PREREQUISITES FOR CHEMICAL THERMODYNAMIC MODELS OF LIVING SYSTEMS

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A few years ago, several of us at The RAND Corporation were motivated to experiment with a method for quantifying chemical aspects of biological systems. The motivation was twofold. First, greatly improved mathematical and numerical techniques for the solution of chemical composition problems had been developed at RAND for other purposes. Second, the emerging space age presented an especially difficult challenge to the quantitative aspects of the biological sciences and medical arts.

The methodology that emerged from these encouraging first experiments involves the construction of mathematical models of biological subsystems or functions and their programming for solution by computers. In particular, the selected physiological functions are described in detail in terms of their essential biochemical reactions. These chemical reactions are rigorously interrelated through the laws of mass action and conservation of mass and charge. What is new are the mathematical techniques by which the hundreds of chemical reaction equations are interrelated and the programs developed that allow the problems to be solved by use of the computer.

At RAND and elsewhere, these methods have been used to construct and study models of increasingly complex physiological functions. Published reports of such studies and of the advances made in the mathematical bases and numerical
procedures related to the models* have thus far not included a general introduction and exposition of the subject directed at those who are unfamiliar with certain physiological or chemical concepts, yet wish to use the techniques or have a curiosity about them. This Memorandum attempts to provide such an introduction.

*See references.
SUMMARY

Certain physiological and numerical concepts are discussed which are helpful in constructing mathematical models of the chemistry of some functions or subsystems of living organisms, in particular of the human body. The ideas of models, systems, and subsystems are considered in the context of simulating physiological reality. Body compartments are seen as identical to the idea of chemical phases with the result that powerful analytical and computational methods can be used to determine the changing composition of large multicompartmented biological systems as they are stressed in various ways. Relations between compartments are described in conventional physiological terms and translated into more rigorous physicochemical statements, a prerequisite for constructing chemically interrelated models for solution on a computer.

Many problems facing the builders of physiological models are analogous to problems encountered by the laboratory or clinical investigator. Variability of individuals, design of preparations, and influences of difficultly controllable factors are examples of areas where the model builder can use stratagems evolved by clinical investigators to circumvent problems.

The proper understanding and relating of scales of concentration used in physiology and in biophysicochemical models are crucial for constructing internally consistent
and correct models. An illustrative model of respiratory gases interacting with an aqueous phase is constructed as an indoctrination in the appropriate determination of model parameters and in the interpretation of computer outputs. This model is subsequently used to solve relatively simple chemical problems related to physiology.
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CONTENTS

PREFACE ........................................ iii
SUMMARY ......................................... v
ACKNOWLEDGMENTS ................................. vii

Section
1. INTRODUCTION .................................. 1
   1.1. Computers and Physiology .................. 1
   1.2. Systems Analysis and Physiology ........... 3

2. THERMODYNAMICS VS KINETICS ................. 6
   2.1. Transient States ........................... 8
   2.2. Devices for Extending the Scope of Thermo-
       dynamic Methods for Simulating
       Physiological Systems ...................... 10
   2.3. Energy, Work and Power ................... 14

3. "STANDARD MAN" VS STATISTICAL VARIATION .... 17

4. BODY COMPARTMENTS ............................ 22

5. ADDITIONAL PERTINENT CHEMICAL CONCEPTS: COM-
   PONENTS, SPECIES, SUBSTANCES ................. 25

6. CHEMICAL GRADIENTS BETWEEN COMPARTMENTS .... 28
   6.1. Osmotic Equilibrium and Tonicity ......... 28
   6.2. Metabolic Processes ....................... 32
   6.3. Possible Influence of Temperature Gradi-
        ents ...................................... 34
   6.4. Gibbs-Donnan Equilibrium .................. 37
   6.5. Active Transport in Relation to Gibbs-
        Donnan Phenomena ........................... 39
   6.6. Activity Coefficients and Distribution
        of Uncharged Substances Between
        Compartment ................................ 46
   6.7. Some Influences of Large Molecular
        Species .................................... 49
   6.8. Starling's Hypothesis of Capillary
        Equilibrium ............................... 51

7. INFLUENCE OF ENZYMES AND HORMONES .......... 55

8. PHYSICAL PHENOMENA AND CHEMICAL MODELS .... 59

9. NUMERICAL PREREQUISITES FOR BUILDING A CHEMICAL
    MODEL ........................................ 62
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.1. The Mole as the Unit of Measure of Chemical Substance.</td>
<td>63</td>
</tr>
<tr>
<td>9.2. Molar and Molal Scales of Relative Composition and Concentration.</td>
<td>67</td>
</tr>
<tr>
<td>9.3. The Mole Fraction, Mole Ratio, and Miscellaneous Scales of Relative Composition and Concentration</td>
<td>72</td>
</tr>
<tr>
<td>9.4. Solubility Coefficients and Henry's Law Constants.</td>
<td>82</td>
</tr>
<tr>
<td>9.5. Activity Coefficients and Activity in Relation to Gas Solubility.</td>
<td>85</td>
</tr>
<tr>
<td>9.6. Additive Qualities of Chemical Stoichiometric Expressions.</td>
<td>87</td>
</tr>
<tr>
<td>10. CONSTRUCTING A MODEL OF SODA WATER AND GASES IN EQUILIBRIUM.</td>
<td>91</td>
</tr>
<tr>
<td>10.1. Selecting the Significant Chemical Species</td>
<td>92</td>
</tr>
<tr>
<td>10.3. Establishing Appropriate Standard Free Energy Parameters.</td>
<td>97</td>
</tr>
<tr>
<td>10.4. Output Format and Solution of Soda-Water Problem.</td>
<td>103</td>
</tr>
<tr>
<td>10.5. Experimenting with the Model--The Construction of Carbon Dioxide Dissociation Curves</td>
<td>111</td>
</tr>
<tr>
<td>11. EXPANDING THE SODA-WATER MODEL.</td>
<td>121</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>123</td>
</tr>
</tbody>
</table>
PREREQUISITES FOR
CHEMICAL THERMODYNAMIC MODELS OF LIVING SYSTEMS

