaF: sodium fluoride

Na, K-activated ATPase: First isolated from the membrane fraction of crab nerve homogenate, this enzyme requires the presence of both K^+ and Na^+ for maximum activity in splitting off the terminal phosphate group of, or dephosphorylating ATP.

Na-selective glass electrode: a glass electrode which shows pronounced specific sensitivity to Na^+ With such an electrode one can measure the *free* Na^+ concentration (or more precisely, activity) in a solution. If one also determine the *total* Na^+ concentration in the solution, one can also obtain the concentration of *adsorbed* or otherwise tethered Na^+ by subtraction.

Nathanson's mosaic membrane theory: a theory of cell membrane structure by A. Nathanson at the beginning of the 20th century In this theory, the cell membrane is a mosaic of cholesterol and proteins (protoplasm).

native protein: As the name suggests, native proteins should be proteins as they exist in Nature. For most proteins this means as they exist inside living cells. However, in reality, the term native protein has gradually become identified with proteins in crystalline form. Evidence exist which show that major proteins examined do not exist in the so-called native state in living cells. {See [16.6(1.3)].}

native (globular) protein: See native protein.

near-neighbor interaction: interaction between near neighbors in a cooperative assembly

nearest neighbor interaction energy, $-\gamma/2$: The extra energy generated each time a new i-j neighboring pair is created in a cooperative assembly of infinitely long chains of similar sites on which one or the other of two alternative species i and j are adsorbed.

Necturus: salamander, mud puppy, an amphibian superficially resembling lizards but without scales and covered with soft skin, breathing by gills in the larval stage

negative energy: The energy between a pair of interacting positive charge (+e) and negative charge (–e) at a distance, r, apart in vacuum is equal to $[(+e) \times (-e)]/ r$ and the value is always *negative*. Thus the larger the magnitude of the (absolute) values of the charges and the closer the distance r, the higher is not the energy but the *negative energy* of the interaction.

negative entropy: Roughly entropy is a measure of randomness. Negative entropy is a measure of order or organization.

nerve fiber: a common name for the long process of a nerve cell also called a nerve axon, along which nerve impulses are conducted

neuron: a nerve cell

NH group: an imino group. In proteins an imino group and a carbonyl group are parts of the peptide group (-NHCHRCO-) making up the polypeptide chain. R is the side chain.

NH_4^+: ammonium ion

Nitella: genus name of a kind of giant algae belong to the family Characeae

Nitella clavata: a species of *Nitella*

nmoles: nanomoles, 10^{-9} mole

NMR, nuclear magnetic resonance: When atoms like hydrogen, carbon isotope C^{13}, and sodium isotope Na^{23} in molecules or in solids are placed in a strong homogeneous magnetic field, H_o, they absorb energy from oscillating electromagnetic pulses of a specific (radiowave) frequency applied perpendicular to the direction of H_o. This specific frequency, called the resonance frequency, varies directly with field H_o applied

and with what is called magnetogyric ratio of the atom under study. For hydrogen atom the resonance frequency is 42.576 megacycles per second at a field of 10,000 gauss or 1 tesla. However, this is true only if all the secondary interfering factors are ignored. In fact, it is precisely these secondary interfering factors that make the study of nuclear magnetic resonance highly valuable, for these variations tell us about the environment of the atoms involved and the physical condition of the molecules containing the atom, e.g., Are these molecules free or fixed? NMR was originally used by physicists to measure the nuclear moments of the elements. Later, chemists found ways to use NMR to determine molecular structures, etc. After the introduction of the PM theory, NMR was used to test this theory of cell water.

NMR resonance peak: When the absorption of energy of a sample in an NMR spectrometer is plotted against the frequency of the radiowave applied, a peak is observed corresponding to the resonance frequency of that atom.

NMR rotational correlation time, τ_r: a characteristic time for the molecule to rotate through a radian or move over a distance comparable with its own dimension

NMR spectrometer: an instrument that measures and records the NMR spectrum of a sample

nonactin: an antibiotic which reversibly and preferentially binds K^+ and has been used as a model ionophore ferrying K^+ across phospholipid bilayers

non-dialyzable: A substance is non-dialyzable if it cannot pass through a semipermeable membrane within a reasonably long period of time.

nonelectrolytes: a substance like sugar and benzene which is not appreciably ionized

(NO-NO-NO)^n: a colloidal system in which each chain of linear polymers carrying alternatingly negatively-charged sites and vacant sites at suitable distances apart is surrounded by n similar chains

nonsolvent: a medium which does not dissolve substances normally dissolved in other similar media

nonsolvent water: an early idea that water associated with colloidal materials does not dissolve substances which dissolve in normal liquid water This idea is at least partly incorrect.

NP-NP system: two juxtaposed surfaces each carrying a checkerboard of alternatingly positively-charged P sites and negatively-charged N sites at suitable distance apart

NP-NP-NP system: a matrix of linear chains carrying alternatingly negatively-charged sites and positively charged sites at suitable distances apart

(NP-NP-NP)^n: a colloidal system in which each chain of linear polymers carrying alternatingly negatively-charged sites and positively-charged sites at suitable distances apart is surrounded by n similar chains

N site: a negatively charged site

nuclear magnetic resonance spectrometer: an instrument which can exhibit and record the NMR spectrum of samples containing nuclides with non-zero nuclear spin quantum numbers like H, C^{13}, Na^{23}

nuclear membrane: membrane covering the nucleus of a cell

nucleation center: the focus or center usually on a solid surface where crystals of a chemical substance begins to form from that substance in a non-solid form

nucleus (of a cell): a cellular organelle composed of nuclear sap and nucleoprotein complex from which chromosomes and nucleoli arise and is enclosed in a nuclear membrane

nucleus (of an atom): the positively charged central body of an atom containing nearly all the atom's mass and both protons and neutrons except in hydrogen which contains only a proton

null-point method: a method introduced in 1987 by Ling and Hu[163] for the determination of the amount of water adsorbed on a protein or polymer at near-saturation vapor pressure

O-site: an empty or vacant site

Occam's razor: a scientific and philosophic rule that the simplest of competing theories be preferred over the more complex or that explanation of unknown be first sought among the known

occlusion: the act or state of being occluded To occlude means to shut off, block or plug up.

occlusion water: name introduced by Bungenberg de Jong to describe water filling the

space in wads of interweaving protein chains

oedema: same as edema

(upward-oriented) OH group: In the perspective representation of Hayworth modified by Pigman, the D-glucose molecule is shown lying on a plane so that the edge of the molecular formula closest to the observer appears as the bottom line of a hexagon with the oxygen atom occupying one corner. Counting from that oxygen atom in a clockwise direction, are the first carbon (C_1), the second carbon (C_2) and so on in that order. In the D-glucose molecule, the OH group on the third carbon atom (C_3) is oriented upward.

oil potential: the electrical potential difference measured across a layer of oil

(olive) oil-water distribution coefficient: the equilibrium distribution coefficient of a chemical substance between an olive-oil phase and water phase

one-dimensional Ising model: a rigorous statistical mechanical treatment of order-disorder transition in one-dimensional crystals introduced by E. Ising in 1925[353]

one-on-one, close-contact adsorption: an adsorption, in which the adsorbing site and the adsorbed molecule or ion are in close steady contact and are stoichiometric, i.e., following the law of definite proportion: one monovalent ion on one monovalent anionic site

oocyte, ovarian egg: an egg before maturation and still in the ovary Mature frog oocytes are big single cells measuring 1 mm or more in diameter.

oppositely-oriented water dipoles: The asymmetric position of the two hydrogen atoms in a water molecule endows the water with a permanent dipole moment. If the positive end of such a water dipole is placed next to the negative end of a neighboring water molecule, they constitute what may be called a pair of oppositely oriented water dipoles.

ordinate: To French mathematician and philosopher, René Descartes (1596–1650) is commonly given the credit for introducing analytical geometry. In this system the location of a point in a plane (or space) is determined by distances measured respectively along two (or three) Cartesian coordinates from their point of intersection. Of these, the vertical or y coordinate is conventionally referred to as the ordinate while the horizontal of x coordinate is referred to as abscissa.

organelles: See subcellular particles.

organic phosphates: important organic phosphates in living cells include adenosine triphosphate (ATP), phosphocreatine or creatine phosphate (CrP) and various phosphate intermediates in carbohydrate metabolism including glucose-1-phosphate, glucose-6-phosphate, fructose-1,6-diphosphate etc.

orientation: the act or state of being positioned at a specific spatial relationship to a fixed object

osmometer: a device comprising aqueous solution of an osmotically active free solute separated by a semipermeable membrane from a similar solution containing the same solute but at a lower concentration exerting an osmotic pressure equal to that predicted by the van Hoff equation (Equation 1)

osmotic activity: The osmotic pressure is directly related to the partial vapor pressure (Equation 2). Osmotic activity of a dissolved substance is the activity of that solute in lowering the partial vapor pressure of the water in which it is dissolved.

osmosis: the passage of the solvent (e.g., water) from one enclosed phase like a living cell to an adjoining phase or *vice versa.*

osmotic pressure: the mechanical pressure that must be applied to the phase containing a higher concentration of osmotically active solute (e.g., sucrose) to prevent a gain in the amount of the solvent (e.g., water) in that phase

osmotically active: A substance is considered as osmotically active if a concentrated solution of that substance enclosed in a semipermeable membrane draws water from another contiguous phase containing either pure water or a solution of the same substance but at a lower concentration.

ouabain: a cardiac glycoside with exceptionally high solubility in water

ovarian egg: an egg that has not yet been discharged from and thus remains in the ovary

oxidation: the act or process of oxidizing

oxidation-reduction potential (standard oxidation-reduction potential): the potential established at an inert electrode when dipped into a solution containing equimolar amount of an ion or molecule in two states of oxidation

oxidative activity: an activity that removes electrons from an atom, ion or molecule

oxidize: to remove one or more electrons from (an atom, ion or molecule)

oxidized collodion-coated glass electrode: a glass electrode covered with a layer of collodion that has been oxidized by exposure to HBr or NaOH, thereby endowing the electrode surface with fixed carboxyl groups

oxyacid: an acid whose functional group contains negatively charged oxygen atoms, e.g., carboxyl group of acetic acid

oxygen tension: partial pressure of oxygen (in the surrounding environment) equivalent to the concentration of oxygen in the system

oxygenated state: Hemoglobin can exist in two states: an oxygenated state in which oxygen molecules adsorb on the four heme sites; a deoxygenated state in which the oxygen molecules are removed.

~P: a symbol for a high-energy phosphate bond

P site: positively-charged site

ρ-value: ρ is Greek alphabet for r and pronounced rho like in row. See under r section.

paraldehyde: a colorless liquid polymeric modification of acetaldehyde used as a hypnotic

para-substitution: In a six-membered aromatic nucleus of say, phenol molecule, a substitution of the H atom on the carbon atom that are separated from the OH-bearing carbon atom by two carbon atoms is called a *para*-substitution. A substitution on the carbon atom separated from the OH-bearing carbon atom by one carbon atom is called a *meta*-substitution. A substitution on the carbon atom immediately next to the OH-bearing carbon atom is called an *ortho*-substitution

pari passu: Latin at an equal rate or pace

partial molar volume: In a mixture of n_1 moles of substance 1 and n_2 moles of substance 2, the volume of the mixture is V, and $V = n_1 V_1 + n_2 V_2$, where V_1 is the partial molal volume of substance 1 and V_2 is the partial molal volume of substance 2.

partially resonating structure: The C-N bond of a polypeptide chain resonates between 60% single bond and 40% double bond structure but the C-C bond is a simple single bond. Therefore as a whole, the polypeptide chain is a partially resonating structure.

partial vapor pressure: According to Dalton's Law of partial pressure, the total pressure of a mixture of gases is equal to the sum of the partial pressure of the constituent gases. The partial pressure is defined as the pressure each gas would exert if it alone occupies the whole volume of the mixture at the same temperature. Thus the partial vapor pressure is the pressure of water vapor in a mixture of water vapor and the gases of the air.

partition coefficient: the true equilibrium distribution coefficient

patent system: an official system which offers to an inventor for a term of years the exclusive right to make, use or sell his or her invention

PCG electrode: short for polylysine-treated collodion-coated glass electrode

peer review system: a system to evaluate the merit of scientific work or a scientist by a panel of peers and relies on their majority opinion to award or deny

PEG: poly(ethylene glycol), $H(OCH_2CH_2)_n OH$, a large water-soluble oxygen-containing polymer

PEG-8000: PEG with an average molecular weight of 8000

PEG-4000: short for poly(ethylene glycol) with an average molecular weight of 4000

PEI: poly (ethylene imine), $(CH_2NHCH_2)_n$, a large water soluble, nitrogen-containing polymer

pentose: various monosaccharides that contain five carbon atoms in the molecules Examples, D-xylose

PEO: poly (ethylene oxide), $(CH_2OCH_2)_n$, a large water-soluble polymer

peptide bond: the chemical bond between the carbon and nitrogen atom of a peptide linkage

peptide linkage: the CONH group that unites the amino acid residues in a peptide

perfect gas: also known as ideal gas, defined as a gas to which the Laws of Boyle, Gay-Lussac, and Avogadro are applicable at all temperatures

perfused dog gastrocnemius muscle: After cannulating the blood vessels supplying the dog gastrocnemius muscle, a Ringer-Locke solution is used to perfuse the muscle so that the gain and loss of K^+ during tetanic contraction of the muscle could be determined from aliquots of the perfusate.

permanent dipole moment: A molecule like H_2O, due to the asymmetric location of the two positively charged H atoms, is positive on one end and negative on the other end, and for this reason, it possesses a *permanent* dipole moment.

permeability: the specific property of a membrane or surface which determines the rate of entry or exit of a particular ion or molecule

permeant: being able to permeate

permittivity: a scalar quantity ε defined by $\mathbf{D} \equiv \varepsilon \mathbf{E}$, where **D** is the (vector electric) *displacement* and **E**, the (vector electric) *field intensity* The more familiar *dielectric constant* of a substance is a relative constant, equal to the permitivity of that substance divided by the permitivity of free space.

Permutite: a silicate which acts as an ion exchanger and used as a water softener, giving off Na^+ in exchange for Ca^{2+} or Mg^{2+}

pH: the negative logarithm of the hydrogen ion concentration

pharmacology: the science of drugs including materia medica, toxicology and therapeutics

pharmacon: a drug

phase transition: The change of liquid water into steam or that of liquid water into solid ice exemplifies the discontinuous changes occurring in a narrow range of temperature called phase transitions. It was recognized early that this type of discontinuous change occurs only in condensed systems and it arises from interaction among large number of the microscopic component(s) of the system. Phase transitions are cooperative phenomena.

phase-boundary potential: the steady electric potential difference across the boundary of two contiguous phases

phenol group: a hydroxyl group on an aromatic ring, e.g., the OH group of phenol

phenylalanine: a common α-amino acid found in most protein hydrolysates

phloem: a complex system in the vascular system in higher plants which is engaged in translocation and in support and storage

phlogiston: a hypothetical substance presumed to exist in all combustible materials and it is liberated from the material during burning

phospholipase a (lecithinase a): an enzyme which hydrolyzes the β-ester bond of zwitterionic glycophospholipids This enzyme is found in cobra venom.

phospholipid bilayer: When a solution of phospholipids in a chloroform-methanol mixture is painted over a loop and the loaded loop then immersed in a salt solution, dispersion of the solvent into the medium eventually thins out the membrane into a bimolecular layer, called phospholipid bilayer.

phospholipid bilayer theory of cell membranes: a theory according to which a phospholipid bilayer forms the continuous phase of all cell membranes

photoemulsion: a suspension of photosensitive silver salt in a viscous medium (e.g., gelatin solution) It can be applied as a coating onto glass plates carrying specimen "stained" with radioactive materials as in autoradiography.

photon: a quantum of radiant energy

physiological activities: normal activities of living cells and aggregates of cells

pinwheel type of triplet route: When an entrant cation, say Rb^+, is strongly adsorbed on the fixed anionic site on the cell surface, its entry into the cell is very slow unless it is facilitated by the participation of a second cation, say K^+, whose approach to the fixed anion-Rb^+ pair lowers the activation energy for the desorption of the Rb^+ and entry into the cell. If the activating cation, K^+, comes from inside the cell, this triplet route is called the pin-wheel type. On the other hand, if the activating K^+ comes from the same side the entrant Rb^+ came from, namely the outside, the triplet route is called the billiard type. For clarity, only cation entry into the cell is discussed. But the same principle applies to exit of free cation (or even free anion) from the cell as well. (For diagrammatic illustrations, see Fig. 36.)

pith: loose spongy tissue occupying the center of the stem of dicotyledonous plants

pK: the negative logarithm of the acid dissociation constant K of an acid or base. See acid dissociation constant.

pK_a: same as pK as used in this volume

pK determination from titration curve: To determine the pK of an unknown acid, one mixes equal volumes of solutions containing a fixed (rather small) amount of the unknown acid and a varying amount of an acid of known concentration. And measure the pH of each of the solutions after equilibrium has been reached. Plotting the amount of acid (as ordinate) against the pH (as abscissa) one obtains the titration (or neutralization) curve of the unknown acid. Now, according to the equation of L. J. Haldane,[13] p 1003 $pH = pK + \log$ ([salt]/[acid]), where [salt] and [acid] represent respectively the concentration of the acid that has been neutralized (and thus turned into "salt") and the acid that has not been neutralized yet (and thus remains as acid). At the mid-point of the titration curve, [salt] and [acid] are equal (their ratio equal to unity). Since logarithm of one is zero, the pH corresponding to the midpoint of the titration curve is equal to the pK of the acid.

plasma membrane: same as Plasmahaut or cell membrane

Plasmahaut: the name Wilh. Pfeffer offered in 1877 for what is now called cell membrane Literally it means protoplasmic skin.