1. INTRODUCTION

1.1. Computers and Physiology

Biophysical chemists, biologists, physiologists and research physicians are beginning to appreciate the potential of large computers. Computers have been less rapidly accepted in the natural sciences than in the physical sciences, business, and military analysis largely because of the complexity of living systems and the lack of suitable mathematical bases for constructing models of these systems that are sufficiently "robust" to reflect accurately the performance of the real system under changing conditions. The idea of building mathematical models of biological systems is, of course, not new. Lotka, Henderson, Michaelis, and other early investigators proposed formal mathematical models of biological systems or pioneered in the quantification of parts of such systems. There is a growing literature on the use of computers in association with such models.

Certain fundamentals of our approach to the modeling of physiological functions are not new but are similar to those described by L. J. Henderson in 1928 [1], who held that physiology's entrance to the society of the exact sciences could be accomplished by combining Claude Bernard's concepts of general physiology with the work of the mathematician Willard Gibbs. In his attempts to perform this
synthesis, Henderson developed many graphical and numerical techniques still in use today. He was, however, severely limited by the inadequacy of the scientific tools then available, in particular by the absence of the general-purpose computer and mathematical methods for optimization.

Underlying all our work is the dominant idea that the laws of chemistry when correctly formulated apply to a significant extent to physiological systems; that is to say, matter in a laboratory beaker, in the sands of the Sahara or in living protoplasm obeys the same laws to the extent that useful predictions can be made about the states of matter in all three systems as various state-determining parameters are altered. We grant that these laws may not be completely known and that their detailed application to these varied systems may be quite different. We also grant that the complexity of living systems is far greater than any other type of system which might be investigated and hence that the application of these chemical laws is apt to be difficult. It is a not uncommon experience of laboratory investigators that apparently "identical" objects* behave differently in the living subject (in vivo) than outside the living subject (in vitro). We would argue here that in most if not all of these cases the objects are not identical.

Even among the author and his associates there is

*Such "objects" outside the living system might be blood samples, cell cultures, or any of a number of physiological preparations.
a varying degree of hardness in the faith that all of nature can be quantified. On one extreme is the credo that "number rules the universe, and man, earthworm, and dandelion are of the universe." A more moderate view is that these quantitative techniques should be pushed to the biological or mathematical limit—whichever comes first—in the hope that mathematical models for increasingly large portions of the human system can be constructed.

1.2. Systems Analysis and Physiology

To study physiological problems we have attempted to simulate specific, carefully defined physiological functions or subsystems of the body rather than the operation of one or another organ or some class of chemical compounds or reactions. The definition of a proper subsystem for study poses problems that often require both judgment and empirical trial. In designing a mathematical experiment to be conducted on a computer one is forced to be absolutely explicit and quantitative about all features of the system under investigation—more so than for most other types of studies. There are guidelines, however, that can be used to aid in the selection and definition of the subsystem.

An obvious point in the selection of a system or problem for mathematical experimentation is that the system lie within the capabilities of the mathematical techniques and computer capacity available to the investigator. A judicious model builder would not select the simulation of the
whole human body in relation to the environment as his first experiment because such a task is probably beyond the present abilities of the mathematical art and capacity of computers.

One is forced, therefore, to select and define for study a part or subsystem of the whole which lies within the scope of the present technology. It is important that the subsystem be conveniently factorable from the larger system so that the subsystem can be studied over an interesting range of conditions when more or less removed from its normal environment. "More or less removed" means that if connections to the whole system--including the environment--cannot be completely severed, they may at least be approximated in the simulation. It is also important that the factored subsystem be selected so that changes induced in the subsystem do not cause significant alterations in related subsystems that are not represented in the simulation. Such alterations in related subsystems (or in the environment) can be such as to have important feedback effects on the subsystem under investigation through means not represented in the model. The simulated results obtained under these conditions may not, therefore, be a realistic representation of the in situ subsystem. Thus, individual organs are not often appropriate factorable subsystems for study by simulation.

Clearly one criterion for the selection of an appropriate
subsystem is that enough must be known about it so that there is some hope of defining its extent, its important features, and connections with other subsystems and with the environment. In gaining experience with mathematical simulations and developing techniques, well established data are crucial for determining the validity of one's methods. The final test of a proper choice and definition of a factorable subsystem is experimental. The important characteristics must be adequately represented by the model when parameters of the problem are altered realistically.

Modeled systems are conceptualizations of the real world. The type of models built will thus strongly reflect the capabilities, interests, and facilities available to their creators. As will become apparent, our methodologies have definite and specific limitations. There are certain types of investigations for which they are well suited and other types for which they are completely inappropriate. However, models such as those described here should become important additional tools for the understanding and quantification of biological phenomena. The application of systems analysis will not supplant the skills of the laboratory worker nor the judgment of the clinician. In other fields where such analysis techniques have been applied, they have enhanced present skills and provided bases for judgment.
2. THERMODYNAMICS VS KINETICS

Physiologists and research physicians are accustomed to use kinetics as the quantitative basis for the purely chemical aspects of their science. Because of the emphasis on kinetics in physiological research during the past several decades, attention has been directed to detailed biological mechanisms rather than to the chemical characteristics of states of systems.

In the study of membrane transport phenomena, radioactive tracers have provided a large amount of information about the flux of various chemical species into or out of the cell and through the membrane. Changes in these flux rates have been related to factors such as alterations in extracellular fluid composition, temperature, and the addition of foreign ions and metabolic poisons. A number of hypotheses have been proposed to explain the mechanisms involved in these transport processes. However, these kinetic studies fail in giving important quantitative answers about the changes that occur in the total chemical states of the intracellular media as the result of various alterations. For example, the net effect of the changes of the different fluxes on the Gibbs-Donnan ratios or on the water content or on the cellular molality are not forthcoming. An important reason for this deficiency is related to the computational difficulty of calculating the net fluxes over time and accounting for the changes in
specific and total concentrations over time as related to the individual one-way flux rates.

The pioneering work of Britton Chance and his colleagues indicates that the computer may so improve computation with respect to enzyme kinetics that this gap can be overcome. [2, 3].

The steady-state approach taken here stems from an appreciation that the scientific basis of thermodynamics is better developed than that of kinetics and the mathematical treatment is easier. We are impressed by the tremendous kinetic complexity exhibited by even the simplest gas-phase reactions, and by the great importance of minute amounts of ephemeral free radicals and of wall effects in determining overall time rates of reactions [4]. We recognize the greater difficulty of rigorously characterizing analogous aspects of the reaction kinetics of biological phenomena. Moreover, data needed for this purely kinetic approach to physiologic understanding are sparse and difficult to collect, and many more kinetic than thermodynamic equations are required to properly characterize the same system. Each of these additional equations requires the determination of extra parameters. We recognize that certain aspects of biological systems, relating particularly to details of reaction mechanisms and to certain transient states, may be beyond study by our methods. Moreover, when it is possible to apply the kinetic approach, the
answers obtained are better in the sense that they contain greater richness of detail about the mechanisms.

2.1. Transient States

No biological system is ever in true equilibrium—that is, isolated from its environment with respect to transfer of matter and with its parameters invariant over time. The simplest case is a steady state in which there are fluxes between the system and its environment and the state-determining parameters are invariant over time.* More realistically, biological systems are in transient states in which there are variations in exchanges with the environment and in which the state-determining parameters vary over time. Consider the changes with time from birth to death, from day to night, between eating and fasting, in exercise and rest, in disease, between breaths, after intravenous infusions. All of these invoke alterations in exchanges with the environment or in state-determining parameters over time, that is, in chemical inputs and outputs, in pressure or temperature.

The problems posed by these transients are not something new to physiology and medical research. Where long-term transients associated with age are involved, the investigator conventionally collates his data by age groups; fasting is dictated before making certain measurements; experimental animals are examined for disease; controlled

* Distinction is sometimes made between steady state and stationary state. Steady states include processes that are purely periodic within the constraints given in the text [5].
and measured exercise states are carefully distinguished from resting, etc. These same expedients for minimizing the effects of certain types of transients are available to and indeed must be used by the model builder.

The cases of intravenous, intra-arterial, or intra-cardiac injections may be used to illustrate some general principles relating to transient states. Where a rapid change in a state parameter—caused for example by a rapid intravenous injection of a tracer or drug—results in a rapid transient in the system, and where the nature of this rapid response is important in studying the characteristics of the system or in establishing the new steady state to be reached, both the laboratory investigator and the model builder must have tools sufficiently keen to follow this rapid transient. Examples of this are one-shot injection techniques for determining circulatory characteristics or lung performance or studies of selective chemotherapeutic agents [6, 7]. In the latter case, the mathematical model for an interesting number of organs becomes quite complex. Fortunately, the availability of high-speed computers makes it feasible to attempt to simulate important transients of this type.

There are, however, a number of interesting cases, even some involving rapid injection, where the form of the rapid transient is not important in establishing the characteristics of the new state. In these cases, the phenomena
may be simulated as having resulted in moving from one steady state to another. Consider the injection of saline solution into a laboratory animal or a man. Here there will be a rapid transient in, say, blood concentrations of certain ions lasting for perhaps three minutes as mixing occurs in the blood stream. During the next fifteen minutes to two hours, depending on the animal, electrolytes and water will redistribute themselves in the readily exchangeable body spaces (see Sec. 4). Then for a period of some hours the system will change so slowly that for many purposes it may be considered to be in a new steady state. The model builder can thus have some assurance that mathematical techniques suitable for representing steady-state conditions will be appropriate.

2.2. Devices for Extending the Scope of Thermodynamic Methods for Simulating Physiological Systems

Our basic tools are a mathematical method and a computer program for calculating efficiently the composition of multiphased chemical systems, given the values for certain combinations of state-determining parameters. With little modification, this program can establish the changing compositions of a closed system in conformity with alterations in the state-determining parameters. Thus we can compute the detailed chemical alterations such as those that occur in a specimen of blood in a laboratory tonometer when the partial pressure of oxygen in the vessel is changed [8, 9].
In the problem described above, a new solution must be computed for each value of oxygen partial pressure. It is often useful to employ a device that can predict with fair accuracy the local changes that will occur with alteration of parameters without requiring additional solutions of the problem. To this end, we employ subroutines that compute a system of partial derivatives (Jacobian matrices) for the problem. In many cases these partial derivatives can be used to obtain a good approximation for both the direction and amount of change that will be brought about in a system by small changes in state-determining parameters. Among their other uses, these Jacobian matrices can serve as a convenient index of the sensitivity of the system to changes in various parameters.

For large changes it is better to change the values of independent parameters and re-solve rather than rely on the predictive power of the Jacobians. However, in physiological applications, large usually means outside the physiological range of changes. Reference [10] describes the details of this Jacobian package and [11] presents a practical application to a real problem.

Thus far we have considered the response of models to a given stress as instantaneous. This view depends on the system either being closed or in a steady state so that the algebraic sum of all additions or removals from the system is zero and hence may be ignored. In physiological
experiments, however, if a stress alters the normal input-output pathways, this algebraic sum may not be zero. Under these circumstances the net effect of these altered normal pathways may produce an additional stress, which in turn may further alter the input-output routes, etc. Therefore, even if we assume an instantaneous initial response to a stress, there may be a continuing response over time to a stress of a physiological system which is continuing to exchange chemical substances with its environment.