PM theory: acronym of the polarized multilayer theory of cell water and model systems

polarizability: the dipole moment produced by a unit electric field acting on a molecule In the absence of permanent dipoles, the polarizability of a molecule is the sum of separate electronic polarizabilities of its constituent atoms with slight corrections due to the bond structure. When a moment m is induced by a field of intensity, F, the polarizability of the molecule, α, is equal to m/F.

polarization: The total polarization of a molecule possessing a *permanent dipole moment* (as in water), is the sum of two terms. One, sometimes called *distortion polarization*, arises from the electric polarization or induction. The other, sometimes called orientation polarization, arises from the orientation of the permanent dipole.

polarization theory, de Boer-Zwikker polarization theory: a theory of multilayer polarization and condensation of noble gas and other molecules on solid surface presenting a checkerboard of positively and negatively charged sites by de Boer and Zwikker {For more details, see [11.2].}

polarized multilayer theory of cell water (PM theory): theory of cell water and model systems introduced by Ling in 1965 as part of the association-induction hypothesis

polyacrylic acid, poly(acrylic acid): $[\text{-}CH_2CH(COOH)\text{-}]_n$

polyatomic molecules: molecules containing many atoms

polyethylene glycol, poly(ethylene glycol), PEG: $H(OCH_2CH_2)_nOH$, a water soluble polymer with average molecular weight ranging from 200 to 10,000. PEG-4000, for example, refers to poly(ethylene glycol) with average molecular weight of 4000

poly(ethylene oxide), PEO, Polyox: $(\text{-}CH_2CH_2O\text{-})_n$, a water soluble polymer

polyethylenimine, PEI: $H(\text{-}NHCH_2CH_2)NH_2$, water-soluble polymer

poly-L-glutamate: a polypeptide of (pure) L-glutamic acid

poly-L-glutamate probe method: a method introduced in 1967 by Ling and Kromash[336] for estimating the size of extracellular space in frog muscle and other tissues

polylysine: polypeptide of pure L-, D- or D,L-lysine

polylysine-treated-collodion-coated glass electrode, PCG electrode: a glass electrode first coated with collodion and (before it is dried) soaked overnight in a solution of polylysine hydrochloride (3 mg/ml) before drying in 43% humidity provided by a saturated K_2CO_3 solution

polymer: a large chemical compound formed by the joining together (polymerization) of a sequence of repeating units called monomers

polyol, polyhydric alcohols: Unlike sugars which are polyhydroxy aldehydehydes and ketones, polyols contain only alcohol groups. Polyols can be divided into (i) "straight-chain" or *acyclic* polyols which include ethylene glycol, glycerol, erythritol, xylitol, D-mannitol and sorbitol; (ii) ring-

structured, or *alicyclic* polyols which include various inositols, e.g., myo-inositol.

polypeptide: a molecular chain of amino-acid residues joined through peptide linkages

polypeptide backbone, "backbone", backbone: the linked portion of a polypeptide chain *exclusive of* the side chains, **R, R′, R″** etc. in -NHCOCH**R**-NHCOCH**R**′-NHCOCH**R**′′-

polypeptide chain: See polypeptide.

polysaccharide: a large carbohydrate that can be decomposed by hydrolysis into two or more molecules of monosaccharides like D-glucose Examples include starch, cellulose, glycogen.

polystyrene sulfonate: Polystyrene, a polymer produced by joining styrene ($C_6H_5CH_2{=}CH_2$) monomers into a linear chain, that carries a sulfonate group When polystyrene sulfonate is cross-linked into a three-dimensional network, it becomes one of the earlier form of cation exchange resin (e.g., Dowex 50).

polyvinylmethylether, PVME: $(\text{-}CH_2CHOCH_3\text{-})_n$, a water-soluble high molecular weight polymer

polyvinylpyrrolidone, PVP: water-soluble polymer with medium molecular weight of 25,000

potassium ferrocyanide: $K_4Fe(CN)_6.\ 3H_2O$, yellow crystals soluble in water

potassium picrate: $C_6H_2(NO_2)_3OK$, the potassium salt of picric acid Yellow, reddish or greenish lustrous crystals

precipitate: a substance, which has separated from a solution or suspension in response to chemical or environmental change, usually in the form of insoluble amorphous or crystalline solids

preexistence theory: A basic cell physiological theory advocated by Emil DuBois-Reymond (1818–1896) that the cellular resting potential (known at that time only in the form of a grossly diminished and distorted version (aptly) called the injury potential or demarcation potential) exists across the surface of normal resting (nerve and muscle) cells and is not an artifact from injury as DuBois Reymond's student, Ludimar Hermann, asserted in Hermann's alteration theory.

preincubate: to prepare a population of cells (e.g., a muscle) for a specific experimental study, the cells are incubated with shaking prior to the experiment for a length of time in a well-defined environment so that "contaminants" (e.g., insulin) present in the cells originally are removed beforehand and their possible undesirable impact on the experimental results thus forestalled

primary structure of proteins: describing the kind, number and sequential order of amino-acid residues in a protein

primer: A concept introduced by Ling, Will and Shannon in 1969[480] that to bring about the full accumulation in frog muscles of D-glucose at 0°C, prior incubation of the tissues at a higher temperature (e.g., 25°C) and in the presence of insulin and a sugar like D-glucose, D-xylose at a suitable concentration is essential. D-glucose, D-xylose and five other sugars bearing structural similarity to D-glucose are called primers because their presence are essential for the accumulation of more molecules of similar structures. (Not to be confused with DNA primers in the further formation of DNA production though the underlying mechanisms may bear similarities.)

principal cardinal adsorbent: a cardinal adsorbent like ATP, which has supreme controlling authority

principle of enhanced association with site fixation: See theory of association through site fixation.

proline: a common α-amino acid found in most protein hydrolysates Like hydroxyproline, a proline residue in a polypeptide chain or protein cannot form intra-, or intermacromolecular H bonds because its peptide nitrogen, being a part of a peptide-linked pyrrolidine ring, lacks a (second) H atom.

n-propanol, n-propyl alcohol: $CH_3CH_2CH_2COOH$

protein: any one of a group of complex organic nitrogenous compounds, widely distributed in plants and animals, which form the principal constituent of cell protoplasm They are essentially linear combinations of α-amino acids, possessing in each a polypeptide chain.

proteinate: a compound of a protein

proteinoid microsphere: microscopic spheres of materials containing mostly protein-like materials (see Fig. 5E)

proton: positive ion of (gaseous) hydrogen atom or a hydrogen atom that has lost its electron. Name given by E. Rutherford from the Greek word, protos meaning "first"

protoplasm: all matter of which life is a manifestation; the ubiquitous and essential material of animal and plant cells

Protoplasmic doctrine: a principle announced by Max Schultze in 1861 that living cells are a membrane-less lump of protoplasm surrounding a nucleus

protoplasmic droplet: The more fluid protoplasm like the endoplasm of internodal cells of giant alga *Nitella* can be "poured out" of the cell through a cut end and collects in an aqueous solution as one or more protoplasmic droplets (for illustration, see Fig. 3b).

protoplast: When a mature plant cell is immersed in a hypertonic solution of sucrose or NaCl, the cell content from the plasma membrane inward shrinks away from the rigid enclosing cell wall. Protoplast is the name von Hanstein gave to the shrunken body of protoplasm thus formed.[27] (See Fig. 1B.)

protozoa: the phylum of one-celled animals, usually microscopic in size, which forms the lowest division of the animal kingdom

proximal functional group: functional groups of a protein molecule, which are on, or close in distance from the polypeptide chain

proximal site: a site that is close-by (usually measured from the starting point of the polypeptide chain of a protein)

psyche: soul or mind in early Greek philosophy

Purkinje muscle fiber: any of the modified cardiac muscle fiber that have few nuclei, granulated cytoplasm, sparse peripheral striation and make up a network of conducting tissue in the myocardium

PVME: See polyvinylmethylether.

PVP: See polyvinylpyrrolidone.

PVP 360: polyvinylpyrrolidone with an average molecular weight of 360,000

pyrrolidine nitrogen atom: The nitrogen atom of a proline or hydroxyproline residue in a polypeptide or protein is part of the five-membered pyrrolidine ring structure.

q-value: the (true) equilibrium distribution coefficient or partition coefficient It is equal to the equilibrium concentration of a dissolved substance in cell water (or that of a model system) over that in the external bathing solution.

quadrupolar broadening: the broadening of an NMR signal (due to signal splitting) of a nuclide with a nonspherical nuclear charge distribution when the nuclide is placed in an asymmetrical electric field

quaternary ammonium compound: any of the numerous strong bases and their salts derived from ammonium by replacement of one of the hydrogen atoms with organic radicals

quasi-elastic neutron scattering: When slow neutrons (speed. $\sim 10^5$ cm/sec, compared to that of X-ray, 3×10^{10} cm/sec) impinge on liquid like water, they are scattered largely by the H atoms of the water molecules and on a time scale comparable to the time for water molecules to jump from one position to another. As a result, the scattering of the slow neutron observed contains information about the diffusive motions of the water involved.

Queen of cardinal adsorbent: As the supreme principal cardinal adsorbent in living cells, ATP is also referred to as the Queen of cardinal adsorbents.

Quellungswasser: swelling water or imbibition water

quenching: extinguishing. the reduction of the fluorescence yield

R-state: relaxed (oxygenated) state of hemoglobin, compared to the tense or deoxygenated T-state

RT: product of the gas constant R (1.987 cal/degree/mole) and the absolute temperature T At room temperature of 25°C or 25 + 273 = 298°K, RT, the average kinetic energy of a mole of chemical substance is equal to 1.987 × 298 = 592 cal/mole.

ρ-value: the apparent equilibrium distribution coefficient It differs from the true equilibrium distribution coefficient or q-value in that the ρ-value may or may not include solute adsorbed or otherwise complexed to proteins or macromolecules in living cells or model systems, whereas the q-value refers exclusively to solute dissolved in the water within living cells or model systems.

radioactive: of, or exhibiting radioactivity, i.e., emitting α-, β- or γ-rays by certain atoms

radioactive tracer technique: A small amount of a radioisotope of a substance is added to the non-radioactive substances so that the path, reaction, or position of the substance may be followed or detected.

radioisotope: an isotope of an element which is unstable, producing α, β or γ radiation

raffinose: a trisaccharide containing one each of the following three monosaccharides: D-glucose, D-fructose and D-galactose

random-coil conformation: the hypothetical conformation of a protein molecule in which the protein molecule in solution behaves like a randomly-moving coiled chain unrestricted by inter- or intra-macromolecular H bonds

rank order of ion selectivity: the sequential order in the preference of, say a β-, of γ-carboxyl group for members of the alkali metal ions due to a corresponding rank order in their respective adsorption energies

Rb^+: rubidium ion It is the most strongly adsorbed among 5 alkali-metal ions usually studied on the β- or γ-carboxyl groups within and at the surface of normal resting frog muscle cells.

receptor sites: a chemical group or ensemble of chemical groups which function as a point of contact or engagement for biological signals in the form of drugs, hormones or other cardinal adsorbents

reciprocal: In this volume and other related scientific literature (see reciprocal plot following), the reciprocal of a concentration s is 1/s.

reciprocal plot: In enzyme kinetics and other related field of quantitative biology and chemistry, the term reciprocal plot means a plot of the variables on the ordinate and abscissa in their respective upside-down inverted form. For example, see Equation A11 in Appendix 1.

rectilinear: characterized by straight lines

rectilinear distribution curve, significance of: When a plot of the equilibrium concentration of a solute S in a cell or model system against the external concentration of that solute (S_{ex}) shows a straight-line or rectilinear relationship (as in Figs. 24, 26, 27), it indicates that the solute is found only or virtually only in the cell water. Thus existing evidence show that given sufficient time, virtually all solutes investigated enter the cells and are found in the cell water (at concentration, S_{in}). The Berthelot-Nernst distribution law demands that there is a rectilinear relationship between S_{in} and S_{ex}. The presence of an additional fraction of adsorbed or complexed S in the cell will as a rule add a "hyperbolic" or sigmoid component making the full plot no longer rectilinear. (See top curves of Fig. 54 and Fig. 59A.)

rectus muscle: a flat muscle extending the length of the abdomen from the xiphoid process of the breastbone or sternum to the pubis In the frog it is a slow muscle, responding to a stimulus by graded contracture.

red blood cells: the oxygen transporting cells of mammalian blood In humans, the red blood cells are shaped like biconcave disks about 7 Å in diameter and 2 Å thick with a depression in the middle.

red cell ghosts: When red blood cells are incubated in a large body of a hypotonic solution, hemoglobin, K^+ etc. leave the cells. The largely discolored remains of the red blood cells are called red cell ghosts.

relative vapor pressure: vapor pressure expressed as a percentage of the vapor pressure at full saturation under identical conditions

relaxation time: the time required for the excess population of individual components of a system at the excited state to fall to 1/e (1/2.718) or roughly 37% of its initial size

Renaissance: a transitional movement in Europe between medieval and modern times beginning in the 14th century in Italy lasting into the 17th and marked by a humanistic revival of classical influences expressed in a flowering of the arts and literature and by the emergence of modern science

resealed red cell ghosts: Red blood cells turn into "ghosts" when incubated in a hypotonic solution. If to such a suspension of red cell ghosts is added enough sucrose (and ATP) to restore isotonicity of the solution, the red cells may regain more or less their original size. They are seen by some investigators at least as having resealed the leaky holes in the cell membrane and become resealed red cell ghosts.

residue: When an amino acid is incorporated into a polypeptide or protein that amino acid becomes an amino-acid residue. Examples: glycine residue, lysine residue.

respiratory proteins: proteins of which the primary physiological functions are in facilitating the gaseous exchange between the respiratory organs like the lungs and gills and other body cells remote from these respiratory organs Examples: hemoglobin, hemocyanin.

resting living state: the state of a normal living cell or protoplasm when it is not in activity

resting potential: the steady electrical potential difference measured across the surface of resting living cells including muscle and nerve

Rhodnius: genus name of a blood-sucking bug

ribonucleic acid, RNA: any of the nucleic acids that contain ribose and uracil as structural components

rigor: rigidness of organ

rigor mortis: temporary rigidity of muscles immediately following death

Ringer's solution: a solution resembling the blood serum in its salt concentrations, first devised by and named after S. Ringer

RNA: ribonucleic acid

rotational diffusion coefficient: D_r, given in units of sec^{-1}, is defined as $\langle\theta^2\rangle$ divided by 6τ, where $\langle\theta\rangle$ is the mean square displacement in time τ.[166 p/929]

rotational entropy: the entropy possessed by a substance due to the rotatory movements of its molecules

Royal Institution: the first research institution founded in 1799 by Count von Rumford (Sir Benjamin Thompson) who was born in Massachusetts and a scientist as well as statesman. Married for four years to Antoine Lavoisier's widow, Marie Lavoisier. The Royal Institution was made famous by Michael Faraday. Despite its name, it was not financially supported by the Royalty.

Ruhland's ultrafilter theory: a theory of cell membrane offered by W. Ruhland in the first decade of the 20th century In this theory, the cell membrane acts like a sieve with pores of small size. Ruhland believed that the speed of entry of various substances should vary with the solute's molecular volume. (See also the size rule.)

S.D. or s.d.: See standard deviation.

S.E. or s.e.: See standard error of the mean.

salt linkage: the ionic bond between fixed ions of opposite charges on proteins due to Coulombic interaction

salt-linkage hypothesis: a hypothesis offered by G.N. Ling in 1952 to account for the failure to demonstrate selective adsorption of K^+, Na^+ in isolated proteins This hypothesis was confirmed by Ling and Zhang in 1984.[114]

saltatory route: a mode of migration of ions in fixed charge systems in which the ion penetrates the cell through the polarized-oriented surface water between fixed sites without undergoing an adsorption-desorption process

sarcode: the name given by Felix Dujardin to what was later called protoplasm

sarcomere: the basic unit of the contractile machine in striated muscle A longitudinally oriented sequence of sarcomeres makes up a myofibril. A bundle of myofibrils, in turn, makes up the substance of a muscle fiber or cell.

sartorius muscle: a thin, flat muscle extending from the pubis to the side of the knee. In the frog, the sartorius muscle contains about 1000 single muscle fibers, each running parallel to one another all the way from one end of the muscle to the other end.

satellite peaks: Due to the nuclear Zeeman effect, the NMR peak of a nuclide like Na^{23} with non-spherical distribution of nuclear charge splits into three peaks, a tall one in the middle and two flat ones one on each side. These side ones are called satellite peaks.

saturated hydrocarbon: a hydrocarbon molecule with neither free valence, nor double bond nor triple bond

scalar quantity: a quantity that has magnitude but no direction (The opposite is vector.)

Schwann's cell theory: the theory offered in 1839 by Theodor Schwann that living cells are the basic units of all living matter

scintillation counter: See γ-scintillation counter.

SDS: See sodium dodecyl sulfate.

second order quadrupole broadening: In an exaggerated quadrupole splitting of NMR signal, the center peak falls below the theoretically expected value and the phenomenon is then known as second order quadrupole broadening.

secondary structure of protein: the folding (or non-folding) pattern of a protein

semipermeability: a name originally introduced by van't Hoff describing the (assumed) properties of membranes that allow only the passage of water but not the solute dissolved in the water As time went by, a much looser definition became accepted in which quantitatively different permeability toward water and solutes are also considered as indicative of semipermeability.

semipermeable membrane: Now taken more to mean that a membrane is semipermeable if some constituent of a given solution passes through rapidly while others do not. (See semipermeability.)

Sephadex column: a column packed with beads of cross-linked dextran—high molecular weight polysaccharides containing exclusively D-glucose or dextrose monomeric units—used in gel filtration, desalting and in concentrating protein solutions

serine: a common α-amino acid found in most protein hydrolysates When incorporated into a protein, a serine residue offers a hydroxyl group on a short side chain.

serosal membrane: the membrane of a bifacial epithelial cells like that of the intestine which faces the side opposite to that of the intestinal lumen

SH group: sulfhydryl group, found in proteins on cysteinyl residues

"shell of high probability of association": Ants are attracted to a lump of sugar lying on the ground. If one draws imaginary concentric circles around the sugar lump, and take successive photos of the sugar lump and visiting insects, you will find that the circular band immediately next to the sugar lump will have more visitor-ants recorded than the next band, and so on. Thus the circular band nearest to the sugar may be called the "band of high probability of visits." If we now move to a three-dimensional world of atoms and ions, replace the sugar lump with an attractive fixed oxyacid anion and the visiting ants with free cations like K^+, draw imaginary concentric spherical shells around the fixed anion, and again take photos repeatedly, then one will find that the shell closest to the fixed anions will have the high probability of receiving visiting K^+, the next shell less and so on. The closest shell is what is called the "shell of high probability of association."

short-range attributes: properties which cannot be perceived without direct contact and thus opposite to long-range attributes which can be perceived from a distance

short-range influence: influence which can only reach sites close-by

sigmoid curve: an S-shaped curve

sigmoidity: being S-shaped

silicone rubber: rubber made from silicone elastomers noted for its retention of flexibility, resilience and tensile strength

silicone rubber gasket: packing of silicone rubber that makes a frog muscle in an EMOC preparation fluid-tight

silver electrode: an electrode when used in conjunction with a reference electrode and a voltage-measuring instrument or pH meter can determine the silver ion concentration (or more accurately, activity) in a solution, just as a pH electrode can measure the activity of H^+ in a solution

silver grains: Photographic film contains light-sensitive silver salt as part of an emulsin also containing gelatin. On exposure to light, metallic silver grains are formed at the location of light exposure and "paints" the darker touches of the image.

sine qua non: an absolutely indispensable or essential thing

Singer-Nicolson fluid mosaic membrane theory: a theory of cell membrane structure offered by S.J. Singer and G.L. Nicolson in 1960 in which a phospholipid bilayer forms the continuous phase of the membrane with islands of protein molecules fully or partly submerged

single fiber sucrose space method: A method introduced by G. N. Ling, S. Will and P. Shannon to measure the size of the extracellular space in frog muscle by quantitative comparison of the concentration of radioactively labeled sucrose in single muscle fibers which have no extracellular space and whole muscles which do.

size rule: the q-value of a solute in cell water and water in model systems varies inversely with the molar volume or size of the solute

Sloan-Kettering Institute: Memorial Sloan-Kettering Cancer Center located at 1275 York Ave. in Manhattan, New York

SO_4^{2-}: the sulfate anion

sodium azide: NaN_3, a poison

sodium channel, Na channel: a hypothetical pore-like structure in the cell membrane based on the membrane pump theory It is supposed to be closed when the cell is at rest but opens momentarily during activity when it would allow only Na^+ ion to go through but nothing else.

sodium citrate: $C_6H_5O_7$, Na_3. 3 H_2O, the sodium salt of citric acid, to which citrus fruits owe their acidity

sodium dodecylsulfate: a long-chain anionic detergent

sodium iodoacetate: the sodium salt of iodoacetic acid, a metabolic poison

sodium pump: a hypothetical mechanism located in the cell membrane, which ceaselessly pumps Na^+ out of the cell at the expense of metabolic energy and in spite of constant inward diffusion so that a suitable low concentration of this ion is maintained. {See Chapter 12 and also [15.4].}

sodium pump hypothesis: a hypothesis for the existence and function of the sodium pump

sol: liquid colloidal system as defined by Thomas Graham

soleus muscle: a broad, flat muscle of the calf of the leg, situated under the gastrocnemius muscle

solute: a dissolved substance

Solution 731: a special frog Ringer's solution containing vitamins, free amino acids and other nutrients as well as antibiotics in which isolated frog muscle could survive as long as 9 days at room temperature. See Ref. 381.

solvate: a complex of an ion and solvent molecules

solvency: the quality or state of being able to dissolve

soma: all of an organism except the germ cells

sorb: to take up and hold by either adsorption or absorption

sorbitol: one of the six-carbon sugar alcohols (hexitols), also known as D-sorbitol or D-glucitol. It is one of the most wide-spread naturally occurring polyols found exclusively in plants.

sorption: the process, or state of being sorbed

sorption theory: A theory of solute distribution in living cells presented formally and in depth in book form by A.S.Troshin in 1958[91] (possibly in 1956[90]), in which a solute may exist as free solute in cell water and adsorbed or otherwise complexed to cell proteins. According to Troshin himself, basic ideas of this theory were introduced earlier by M. Fischer, W.W. Lepeschkin, D. Nasonov, and others. (See Chapter 7 for details.)

spatial fixation: the act of holding motionless at a location in space

spin quantum number: The rotation of an electron along its axis is called spin and as such contributes to the total angular momentum. The contribution is supposed to be quantized. The spin quantum number of electrons represented by s, can have only two alternative values, *viz.*, +1/2 and –1/2 (in units of angular momentum, $h/2\pi$ where h is the Planck constant equal to 6.625×10^{-27} erg second) with one spin rotating in one direction and the other in the opposite direction. In the case of atomic nuclei, the nuclear spin quantum number is zero or an odd or even multiple of ½. As a general rule, elements of even atomic weight have zero nuclear spin (with the exception of deuterium and nitrogen). Hydrogen nucleus has spin quantum number of ½. Na^{23}, 3/2.