Techniques for simulating such continuing response stem from the following two general principles. First, although it is theoretically necessary to evaluate the instantaneous net effect on the system of all input-output exchanges at each moment of time, in practice this is only necessary at multiples of an appropriate, basic time period $\Delta t$. The magnitude of $\Delta t$ depends upon the purpose of the simulation and on the nature of the situation being studied. For simulations accomplished by this device so far, $\Delta t$'s varying from less than a minute to an hour have been used. The following diagram grossly illustrates this general simulation process.
Start with normal system.

Solve*

Apply one-time externally generated stress (if any).

Solve.

Apply net effect of changes in normal input-outputs for time period, \( \Delta t \).

Second, this simulation technique merely requires determining and applying the net effect of changes in normal input-output pathways. Changes in the individual input-output pathways need not be separately determined or applied. This principle is important in that the net effect of all normal pathways is zero in the unstressed state.

Reference [11] describes the application of this device to the simulation of the renal excretory system.

*"Solve" means to compute the chemical composition of the system.
In that study, the net chemical inputs were altered with \( \Delta t \) to represent changes occurring in the system as the result of metabolism and experimental chemical stress and changes in the outputs as from micturition. Currently we are studying models in which not only the chemical inputs are altered with time as above, but other state-determining parameters, the free-energy values, are changing as well. Using this device we have obtained excellent simulations of the chemical alterations occurring over time in body and urine after the rapid ingestion of a large amount of water, an experimental chemical stress that causes a major alteration in the levels of circulating antidiuretic hormone.

2.3. Energy, Work and Power

In discussing the physiocochemical characteristics of biological systems we stress the compositional aspects—mole number, concentration, or activity of chemical species—rather than the energetic aspects. Thermodynamic models of the type we shall describe cast little light on the true energetics of real systems. It is not clear that this deficiency is particularly serious. Our work has demonstrated that biophysicochemical models can predict, for example, the fluid and electrolyte shifts that occur between the interstitial and intracellular spaces in the human body or the intracellular composition of fetal red cells in utero. However, these models can only approximate the minimum work required to bring about the shifts or the minimum power.
required to maintain the fetal erythrocyte interior over time, not the actual work or power. There may be great difference between the minimum and the actual.

For both practical and theoretical reasons we are more confident in modeling the compositional aspects of physiological systems than their energetic aspects. In chemical processes it is relatively easy to measure the chemical substance in a system and to keep track of it moving into and out of the system from associated sources and sinks. In contrast, energy can be rapidly changed from one form to another, each of which may be difficult to discern and to measure, especially during transients. Thus even if it were theoretically feasible to incorporate the computation of work and power into a biological model, it would be extremely difficult to validate such a model by comparison with measurements made on the real system.

There are many well known inorganic analogies. Consider a lead storage battery. Again the compositional features are more reliably subject to calculation than the energetic. We can figure how a change in fluid electrolyte composition or a decrease in temperature will affect the composition of the active electrodes or vice versa (given enough time for the new state to be realized), and we can compute the effects of these changes on the open circuit voltage of the battery [12]. But we cannot calculate how much actual power is available from the system. This will
vary for each condition under which work is removed from the battery. The power rating is therefore established for each quality of battery on the basis of empirical tests.

A careful distinction must be made between the actual work available from a system, or the actual power required to maintain a system in a specific state, and the theoretical concept of free energy or Gibbs' electrochemical potential. A standard free energy parameter [13] is associated with each chemical species as an inherent characteristic of that chemical species. The absolute free energy parameters are not definable, so they must be established in relation to some empirical standard. (There are also practical problems of fixing the appropriate free energy for a species in non-ideal solutions; these matters will be considered later.)

A key assumption in our work is that many biological systems are either in or closely approach steady states with respect to their chemical composition so that the modified free energy of the system is at a minimum. Results obtained so far are compatible with this thesis.

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\*The manner in which standard free-energy parameters may be modified to reflect the operation of steady-state processes will be described briefly in a later section. The free energy of the system can be determined, if desired, from the modified standard free-energy parameters of the species present and their amounts.
3. "STANDARD MAN" VS STATISTICAL VARIATION

Because biologists have not generally been able to define the state of a living system or subsystem in the rigorous mathematical way that the state of a mechanical structure or the state of a hot, ionizing gas can be defined, they have relied heavily on statistical correlations in attempting to relate cause and effect in living systems. For example [14], statistical correlations among various blood plasma electrolytes have been based on measurements of a large number of individuals with different electrolyte patterns. Our analytic technique based on chemical thermodynamics demonstrates these same electrolytic relationships in a much more rigorous and comprehensive fashion [8, 9].

If each individual varies from others of the species in hundreds of thousands of biochemical detail, is it possible to construct a biochemical model of a physiological function (subsystem) that meaningfully describes specific individuals or groups of individuals? This problem is not specific to the model builder. All biologists are confronted with this same problem. The medical clinician examining a laboratory report on the blood analysis of a patient intuitively is forced to recognize a conceptual norm for the measured values.

In a similar fashion, the biochemical model builder can base his formulation of a system on the normal values
of the chemistry of the system for the class of individual and species of animals to be represented. Our model of the respiratory system [8, 13], for example, was based on the chemical and physical characteristics of a normal resting young adult human male. The model of a young adult human female would be somewhat different in chemical makeup and in response to experimental stresses. In these models, if all of the chemical reactions essential for the definition of the system are present and are rigorously interrelated through the appropriate chemical laws, then the model should react to changes in its state-determining parameters (chemical inputs, temperature, pressure) in exactly the same way as the real-life system reacts.