spinal cord: the longitudinal cord of nervous tissue extending from the brain along the back inside the cavity within the column of vertebrae called the spinal canal

"sponge protein": nickname of the intracellular protein(s) in bifacial epithelial cells, which sops up ions and/or other transported solutes and discharges them in cycles as part of the hypothetical mechanism for true active transport according to the AI Hypothesis

squid axon, squid giant axon: Giant nerve fiber measuring some 400 to 800 μ in diameter can be dissected from North Atlantic squids (*Loligo*) and is a very useful nerve preparation for the investigations of the nerve impulses and their underlying mechanisms. Similar giant axons are obtainable from cuttlefish (*Sepia*). Chilean squid axons may have diameters as large as 1.5 mm.

standard deviation, S.D. or s.d.: a statistical parameter to measure the degree of scattering or dispersion in a population of measurements or values It is equal to the quadratic means of the deviations of values from the

arithmetic mean, or put differently, it equals the square root of the sum of the squares of the deviations from the arithmetic mean divided by the degree of freedom or sample size.

standard error of the means, S.E. or s.e.: a statistical parameter measuring dispersion and equal to the standard deviation of the sample divided by the square root of the sample size

standard free energy of adsorption: In the context of this document, the standard free energy of adsorption (ΔF°) refers to the free energy change associated with the (close-contact) adsorption of an ion, molecule or other solute on an adsorption site. And it is related to the equilibrium adsorption constant, K, by the relation: $\Delta F^{\circ} = - RT \ln K$, where R and T are the gas constant and absolute temperature respectively. (See van't Hoff equation for the historical derivation of this equation.)

standard free energy of solute distribution, ΔF°: The standard free energy change (ΔF°) in the distribution of a solute between two contiguous but largely immiscible phases is described by the equation: $\Delta F^{\circ} = - RT \ln q$ (which can be derived from van't Hoff equation for chemical reactions), where R and T are the gas constant and absolute temperature respectively. q is the true equilibrium distribution coefficient of the solute.

standing K potential: a concept introduced in the Hodgkin-Huxley theory of action potential It refers to the steady or standing potential generated by the much higher (assumed free) K^+ concentration within living cells than that in the free K^+ concentration in the bathing medium. {For evidence intracellular K^+ is in fact not free, see [10.2].}

standing Na potential: a concept introduced in the Hodgkin-Huxley theory of action potential It refers to the steady or standing potential generated by the much higher free Na^+ concentration in the surrounding medium than the much lower Na^+ concentration in (the assumed free) cell water. {For evidence that cell water is not free, see Chap. 11}

static: of or relating to body at rest and exerting influence without motion

static structure: of or relating to structure at rest

statistical mechanics: a branch of mechanics dealing with the application of the principle of statistics to the mechanism of a system consisting of a large number of parts having motions that differ by small steps over a large range

steady state: a condition of a system where no changes are observable in a particular parameter due to the fact that any process occurring in one direction is exactly balanced by an equivalent process in the opposite direction The shape of the flame of a steadily burning candle represent such a steady state. In its common usage, a steady state is maintained by a continual supply of energy and in this way it differs from an equilibrium state

Stentor: any of a widely distributed Genus (*Stentor*) of ciliated protozoa that have a trumpet-shaped body attached to the substrate at the small end and with the mouth at the large end

steroid: any of the numerous compounds containing the ring system of sterols

stoichiometric: of, or relating to, quantitative chemical properties and composition, esp. as a factor in chemical or physical change

straight-line distribution curve, significance of: See rectilinear distribution curve, significance of.

striated muscle: muscle tissue that is marked by transverse dark and light bands and under voluntary control (in contrast to smooth muscle which does not show striation nor under voluntary control)

strontium: a malleable bivalent metal belonging to the alkali earths, its ion represented as Sr^{2+}

strychnine (sulfate): alkaloid present in seeds of *Strychnos nux vomica* and other species of *Strychnos* genus; a nerve poison, causing intense convulsion

subcellular particles (organelles): specialized structures in living cells like mitochondria

substrate: a substance acted upon (as by an enzyme); the base on which an organism lives; an underlying support

sucrose, cane sugar: a sweet-tasting disaccharide from sugar canes and beets When hydrolyzed, it yields an equimolar mixture of D-glucose and D-fructose.

sulfonate group: SO_3^-, the univalent anionic group of the dissociated sulfonic acid, SO_3H

superconducting magnet: In certain metals (e.g., niobium-titanium), electric resistance drops

to zero when the temperature is brought to near absolute zero. A superconducting magnet is made from coils of wire of these metals, where rapid circular electric current produces strong magnetic field while the coils are kept at liquid helium temperature (–268.9°).

supercool: to cool below the freezing point without crystallization or solidification

surface adsorption theory, SA theory: older name of the theory of cellular electrical potential according to the association-induction hypothesis, now known as the close-contact surface-adsorption (CSA) theory

surface component of the polarization energy (U_s): the portion of the water-to-water interaction energy in polarized and oriented water which arises from the enhanced interaction of a solute molecules with the immediately surrounding water molecules

surface-limited diffusion: If the slowest step in a diffusion process from one phase (say a solid) to another phase (say a liquid) is across the boundary between the phases, the process is surface-limited.

surrogate ion: an ion that serves as a substitute for another ion

swelling and shrinkage: increasing and decreasing of the volume of a body, usually as a result of the gain or loss of its water content

swelling water: water in a swollen tissue, cell or colloid

switch protein: nickname of a hypothetical protein, which turns on or off a physiological process

switching: making a shift or exchange

synapse: the point at which a nerve impulse passes from one neuron to another

$t_{1/2}$: the half time of a process

T_1: the (nuclear-magnetic resonance) spin-lattice relaxation time, or longitudinal relaxation time The difference between the excess population at an elevated energy level in a static uniform magnetic field and its equilibrium value, is reduced by a factor of e (2.718) after a time, T_1.

T_2: the (nuclear magnetic resonance) spin-spin relaxation time or transverse relaxation time Defined approximately as the lifetime or phase memory time of a nuclear spin state.

T-state: the more rigid or tense deoxygenated sate (of hemoglobin)

Taft's induction constant: a set of empirically determined constants of substituents applicable to aliphatic compounds introduced by R. W. Taft in 1953

target: something to be affected by an action or development

tesla: unit of magnetic induction equal to 10^4 gauss, named after inventor, Nikola Tesla

tethered: to be fastened or restrained by

theorem: an idea accepted or proposed as a demonstrable truth often as a part of a general theory

theory of enhancement of association through site (or charge) fixation: the theory that charge- or site fixation enhances the degree of association of an adsorbent for two kinds of reasons: one electrostatic and the other kinetic {For details, see [10.1(1)].}

Theory of light: theory of light as propagated electromagnetic waves introduced by James Clerk Maxwell in 1864

theory of thermostability of Perutz: Max Perutz suggested in 1978 that the cause some living organisms can survive in high temperature is due to the presence of extra structure-stabilizing salt-linkages in their enzymes and other proteins.

thermodynamics: the science that deals with the relationship of heat and mechanical energy and the conversion of one into the other

THO: tritiated water Water molecules in which one of the hydrogen atoms has been replaced by a tritium atom

threonine: $CH_3CH(OH)CH(NH_2)CHCOOH$, an α-amino acid found in most protein hydrolysates Its presence as threonine residue in a protein endows the protein with a hydroxyl group on a short side chain.

tibial end: the distal end of (a sartorius muscle) that is attached to the tibial bone, which is located between the knee and the ankle

titration curve: Titration is a process of determining the strength of a solution or the concentration of the substance in solution in terms of the smallest amount of a reagent of known concentration required to bring about a given effect in reaction with a known volume of the test solution. A most common type of titration is to determine the acid binding curve of a substance. e.g., protein.

Tl^+: thalium cation, a surrogate ion for K^+ but poisonous Used to poison rats.

tonoplast: the membrane surrounding the central vacuole in mature plant cells, also known as vesicular membrane

toxicological action: the specific toxic activity of a poison

Tradecantia elongata: spiderwort, an American herb

transcription: a process involving base pairing, by which the genetic information contained in a DNA molecule is used to produce a complementary sequence of bases in an RNA chain

translation: the process whereby the genetic information contained in a messenger RNA (mRNA) molecule directs the order of the specific sequential order of amino-acid residues during the synthesis of a specific protein (See also transcription.)

transmissivity, transmission factor: the fractional number or percentage which describes the attenuation each passage across an atom imposes on the transmitted inductive effect

trehalose: a disaccharide of two D-glucose found in fungi instead of starch Also found in insects.

trichloroacetic acid (TCA): Cl_3CCOOH, a strong acid and protein denaturant, an acetic acid in which all three of the methyl protons have been replaced by (three) chlorine atoms

triplet adsorption-desorption route: a way by which an external or internal cation, for example, may enter (or leave) the cell, which involves the participation of a second free cation

triplet migration: a mechanism by which an ion can move across a fixed site at the surface or within a fixed charge system. This process involves the occupation of the site by the ion followed by its desorption with the participation of a second free ion.

trisaccharide: a sugar that upon hydrolysis yields three monosaccharides Example, raffinose

tritiated water: H^3HO, a radioactively labeled water molecule

Troshin equation: Equation A1 in Appendix 1.

true active transport: refers to the active transport across bifacial cell layers and similar systems

true equilibrium distribution coefficient (q-value): the ratio of the equilibrium concentration of a solute (exclusively) in the water of a living cell or model system over that in the external bathing medium

tryptophane: a common α-amino acid found in most protein hydrolysates Its presence in a protein endows the protein with an indole group carried on a short side chain.

Tyndall phenomenon: Discovered by Michael Faraday, the scattering of light by a solution of colloidal particles was studied by J. Tyndall and is known as the Tyndall phenomenon.

tyrosine: an aromatic α-amino acid found in most protein hydrolysates Incorporated into a polypeptide or protein it provides a phenolic group on a short side chain.

μ: Greek alphabet for m See at beginning of m section.

U_s: See surface component of the interaction energy.

U_{vp}: the exclusion intensity incorporating the volume contribution and the entropy contribution to solute exclusion

ultracentrifuge: a high-speed centrifuge which can sediment and thus separate particles and large molecules that conventional centrifuge cannot Ultracentrifuge is used to separate different fractions of cellular components from cell homogenate and in determining molecular weights of proteins.

ultramicroscope: a microscope designed for the visualization of colloidal materials based on the Tyndall phenomenon

unifacial cells: cells like muscle, nerve, red blood cells each possessing a single type of cell membrane throughout its entire cell surface

unifying theory: a theory that makes into a unit or coherent whole

UO_2Cl_2: uranyl chloride. bright yellow crystals

uranium and lead stained EM sections: conventional EM sections stained with electron-dense uranium and lead

urea: $(NH_2)_2CO$, a product of protein metabolism At high concentration, it acts as a protein denaturant.

utricle: any of the small pouches of animal or plant body

V = 1/C: the concentration C in moles per liter is the reciprocal of volume in liters containing 1 mole of solute. Or vice versa as shown here.[13 pp 662–663]

valence, valency: an expression of the combining capacity of an element as measured by the number of hydrogen atoms or their equivalents, with which one atom of the element can combine

van't Hoff equation for chemical reactions: In 1886, J. H. van't Hoff introduced the following equation for a chemical reaction: $aA + bB \rightleftharpoons cC + dD$: $\Delta F = \Delta F^{o} + RT \ln Q$, where Q, called *reaction quotient,* is equal to $\{[A]^{a} \times [B]^{b}\} / \{[C]^{c} \times [D]^{d}\}$. $[A]^{a}$ is the concentration of A raised to the power of a, etc. When the reaction has reached equilibrium, ΔF is zero, and Q equals K, the equilibrium constant for the reaction. And $\Delta F^{o} = -RT \ln K$.

valinomycin: an antibiotic isolated from *Streptomyces fulvissimus*. Active *in vitro* against *Mycobacterium tuberculosis*. Acts as a highly specific ionophore for K^{+} across phospholipid bilayer.

vector: a quantity that has magnitude and direction

vitalism: a doctrine that the process of a living organism are due to a vital principle; distinct from physicochemical forces

Volta chain: an electrical-current-producing setup containing a pair of metal electrodes each immersed in a solution of electrolyte in compartments which may be separated by a semipermeable membrane It is named after Italian physicist, Count Alessandro Volta (1745–1827) who first discovered that two different metals separated by an electrolyte solution generates a potential difference and electric current.

water-immiscibility: being water immiscible

well type γ-scintillation counter: a device for detecting and registering individual scintillations as in radioactive emissions in which the sample is placed within a deep hole or well inside the scintillation detector

White blood cell, leucocyte: any of the colorless nucleated cells in blood

white ghosts: red cell ghosts that have lost all or nearly all its red hemoglobin pigments

X-ray absorption-edge fine structure: When monochromatic X-ray beam shines through a thin film of an aqueous solution, different proportions of the energy of the X-ray are absorbed, depending on the photon energy of the beam and the nature of the atoms or ion present in the solution (e.g., K^{+}). By expressing the ratio of the intensity of the incident beam over that of the emerging transmitted beam at different photon energy, an X-ray absorption edge fine structure is obtained.

xylem: a complex tissue in the vascular system of higher plants that consists of vessels and long tubules with tapering blind ends called tracheids. Xylem functions chiefly in conduction but also in support and storage.

xylose: See D-xylose.

y-gene: often refers to one of the three genes in the Lac operon of E. coli, which specifies the protein called lactose permease

Yang-Ling cooperative adsorption isotherm: See Equation A4 in Appendix 1.

Z-line: the dense line at the end of a sarcomere

Zeeman effect: In a strong magnetic field, the lines of the emission spectrum of atoms are split up. This is known as the Zeeman effect reported by P. Zeeman in 1896.

List of Abbreviations and Selected Symbols

a_1, a_2: activities of ionic species 1 and 2 respectively
ADP: adenosine diphosphate
AI Hypothesis: association-induction hypothesis
AIH: association-induction hypothesis
Ala: alanine residue
Arg: arginine residue
Asn: asparagine residue
Asp: aspartic acid residue
ATP: adenosine triphosphate

CG-electrode: collodion-coated glass electrode
Cl: chlorine atom
Cl^-: chlorine ion
CO group: carbonyl group
CONH: peptide bond
CrP: creatine phosphate, phosphocreatine
Cs^+: cesium ion
CSA: close-contact surface adsorption theory of cellular electric potentials
Cys: cysteine residue

D_K: diffusion coefficient of K^+
DPG: diphosphoglycerate, an organic phosphate found in red blood cells

E. coli: see Escherichai coli in Glossary
EDC: electron-donating cardinal adsorbent
EIC: electron-indifferent cardinal adsorbent
EMOC: effectively membrane-pump-less open-ended cell preparation
EPPI: Eastern Pennsylvania Psychiatric Institute
EWC: electron-withdrawing cardinal adsorbent

f^-: fixed monovalent anion
f^+: fixed monovalent cation
f^+Y^-: associated fixed monovalent cation-free monovalent anion pair
f^-X^+: associated fixed monovalent anion-free monovalent cation pair

$-\gamma/2$: nearest neighbor interaction energy
γ_K: activity coefficient of potassium ion (K^+)
Gln: glutamine residue
Glu: glutamic acid residue
Gly: glycine residue

H: hydrogen atom
H^+: hydrogen ion, or proton
His: histidine residue
HKKP: Hodgkin-Keynes-Kushmerick-Podolsky
H_2O: normal water molecule

IAA: iodoacetic acid or iodoacetate ion
Ile: isoleucine residue

K^+: potassium ion
K_K: adsorption constant of K^+
K_{Na}: adsorption constant of Na^+
$K^{oo}_{Na\to K}$: intrinsic equilibrium constant between Na^+ and K^+
$K^{oo}_{i\to j}$: intrinsic equilibrium constant between ith and jth ion or molecule

Leu: leucine residue
LFCH: Ling's fixed charge hypothesis
Li: lithium atom
Li^+: lithium ion
Lys: lysine residue

μ: micron, 10^{-4} cm
Met: methionine residue

N sites: negatively charged site
N^+: sodium ion

NaOH: sodium hydroxide
Na_2SO_4: sodium sulfate
NH_4^+: ammonium ion
NH group: imino group
NO-NO-NO: a chain of alternatingly negatively-charged N sites and vacant O sites
$(NO\text{-}NO\text{-}NO)^n$: parallel array of NO-NO-NO chains where each NO-NO-NO chain is immediately surrounded by n similar chains
NO-NO-NO system: a system of NO-NO-NO chains
NP-NP-NP: a chain of alternatingly positively-charged P sites and vacant O sites
NP system: a two-dimensional array or checkerboard of alternatingly negatively-charged N sites and positively-charged P sites
NP-NP system: juxtaposed checkerboard of two dimensional array of alternating negatively-charged N sites and positively-charged P sites
$(NP\text{-}NP\text{-}NP)^n$: parallel array of NP-NP-NP chains where each NP-NP-NP chain is immediately surrounded by n similar chains

O sites: vacant sites

π: osmotic pressure
(ρ: Greek rho, see under R)
P~: high energy phosphate bond
$P_{\text{cellulose acetate membrane}}$: permeability through a layer of heat-activated cellulose acetate membrane
P_{Cl}: permeability constant of chloride ion
$P_{\text{frog skin}}$: permeability through a sheet of inverted frog skin
p^o/p: reciprocal of partial vapor pressure where p is the existing vapor pressure and p^o is the vapor pressure at saturation under the same condition
P_K: permeability constant for K^+
P_{Na}: permeability constant for Na^+
Pro: proline residue
P sites: positively-charged sites
PEI: poly(ethylene imine), a linear polymer carrying fixed positively charged imine groups
PEO: poly(ethylene oxide), a linear polymer carrying fixed oxygen atoms serving as an extrovert model
Phe: phenylalanine residue
PM theory: polarized multilayer theory of cell water and model systems
PVME: polyvinylmethylether, another highly useful extrovert model
PVP: polyvinylpyrrolidone, another useful extrovert model

q: true equilibrium distribution coefficient or q-value

R: the gas constant, equal to 1.987 cal per degree per mole
ρ: apparent equilbrium distribution coefficient or ρ-value
R′: an empirically determined constant that approaches the value of R
Rb: rubidium atom
Rb^+: rubidium ion
Residue: each amino acid when incorporated into a polypeptide or protein is referred to as an amino-acid residue Example: glycine residue comes from the amino acid, glycine

SA theory: the surface adsorption theory of cellular electric potential was a term introduced with the AI Hypothesis but later replaced by the name close-contact surface adsorption theory (CSA theory)
SDS: sodium dodecyl sulfate, an anionic detergent
S.D. or s.d.: standard deviation
S.E. or s.e.: standard error of the mean
Ser: serine residue

T: absolute temperature

$t_{1/2}$: half time of exchange
τ_r: rotational correlation time
Thr: threonine residue
Tl^+: thalium cation
Trp: tryptophane residue
Tyr: tyrosine residue

U_s: the surface component of solute-water interaction energy
U_{vp}: exclusion intensity of solute-water interaction energy It includes both an energy component and an entropy component.
V: volume
V_1: partial molal volume It represents the actual volume a mole of a substance occupies when it constitutes one of two (or more) components of a solution.

Val: valine residue

X^+: a monovalent free cation
x/l: length x expressed as a fraction of the total length l

Y^-: free monovalent anion

Z-line: the dense line at the either end of a sarcomere

ψ: symbol used to designate the magnitude of the resting or action potential
ψ^0: a constant used in Nicolsky's equation (equation 9) for glass electrode potential

List of Figures, Tables and Equations

EQUATIONS

FIGURES

TABLES

References

(1) Schwann, T. 1839 *Mikroskopische Untersuchungen über die Übereinstimung in der Struktur and dem Wachstum der Thiere und Pflanzen* (Berlin); republished in Ostwald's "Kassiker" (Leipzig, 1910), pp, 60–158. See also Ref. 3 for valuable review in English on Schwann's work.

(2) Dujardin, F. 1835 Annales des sciences naturrelles; partie zoologique, 2d sér., 4:364.

(3) Hall, T.S. 1969 *Ideas of Life and Matter; Studies in the History of General Physiology 600 B.C.–1900 A.D.,* Vol 2, The University of Chicago Press, Chicago.

(4) Ledbetter, M.C. and Porter, K.R. 1970 *Introduction to the Fine Structure of Plant Cells,* Springer, New York.
Dodge, J.D. 1973 *The Fine Structure of Algal Cells*, Academic Press, New York.

(5) Oken, L. 1805 Die Zeugung (cited by C. Singer in his *A History of Biology to About the Year 1900: A General Introduction to the Study of Living Things*, Abelard-Schuman, London.)
———. 1810 *Lehrbuch der Naturphilosophie*, Jena.

(6) von Nägeli, K. 1844 Zeitschr. wiss. Bot. 1: 34.

(7) von Mohl, H. 1846 Bot. Z. 4:73, 84.

(8) Cohn, F. 1847 *Nova Acta Academiae Caesareae Leopoldino-Carolinae* [of Halle] 22: 605. For partial English transl. See Huxley, T.H., 1872, The Contemporary Review 19: 34.

(9) Remak, R. 1852 Müller's Arch f. Anatomie, Physiologie, und wissenschaftlische Medizin (Berlin) p. 49.

(10) Leydig, F. 1857 *Lehrbuch der Histologie des Menschen und der Thiere*, (Frankfurt) pp. 14–15.

(11) Schultze, M. 1861 Müller's Archiv f. Anatomie. Physiologie und wissenschftliche Medicin (Berlin) p. 1. For transl. [of a part], see M. and V. Hamburger and T.S. Hall, in T.S. Hall, *A Source Book in Animal Biology* (New York, 1951, 1964), p. 449.

(12) Wilson, E.B. 1928 *The Cell in Development and Heredity*, 3rd ed.Macmillan, New York.

(13) Glasstone, S. 1946 *Textbook of Physical Chemistry*, 2nd ed., D. Van Nostrand, New York.

(14) van't Hoff, J.H. 1887 Z. phys. Chem. 1: 481.
———. 1888 Phil. Mag. 26: 81.

(15) Ling, G.N. 1984 *In Search of the Physical Basis of Life*, Plenum Publ. Co., New York.

(16) Dutrochet, R.J.H.1824 *Recherche anatomiques et physiologiques sur la structure intime des animaux et des végétaux et sur la motilité*, (Paris).
———. 1827 Ann. Chim et Physique. 34:393; 35: 393.

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

(18) Pfeffer, M. F. 1877 (1st ed.) 1921 (2nd ed.) *Osmotische Untersuchungen: Studien zur Zell-Mechanik*, Engelmann, Leipzig.
———. 1985 *Osmotic Invesigations: Studies on Cell Mechanics* (Eng. Transl. by G.R. Kepner and Ed. J. Stadelmann), Van Nostrand Reinhold, New York.

(19) Bernstein, J. 1902 Pflügers Arch. ges. Physiol. 92: 521.

(20) Donnan, F. 1911 Z. Elektrochem. 17: 572.
———. 1924 Chem. Rev, 1: 73.

(21) Overton, E. 1899 Vierteljahrschr. Naturforsch. Ges. Zürich 44: 88.

(22) Chambers, R. and Chambers, E.L. 1961 *Explorations into the Nature of the Living Cell*, Harvard Univ. Press, Cambridge, Mass. p. 9.

(23) Ling, G.N. 1973 Physiol. Chem. Phys. 5: 295.

(24) de Vries, H. 1871 Arch. néerl. sci. 6: 117.

(25) Overton, E. 1902 Pflügers Arch ges Physiol. 92: 115.

(26) Hamburger, H, J. 1887 Arch. für Anatomie und Physiologie S 41.

———. 1889 Z. Biol. 26: 414.
(27) von Hanstein, J. 1880 *Das Protoplasm als Träger des pflanzlichen und thierischen Lebensverrichtungen*, Sammlung von Vorträgen für das deutsche Volk, Heidelberg, p. 169.
(28) de Vries, H. 1884 Jahrb. wiss. Bot. 14: 427.
(29) Höfler, K. 1918 Ber. deutsch. bot. Ges. 36: 414.
(30) Lucké, B. and McCutcheon, M. 1932 Physiol. Rev. 12: 68.
(31) Höfler, K. 1926 Planta 2: 454.
———. 1930 Jahrb. wiss. Bot. 73: 300.
———. 1931 Ber. dtsch. bot. Ges. 49: 19.
———. 1932 Protoplasma 15: 462.
(32) Plowe, J. Q. 1931 Protoplasma 12: 196, 221.
Strugger, S. 1932 Ber. dtsch. bot. Ges. 50: 24.
Ullrich, H. 1939 Arch. exp. Zellforsch. 22: 496.
(33) Nasonov, D. N. and Aizenberg, E. I. 1937 Biol. zh. (In Russian) 6: 165, see also Ref. 92, p. 48 and Ref. 86, p. 111.
(34) Kamnev, I. Ye. 1938 Arkh. anat. gistol. i embr. 19:145 (also Ref. 92, p. 111, Ref. 86, p.115).
(35) Ling, G.N., Neville, M.C., Will, S. and Shannon, P. 1969 Physiol. Chem. Phys. 1:85.
Ling, G.N., Walton, C.L. and Ochsenfeld, M.M. 1981 J. Cell. Physiol. 106: 385.
(36) Gérard, P. 1912 C.R. 154: 1305.
Wu, H. and Yang, E.F. 1931 Proc. Soc. Exp. Biol. Med. 29: 248.
Kaplanskii, S. Ya. and Boldyreva, N. 1934 Fisiol. Zh. 17: 96.
Cohn, W. and Cohn, E. F. 1939 Proc. Soc. Exp. Biol. Med. 41: 445.
Heppel, L. A. 1939 Amer. J. Physiol. 127: 385.
Steinbach, B. 1940 J. Biol. Chem. 133: 695.
(37) von Nägeli, K. 1855 *Pflanzenphysiol. Untersuchungen* (C. Nägeli and C. Cramer, eds.) Hefl. I, Schulthese, Zürich.
(38) Kühne, W. 1864 *Untersuchungen über das Protoplasma*, Engelmann, Leipzig.
(39) Pfeffer, Wilh. 1897 *Pflanzenphysiologie*, 2te Aufl., 2 vols., Engelmann, Leipzig.
(40) Kite, G.L. 1913 Biol. Bull. 25: 1.
(41) Lillie, R. S. 1923 *Protoplasmic Action and Nervous Action*, 1st ed., Univ. of Chicago Press, Chicago.
(42) Cameron, I. L. 1988 Physiol. Chem. Phys. and Med. NMR 20: 221.
(43) Ostwald, Wilh. 1890 Z. physik. Chem. 6: 71.
(44) Boyle, P.J. and Conway, E.J. 1941 J. Physiol.(London) 100: 1.
(45) Hutter, O. F. and Padsha, S. M. 1959 J. Physiol. (London) 146: 117.
(46) Lorkovic, H. and Tomanek, R.J. 1977 Amer. J. Physiol. 232: C109.
(47) Hodgkin, A.L. 1951 Biol. Rev. 26: 339.
———. 1971 *The Conduction of the Nervous Impulse*. Liverpool Univ. Press, Liverpool.
(48) Ling, G. N. 1982 Physiol. Chem. Phys. 14: 47.
(49) Ling, G. N. 1997 Physiol. Chem. Phys. & Med. NMR 29:123.
(50) Netter, H. 1928 Pflüger's Arch. ges. Physiol. 220: 107.
(51) Mond, R. and Amson, K. 1928 Pflüger's Arch. Ges. Physiol. 220: 69.
(52) Skou, S.C. 1957 Biochem. Biophys. Acta 23: 394.
———. 1965 Physiol. Rev. 45: 596.
———. 1988 Methods of Enzymol. 156: 1.
———. 1989 Biochem. Biophys. Acta 1000: 435.
(53) Graham, T. 1861 Philos. Trans. Roy. Soc. (London) 151: 183.
(54) Williams, L. P. 1965 *Michael Faraday: A Biography*, Da Capa Press, Plenum Publ. Co., New York.
(55) Bigelow, S. L. and Bartell, F. E. 1909 J. Amer. Chem. Soc. 31: 1194.
Bigelow, S.L. and Hunter, F. W. 1911 J. Phys. Chem. 15: 367.
Bartell, F.E. 1911 J. Phys. Chem. 15: 318.

(56) Findlay, A. 1919 *Osmotic Pressure*, Longmans, Green & Co., London.
(57) L'Hermite, M. 1855 Annales chim. phys. 43: 420.
(58) Pauli, W. and Rona, P. 1902 . Beitr. Chem. Physiol. Pathol. 1902: 114.
(59) Bungenberg de Jong, H, G. and Kruyt, H. R. 1929 Proc. Koninkl. Nederland. Akad. Wetenschap., Amsterdam 32: 849.
(60) Glicksman, M. 1969 *Gum Technology in the Food Industry*, Academic Press, New York.
(61) Bungenberg de Jong, H. G. 1949 in *Colloid Science, Vol. II Reversible Systems* (H. R. Kruyt, ed.) Elsevier Publ. Co., New York.
(62) Lepeschkin, W.W. 1926 Ber. dtsch. bot. Ges. 44: 7.
———. 1930 Protoplasma 9: 269, p. 275.
(63) Kuroda, K. 1964 in *Primitive Motile Systems in Cell Biology* (R.D. Allen and N. Kamiya eds.), Academic Press, New York, p. 31.
(64) Gortner, R. A. 1938 *Outline of Biochemistry*, 2nd ed., John Wiley & Sons, New York.
(65) Houwink, R. 1949 in *Colloid Science* Vol. II *Reversible Systems*, Elsevier Publ. Co., New York, pp. 19–45.
(66) Staudinger, H. 1940 *Organische Kolloidchemie*, Braunschweig.
(67) Staudinger, H. 1920 Ber. 53: 1073.
(68) Ling, G. N. 1994 Physiol. Chem. Phys. & Med. NMR 26: 121.
(69) Negendank, W. and Shaller, C. 1982 Bioch. Biophys. Acta 688: 316.
(70) Holleman, L.W.J., Bungenberg de Jong, H. G. and Modderman, R.S.T. 1934 Kolloid Beih. 39: 334.
(71) Bungenbeg de Jong, H. G. 1932 Protoplasma 15: 110.
(72) Huxley, T.H. 1869 "On the Physical Basis of Life", Fortnightly Review 5: 129.
(73) Abderhalden, E. 1898 Z. physiol. Chem. 25: 65.
(74) Ponder, E. 1948 *Hemolysis and Related Phenomena*, Grune & Stratton, New York.
(75) Katz, J. 1896 Arch. ges. Physiol., 63: 1.
(76) Hamburger, H.J. 1904 *Osmotische Druck und Ionenlehre*, Vol. 3, Bergmann, Wiesbaden.
(77) Moore, B. 1906 *Recent Advances in Biochemistry* (L. Hill, ed.) Arnold, London, pp. 139–195.
Moore, B. and Roaf, H. E. 1908 Biochem. J. 3: 55.
(78) Fischer, M. H. 1909 *Oedema*, Trans. College Physicians, 3rd Ser. Vol. 31, Philadelphia, p. 457–657.
———. 1910 *Das Oedem*, Steinkopf, Dresden.
———. 1913 *Introduction to Colloidal Physiology* I. *Oedema* [in Russian], Moscow, pp. 1–331.
———. 1921 *Oedema and Nephritis,* 3rd ed., Wiley, New York.
(79) Ostwald, Wo. 1940 "Martin Fischer upon His Sixtieth Birthday" J. Med. 20: 436.
(80) Gortner, R. A. 1930 Trans. Faraday Soc. 26: 678.
(81) Hill, A. V. 1930 Proc. Roy. Soc. (London) Ser. B. 106: 477.
Hill, A. V. and Kupalov, P. S. 1930 Proc. Roy. Soc. (London) Ser. B. 106: 445.
(82) McLeod, J. and Ponders, E. 1936 J. Physiol. (London) 86: 147.
Hunter, F. R. and Parpart, A. K. 1938 J. Cell. Comp. Physiol. 12: 309.
(83) Ernst, E. 1963 *Biophysics of the Striated Muscle*, Hungar. Acad. Sci., 2nd ed., Budapest.
(84) Macallum, A.B. 1905 J. Physiol. (London) 32: 95.
Menten, M. L. 1908 Trans. Canad. Inst. 8: 403.
(85) Tigyi, J., Kellermayer, M. and Hazlewood C.F. 1991 *The Physical Aspect of the Living Cell*, Akad. Kiadó, Budapest.
(86) Nasonov, D. N. 1962 *Local Reaction of Protoplasm and Gradual Excitation* (English Transl. by Y.S. Halpern), National Science Foundation, available at Office of Technical Services, US. Department of Commerce, Washington, D.C.
(87) Hermann, L. 1871 Pflügers Arch. ges. Physiol. 4: 163.
———. 1879 *Handbuch der Physiologie*, Vogel Verlag, Leipzig, Band II Theil I
(88) Graham, J. and Gerard, R.W. 1946 J. Cell. Comp. Physiol 28: 99.

Ling, G. N. and Gerard, R. W. 1949 J. Cell. Comp. Physiol. 34: 383.
Weidemann, S. 1971 in *Research in Physiology*, (F. F. Kao, K. Koizumi and M Vassale, eds.), Aulo Gaggi Publ., Bologna, pp. 3–25.
(89) DuBois-Reymond, E. 1848-1849 *Untersuchungen über Thierische Elektrizität*, Vol. I and II, Reimer, Berlin.
(90) Troshin, A. S. 1956 *Problema kletochnoi pronitsaemosti*, Moskva-Leningrad.
(91) Troshin, A.S. 1958 *Das Problem der Zellpermeabilität*, Gustav. Fischer Verlag, Jena.
(92) Troshin, A.S. 1966 *Problems of Cell Permeability*, (English Transl. by M.G. Hell and N. D. Widdas), Pergamon Press, London.
(93) Troshin, A.S. 1948 Biokhimiya 13: 253.
———. 1951a Byull. eksp. biol. med. 31: 180.
———. 1951b Byull. eksp. biol. med. 31: 285.
———. 1951c Byull. eksp. biol. med. 32: 162.
———. 1951d Byull. eksp. biol. med. 33: 228.
———. 1951e Biokhimiya 16: 164.
———. 1952 Byull. eksp. biol. med. 34: 59.
(94) Ling, G. N. 1951 Amer. J. Physiol. 167: 806.
(95) Ling, G. N. and Gerard, R. W. 1949a J. Cell. Comp. Physiol. 34: 383.
———. 1949b J. Cell. Comp. Physiol. 34: 397.
———. 1949c J. Cell. Comp. Physiol. 34: 413.
Ling, G.N. and Woodbury, J. W. 1949 J. Cell. Comp. Physiol. 34: 407.
(96) Ling, G. N. 1952 in *Phosphorus Metabolism,* Vol. II, (W.D. McElroy and B. Glass, eds.), The Johns Hopkins Univ. Press, Baltimore, p. 748.
(97) Guggenheim, E. A. 1950 *Thermodynamics, An Advanced Treatise for Chemists and Physicists*, Interscience Publ., Inc., 2nd ed., New York, pp. 330–331.
(98) Ling, G. N. 1962 *A Physical Theory of the Living State: the Association-Induction Hypothesis*, Blaisdell Publ. Co., Waltham, Mass.
(99) Bugarszky, S. and Liebermann, L.1898 Arch. ges. Physiol. 72: 51.
Michaelis, L. and Rona, P. 1908 Biochem. Z. 14: 476.
Pauli, W. and Samec, M. 1909 Biochem Z. 17: 235.
Erdös, T. 1946 Hung. Acta Physiol. 1: 33.
Beatley, E. H. and Klotz, I. M. 1951 Biol. Bull. 101: 215.
Carr, C.W. 1956 Arch. Biochem. Biophys. 62: 476.
Lewis, M. S. and Saroff, H. A. 1957 J. Amer. Chem. Soc. 79: 2112.
(100) Bethe, A. and Toropoff, T. 1914 Z. Phys. Chem. 88: 686.
———. 1915 Z. Phys. Chem. 89: 597.
Mond, R. 1927 Pflüger's Arch. Ges. Physiol. 217: 618.
Teorell, T. 1953 Progr. Biophys. Biophys. Chem. 3: 305.
Meyer, K. H. and Sievers, J. F. 1936 Helv. chim. Acta 19: 649, 665.
Sollner, K. 1949 J. Phys. Colloid. Chem. 53: 1211.
(101) Gunn, R. B. 1979 in *Membrane Transport in Biology*, Volume II (D. C. Tosteson, ed.), Springer-Verlag, New York, Chapt. 2, p. 61.
(102) Arrhenius, S. 1887 Z. physk. Chem. 1: 631.
(103) Debye, P. and Hückel, W. 1923 Phys. Z. 24: 185.
(104) Ives, D.J.G. and Janz, G. J. 1961 *Reference Electrodes* , Academic Press, New York.
(105) Sollner, K. 1949 J. Phys. Colloid. Chem. 53: 1211.
(106) Ling, G. N. 1960 J. Gen. Physiol. 43: 149.
(107) Ling, G. N. 1992 *A Revolution in the Physiology of the Living Cell*, Krieger Publ. Co., Malabar, Fl.
(108) Speakman, J. B. and Hirst, M. C. 1931 Nature 127: 665.
(109) Riseman, J. and Kirkwood, J. G. 1948 J. Amer. Chem. Soc. 70: 2820.
(110) Debye, P. and Pauling, L. 1925 J. Amer. Chem. Soc. 47: 2127.

Webb, T. J. 1926 J. Amer. Chem. Soc. 48: 2589.
(111) Gorin, M. 1939 J. Chem. Phys. 7: 405.
Ling, G.N. 1957 in *Metabolic Aspect of Transport across Cell Memebrane*, (Q.R. Murphy, ed.), Univ. Wisconsin Press, Madison, pp. 181–186.
(112) Ling, G. N. 1978 J. Physiol. (London) 280: 105.
(113) Ling, G. N. 1973 Physiol. Chem. Phys. 5: 295.
(114) Ling, G. N. and Zhang, Z. L. 1984 Physiol. Chem. Phys. & Med. NMR 16: 221.
(115) Huang, H. W., Hunter, S.H., Warburton, W. K. and Moss, S.C. 1979 Science 204: 191.
(116) Ling, G. N. and Ochsenfeld, M. M. 1966 J. Gen. Physiol. 49: 819.
(117) Langmuir, I. 1916 J. Amer. Chem. Soc. 38: 2221.
———. 1918 J. Amer. Chem. Soc. 40: 1361.
(118) Hoare, D. G. and Koshland, D. E. 1967 J. Biol. Chem. 242: 2447.
Khorana, H. G. 1953 Chem. Rev. 53: 145.
(119) Ling, G. N. 1989 Physiol. Chem. Phys. & Med. NMR 21: 13.
(120) Stecher, P. G., Windholz, M., Leahy, D. S., Bolton, D. M. and Eaton, L. G., (eds) 1968 *The Merck Index,* 8th ed. Merck and Co., Rahway, N.J.
(121) Tanford, C. 1962 Adv. Protein Chem. 17: 69.
(122) Ling, G.N. and Ochsenfeld, M.M. 1991 Physiol. Chem. Phys. & Med. NMR 23: 133.
(123) Engelmann, T. W. 1873 Pflüger's Arch. Ges. Physiol. 7: 155.
Hanson, J. and Huxley, H. E. 1953 Nature 172: 530.
(124) Ling, G. N., 1977 Physiol. Chem. Phys. 9: 319.
(125) Edelmann, L., 1977 Physiol. Chem. Phys. 9: 313.
(126) Edelmann, L. 1978 Microsc. Acta Suppl. 2: 166.
———. 1980 Physiol. Chem. Phys. 12: 509.
———. 1980a Histochem. 67: 233.
———. 1980–1981 Intern. Cell Biol. (H. G. Schweiger, ed.) Springer, Berlin, p. 941.
———. 1983 Physiol. Chem Phys. & Med. NMR 15: 337.
———. 1984 Scanning Electron Microscopy II: 875.
———. 1986 in *Science of Biological Specimen Preparation*, (M. Müller, R. P. Becker, A. Boyde and J. J. Wolosewick, eds.) SEM Inc., AMF O'Hare, Chicago, Ill., p. 33.
———. 1886a Scanning Electron Microscopy, SEM Inc., Chicago, Ill. IV: 1337.
———. 1988 Scanning Microscopy 2: 851.
———. 1991 Scanning Microscopy 5: S-75.
———. 1994 Scanning Microscopy 8: 67.
———. 1996 Scanning Microscopy 10: 295.
———. 1998 Scanning Microscopy 12: 1.
(127) Gregor, H. 1948 J. Amer Chem. Soc. 70: 1293.
———. 1951 J. Amer. Chem.Soc. 73: 642.
(128) Fletcher, W.M. and Hopkins, F.G. 1907 J. Physiol.(London) 35: 247.
(129) Edelmann, L. 1989 Scanning Microscopy 3: 1219.
(130) Gulati, J., Ochsenfeld, M.M. and Ling, G. N., 1971 Biophys. J. 11: 973.
(131) Ling, G. N., Miller, C. and Ochsenfeld, M. M. 1973 Ann. N.Y. Acad. Sci. 204: 6.
(132) Lipmann, F. 1941 Adv. Enzymol. 1: 99.
(133) Podolsky, R. J. and Morales, M. F. 1956 J. Biol. Chem., 218: 945.
(134) George, P. and Rutman, R. J. 1960 Prog. Biophys. Biophys. Chem. 10: 1.
(135) Baker, P. F., Hodgkin, A. L. and Shaw, T. I. 1961 Nature 190: 885.
Oikawa, T., Spyropoulos, C. S., Tasaki, I. and Teorell, T. 1961 Acta Physiol. Scand. 52: 195.
(136) Ling, G. N., 1965 Persp. Biol. Med. 9: 87.
(137) Baker, P. F., Foster, R. F., Gilbert, D.S., and Shaw, T.I. 1971 J. Physiol. (London) 219: 487.
(138) Ling, G. N. and Negendank, W. 1980 Persp. Biol. Med. 23: 215.
(139) Ling, G. N., and Tucker, M. 1983 Physiol. Chem. Phys. & Med. NMR 15: 311.
(140) Ling, G. N., Zodda, D. and Sellers, M. 1984 Physiol. Chem. Phys. & Med. NMR 16: 381.