This realism describes a good or "robust" model. Under certain conditions the arithmetic processing ability of computers allows the clever use of empirical "fits" to given data, so that only modest effort is required to set up an empirical model that will superficially look like the real system. Such an empirical model may even be programmed to follow a predetermined path to a new state. Empirical computations of this nature have their place when more rigorous methods are not available.

The use of rigorous models, based on well established scientific principles, yields more than the empirical models. In particular, such models yield new biological facts and insights which in turn lead to new experiments and further
verification. For this reason we believe that computers and models will shortly become common, necessary adjuncts to the laboratory for biological research.

If the essential reactions are properly incorporated, the rigorous models may be stressed in any appropriate fashion with some confidence that the new state so obtained will correspond in each relevant chemical detail with the corresponding detail of the real system. Conversely, working from the chemical detail of an abnormal state, the stresses that moved the system from normal may be determined. Each and every chemical species is related to all of the others and changes in appropriate amount and concentration as any one of the state parameters is altered even slightly.

The answers obtained with our models under a given set of conditions are a unique numeric solution. At the present state of development we can only hypothesize that this same situation obtains for an interesting number of real biological systems. There are still incompletely explored theoretical conditions of fluxes and reaction rates that may result in more than one feasible steady-state condition, or that may result in a periodic steady state rather than in a stationary state [15, 16]. Practical experiments made so far to compare our models with laboratory results indicate that models of a type yielding unique solutions and stationary states give satisfactory answers for a variety of useful cases.
If a rigorous model does not have characteristics which correspond to the \textit{in vivo} system, then one or more of the following conditions must obtain:

1. Some essential reaction must have been overlooked, either by the model builder or by physiologists.

2. The reported reaction constants or mechanisms for important chemical phenomena are in error.

3. The model is being stressed in an inappropriate fashion, i.e., it is being asked questions it was not designed to answer.

Condition (3) above deserves special comment. For example, the incorporation in a model of the specific mechanisms by which the enzyme carbonic anhydrase increases the rate of hydration of carbon dioxide in blood are not essential for many investigations. The model, if otherwise correct, will respond properly to stresses or simulated changes in the environment. Apparently there is enough carbonic anhydrase in the system under most circumstances to cope with its catalytic job. If, however, one is studying the role of hemoglobin carbamino compounds in the transport of carbon dioxide \cite{17} he may wish for certain purposes to inhibit the action of carbonic anhydrase. The model must then be provided with some mechanism that reflects the resulting alterations in the carbon dioxide reactions as influenced by the absence of the enzyme.

Pathophysiologic states such as marked depletion of any of the elements may lead to marked deviation of chemical
results from those predicted by a biochemical model of a normal man. Following a large loss of blood, a patient may have a different response to saline than would a mathematical model from which a corresponding amount of "blood" is arbitrarily subtracted. The reasons for these differences reside in our yet incomplete description of aerobic and anaerobic metabolism, mechanical factors of tissue perfusion, altered enzyme and hormonal activity, etc. One advantage of the model is to sharpen our perception of these experimental gaps. However, despite incompleteness, hundreds of laboratory determinations have been compared with our present biochemical models of man, and good comparisons have resulted under what may be termed "moderate stress." The present models appear sufficiently descriptive of human biochemical functions to allow accurate insight into phenomena such as acid-base and fluid-electrolyte balance and some of the hemodynamic and respiratory functions even in some rather severe illnesses. Some of the success to date has been in the clarification of factors that relate one body compartment to another.
4. BODY COMPARTMENTS

One may consider chemical systems, including physiological systems, to be composed of a finite number of homogeneous phases or compartments [13, 18].* The homogeneity refers to chemical composition, pressure and temperature. The degree of homogeneity required can only be determined in relation to the specific model being considered. A phase need not occupy contiguous space. Thus it is that one may speak of the "red cell compartment" although red cells are individually dispersed throughout the blood. However, one red cell is sufficiently like another in chemical composition that for many purposes they are inseparable when viewed as to their function. The plasma of the blood is chemically distinct and so different from the red cell that it constitutes a separate chemical phase or compartment. Each body compartment is often viewed as being separated from other body compartments by at least one membrane, and a compartment itself may be divided by membranes as are the red cell and the muscle cell compartments.

The nature of these membranes and the metabolic machinery they enclose contribute largely to the complexity of study of the real system. On the other hand, the possibility of aggregating the constituents of millions of

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*The idea of a phase as defined by Gibbs is identical with the concept of compartment as used by many physiologists to designate a body space. To emphasize this similarity the words "phase" and "compartment" are used as synonyms.
individual cells into a single compartment through the application of Gibbs' concept of phases presents a powerful tool for coping with this complexity.

Using the red cell as an example, it is postulated that a chemical "machine" capable of doing work exists in this cell, and a membrane that encloses the cell is capable of affecting the entrance and exit of specific chemical species. The red cell machinery and membrane are different, at least in degree, from other compartments. Although exact mechanisms may not be known, models of the red cell compartment may be developed by describing in compositional terms the work necessary to maintain the chemical uniqueness of the cell. It then does not matter whether this work is done internally or at the membrane, nor, for many purposes, does it matter exactly how the work is done. The proper quantitation of the compositional gradients between the cell and its environment is sufficient for the construction of certain classes of models.