(141) Cope, F. W. 1967 Bull. Math. Biophys. 29: 583.
(142) Murphy, Q. R. (Ed.) 1957 *Metabolic Aspects of Transport across Cell Membrane*, Univ. of Wisconsin Press, Madison.
(143) Bregman, J. I. 1953 Ann. N. Y. Acad. Sci. 57: 125.
(144) Teunissen, P. H., Rosenthal, S. and Zaayer, W. H., 1938 Rec. Trav. Chim. Pays Bas. 57: 929.
(145) Ling, G. N., 1955 Fed. Proc. 14: 93.
(146) Eisenman, G., Rudin, D. O. and Casby, J. U. 1957 Paper presented at the 10th Annual Conference on Electrical Techniques in Medicine and Biology of the A.I.E.E., I.S.A. and I.R.E., Boston, November 1957, not printed.
Rudin, D.O., and Eisenman, G. 1959 Abstract, 21st Intern. Congress Physiol. Sciences, p. 237.
(147) Ling, G. N. 1960 J. Gen. Physiol. 43 (Suppl).: 149.
(148) Eisenman, G. (ed.) 1967 *Glass Electrodes for Hydrogen and Other Cations; Principles and Practices*, Marcel Dekker, New York.
(149) Anfinson, C. B. 1962 Brookhaven Symp. Biol. 15: 184.
———. 1967 Harvey Lectures 61: 95.
(150) Ling, G. N. 1986 Physiol. Chem. Phys. & Med. NMR 18: 3.
(151) Chou, P. Y. and Fasman, G. D. 1978 Adv. Enzymol. 47: 45.
(152) Tanaka, S. and Scheraga, H. A. 1976 Macromol. 9: 168.
(153) Garnier, J., Osguthorpe, D. J. and Robson, B. 1978 J. Mol. Biol. 120: 97.
(154) Ling, G. N. 1965 Ann. N. Y. Acad. Sci. 125: 401.
(155) Ling, G. N. 1972 in *Water and Aqueous Solutions, Structure, Thermodynamics, and Transport Processes* (A. Horne, ed.) Wiley-Interscience, New York, pp. 663–699.
(156) Ling, G. N., Niu, Z. and Ochsenfeld, M.M. 1993 Physiol. Chem Phys. & Med. NMR 25: 177.
(157) Veis, A. 1964 *The Macromolecular Chemistry of Gelatin*, Academic Press, New York.
(158) Ling, G. N. 1985 in *Water and Ions in Biological Systems* (A. Pullman, V. Vasilescu and L., Packer, eds.), Plenum Press, New York. (2nd Intern. Conf. Bucharest, Sept. 6–11,1982) pp. 79–94.
(159) Ling, G. N. and Negendank, W. 1970 Physiol. Chem. Phys. 2: 15.
(160) Ling, G. N. 1983 Physiol. Chem. Phys. & Med. NMR 15: 155.
(161) Ling, G. N. and Ochsenfeld, M. M. 1987 Physiol. Chem. Phys. & Med. NMR 19: 177.
(162) Ling, G. N., and Zhang, Z. L. 1983 Physiol. Chem. Phys. & Med. NMR 15: 391.
(163) Ling, G. N. and Hu, W. H. 1987 Physiol. Chem Phys. & Med. NMR 19: 251.
(164) Ling, G. N. and Murphy, R. C. 1983 Physiol. Chem. Phys. & Med. NMR 15: 137.
(165) Clegg, J. S., Szwarnowski, S., McClean, V.E.R., Sheppard, R. J. and Grant, E. H. 1982 Bioch. Biophys. Acta 721: 458.
Clegg, J. S., McClean, V.E. R., Szwarnowski, S. and Sheppard, R. J. 1984 Phys. Med. Biol. 29: 1409.
(166) Trantham, E. C., Rorschach, H. E., Clegg, J. C., Hazlewood, C. F. Nicklow, R M. and Wakabayashi, N. 1984 Biophys. J. 45: 927.
Heidorn, D. B., Rorschach, H. E., Hazlewood, C. F., Ling, G. N. and Nicklow, R. M. 1986 Biophys. J. 49: 92A.
(167) Ludwig, C. 1849 Z. ration. Med. von Henle 8: 1.
(168) Ling, G. N. 1993 Physiol. Chem. Phys. & Med. NMR 25: 145.
(169) Ling, G. N. 1987 Physiol. Chem. Phys. & Med. NMR 19: 193.
(170) Ling, G. N. and Hu, W. 1988 Physiol. Chem. Phys. & Med. NMR 20: 293.
(171) Ling, G. N. and Hu, W. (to be published).
(172) Ling, G. N. and Ochsenfeld, M. M. 1983 Physiol. Chem. Phys. & Med. NMR 15: 127.
(173) Ling, G. N. 1969 Intern. Rev. Cytology 26: 1.
(174) Ling, G. N. 1988 Physiol. Chem Phys. & Med. NMR 20: 281.
(175) Ling, G. N. and Ochsenfeld, M. M. 1989 Physiol. Chem. Phys. & Med. NMR 21: 19.
(176) Luyet, B. J. and Hartung, M. C. 1941 Biodynamica 3: 353.
Polge, C., Smith, A. U. and Parks, A. S. 1949 Nature 164: 666.

Rall, W. F. 1987 Cryobiology 24: 387.
(177) Szent-Györgyi, A. 1978 *The Living State and Cancer*, Marcel Dekker Inc. New York.
(178) Rushbrooke, G. S. 1949 *Introduction to Statistical Mechanics*, Clarendon Press, Oxford.
Myers, J. E. and Meyer, M. G. 1940 *Statistical Mechanics*, John Wiley & Sons, New York.
Fowler, R. and Guggenheim, E. A. 1960 *Statistical Thermodynamics: A Version of Statistical Mechanics for Students of Physics and Chemistry*, Cambridge University Press, Cambridge.
(179) Ernst, E. and Hazlewood, C.F. 1979 Inorganic Perspectives in Biol and Med 2: 181.
(180) Pollack, G. H. 1990 *Muscle and Molecules: Uncovering the Principles of Biological Motion*, Ebner & Sons, Seattle.
———. 2001 *Cells, Gels and the Engine of Life, A New Unified Approach to Cell Function*, Ebner & Sons, Seattle, WA, USA.
(181) Barcroft, J. 1928 *The Respiratory Function of the Blood*, Part II. *Hemoglobin*, The Cambridge University Press, London.
(182) Forbes, W. H. and Roughton, F. J. W. 1931 J. Physiol. (London) 71: 229.
(183) Hill, A. V. 1910 J. Physiol. (London) 40: iv.
Hill, R. and Wolvekamp, H. P. 1936 Proc. Roy. Soc. (London) B120: 484.
(184) Wyman, J. 1964 Adv. Protein Chem. 19: 223.
(185) Yang, C. N. and Ling, G. N., in Ling, G.N. 1964 Biopolymers (Biophys. Symp. Issue) 1: 91.
(186) Ling, G. N. 1970 Proc. Nat. Acad. Sci. 67: 296.
(187) Bohr, C. 1890 Z. physiol. Chem. 4: 249.
(188) Manwell, C. 1958 Science 127: 593.
(189) Chanutin, A. and Curlish, R. R. 1967 Arch, Biochem. Biophys. 121: 96.
Chanutin, A. and Hermann, E. 1969 Arch. Biochem. Biophys. 131: 180.
Klinger, R. G. , Zahn, D. P., Brox, D. H. and Frunder, H. E. 1971 Europ. J. Biochem 18: 171.
(190) Ling, G.N. and Bohr, G. 1971 Physiol. Chem. Phys. 3: 431.
(191) Miseta, A., Bogner, P., Berényi, E., Kellermayer, M., Galambos, S. and Wheatley, D. N. and Cameron, I. L. 1993 Biochem. Biophys. Acta 1175: 133.
(192) Bull, H. 1944 J. Amer. Chem. Soc. 66: 1499.
Dole, M. and Faller, I. L. 1950 J. Amer. Chem. Soc. 72: 414.
Katchman, D. and McLaren, A. D. 1951 J. Amer. Chem. Soc. 73: 2124.
McLaren, A. D. and Rowen, J. W. 1951 J. Polymer Sic. 7: 289.
Hnojewyj, W. S. and Reyerson, L. H. 1959 J. Phys. Chem. 63: 1653.
Eley, D. D. and Leslie, R. B. 1964 in *The Structure and Properties of Biomolecules and Biological Systems*, pp. 238–258.
D'Arcy, R. L. and Watt, I. C. 1970 Trans. Farad. Soc. 66: 1236.
Leeder, J. D. and Watt, I. C. 1974 J. Coll. Interfac. Sci. 48: 339.
(193) Speakman, J. B. 1944 Trans. Farad. Soc. 40: 6.
Leeder, J. D. and Watt, I. C. 1965 J. Phys. Chem. 69: 3280.
(194) Katz, J. R. 1919 Kolloidchem. Beih. 9: 1.
(195) Ling, G. N. 1987 Physiol. Chem. Phys. & Med. NMR 19: 159.
(196) von Korösy, K. 1914-1915 Z. physiol. Chem. 93: 154.
(197) Hodgman, C. D., Weast, R.C. and Selby, S.M. (Ed.) 1961 *Handbook of Chemistry and Physics*, 43rd Ed., The Chemical Rubber Publ. Co., Cleveland, Ohio.
(198) Ling, G. N. and Peterson, K. 1977 Bull. Mathem. Biol. 39: 721.
(199) Ling, G. N. and Kwon, Y. 1983 Physiol.. Chem. Phys. & Med. NMR 15: 239.
(200) Singer, S. J. and Nicolson, G. L. 1972 Science 175: 720.
(201) Ling, G. N., Ochsenfeld, M.M. and Karreman, G. 1967 J. Gen. Physiol. 50: 1807.
(202) Ling,. G. N. and Reisin, I. L. 1973 Physiol. Chem. Phys. 5: 183.
(203) Caillé, J. P. and Hinke, J. A. M. 1974 Canad. J. Physiol. Pharmacol. 52: 814.

(204) Abetsedarskaya, L. A., Miftakhutdinova, J. G. and Fedotov, V. D. 1968 Biofizika 13: 630; Biophysics (English Transl.) 13: 750.
(205) Finch, E. D. , Harmon, J. F. and Muller, B. H. 1971 Arch. Biochem. Biophys. 147: 299.
(206) Walter, J. A. and Hope, A. B. 1971 Austral. J. Biol. Sci. 24: 497.
(207) Bunch, W. H. and Kallsen, G. 1969 Science 164: 1178.
(208) Ling, G. N. 1987 Physiol. Chem. Phys. & Med. NMR 19: 199.
(209) Miyamoto, V. K., and Thompson, T. E. 1967 J. Coll. Interf. Sci. 25: 16.
Miyamoto, V. K. 1966 *DC Electrical Properties of Lipid Bilayer Membranes*, Ph.D Thesis, The Johns Hopkins University, Baltimore.
(210) Andreoli, T. E., Bangham, J. A. and Tosteson, D. C. 1967 J. Gen. Physiol. 50: 1729.
Andreoli, T. E., Tiffenberg, M. and Tosteson, D. S. 1967 J. Gen. Physiol. 50: 2527.
(211) Ling, G. N. and Ochsenfeld, M.M. 1986 Physiol. Chem. Phys. & Med. NMR 18: 109.
(212) Stillman, I. M., Gilbert, D. L., and Robbins, M. 1970 Biochem. Biophys. Acta 203: 338.
(213) Maloff, B. L., Scordillis, S. P., Reynold, C. and Tedeschi, H. 1978 J. Cell Biol. 78: 199.
(214) Fleischer, S., Fleischer, B. and Stoeckenius, W. 1967 J. Cell Biol. 32: 193.
(215) Napoliano, L., Le Baron, F., and Scaletti, J. 1967 J. Cell Biol. 34: 817.
Morowitz, H. J., and Terry, T.M. 1969 Biochim. Biophys. Acta 183: 276.
(216) Fox, S. W. 1973 Naturwissenschaften. 60: 359.
(217) Ling, G. N. 1973 Biophys. J. 13: 807.
(218) Ling, G. N. 1953 Proc. 19th Intern. Physiol. Congr., Montreal, Canada, p. 566.
(219) Ling, G. N. 1955 Amer. J. Phys. Med. 34: 89.
(220) Epstein, E. and Hagen, C.E. 1952 Plant Physiol. 27: 457.
(221) Conway, E., J. and Duggan, F. 1958 Biochem. J. 69: 265.
(222) Ling, G.N. and Ochsenfeld, M.M. 1965 Biophysical J. 5: 777.
(223) Ling, G.N. and Ochsenfeld, M.M. 1970 Physiol. Chem. Phys. 2: 189.
(224) Müller, P. 1975 Ann. N.Y. Acad. Sci. 264: 97.
(225) Ling, G.N. 1981 Physiol. Chem. Phys. 13: 356.
Ling, G.N. 1990 Scanning Microscopy 4: 723.
(226) Koefoed-Johnson, V. and Ussing, H.H. 1958 Acta Physiol. Scand. 42: 298.
(227) Maddrell, S.H.P. in *Membrane Transport in Biology* Vol. 3, pp. 250–251 (G. Giebisch, ed.) Springer, New York, p. 239.
(228) Spring, K.R. and Giebisch, G. 1977 J. Gen Physiol. 70: 307.
(229) Morel, F. 1961 in *Proc. Intern. Congr. Nephrology,* Karger, Basel, p. 16.
(230) Hodgkin, A.L. and Katz, B. 1949 J. Physiol (London) 108: 37.
(231) Goldman, D.E. 1943 J. Gen. Physiol. 27: 37.
(232) Bernstein, J. 1912 *Elektrobiologie*, F. Vieweg und Sons, Braunschweig.
(233) Hodgkin, A.L. and Katz, B. 1949 J. Physiol. (London) 109: 240.
(234) Adrian, R. H. 1956 J. Physiol. (London) 133: 631.
(235) Hodgkin, A. L. and Horowicz, P. 1960 J. Physiol. (London) 153: 404.
(236) Hodgkin, A.L. 1958 Proc. Roy. Soc. (London) Ser. B 148: 1.
(237) Katz, B. 1966 *Nerve, Muscle and Synapse*, McGraw Hill, New York.
(238) Kernan, R.P. 1970 in *Membranes and Ion Transport*, Vol. 1 (E. E. Bittar ed.) Wiley-Interscience, New York, p. 395.
(239) Baur, E. 1913 Z. Elektrochem. 19: 590.
(240) Akaike, N. 1975 J. Physiol. (London) 245: 499.
(241) Baur, E. and Kronmann, S. 1917 Z. physik. Chem. 92: 81.
(242) Ehrensvard, G. and Sillen, L. G. 1938 Nature 141: 788.
(243) Colacicco, G. 1965 Nature 207: 936.
(244) Ling, G. N. 1966 Fed. Proc. (Symposium Issue) 25: 958.
(245) Ling, G. N. 1959 Fed. Proc. 18: 371.
(246) Edelmann, L.1973 Ann. N.Y. Acad. Sci. 204: 534.
(247) Ling, G.N. <http://www.gilbertling.org>

(248) Ling, G.N. 1979 Physiol. Chem. Phys. 11: 59.
(249) Armstrong, C. M. 1975 in *Membranes: A Series of Advances*. Vol. III (G. Eisenman ed.) Academic Press, New York, p. 325.
(250) Hille, B. 1975 in *Membranes: A Series of Advances*. Vol. III (G. Eisenman, ed.) Academic Press, New York, p. 255.
(251) Hodgkin, A.L. and Huxley, A. F. 1952 J. Physiol. (London) 116: 449.
(252) Hodgkin, A.L. , Huxley, A. F. and Katz, B., 1952 J. Physiol. (London) 116: 424.
(253) Hodgkin, A.L. and Huxley, A. F. 1945 J. Physiol. (London) 104: 176.
(254) Shores, L. 1961 *Collier's Encyclopedia* Vol. 16, p. 217 Cromwell-Collier Publ. Co., New York.
(255) Chandler, W. K. and Meves, D. E. 1965 J. Physiol (London) 180: 788.
(256) Ling, G. N. 1957 Fed. Proc. 16: 81.
(257) Ling, G. N. 1982 Physiol. Chem. Phys. 14: 47.
(258) Hille, B. 1968 J. Gen. Physiol. 51: 221.
(259) Hille, B. 1975 Fed. Proc. 34: 1318.
(260) Stillman, I. M., Gilbert, D. L. and Lipidsy, R.J. 1971 Biophys. J. 11: 55a.
(261) Khodorov, B. I. 1978 in *Membrane Transport Process* Vol. 2 (D.T. Tosteson, A. Yu. Ovchinnikov and R. Latorre, eds.) Raven Press, New York, p. 153.
(262) Villegas, R., Blei, M. and Villegas, G. M., 1965 J. Gen. Physiol. 48: 35.
(263) Hodgkin, A. L. and Keynes, R. D. 1953 J. Physiol (London) 119: 513.
(264) Kushmerick, M. J. and Podolsky, R. J. 1969 Science 166: 1297.
(265) Höber, R. 1913 Pflüger's Arch. Ges. Physiol. 150: 15.
(266) Thomson, D. L. 1928 J. Physiol. (London) 65: 214.
Hartree, W. and Hill, A. V. 1921 Biochem. J. 15: 379.
(267) Edelmann, L. 1986 Science of Biological Specimen Preparation, SEM Inc., AMF O'Hare, Chicago, pp. 33–42, Fig. 4.
(268) Levi, H. and Ussing, H.H. 1948 Acta Physiol. Scand. 16: 232.
(269) Robertson, J. D. 1950 Progr. Biophys. Biophys. Chem. 10: 343.
Sjöstrand, F.S. 1990 *Deducing Function from Structure, Vol. 1: A Different View of Membranes,* Academic Press, San Diego, p. 224.
(270) Kushmerick, M. J. 1979 Trends Biochem. Sci. Lett. Ed. (TIBS) 3: N210.
(271) Ling, G.N. 1979 Trends Biochem. Sci. Lett. Ed. (TIBS) 4: N134.
(272) Lohmann, K. 1929 Naturwissenschaften 17: 624.
———. 1931 Biochem. Z. 233: 460.
(273) Lehninger, A. L. 1975 *Biochemistry*, 2nd ed. Worth Publishers, New York.
(274) Jones, A. W. 1965 Ph. D. Thesis, Univ.of Pennsylvania, Philadelphia.
(275) Minkoff, L. and Damadian, R.V. 1973 Biophys. J. 13: 167.
———. 1974 Biophys. J. 14: 69.
(276) Ling,. G. N. and Cope, F. W. 1969 Science 163: 1335.
(277) Crank, J. 1956 *The Mathematics of Diffusion*, Clarendon Press, Oxford.
(278) Bradley, R.S. 1936 J. Chem. Soc. 1936: 1799.
(279) Ling, G. N. and Bohr, G. 1970 Biophys. J. 10: 519 .
(280) Ling, G. N. and Bohr, G. 1971 Physiol. Chem. Phys. 3: 573.
(281) Skou, J. C. 1965 Physiol. Rev. 45: 596.
(282) Edelmann, L. 1991 Scan. Microsc. 5: S-75.
(283) Engelhardt, V. A. and Ljubimova, M.N. 1939 Nature 144: 668.
(284) Morales, M. and Botts, J. 1953 Disc. Farad. Soc. 13: 125.
(285) Terner, C., Eggleston, L. V. and Krebs, H.A. 1950 Biochem. J. 47: 139.
(286) Koechlin, B.A. 1955 J. Biophys. Biochem. Cytol. 1: 511.
(287) Goody, R.S., Hofmann, W. and Mannherz, H.G. 1977 Europ. J. Biochem. 78: 317.
Cardon, J. W. and Boyer, P.D. 1978 Europ. J. Biochem. 92: 443.
(288) Lowey, S. and Luck, S.M. 1969 Biochem. 8: 3195.
Marsh, D.J., De Bruin, S.H. and Gratzer, W. B. 1977 Biochem. 16: 1738.