We must emphasize that physiological compartments are a man-made concept, not necessarily a reality of nature. That is, a physiological system does not possess a unique, intrinsic compartmentalization. Rather, compartmentation is a useful device, an option of the model maker who must formulate his compartments with a view to the nature of his model and to the ways it will be applied. For example, for certain types of models it would not be judicious to
consider only a single red-cell compartment; it would be necessary to formulate several such compartments in which the cells were grouped by age. For other types of models it would be expedient to formulate a single intracellular compartment that would include the interiors of all red cells as well as the interiors of all other body cells.
5. ADDITIONAL PERTINENT CHEMICAL CONCEPTS: COMPONENTS, SPECIES, SUBSTANCES

Following the discussion of phases or compartments, it is appropriate to describe briefly certain related chemical concepts that are used throughout in the remaining sections of this Memorandum. These are the ideas of components, species and substances.

Components are the fundamental, conceptual building blocks that one chooses to construct the chemicals occurring in his model. A great deal of freedom is possible in selecting these components. They may consist of molecules, portions of molecules, ions, electrons, protons, etc., as may be conveniently used in assembling the various chemicals of the system. It should be possible to assemble the chemicals of the system from small, integral combinations of the components. Components also serve, in the models to be described later, as the means for establishing the "mass" balance of the system.

The actual chemical compounds occurring in the system are called substances. Each phase contains one or more substances. Each substance in each phase or compartment is called a species and has a specifically defined molecular or ionic composition in terms of the components of the system. For mathematical convenience, a substance that occurs in, say, two different phases will be regarded as representing two different species. Thus the substance H₂O occurs in both liquid and ice phases in equilibrium,
and \( \text{H}_2\text{O} \) in liquid and \( \text{H}_2\text{O} \) in ice would be regarded as representing two different species. When molecules of substances move from one phase to another (for example, by evaporation, condensation, or migration across a semi-permeable membrane), this movement is regarded as if a type of chemical reaction had occurred—a reaction in which one species is transformed into another species \[18\].

To illustrate these concepts, consider the small tableau below which represents pure liquid water and its ionization products (one phase) \[13, 19\].

**Compartment: Liquid Water**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Species</th>
<th>( \text{H}^+ )</th>
<th>( \text{OH}^- )</th>
<th>( \text{H}_2\text{O} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}^+ )</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>( \text{OH}^- )</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>( \text{c}_1 )</td>
<td>( \text{c}_2 )</td>
<td>( \text{c}_3 )</td>
<td>Free Energy Parameters</td>
<td></td>
</tr>
</tbody>
</table>

It is clear that the chemical reaction

\[ \text{H}^+ + \text{OH}^- = \text{H}_2\text{O} \]

is simply a statement about the corresponding column vectors in the tableau, namely,

\[ \begin{bmatrix} 1 \\ 0 \end{bmatrix} + \begin{bmatrix} 0 \\ 1 \end{bmatrix} = \begin{bmatrix} 1 \\ 1 \end{bmatrix} . \]

Note that the components or elemental building blocks for this model are \( \text{H}^+ \) and \( \text{OH}^- \). Also, the species \( \text{H}^+ \), \( \text{OH}^- \), and
H$_2$O are formed from these components where the defining entry $a_{ij}$ in row i and column j is the amount of component i found in species j. As mentioned earlier, free energy parameters are characteristics of each species or column in the phase. They are indicated as $c_j$ at the bottom of the tableau.

Most frequently one is interested in solving for the composition of a system where the amounts of the components and values of the free energy parameters are all given. However, only certain combinations (whose definition need not concern us here) of the values for components, species, and free energy must be known to provide a valid problem. It is therefore possible to solve to determine certain free energy values, given information about amounts or concentrations of certain species and components. Alternatively, for example, one might wish to determine the changing amount of components required to supply a system having changing composition as brought about by, say, alteration in free energy parameters.
6. CHEMICAL GRADIENTS BETWEEN COMPARTMENTS

The following sections are intended to describe briefly certain biochemical and physiological notions of gradients that have important relations both to the construction of adequate models of biological systems and to the interpretation of the results obtained with such models. There is no pretense of completeness in these discussions; otherwise each section would become a monograph of large size. Rather, the intent is to highlight specific features of each of the concepts to be discussed that from our experience require clarification if error is to be avoided.

6.1. Osmotic Equilibrium and Tonicity

Whole philosophies of physiology have been tied importantly to the concept of osmotic pressure [20]. Until quite recently it was generally believed that the body fluids were all osmotically equivalent. Now it is considered by some that metabolically active processes, especially relating to secretion and excretion, can produce body fluids that are osmotically different from an average fluid represented by blood plasma ([21], p. 881). A qualitative discussion of the subject now may be helpful in understanding the later quantitative treatment and in clarifying some of the obscurity that still surrounds osmotic relations in the body.

First, and in line with the suggestions of Chinard and Enns [22], nothing can be gained and confusion may
be added by thinking of osmotic phenomena in terms of some mechanism such as the impact of solute molecules on a membrane, or as the effect of solute molecules on the escaping tendency of water. As discussed by Glasstone ([23], pp. 662-668), there is no satisfactory theory explaining osmotic pressure, and nothing much is lost from the computational view as a result of this mechanistic deficiency. We should consider that the chemical potential of an un-ionized species in a homogeneous mixture (say, a solution) is determined as the combined effect of the amount of that species and of all the other species that are present in the mixture and of the other state-determining parameters (for the present, only pressure and temperature). If two such mixtures at the same pressure and temperature are separated by a completely permeable and symmetric membrane, then equilibrium states will be reached when the chemical potentials of all species are equal on both sides of the membrane.* The plural ("states") is properly used here because under these circumstances there will be an infinite number of ways that the amounts of the species can be distributed on both sides of the membrane so that their chemical potentials will be equal. From a different view, imagine that the membrane can be removed and replaced at any position in the container holding the two mixtures. No position of the membrane will

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*In this discussion it is assumed there are no reactions occurring within the compartments separated by the membrane.
result in the net movement of species from one part of the container to the other and for each position of the membrane the free energy of the system will be at the same minimum.

This situation of complete permeability is not of great practical import in biology. However, semipermeable membranes which larger molecules cannot penetrate are widely present in biological systems. Suppose now that such an impermeable species is present in the mixture on one side of the membrane (Compartment 1) and not on the other (Compartment 2). If pressure and temperature are maintained equal in both compartments there is a unique equilibrium state in which all species are in Compartment 1, which contains the impermeable species. Under these conditions, there is no way that the chemical potential of all species can become equal if a mixture without the nondiffusible species exists on one side of the membrane. Or, the minimum free energy for the entire system occurs when Compartment 2, which has no large molecules, disappears entirely.*

If, however, energy is transmitted to this system, for example by applying sufficiently large pressure to Compartment 1, diffusible species will move into Compartment 2, causing it to reappear. Pressure will increase the chemical potential of all species in Compartment 1 until the new minimum free energy state for the entire system occurs and the other compartment reappears. As pressure

*It is recognized that practical difficulties and the possible presence of salting-out phenomena may prevent the complete disappearance of one phase in real systems under these circumstances.
is increased, the amount of substance in Compartment 2 will enlarge further at the expense of Compartment 1 until the pressure exceeds the bursting strength of a practical semipermeable membrane. When nondiffusible species exist on both sides of the membrane, there is a unique equilibrium state where the two compartments coexist without the requirement of a pressure gradient. In this case, the total amount of species in each compartment will be proportional to the amount of nondiffusible species in each compartment.

The term "tonicity" has been widely used in the biological sciences to designate certain properties of body fluids and infusion solutions. A solution of sodium chloride of the same total molarity as blood plasma is spoken of as a solution isotonic to plasma. Isotonicity thus designates fluids that should have equal osmotic pressures. Because osmotic pressure is difficult to measure, equal reduction in freezing point is frequently used as the measure of equal osmotic pressure. If such a sodium chloride solution is isotonic with plasma, the activity of water is supposedly the same for both mixtures, at least at temperatures near the freezing point.

Throughout the literature, an idea frequently expressed or implied is that equal activities of water in two body fluids or between an infusing solution and a fluid is a

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*Molarity is moles of solute per liter of solution; molality is moles of solute per kilogram of water in the solution.*
necessary and sufficient condition to prevent movement of substance between the two mixtures. Equal water activity is certainly a necessary condition, but not sufficient to prevent such movement across membranes. In addition, as described earlier in this section, the chemical potentials of all diffusible species must be equal in the two mixtures to prevent net movement across a semipermeable membrane.

One gains the impression that use of the concept of osmotic pressure and of the terms osmolarity and tonicity has been rather overextended in the biological sciences. The concepts can be expressed in other, more rigorous ways that do not lead as readily to misunderstanding. In addition to theoretical difficulties with the concept of osmotic pressure as a measure of the chemical activity of water, there are practical, computational problems involved in its use. These computational difficulties will be discussed later.

6.2. Metabolic Processes

Conventional treatments of membrane equilibria may include pressure but frequently ignore other phenomena that can provide energy transfer and thus alter the chemical potential of species in a compartment. Of these, metabolic sources of energy and possible temperature gradients are important in normal physiological situations, while gravitational and electromagnetic field gradients may enter into certain stressful situations. In other cases, the energy
Transfer may occur through rather obscure physicochemical means whereby the activity coefficients and thereby the chemical potentials of species are altered in the two phases. The activity coefficients might be influenced, for example, through changes in dielectric constants of phases with resulting occurrence of salting-in or salting-out [24].

The metabolic sources of energy and their influences on maintaining membrane equilibrium (or steady state) are important factors in determining the distribution of species across membranes in the body, especially between intracellular and extracellular spaces, and even in the vascular system between plasma and interstitial space [25]. Whatever pathways this metabolic influence may take, it can be shown that for certain computational purposes this metabolic component can be treated as an appropriate change in the standard free-energy parameter for the species affected. Just as electrochemical potentials must be considered in defining equilibrium when charged species and electrical forces are present, so metabolochemical potentials might be considered in defining equilibrium in systems where metabolic forces are present.*

Whatever the value of this concept for other than computational purposes, metabolically activated processes

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*So far in this discussion only un-ionized species have been considered. If ionized species are present, the appropriate term might be something like "metabolo-electrochemical potential," although this has the objectionable sound of a third-rate German opera.
apparently play an important role in establishing the distribution of chemical species on either side of biological membranes. In many real situations the role of metabolic processes in determining this distribution exceeds that of impermeable species and pressure gradients. Returning to our example, metabolic processes can so increase the chemical potentials of certain species on the full side (Compartment 1) of the system that substance will move to the empty side (Compartment 2) in such amount as will provide for a new equal potential, including the metabolochemical components, for all species and a new minimum free energy for the whole system. The new system will consist of two compartments, one with and the other without an impermeable species present. The total size of Compartment 2 will be determined by the magnitude of the metabolic processes and by the amount of the impermeable species present in Compartment 1. This same shift of substance through metabolic activity can and does occur in real systems where there are nonpermeable species on both sides of the membrane.

6.3. Possible Influence of Temperature Gradients

A factor seldom discussed in animal physiology is the possible influence of temperature gradients in establishing the relative amounts of chemical species on the two sides of a semipermeable membrane. Spanner [26] describes the thermodynamic basis for the phenomenon and speculates on
its possible influence in physiology. He makes the important point that temperature gradients of small magnitude can have very large effects in transferring substance from one side of a membrane to the other. Consider as an example two small containers, both enclosed in a sealed larger vessel. If pure water is in both containers and if one container is maintained at a slightly elevated temperature, all the water in the warmer container will evaporate, moving to the cooler container. If there is a salt solution, say sea water, in each container, when one container is warmed slightly, water will move to the cooler container. The result at steady state will be a difference in concentration of both water and salts in the two containers.