(289) Ling,. G. N. 1981 Physiol. Chem. Phys. 13: 29.
(290) Ling, G.N. and Woodbury, W. 1949 J. Cell. Comp. Physiol. 34: 407.
Coraboeuf, E. and Weidemann, S. 1954 Helv. Physiol. Acta 12: 32.
(291) Hodgkin, A. L. and Katz, B. 1949 J. Physiol. (London) 109: 240.
(292) McDonald, J. S. 1900 Proc. Roy. Soc. (London) 67: 310.
Curtis, H. J. and Cole, K. S. 1942 J. Cell. Comp. Physiol. 19: 135.
Ling, G.N. and Gerard, R. W. 1950 Nature 165: 113.
(293) Huxley, A.E. and Stämpfli, R. 1951 J. Physiol. (London) 112: 496.
(294) Nastuk, W. L. and Hodgkin, A. L. 1950 J. Cell. Comp. Physiol. 35: 39.
(295) Ling, G. N. 1980 in *Cooperative Phenomena in Biology* (G. Karreman, ed.) Pergamon Press, New York.
(296) Ling, G. N. and Walton, C. L. 1976 Science 191: 295 .
(297) Miller, C. and Ling, G. N. 1970 Physiol. Chem. Phys. 2: 495.
(298) Luyet, B. and Rapatz, G. 1956 Biodynamica 8: 1.
(299) Odeblad, E., Bhar, B. N. and Lindstrom, G. 1956 Arch. Biochem. Biophys. 63: 221.
Bratton, C. B. Hopkins, A. L. and Weinberg, J. W. 1965 Science 147: 738.
(300) Cope, F. W. 1969 Biophys. J. 9: 303.
(301) Hazlewood, C.F., Nichols, B. L. and Chamberlain, N. F. 1969 Nature 222: 747.
Hazlewood, C. F. 1979 in *Cell Associated Water* (W. Drost-Hanson and J.S. Clegg, eds.) Acad. Press, New York, p. 165.
Hazlewood, C.F. 1973 Ann. N.Y. Acad. Sci. 204: 593.
(302) Damadian, R. 1971 Science 171: 1151.
(303) Kaatze, U. 1975 Adv. Molecular Relaxation Process 7: 71.
(304) Rorschach, H. E. 1984 in *Water, Ions in Biological Systems*, (V. Vasilescu Ed.) Plenum Press, New York.
(305) Kleinfield, S. 1985 *A Machine Called Indomitable*, Times Books, New York.
<http://www.fonar.com>.
(306) Ling, G. N., Ochsenfeld, M.M., Walton, C. and Bersinger, T. J. 1980 Physiol. Chem. Phys. 12: 3.
Ling, G. N., Walton, C. and Bersinger, T. J. 1980 Physiol. Chem. Phys. 12: 111.
(307) Ling, G. N., Walton, C.L. and Ochsenfeld, M.M. 1981 J. Cell. Physiol. 106: 385.
(308) Lamarck, J. B. 1830 *Philosophie zoologique au exposition des considérations relatives à l'histoire des animaux* (Paris, Nouvelle éd.) (First published in 1809). 1: 403.
Landrieu, M. 1797 *Lamarck* p. 161, excerpting his *Memoires de physique et d'histoire naturelle*.
(309) Hall, T. S. 1969 *Ideas of Life and Matter. Studies in the History of General Physiology: 600B.C.–1900 A.D.*, Univ. of Chicago Press, Chicago, Vol. 1.
(310) Hill, D. K. 1962 J. Physiol. (London) 164: 31.
(311) Renkin, E.M. 1954 J. Gen. Physiol. 38: 225.
(312) Ling, G.N. and Ochsenfeld, M.M. 1973 Science 181: 78.
(313) Ling, G. N. and Ochsenfeld, M.M. 1973 Ann. N. Y. Acad. Sci. 204: 325.
(314) Ling, G. N., Baxter, J. D. and Leitmann, M.I. 1984 Physiol. Chem. Phys. & Med. NMR 16: 405.
(315) Chambers, R. 1922 J. Gen. Physiol. 5: 189.
(316) MacDonald, J.S. 1900 Proc. Roy. Soc. (London) 67: 310.
(317) Ling, G.N. 1981 *Die Zelle, Struktur und Funktion* (H, Metzner, ed.), 3rd ed., Wissenschft. Verlag, Stuttgart, Germany, pp. 356–389.
(318) Meyerhof, O. and Lohman, K. 1932 Biochem. Z. 253: 431.
(319) Teunissen, P. H. and Bungenberg de Jong, H.G. 1949 Kolloid Beih. 48: 33.
(320) Collander, R. 1959 in *Plant Physiology*, vol. 2 (F.C. Steward, ed.) Academic Press, New York, p. 3.
(321) Bungenberg de Jong, H. G. 1932 Protoplasma 15: 110.
(322) Perutz, M. 1978 Science 201: 1189.

(323) Müller, O. F. 1786 *Animalcula infusioria*, Hausiae.
(324) Lepeschkin, W.W. 1930 Protoplasma 9: 269.
Duclaux, J. 1934 *Actualités scientif.*, Herman édit., Paris, 127, pp. 1–43.
Guilliermond, A. 1941 Chron. Botan. Co., Mass., pp. 1–246.
Oparin, A. I. 1941 *The Origin of Life on the Earth* (in Russian), Moscow-Leningrad, pp. 1–268.
(325) Lepeschkin, W.W. 1936 Biodynamica 19: 1.
(326) Schneider, D. 1997 Scientific American 276: 3.
(327) Edelmann, L. 1989 Scan. Microsc. Suppement 3: 241.
(328) Page, L. and Adams, N.I. 1931 *Principles of Electricity. An Intemediate Text in Electricity and Magnetism,* D. Van Nostrand Co., Inc. New York.
(329) Perutz, M. F., Muirhead, H., Cox, J.M., Goaman, L.C.G., Mathews, F.S., McGandy, E.L., and Webb, L. E. 1968 Nature 219: 29.
Perutz, M.F. 1969 Proc. Roy. Soc. B173: 113.
(330) Sollner, K., Abrams, I. and Carr, C. W. 1941 J. Gen. Physiol. 24: 467; 25: 7.
(331) Neville, M.C. 1973 Ann. NY Acad.Sci. 204: 538.
(332) Kizel', A.P. 1940 *The Chemistry of Protoplasm (Khimiya protoplasmy)*, Moscow-Leningrad, pp. 1–624.
Nasonov, D.N. and Alexandrov, V. Ya. 1940 *The Reaction of Living Matter to External Agencies*, Moscow pp. 1–252.
(333) Dean, R. 1941 Biol. Symp. 3: 331.
(334) Jacobson, B. 1955 J. Amer. Chem. Soc. 77: 2919.
(335) Mendelsohn, E. 1963 *Cell Theory and the Development of General Physiology*, Arch. intern. d'histoire de sciences 6: 419.
(336) Ling, G.N. and Kromash, M.H. 1967 J. Gen. Physiol. 50: 677.
(337) Lewis, G.N. 1923 *Valence and the Structure of Atoms and Molecules*, Chemical Catalogue, New York.
(338) Hammett, L.P. 1970 *Physical Organic Chemistry*, 2nd ed., McGraw Hill, New York.
Branch, G.E.K. and Calvin, M. 1941 *The Theory of Organic Chemistry, An Advanced Course*, Prentice Hall, Englewood Cliffs, New Jersey.
Dewar, M.J.S. 1949 *The Electronic Theory of Organic Chemistry*, Oxford University Press, London.
Smith, R.P., Rhee, T. Magee, J. L. and Eyring, H. 1951 J. Amer. Chem. Soc. 73: 2263.
Ingold, C.K. 1953 *Structure and Mechanism in Organic Chemistry*, Cornell Univ. Press, Ithaca.
Taft, R.W. 1960 J. Phys. Chem. 64: 1805.
Chiang, M.C. and Tai, T. C. 1963 Scientia Sinica 12: 785.
Chiang, M. C. 1987 *The Role of Homologous Linearity of Organic Compounds*, Science Press, Beijing.
(339) Mattson, J. and Simon,. M. 1995 *The Pioneers of NMR and Magnetic Resonance in Medicine: The Story of MRI*, Bar-ilan Univer. Press and Dean Books Co., Jericho, NY.
(340) Ling, G.N. and Tucker, M. 1980 J. Nat. Cancer Inst. 64: 1199.
Ling, G. N. 1983 Physiol. Chem. Phys. & Med. NMR 15: 511.
Ling,. G.N., Kolebic, T. and Damadian, R.V. 1990 Physiol. Chem. Phys. & Med. NMR 22: 1.
(341) Hodgkin, A. L. 1971 *The Conduction of the Nervous Impulse*, Liverpool Univ. Press, Liverpool.
(342) Baker, P. F. and Shaw, T. I. 1965 J. Physiol. (London) 180: 424.
(343) Schultz, R. D. and Asunmaa, S. K. 1969 Recent Progr. Surf. Sci. 3: 291.
(344) Jain, M. K. 1972 *The Bimolecular Lipid Membrane: A System*, Van Nostrand-Rheinhold, New York.
(345) Burger, A. 1960 *Medicinal Chemistry*, 2nd ed., Interscience Publishers, Inc., New York.
(346) Cohen, I.B. 1985 *Revolution in Science*, Harvard Univ. Press, Cambridge.

(347) Engelmann, T.W. 1873 Pflügers Arch.ges. Physiol. 7: 33, 155.
(348) <http://www.gilbertling.org/lp11.htm>
(349) <http://post.queensu.ca/ ~forsdyke/peerrev.htm>
(350) <http://www.gilbertling.org/lp12.htm>
(351) Gary-Bobo, C.M. and Lindenberg, A.B. 1969 J. Coll. Interf. Sci. 29: 702.
(352) Rothschuh, K.E. 1973 *History of Physiology*, (English transl. by G.B. Risse), Krieger Publ.Co., Malabar. Florida.
(353) Ising, E. 1925 Z. Phys. 31: 253.
(354) Landau, L.D. and Lifschitz, E.M. 1969 *Statistical Physics,* 2nd ed. Pergamon, London.
(355) Dyson, F.J. 1969 Commun. Math. Phys. 12: 91.
(356) Griffith, R.B. 1969 *Phase Transitions and Critical Phenomena*, vol 1, pp. 89–94, Academic Press., New York , p. 7.
(357) Hill, A.V. 1932 Physiol. Rev. 12: 56.
(358) Greenlee, R. T. 2000 *Cancer Statistics, 2000*, CA, Cancer Journal for Clinicians 50: 7.
(359) Overton, E. 1902 Pflügers Arch. Ges. Physiol. 92: 346.
(360) Nernst, W. 1892 Z. physik. Chem 9: 137.
(361) Bendall, J. R. 1969 *Muscles, Molecules and Movement: An Essay in the Contraction of Muscles*, Amer. Elsevier Publ. Co, New York.
(362) Lark-Horovitz, K. 1931 Nature 127: 440.
(363) Schrödinger, E. 1944 *What is Life? The Physical Aspect of the Living Cell,* Cambridge Univ. Press, Cambridge (Canto Edition 1992).
(364) Van Doren, C. 1991 *A History of Knowledge, Past Present and Future*, Bird Lane Press/Carol Publ. Group, New York.
(365) Einstein, A. 1956 *Out of My Later Years*, Wings Books, New York.
(366) Williams, L. P. 1965 *Michael Faraday, A Biography*, Da Capo Press, Inc., a Subsidiary of Plenum Publ. Co., New York.
(367) Singer, C. 1915 "The Dawn of Microscopic Discoveries", J. Roy. Microsc. Soc. pp. 317–340.
(368) Hooke, R. 1665 *Micrographia; or, Some Phyiological Descriptions of Minute Bodies Made by Magnifying Glasses with Observations and Inquiries Thereupon*, London.
(369) Selye. H. 1978 *The Stress of Life*, McGraw-Hill, New York (paperback ed.).
(370) Nagy, S., Paál, M., Köszegi, T. Ludány and Kellermayer, M. 1998 Physiol. Chem. Phys. & Med. NMR 30: 141.
(371) Infante, A.A., and Davies, R. C. 1962 Biochem. Biophys. Res. Comm. 9: 410.
(372) Lundsgaard, E. 1930 Biochem. Z. 227: 51.
(373) Nicolsky, B.P. 1937 Acta Physicochimica. U.R.S.S. 7: 597.
(374) Horovitz, K. 1923 Z. Physik. 15: 369.
(375) Horovitz, K. 1925 Z. physik. Chem. 115: 424.
———. 1925 Sitzb. Akad. wiss. Wien, Abt. IIa, 134, 335.
(376) Ling, G. N. 1967 in *Glass Electrodes for Hydrogen and Other Cations* (G. Eisenman, Ed.) Marcel-Dekker, New York, pp. 284–292.
(377) Ling, G. N. and Fisher, A. 1983 Physiol. Chem. Phys. 15: 369.
(378) Menten, M. L. 1908 Trans. Canad. Inst. 8: 403.
(379) Priestley, J. 1767 *The History and Current State of Electricity*, 1st ed. 2 volumes, London.
(380) Horgan, J. 1996 *The End of Science: Facing the Limits of Knowledge in the Twilight of the Scientific Age*, Addison-Wesley, Reading, Mass.
(381) Ling, G.N. and Bohr, G. 1969 Physiol. Chem. Phys. 1: 591.
(382) Conway. E.J. 1957 in *Metabolic Aspects of Transport across Cell Membranes* (Q. R. Murphy, Ed.) Univ. Wisconsin Press, Madison, Wisc., pp. 107–111.
(383) Fick, A. 1882 *Mechanische Arbeit und Wärmeentwickelung bei der Muskelthätigkeit.*, Brockaus, Leipzig.
(384) Jacobson, B. 1953 Nature 172: 666.
(385) Bernal, J.D. and Fowler, R.H. 1933 J. Chem. Phys. 1: 515.

(386) Forslind, E. 1952 Acta Polytechnica 3 No. 5.
(387) Szent-Györgyi, A. 1957 *Bioenergetics*, Acad. Press, New York.
(388) Klotz, I.M. 1958 Science 128: 815.
(389) Chambers, R. and Hale, H.P. 1932 Proc. Roy. Soc (London) Ser. B 110: 336.
(390) Wilde, W.S. and O'Brien, J.M. 1953 Proc. 19th Intern. Physiol. Congr., Montreal, p. 889.
(391) Ernst, E. and Hazlewood, C.F. 1978 Inorg. Persp. Biol. & Med. 2: 27.
(392) Edelmann, L. 1984 Scann. Electron Microsc. II; 875.
———. 1989 Scann Microsc. Suppl. 3: 241.
———. 1991 J. Microsc. 161: 217–228, p. 219, p. 226.
(393) Cannon, C. G. 1955 Mikrochim Acta 2–3, 555.
(394) Mizushima, S., Tsuboi, M., Shimanouchi, T., and Tsuda, Y. 1955 Spectrochimica. Acta 7: 100.
(395) Bamford, C.H., Elliott, A. and Hanby, W.E. 1956 *Synthetic Polypeptides, Preparation, Structure and Properties,* Acad. Press, New York, pp. 197–199.
(396) Wolfenden, W. 1978 Biochem. 17: 201.
(397) Klotz, I. M. 1973 Ann. NY Acad. Sci. 226: 18.
Aizono, Y., Roberts, J.E., Sonnenberg, M. and Swislocki, N. I. 1974 Arch. Biochem. Biophys. 163: 634.
Imae, T., Fasman, G.D., Hinkle, P.M. and Tashjian, A.H. 1975 Bioch. Biophys, Res. Comm. 62: 923.
(398) Ling, G. N. and Fu, Y. 1988 Physiol. Chem. Phys. & Med. NMR 20: 61.
(399) Ernst, E.., Tigyi, J. and Zahorcsek, A. 1950 Acta Physiol. Acad. Sci. Hung. 1: 5.
———. 1951 Acad. Physiol. Acad. Sci. Hung. 2: 78.
(400) Hagen, R.D. 1986 *Windows to the Origins*, Naval Research Reviews 38: 4.
(401) Michaelis, L. 1926 Naturwissenschaften 14: 33.
(402) Fujita, A. 1926 Biochem. Z. 179: 18.
(403) Collander, R. and Bärlund, H. 1933 Acta Botan. Fennica. 11: 1.
(404) Woolf, H. B. (Editor-in-Chief) 1977 *Webster's New Collegiate Dictionary*, G.C. Merriam Co., Springfield, Mass.
(405) Andreoli, T. E., Bangham, J. A. and Tosteson, D.C. 1967 J. Gen. Physiol. 50: 1729.
(406) Müller, P. Rudin, D.O., Tien, H. T. and Wescott, W.C. 1962 Nature 194: 979.
———. 1963 J. Phys. Chem 67: 534.
———. 1964 Rec. Prog. Surface Sci. 1: 379.
(407) Tien, H. T. 1967 J. Phys. Chem. 71: 3395.
Cherry, R. J. and Chapman, D. 1969 J. Mol. Biol. 40: 19.
(408) Cole, K.S. 1932 J. Cell. Comp. Physiol. 1: 1.
(409) Harvey, E. N. 1931 Biol. Bull. 60: 67.
(410) Ueda, T., Muratsugu, M., Inoue, I. and Kobatake, Y. 1974 J. Mol. Biol. 18: 177.
(411) Inoue, I., Ueda, T., and Kobatake, Y. 1973 Bioch. Biophy. Acta 298: 653.
(412) Wood, E.H., Collins, D.A. and Moe, G.K. 1940 Amer. J. ,Physiol. 128: 635.
(413) Miyake, M., Inoue, I. and Kobatake, Y. 1973 Bioch. Biophys. Acta 323: 367.
(414) Inoue, I., Ishida, N. and Kobatake, Y. 1973 Bioch. Biophys. Acta 330: 27.
(415) Inoue, I., Ishida, N. and Kobatake, Y. 1974 Bioch. Biophys. Acta 367: 24.
(416) Koppenhöfer, E. 1974 Pflügers Arch. ges. Physiol. 347: 71 (R36).
(417) Zwaal, R.A., Roelofson, B. and Colley, C.M. 1973 Bioch. Biophys. Acta 300: 159.
(418) Adrian, R.H. 1956 J. Physiol.(London) 133: 631.
Baker, P.F., Hodgkin, A.L. and Shaw,T.I. 1961 Nature 190: 885.
Hagiwara, S., Chichibu, S., Naka, K.I. 1964 J. Gen. Physiol. 48: 163.
Sato, M., Akaike, N. and Nishi, R. 1967 Kumamoto Med. J. 20: 39.
(419) Tobias, J. M. 1950 J. Cell. Comp. Physiol. 36: 1.
Falk, G. and Gerard, R.W. 1954 J. Cell. Comp. Physiol. 43: 393.
Grundfest, H., Kao, C.Y., and Altamirano, M. 1954 J. Gen. Physiol. 38: 245.

Kao, C.Y. 1956 Biol. Bull. 111: 292.
Shaw, F.H. and Simon, S.E. 1955 Austral. J. Exp. Biol. Med. Sci. 33: 153.
Koketsu, K. and Kimura, Y., 1960 J. Cell. Comp. Physiol. 55: 239.
Tasaki, I., and Takenaka, T. 1964 Proc. Nat. Acad. Sci. USA 52: 804.
Hazlewood, C.F. and Nichols, B.L., 1969 Johns Hopkins Med. J. 125: 119.
Thomas, R.C. 1972 Physiol. Rev. 52: 563.
Ling, G.N. and Fisher, A. 1983 Physiol. Chem. Phys. & Med. NMR 15: 369.
(420) Berthelot, M. and Jungfleisch, H. 1872 Ann. Chim. Phys. 26: 396.
Nernst, W. 1891 Z. physik. Chem. 8: 110.
(421) Scheraga, H. A. 1974 Current Topics Biochem. 1973: 1.
Finkelstein, A.V. and Ptitsyn, O.B. 1971 J. Mol. Biol. 62: 613.
(422) Ruhland, W. 1909 Jahrb. wiss. Bot. 46: 1.
———. 1912 Jahrb. wiss. Bot. 51: 376.
Ruhland, W. and Hoffmann, C. 1925 Planta 1: 1.
(423) Ling, G.N., Will, S. and Shannon, P. 1969 Physiol. Chem. Phys. 1: 355.
(424) Fischer, E. 1906 Ber. dtsch chem. Ges. 39: 530.
———. 1907 Sitzb. kgl. preuss. Akad. Wiss. (1907): 35; (1916): 990.
(425) Ling, G.N., Walton, C., and Bersinger, T. J. 1980 Physiol. Chem. Phys. 12: 111.
(426) Watt, I.C. and Leeder, J. D. 1964 Trans. Farad. Soc. 60: 1335.
(427) Ling, G. N., Neville, M., Shannon, P. and Will, S. 1969 Physiol. Chem. Phys. 1: 42.
(428) Ling, G. N. and Will, S. 1969 Physiol. Chem. Phys. 1: 263.
(429) Ling, G. N. and Will, S. 1976 Physiol. Chem. Phys. 8: 115.
(430) Kolber, A. R. and Stein, N. D. 1966 Nature 209: 691.
(431) Trombitás, C. and Tigyi-Sebes, A. 1979 Acta Physiol. Acad. Sci. Hung. 14: 271.
(432) Edelmann, L. 1983 Physiol. Chem. Phys. & Med. NMR 15: 337.
(433) von Zglinicki, T. 1988 Gen. Physiol. Biophys. 7: 495.
(434) Somlyo, A. V., Gonzales-Serratos, H., Shuman, H., McClellan, G. and Somlyo, A.P. 1981 J.Cell. Biol. 90: 577.
(435) Wood, R.E., Wirth, F. P. and Morgan, H. E. 1968 Bioch. Biophys. Acta 163: 171.
(436) Rossini, F.D., Knowlton, J.W. and Johnston, H.L. 1940 J. Res. Nat. Bur. Stand. 24: 369.
(437) Woessner, D.E. and Snowden, B.S. 1973 Ann. N.Y. Acad. Sci. 204: 113.
(438) Nathanson, A. 1904 Jahrb. wiss. Bot. 39: 607; 40: 403.
(439) Ling, G.N. 1966 Fed. Proc. (Symposium) 25: 958.
(440) Eastman, Geiling and DeLawder 1961 quoted in *Function of the Blood* (Macfarlane, R.G. and Robb-Smith, A.H.T.) Academic Press, New York, Fig. 18 in Chapter 1.
(441) Hagiwara, S. 1960 in *Electrical Activity of Single Cells* (Y. Katsuki, ed.), Igaku Shoin Ltd, Tokyo, p. 145.
(442) Matsuda, K. 1960 in *Electrical Activity of Single Cells* (Y. Katsuki, ed.), Igaku Shoin, Ltd., Tokyo, p. 283.
(443) Lassen, U. V. and Rasmussen, B.E. 1978 in Membrane Transport in Biology (D.C. Tosteson, ed.), Vol. 1, Springer Verlag., New York, p. 201.
(444) Fischer, E. 1894 Ber. dtsch. chem. Ges. 27: 2985.
(445) Henri, V. 1902 C.R. Acad. Sci., Paris 135: 916.
(446) Dixon, M. and Webb, E.C. 1958 *Enzymes*, Academic Press, New York.
(447) Clark, A.J. 1926 J. Physiol. (London) 61: 547.
———. 1933 *The Mode of Action of Drugs on Cells*, 3rd ed., Edward Arnold, London.
———. 1937 Quart. J. exp. Physiol. 27: 375.
(448) Ariëns, E.J., van Rossum, J.M. and Simonis, A.M. 1956 Arzneimittel-Forsch. 6: 282, 611, 737.
(449) Bowen, W. J. and Kerwin, T.D. 1955 Proc. Soc. Exp. Biol. Med. 89: 515.
(450) Mommaerts, W.F.H.M. and Green, I. 1954 J. Biol. Chem. 208: 833.
(451) Wakabayashi, M. and Fishman, W.H. 1961 J. Biol. Chem. 236: 996.

(452) Reichard, P., Baldesten, A. and Rutberg, L. 1961 J. Biol. Chem. 236: 1150.
(453) Ling, G. N. 1964 Texas Report Biol. Med. 22: 244.
(454) van Rossum, J. M. and Ariëns, E. J. 1958 Arch. Intern. Pharmacodynam. 118: 393.
(455) Kühne, W. 1878 Unters. physiol. Inst. Univ. Heidelberg, 1: 291.
(456) Cohen, G. N. and Monod, J. 1957 Bact. Rev. 21: 169.
(457) Saladino, A.J., Bentley, P. J. and Trump, B.F. 1969 Amer. J. Pathol. 54: 421.
(458) Noonan, T.R., Fenn, W.O. and Haege, L. 1941 Amer. J. Physiol. 132: 612.
(459) Woods, A. 1820 Athenae Oxonienses, "new" ed. , London 4: col. 628.
(460) Brisseau de Mirbel, C.F. 1809 *Exposition de la théorie de l'organisation Végétale*, Paris.
(461) Treviranus, G. R. 1805 Biologie, oder Philosophie der lebenden Natur für Naturforscher und Aerzte, 3: 233, Göttingen.
(462) Link, H. F. 1809 *Grundlehren der Anatomie und physiolgie der Pflänzen*, pp 11–13, Leipzig.
(463) Wilson, J. M. 1944 "Cellular Tissue and the Dawn of Cell Theory", Isis 35: 168.
(464) Manuel, F.E. 1968 *A Portrait of Issac Newton*, Belknapp Press of Harvard Univ., Cambridge, Mass. Da Capo Press, Plenum, New York (paper-back ed.).
(465) Solomon, A. K. 1952 J. Gen. Physiol. 36: 57.
(466) Cremer, M. 1906 Z. Biol. 47: 562.
(467) Linderstrøm-Lang, K. U. 1924 Compt. rend, trav. lab. Carlsberg, Sér chim 15 No. 7.
(468) Haber, F. and Klemensiewicz, Z. 1911 Z. Physk. Chem. 78: 228.
(469) Kern, W. 1948 Makromol. Chemie 2: 279.
(470) Harkins, W. D. 1945 Science 102: 292.
(471) Freedman, J. C. 1976 Biochem Biophys Acta 455: 989.
(472) Cope, F. W. 1967 J. Gen. Physiol. 50: 1353.
(473) Jardetsky, O. and Wertz, J. E. 1960 J. Amer. Chem. Soc. 82: 318.
(474) Berendsen, H. J. C. and Edzes, H. T. 1973 Ann. N.Y. Acad. Sci. 204: 459.
(475) Abragam, A. 1961 *The Principles of Nuclear Magnetism*, Clarendon Press. Oxford.
(476) Cohen, M.H. and Reif, F. 1957 Solid State Physics 5: 321.
(477) Brand, J. C. C. and Speakman, J. C. 1960 *Molecular Structure: The Physical Approach* Edward Arnold, London.
(478) Ling, G. N. and Zhang, Z. L. 1983 Physiol. Chem. Phys. & Med. NMR 15: 251.
(479) Lindblom, G. 1971 Acta Chem. Scand. 25: 2767.
(480) Ling, G.N., Will, S. and Shannon, P. 1969 Physiol. Chem Phys. 1: 355.
(481) Stone, F.W. and Stratta, J.J. 1967 *Encyclopedia of Polymer Science and Technology*. vol. 6, pp.103–145, John Wiley and Sons, Inc., New York p. 113.
(482) Liebig, J. 1862 Annalen 121: 78.
(483) Davson, H. and Danielli, J.F. 1943 *The Permeability of Natural Membranes*, 2nd ed., Cambridge University Press, London.
(484) Beatley, E.H. and Klotz, I. M. 1951 Biol. Bull. 101: 215.
(485) Pauling, L. 1960 *The Nature of theChemical Bond*, 3rd Ed., Cornell Univ. Press, Ithaca, NY.
(486) Jost, WE. 1960 *Diffusion in Solids, Liquids and Gases*, Academic Press, New York.
(487) Skriver, W., Maunsbach, A.B. and Jørgensen, P.L. 1980 J. Cell. Biol. 86: 746.
(488) Ling, G. N. 1964 Biopolymers (Biophysics Symposium Issue) 1: 91.
(489) Hodgkin, A.L. and Horowicz, P. 1959 J. Physiol. (London) 148: 127.
(490) Ling, G.N. and Schmolinski, A. 1956 Fed. Proc. 15: 120.
(491) Gutknecht, J., Hastings, D.F. and Bisson, M.A. 1978 in *Membrane Transport in Biology*, Vol. 3 , pp. 125–170 (G. Gibiesch ed.) Springer Verlag, New York.
(492) Brooks, S.C. 1939 J. Cell. Comp. Physiol. 14: 383.
———. 1940 Cold Spring Harbour Symp. Quant. Biol. 8: 171.
(493) Chance, B. and Yoshioka, T. 1966 Arch. Biochem. Biophys. 117: 451.
(494) Lardy, H. A. and Graven, S. N. 1965 Fed. Proc. 24: 424.
(495) Pressman, B.C. 1965 Proc. Nat. Acad. Sci. USA 53: 1076.
(496) Ling, G.N. 1981 Physiol. Chem. Phys. 13: 29.

(497) Falcone, A.B. and Hadler, H. I. 1968 Arch. Biochem. Biophys. 124: 91.
(498) Gylkhandanyan, A. V., Evtodienko, Yu. V., Zhabotinsky, A.M. and Kondrashova, M. N. 1976 FEBS Lett. 66: 44.
(499) Hopfer, U., Lehninger, A. L. and Lenarz, W. J. 1970 J. Biol. Med. 2: 41.
Ohki, S. 1972 Biocheim. Biophys. Acta 282: 55.
MacDonald, R.C. and Bangham, A.D. 1972 J. Memb. Biol. 7: 29.
(500) Burnstock, G. 1958 J. Physiol. (London) 143: 183.
(501) Martell, A.E. and Smith, R. M. 1974 *Critical Stability Constants*, Vol. 1, Amino Acids, Plenum Press, New York.
(502) Ling, G.N., Walton, C. and Ling, M.R. 1979 J. Cell. Physiol. 101: 261.
(503) Ling, G. N., Walton, C. and Ochsenfeld, M.M. 1983 Physiol. Chem. Phys. & Med. NMR 15: 379.
(504) Abood, L.G., Koketsu, K. and Noda, K. 1961 Amer. J. Physiol. 200: 431.
Koketsu, K. 1966 Pers. Biol. Med. 9: 54.
(505) Aickin, C. 1986 Ann. Rev. Physiol. 48: 349.
(506) Taft, R.W. 1953 J. Amer. Chem. Soc. 75: 4231.
(507) Chiang, M. C. and Tai, T.C. 1963 Sci. Sin. 12: 785.
(508) Eastoe, J. E. 1955 Biochem. J. 61: 589.
(509) Chang, T. and Penefsky, H. S. 1973 J. Biol. Chem. 248: 2746.
(510) Reiser, A. 1959 in *Hydrogen Bonding* (D. Hadži and H. W. Thomspon, eds.) Pergamon Press, New York, pp. 443–447.
(511) Kossiakoff, A. and Harker, D. 1938 J. Amer. Chem. Soc. 60: 2047.
(512) Coulson, C.A. 1959 in *Hydrogen Bonding* (D. Hadži and H. W. Thompson, eds.) Pergamon Press, New York, pp. 339–360.
(513) Burawoy, A. 1959 in *Hydrogen Bonding* (D. Hadži and H. W. Thompson, Eds.), Pergamon Press, New York, pp. 259–276.
(514) Baur, E. 1913 Zeitshr. f. Elektrochem. 19: 590.
(515) Loeb, J. and Beutner, R. 1913 Biochem. Z. 51: 295, 296.
Beutner, R. 1914 Z. Physik. Chem. 87: 392.
(516) Ling, G.N. and Walton, C. 1975 Physiol. Chem Phys. 7: 215.
(517) Ling, G.N. 1970 Physiol. Chem. Phys. 2: 242.
(518) Michaelis, L. and Perlzweig, W. A. 1927 J. Gen. Physiol. 10: 575.
(519) Ling, G.N. 1967 in *Thermobiology* (A. H. Rose, ed.) Academic Press, New York, pp. 5–24.
(520) Dawson, W.R. and Schmidt-Nielson, K. 1964 in *Handbook of Physiology* (D.B. Dill, ed) Amer. Physiol. Soc., Washington pp. 481–492.
(521) Belding, H. S. 1967 in *Thermobiology* (A. H. Rose, ed.) Academic Press, New York, pp. 479–510.
(522) Hammett, L.P. 1940 *Physical Organic Chemistry*, McGraw Hill, New York.
(523) Tasaki, I. and Iwasa, K. 1980 Biol. Bull. 159: 494.
Tasaki, I. Iwasa, K. and Gibbons, R.C. 1980 Jap. J. Physiol. 30: 897.
(524) Tasaki, I and Byrne, P.M. 1994 Physiol. Chem. Phys. & Med. NMR 26: 101.
(525) Nakao, M., Nakao, T., Yamtzoe, S. and Yoshikawa, H. 1973 J. Biochem. (Tokyo) 49: 487.
(526) Arist. Metaph. 983b.
(527) McKie, D. 1952 *Antoine Lavoisier*, Da Capa Press, a Subsidairy of Plenum Publ. Co., New York.
(528) Dubos, R. 1950 *Louis Pasteur; Free Lance of Science*, Da Capa Press, a subsidiary of Plenum Publ. Co. New York.
(529) Bronowski, J. 1973 *The Ascent of Man*, Little, Brown & Co., Boston.
(530) Ling, G. N. 1994 Physiol. Chem. Phys. & Med. NMR 26: 121.
(531) Weissbluth, M. 1974 *Hemoglobin: Cooperativity and Electronic Properties*, Springer Verlag, New York.

(532) McBain, J. W. 1932 *The Sorption of Gases and Vapors by Solids*, George Routledge and Sons, London.
(533) de Boer, J, H, and Zwikker, C. 1929 Z. Physik. Chem. B3: 407.
(534) Brunauer, S., Emmett, P.H. and Teller, E. 1938 J. Amer. Chem. Soc. 60: 309.
(535) Mellon, E.F., Korn, A.H. and Hoover, S.R. 1948 J. Amer. Chem. Soc. 70: 3040.
(536) Perutz, M.F. 1969 Proc. Roy. Soc. B173: 113.
(537) von Helmholtz, H. 1881 in: *The Modern Development of Faraday's Conception of Electricity*, The Faraday Lecture, delivered before the Fellows of the Chemical Society in London on April 5, 1881.
(538) Schultze, H.E. and Heremans, J.F. 1966 *Molecular Biology of Human Proteins*, Vol 1, Elsevier Publ. Co., New York, p. 4.
(539) Schatzmann, H, J, 1953 Helv. Physiol. Pharmecol. Acta 11: 346.
Dunham, E.T. and Glynn, I. M. 1961 J. Physiol. (London) 156: 274.
Bonting, S.L. and Caravaggio, L.L. 1963 Arch. Biochem. Biophys 101: 37.
Bonting, S. L. in *Membrane and Ion Transport*, Vol. 1 (E.E. Bittgar, ed.) Wiley-Interscience, New York, p. 257.
(540) Ling, G.N. and Palmer, L. 1972 Physiol. Chem. Phys. 4: 517.
(541) Yang, C.N. 1972 in *Phase Transition and Critical Phenomena* (C. Domb and M.S. Green eds.), Academic Press, New York, pp. 1–5.
(542) Quinke, G. 1898 Ann. Physik 64: 618.
(543) Ling, G. N. 1979 in *The Aqueous Cytoplasm* (A.D. Keith, ed.) Marcel Dekker, New York.
(544) Korn, E.D. 1978 Proc. Nat. Acad. Sci. 75: 588.
(545) Ingber, D.E. and Folkman, J. 1989 in *Cell Shape, Determinants, Regulation and Regulatory Role* (W.D. Stein and F.B. Bronner, eds.) Academic Press, San Diego.
(546) Fischer, M.H. and Moore, G. 1907 Amer. J. Physiol. 20: 350.
(547) Ling, G.N. <http://www.gilbertling.org/lp12.htm>
(548) Lauffer, M.A. 1975 *Entropy-Driven Processes in Biology: Polymerization of Tobacco Mosaic Virus, Proteins and Similar Reactions*, Springer Verlag. New York.
(549) Ling, G.N. and Ochsenfeld, M.M. 1977 Physiol. Chem. Phys. 9: 427.
(550) Ling, G.N. 1957 in *Metabolic Aspects of Transport across Cell Membranes* (Q.R. Murphy, ed.) Univ. Wisconsin Press, Madison, Wisc., pp. 181–186.
(551) Bruno, L.D. 2001 Newsday (Long Island, N.Y.), January 15, p. C-17, Section "Not for Profit."
(552) Haseltine, E. 2000 Discover (Twentieth Anniversary Issue), October Issue, p. 84.
(553) Shute, N., 2001 U.S. News and World Report, February Issue p. 44.
(554) McQueen, A. 2000 The Philadelphia Inquirer June 28, page A4 C.
(555) <http://project2061.aaas.org/newsinfo/press/r1000627.htm>
(556) Dawson, R. 1981 *Confucius*, Oxford University Press, Oxford.
(557) Lyster, R.L.J. 1955 Ph.D. Thesis, Cambridge University Cambridge, England.
Rossi-Fanelli, A., Antonini, E. and Caputo, A. 1984 Adv. Protein Chem. 19:73.

Author Index

Subject Index

Acknowledgments

First of all, I want to thank my father (Yentse Ling) and mother (Chilan) with infinite love and boundless sadness—for both have passed away years ago after protracted and agonizing illness of tuberculosis.

Louis Pasteur (1822–1895), the famous French scientist-inventor, seemed such a distant figure from the vantage point of a little boy in China. Yet such is the global nature of science that his invention of the rabies vaccine could very well have saved my life when I was only three-year old. A grown-up friend of the family was also bitten by my pet dog turned rabid, but did not receive the Pasteur vaccine treatment I did. He died a terrible death shortly afterward.

I acknowledge with gratitude generous gifts of more than one kind from the United States. Firstly by returning the Boxer Redemption Fund to China; and with the funds thus made available, America helped to establish what is known as the Boxer Scholarship.[107 p xviii n 1] Through this window of opportunity, fresh college graduate like myself could participate in the (sixth and last) nationwide competitive examination held across all China and win the highly-coveted funding and support to continue graduate study in America. I also thank the United States for accepting me and my wife, Shirley sixteen years later as US citizens and for providing the opportunities for me to pursue full-time basic scientific research with no major interruption to this very day—a privilege very few in this world have enjoyed.

I also want to express my indebtedness for their teaching and guidance to Professor C. Oh-Yang, Head of the Biology Department of the National Central University in Chungking, to Professor Pei-sung Tang of the Tsing Hua University and sponsor of my graduate work at Ta Pu Chi Research Laboratory and to Professor R. W. Gerard of the Department of Physiology at the University of Chicago

I thank Professor C.N. Yang of what is now the C.N. Yang Institute of Theoretical Physics at the State University of New York at Stony Brook, my graduate-school roommate, fellow member of the physics slot of the 6th contingent of Boxer Scholars, Nobel laureate for physics in 1957 (see Preface). He was not only a life-long friend but a generous and dependable source of knowledge on theoretical physics whenever I needed help.

Dr. Frank Elliott, a brilliant neurologist, invited me and my (then) little crew in 1961 to join his staff of researchers at the Pennsylvania Hospital, the Nation's First Hospital, then in the midst of a renaissance. It was under his protective wing, that my associates and I continued our research for 27 years. Financial support for our research at that time came primarily from the Office of Naval Research (ONR) and the National Institute of Health (NIH). To show my appreciation of all his help and friendship, I dedicated my second book,

In Search of the Physical Basis of Life[15] to him and his brilliant and independent-minded wife, Gee, who had since then passed away.

The prevailing way of allotting public funds to support research depends on what is known as the *peer review system*. This system strongly favors researchers pursuing studies along conventional line of thinking. But it has not yet found a way wisely to support those like myself whose research findings dictate a direction contradictory to that of the majority view. Nonetheless, the very fact that my work had received support for as long as it did, was to no small measure due to a pair of intelligent, fair-minded and courageous scientists-administrators, Dr. Arthur Callahan of ONR and Dr. Stephen Schiaffino of NIH. When they eventually left their posts and some lesser successors took their places, I knew the end was near. Both ONR and NIH withdrew all their support in the fall of 1988. Our laboratory, at the height of its productivity, was forced to close. Suddenly I realized that it could be the end of the association-induction hypothesis.

But that was not going to be—due to the intervention of one single man. At this critical juncture, my friend Dr. Raymond Damadian came to our rescue. In seven big trucks, workers of his small NMR-manufacturing Fonar Corporation came and transported all our belongings to Melville on Long Island, New York. Henceforth Fonar, though itself struggling to survive, has been magnanimously providing shelter and support for myself and my two associates, Margaret Ochsenfeld and Dr. Zen-dong Chen.

Needless to say that without this support, not only would this book never come into existence, even the 1992 book[107] would probably never have been published. Thus it was four years after our arrival on Long Island did I succeed in signing a contract with R.A. Krieger Co., to publish the volume while the rest of the forty-some publishing houses I accosted all declined. My thanks to the Krieger Publishing Co., also include their granting me permission to reproduce in the present volume, seven figures from the 1992 book, even though substitutes are used instead.

I dedicated this 1992 book, "A Revolution in the Physiology of the Living Cells" to Dr. Raymond Damadian and his wife, Donna, to express my deep appreciation of all he has done for me and for making possible the survival of the association-induction hypothesis and what it stands for. I also thank the many friendly members of the Fonar Corporation who have made our stay on Long Island welcome, joyful and productive. They include Tim Damadian, President and CEO of HMCA, a health management subsidiary of Fonar, Jevan Damadian and his wife, Louise; Fred Peipman, Henry Myers, Sol Ginzberg, Dave Terry, Dan Culver, Lou Bonanni, Jay Butterman, Jay Dworkin, Joe Colombo, Lou Corradeno, Gregory Eydelmann, Jan Votruba, Guo Ping Zhang, Claudette Chan, Philip Cheang, Frank Vieira, Bob Capon, Doug Warner, Guy Goss, JoAnn Hirsch and many others. In particular, I want to thank Kurt Reimann, Michael Guarino and Bernie LoCurto as well as our librarians, Pat Gitomer and Tony Colella, whose help in the writing and publishing of this book is deeply appreciated.

To continue pursuing my research work at a time when the majority of one's peers subscribe to a scientific view directly opposite, has never been easy. That applies not only to myself but to a number of my friends who too challenged the conventional view. They include (the late) Dr. Freeman Cope, Dr. Carlton Hazlewood, (the late) Dr. George Karreman, Dr. Miklós Kellermayer and Dr. Ludwig Edelmann. To their dedication, and their scientific brilliance, I acknowledge with admiration and gratitude. (For more details on their respective ordeals, see my web site listed as Reference 247). The reader of this volume

will find case after case of their important and often heroic efforts to stand by what they believe to be the truth.

Difficulty in obtaining financial support is only one problem all of us faced. The other difficulty was finding a scientific journal willing to publish our (dissenting) views and findings. The situation had become worse and worse with time after the 1960's. Thus it is with deep gratitude that I acknowledge the introduction in 1969 of the scientific journal, then called Physiological Chemistry and Physics by (the late) Dr. Adam Lis and Diane McLaughlin. From its inception, this journal, differs from many others in allowing the author to dispute on reasonable scientific grounds the (unfavorable) verdict of reviewers . In addition, he or she has the right to publish a short priority note if the paper is eventually rejected. Later the ownership of the journal was transferred to Drs. Raymond Damadian and Freeman Cope. With Cope's passing in 1982, Dr. Damadian became its sole owner, and soon after the journal changed its name to Physiological Chemistry Physics and Medical NMR. [I have been its co-Editor-in-Chief from 1982 to 1985 with Dr. Bill Negendank and its (only) Editor-in-Chief since 1986.]

Then there are another category of scientists who have also made major contributions to this volume for their original figures, tables or written statements reproduced *(verbatim)* in this volume. For most of these reproduced materials I have obtained signed permissions from the copyright owners. In these cases, my acknowledgment of their permission are given either at the end of the legend of the figure or table involved, or on the copyright page for verbatim quotations of written passages. Other materials, however, have presented difficulty—as in the cases where the book in which the copyrighted materials is presented has gone out of print and the author—who automatically received the copyright—has also passed away many years ago. Attempts to locate a possible heir to the deceased author got me nowhere. What should I do?

It seems that I need the advise from a greater wisdom. And for me, it is not far to find. When asked, Confucius's disciple, Tseng Tse answered that the Master's teaching can be summarized in just two words: *Chung* meaning to exhaust everything that is in yourself; *Shu* meaning to put yourself in the other's position. Or, don't do to others what you don't like done to yourself. Chung, we certainly have achieved already. Shu tells me that I should publish the quoted materials as I planned. Here is how I see it.

Lao Tse once told Confucius: "As years go by, even our bones will disappear. The only things that will endure are words." If I were in the position of my departed scientist friend whose "words"are in books long out of print and thus likely on the way to oblivion, would I object to someone wanting to reproduce *verbatim* my words (after giving me full credit for its rightful original authorship)? Of course, Not. More than that. I would be most grateful.

Thus assured, I now cite the names of three unreachable authors and locations of their sayings quoted verbatim in this volume: H.G. Bungenberg de Jong [14.1(1)]; H.E. Schultze and J. F. Heremans [16.3] (and others).

With all these going on in my mind, I was most delighted to come upon the following passage in a book on Confucius written by Raymond Dawson and published by the Oxford University Press.[556] It says that after Jesuits priests brought back what they learned about China, *Confucius himself has been described as the patron saint of the Enlightenment.* Enlightenment, of course, is the intellectual movement that played a major role in promoting the development of modern science. After all, Chung and Shu are not that

different from what the West calls *sportsmanship* and *fair play*, ethical standards that have provided the quidelines for both football (soccer) and science. (See Epilogue.)

I now turn to the many other individuals who have worked hard to produce the body of scientific work under the umbrella of the association-induction hypothesis. It gives me great pleasure to recognize all of them here. First my succession of able secretaries, starting out with Betty Jane Breucker, it continues with Shirley Ripka, Diane Weinstein, Nina Primakov, Nancy Hunt, Janet Malseed, Marilyn DeFeo and Jean Brogan. Then there is my long list of skilled and dedicated research assistants: Arlene Schmolinske, Mary Carol Williams, Margaret Samuel, Margaret Ochsenfeld, Marilyn Welsh, Jeannie Chen, Betty Kalis, Marsha Hurok, Patricia Shannon, Grace Bohr, Ellen Ferguson, Cheryl Walton, (the late) Sandy Will, Ann Sobel, Kim Peterson, T. Jennine Bersinger, Diane Graham, Dee Zoda, Ya-tsen Fu, Ze-ling Niu, Randy Murphy, Marriane Tucker, Joanne Bowes. Diane Graham, John Greenplate, Christopher Reed, and Mark Sellers.

I also thank my postdoctoral fellows: Drs. Ignacio Reisin (from Buenos Aires), William Negendank, Wei-hsiao Hu (from the Chekiang Institute of Technology, China), Zheng-lian Zhang (from the Beijing Institute of Biophysics, China) and Zhen-dong Chen, all having made major contributions.

I am also indebted to my many summer students, who often succeeded in completing a piece of major work in just three months: Edward Rossomando, Harriet Wells, Victor Smolen, E. Denise Campbell, Susan Sneider, Young Kwon, John Baxter, Mark Whalen, Mark Ling, Tim Ling, Ken Weiss, Marion Kelly, David Blackman, Michael Balter, Jim Wood, Curtis Cooke, Michael Leitman, Andrew Fischer, Jim Wood, Bing-hong Cheak, Howard Dubner, Gerri Margavaro, Sharon Horowitz, Thea Kolebic, Jill Wright, Karen Holmes, Lisa DiSanto, Anna Marie Maguire and John O'Leary.

Special mention should be made of two colleagues in particular: Margaret Ochsenfeld and Dr. Zhen-dong Chen. First, Margaret has been working in my group since 1958. She and Dr. Chen are the only two of my staff at the Pennsylvania Hospital that had come to Long Island with me. They are now each a major component of the trio that makes up the staff of the non-profit Damadian Foundation for Basic and Cancer Research inside the Fonar Corporation. As managing editor of Physiological Chemistry Physics and Medical NMR, Margaret Ochsenfeld has taken over much of the work publishing this journal—which is vital to the survival of not only work associated with the AI revolution but work of other dissenting scientists who have done important work but could not get it published.

Both Margaret Ochsenfeld and Dr. Zhen-dong Chen are capable of the most demanding meticulous work which the reader will find throughout this volume often under the authorship of Ling and Ochsenfeld. Dr. Chen's most important work is yet to be published. But when it will be finally published, many would marvel at the phenomenal skill with which it has been achieved.

I am deeply indebted to Howard Bloom, Perry A. Chapdelaine, Sr., Michele Davis, Dr. Tony Chapdelaine, Dr. Ludwig Edelmann, Prof. Carlton Hazlewood, Dr. Tamas Henics, Prof. Harold Hillman, Prof. Miklós Kellermayer, Prof. Gerald Pollack, Dr. Hirohisa Tamagawa, Dr. Dominique Villey for their respective invaluable comments on the manuscript of this volume.

I would like to thank our typesetter, Chris Collins of Farm River Publications, Branford, Connecticut, Steve Kehoe of Cushing-Malloy Printing Co. of Ann Arbor, Michigan in

addition to Marlene Cenotti and Lisa Polizzo of the Advocate Press of New Haven, Connecticut for their patience and excellent skill in making the book a reality.

At last I am to acknowledge with boundless love and appreciation my capable and talented wife, pianist Shirley Ling. Aside from performing all the chores and business of running a household, she is a constant consultant to my scientific work, giving piano concerts at least once a year, year after year, beside teaching and training a truly impressive covey of talented young pianists. After moving my laboratory to Long Island, we soon found out that she had to endure the inconvenience of being alone in a big house in Philadelphia four days a week. She had borne this with courage and grace.

Then I look on with pride and thanks our three wonderful children, their spouses and children. Mark, who graduated from Harvard, had earned both a Ph.D. and M.D. degree at Duke University. With his wife Jenny who holds an MBA from the Wharton School of the University of Pennsylvania, they are now the co-CEOs of a dermatological drug testing firm, MEDAPHASE. Mark also practices Dermatology in suburban Atlanta. Our daughter, Eva, holds a master degree in physical medicine and practices in Philadelphia. Our second son Tim received his geology degree from Cornell, an MBA from Stanford and is now the President of the Unocal Oil company in Los Angeles. He and his wife Kim had made repeated contributions to the operating expenses of the non-profit Damadian Foundation of Basic and Cancer Research—much to the gratitude of myself and my colleagues. Last but the least I want to acknowledge the infinite joy we experienced from the arrivals and charming lives of our six grand children. Sydney and Graham (daughter and son of Mark and Jenny); Stephanie and Kasie (daughters of Eva and Neil Monahan) and Hudson and Tommy (sons of Tim and Kim).

February 28, 2001

About the Author

Born in Nanking, Gilbert Ling grew up and received his early schooling in Beijing and his Bachelor degree in Biology from the National Central University in Chungking in war-torn China. Shortly after college graduation, he took part in a nation-wide competitive examination and won the biology slot to continue education in the US. In early 1946 he began his graduate study in the Department of Physiology at the University of Chicago under the world-famous Professor Ralph W. Gerard. Completing his Ph.D. in 1948, he spent two more years under Prof. Gerard as a Seymour Coman Postdoctoral Fellow. In 1950 he got his first job as an Instructor at the Medical School of the Johns Hopkins University in Baltimore, a job he held until 1953 when he took a new offer back in Chicago again.

Three major events marked this three-year stay in Baltimore. First, on the basis of the results of some new experimental studies completed, he came to the conclusion that the traditional theory of the living cell could be in very serious trouble. Second, he offered a replacement—known later as Ling's Fixed Charge Hypothesis. The third major event began with the acceptance of a dinner-party invitation over the alternative of seeing a movie. Little did he know that at this party, he was to meet, and later marry the beautiful and talented Shirley Wong—once a school-mate of Ling's sister, Nancy, in Shanghai—, then a student at the Peabody Conservatory of Music of Baltimore, studying piano under M. Munz.

Ling's new job in Chicago offered him more opportunity to continue full-time research at the Neuropsychiatric Institute of the University of Illinois Medical School. Beginning as an Assistant Professor, he was promoted two years later to (tenured) Associate Professorship. In 1957 an even more attractive job lured him back to the East again. But before leav-

ing Chicago, he had the opportunity to lay down much of the groundwork for the major thesis he was to complete later.

At that time, the city of Philadelphia was undergoing a major renewal under the leadership of Joseph Clark and Richardson Dilworth. As part of this buildup, a brand-now 9-storied Eastern Pennsylvania Psychiatric Institute (EPPI) was erected, including on its 9th floor with a panoramic view, a superb Basic Research Department—where Ling (and his crew) was to continue his research as a Senior Research Scientist for five more years. At EPPI, he completed the writing of his first book. Warren Blaisdell, President of the newly-formed Blaisdell Publishing Co. (a division of Random House) came by and signed a contract to publish the volume under the title: "A Physical Theory of the Living State: the Association-Induction Hypothesis." The book appeared in print in 1962.

Unfortunately the nearly ideal environment at EPPI was not to endure. In 1961 the wonderful group of scientists gathered at the Basic Research Department was mostly on the way out.

Ling then had to make a critical decision. To accept a tenured full professorship offered him at Tulane University where he would have to spend a substantial part of his time teaching pharmacology. Or to live on uncertain research grants from there on—but to continue full-time research. With the support of his wife, he took the risky alternative—as the director of a still-nonexistent research laboratory at the Pennsylvania Hospital to be supported by research grant money yet to be applied for.

On the plus side of the new situation was Frank Elliott, M.D., a brilliant, witty, and influential research-oriented neurologist. It was under his protective wing, Ling began the next phase of his research career. Another plus for staying in Philadelphia was that the Lings could continue to live in their beloved 100-year-old Victorian house in the suburb, where they have raised their three children and where Dr. Ling had indulged in his love for growing roses—as many as over 70 bushes at one time—and for raising from seeds a vegetable garden every year.

Pennsylvania Hospital, founded by Benjamin Franklin and Thomas Bond, is America's First Hospital. At this juncture, the Hospital was itself undergoing a renaissance. With funds provided by the John A. Hartford Foundation (of the A and P fortune) and under the able guidance of its young and able President, H. Robert Cathcart, noted physicians from all over the world like Frank Elliott were invited to join its staff. So it was in a pervasive atmosphere of adventure and expectation that a first-rate research laboratory materialized in a stand-alone two-storied building—used at the time for storage but was once a part of a Catholic Orphanage. This was how the Department of Molecular Biology came into existence at the corner of 7th and Spruce Street in the Society Hill district of central Philadelphia.

It was here that Ling began his next 27 years of scientific research with his group of research assistants, a succession of secretaries, graduate students, postdoctoral students—from here and abroad. The membership of the group rose every summer with the arrival of summer students, who not only brought with them fresh enthusiasm and new talents but more often than not, actually contributed significantly to basic Science as witnessed by their names listed in the Author Index of this volume.

But to make this 27 years of continued scientific research possible, two exceptional scientist-administrators played key roles. They are Dr. Arthur Callahan of the Office of Naval Research and Dr. Stephen Schiaffino of the National Institute of Health. And their insistence on inviting only neutral reviewers to evaluate Ling's research proposals, for example.

In 1984, Ling published his second book, "In Search of the Physical Basis of Life." (Plenum Publ. Co.), summarizing rapidly gathering new knowledge from his laboratory and other investigators. Unfortunately, both Drs. Callahan and Schiaffino eventually retired. With their departure, the situation deteriorated rapidly. In October 1988, Ling's laboratory was forced to close by the simultaneous withdrawal of all financial support.

It was at this critical juncture, another exceptional person intervened. This is Dr. Raymond Damadian, the inventor of MRI and President of the MRI manufacturing company called Fonar—located at Melville on Long Island in New York. Henceforth, Fonar company has not only been supporting and sheltering Ling himself but also two of his staff: Margaret Ochsenfeld and Dr. Zhen-dong Chen.

Along with all the books, records, office and laboratory equipments, Ling also brought with him to Long Island the editorial office of the scientific journal, Physiological Chemistry & Physics and Medical NMR. He was a co-Editor-in-chief of this journal between 1982 and 1984. Since 1984, he has been its (only) Editor-in-Chief.

Since the teaching part of Mrs. Ling's teaching-concertizing career could not be moved to Long Island, the Lings decided to buy a condo in Kings Park about 20 minutes drive from Fonar. From here on, Dr. Ling has been commuting by car between Long Island and Philadelphia over each weekend.

In 1992 Ling published his third book, "A Revolution in the Physiology of the Living Cell." Five years after that, he began to write the present volume, planning to publish it this time by himself.

Pacific Press, Inc.

http://pacificpressnewyork.com

Quick Order Form

Fax orders:	631-390-0053. Send this form or Xerox copy.
Telephone orders:	877-390-7730 toll-free. Have your credit card ready.
Computer orders:	Email: pacificpressny@aol.com; Website: http://www.pacificpressnewyork.com
Postal orders:	Pacific Press, PO Box 1452-11, Melville, NY 11747

Please send me the following:

_____

_____

Name: _____

Address: _____

City:_____State:_____Zip _____ -_____

Telephone:_____

E-mail address: _____

Sales tax: Please add 8.5 % for products shipped to New York addresses. For a $19.95 book, the total is $21.65.

Priority shipping: US $4.00.

Payment: ❑ Cheque

❑ Credit card

Visa MasterCard AMEX Discover

Card number: _____

Name on card:_____Exp. date:_____ / _____

Signature: _____

Pacific Press, Inc.

http://pacificpressnewyork.com

Quick Order Form

Fax orders:	631-390-0053. Send this form or Xerox copy.
Telephone orders:	877-390-7730 toll-free. Have your credit card ready.
Computer orders:	Email: pacificpressny@aol.com; Website: http://www.pacificpressnewyork.com
Postal orders:	Pacific Press, PO Box 1452-11, Melville, NY 11747

Please send me the following:

_____

_____

Name: _____

Address: _____

City:_____State:_____Zip _____ -_____

Telephone:_____

E-mail address: _____

Sales tax: Please add 8.5 % for products shipped to New York addresses. For a $19.95 book, the total is $21.65.

Priority shipping: US $4.00.

Payment: ❑ Cheque

❑ Credit card

Visa MasterCard AMEX Discover

Card number: _____

Name on card:_____Exp. date:_____ / _____

Signature: _____