Spanner computes that a 0.01°C temperature difference would yield a concentration difference in the two containers that would be equivalent to 0.8 atmosphere of hydrostatic osmotic pressure.

In this situation the atmosphere of the large vessel is analogous to a semipermeable membrane that is pervious to water but not to the saline species in the solution. Through the effect of temperature on the activity coefficient of water, the chemical potential of water is increased in the warm container and a concentration gradient is established. Of course, the activity coefficients of the other species are altered as well (they may increase or decrease), and in most real systems where many such species may diffuse
through a membrane the net effect of the temperature gradient on all species must be considered.

This effect of temperature difference between condensed phases in establishing mass flows has been called the Soret effect [27], although it was first discovered by Ludwig [28] in 1856. Modern treatments of the physicochemical aspects of the phenomena are contained in works by Jost ([29], pp. 521-532) and DeGroot and Mazur ([30], pp. 273-303), among others. An interesting physiological investigation of the eye [31] explores the possible influence of temperature gradients in establishing chemical composition within an organ. Fairly significant temperature gradients may be expected to occur in the eye because it is partially exposed to the external environment, yet largely buried within the head. But temperature gradients may possibly occur within internal organs as well, as shown by a preliminary survey of the temperatures of certain organs of a dog's abdominal cavity [32]. It was found that the gross external temperatures of these organs were higher than the average rectal or blood temperature when the latter was measured in a large vessel. Of the organs measured, the spleen, kidney, and liver had temperatures more than 1.50°C higher than the body average. The tissues of these organs are known to have a high metabolic activity, and the organs themselves have excretory, secretory, or otherwise "fluid-concentrating" functions. On the basis of a reasonable set of assumptions,
computations may be made showing that temperature gradients must exist between the interior and exterior of these organs.

6.4. Gibbs-Donnan Equilibrium

We turn now to the distribution of chemical species across semipermeable membranes when some or all of the species may be ionized. The conventional example of this situation used in many texts starts with ionized sodium proteinate (the proteinate being nondiffusible, completely ionized and having one charge per molecule) and water on one side of the membrane and ionized sodium chloride in water on the other. The problem is to compute the distribution of all species after equilibrium is reached (e.g., \cite{21}, pp. 534-535; \cite{33}, pp. 59-62; \cite{34}, pp. 33-34; \cite{35}, pp. 97-101). In our previous example of ideal un-ionized species under these conditions, all of the substance will move to Compartment 1 which contains the nondiffusible species, and Compartment 2 will tend to disappear. The same thing will happen for the ideal case with ionized species. This difficulty of having the compartment disappear can be circumvented by stipulating that either hydrostatic pressure will be built up in Compartment 1 or that water shall not migrate from Compartment 2 to Compartment 1. In the latter case, the problem becomes one in which there are really three nondiffusible species: proteinate and water in Compartment 1 and water in Compartment 2. As previously noted, there is a unique solution for this latter problem.
where nondiffusible species occur on both sides of the membrane and the two compartments coexist. With the additional stipulation that the sums of the positive and negative charges shall be equal on both sides of the membrane, the diffusing species Na\(^+\) and Cl\(^-\) will satisfy the following relations for the case when there are nondiffusible species in both compartments:

\[
\frac{[\text{Na}^+]}{[\text{Na}^+]}_1 = \frac{[\text{Cl}^-]}{[\text{Cl}^-]}_2
\]

where the square brackets indicate concentration in moles per liter of solution. The relative sizes of the two compartments are fixed in this problem by the assumed fixed amounts of water in each compartment. If pressure is used to maintain the system, this reciprocal relation does not hold, as we shall demonstrate shortly.

Presenting the Gibbs-Donnan relations in this fashion is misleading. It hides the important concept that energy must be transmitted to the system if there are to be ionic gradients. To illustrate this point consider a similar system but with different amounts of sodium proteinate on each side of the membrane, all other species (sodium and chloride ions and water) free to diffuse, and temperature and pressure maintained equal on both sides. We know that this system is completely determined and has a unique solution. The total mass in each compartment will be proportional to the amount of nondiffusible proteinate in that
compartment; the charges will sum to zero, and all species will have equal concentrations on both sides. There will be no ionic gradients between the two sides of the semi-permeable membrane. It is only by transferring energy to this system—for example by imposing a higher pressure on Compartment 1—that an ionic concentration gradient can be obtained.

Suppose that in the above case we gradually increase pressure in Compartment 1 (computationally we may assume that this can be accomplished by increasing the standard free energy parameters by the same amount for all species in Compartment 1 as a function of pressure). All species but proteinate will move from Compartment 1 to Compartment 2, thereby increasing the mole fractions of proteinate, sodium ion, and water, and decreasing the mole fraction of chloride ion in Compartment 1. The reverse situation will pertain in Compartment 2 so that there will be concentration gradients for all species whose magnitudes depend on the pressure exerted, assuming nothing else is changed.

6.5. Active Transport in Relation to Gibbs-Donnan Phenomena

Whole monographs have been written on various aspects of active transport in biological systems [33, 36]. Active transport is defined in many ways in the literature. Generally the term is used to indicate a process in which the energy required to transport species from one compartment to another comes from sources other than simple concentration gradients.
In higher animals a major source of this energy must be metabolic in origin.* Thus, in our last example of Gibbs-Donnan equilibrium, the pressure to maintain the concentration gradients and change the mass distribution between the two compartments may come from the muscular activity of the heart. The metabolic energy of the heart muscle is converted indirectly into a transport of substance and the buildup of concentration gradients across a semipermeable membrane. Such a situation probably occurs, for example, in the glomerulus of the mammalian kidney between the blood and the glomerular filtrate, the precursor of urine.

Ordinarily, Gibbs-Donnan phenomena are not considered in relation to active transport processes. In fact, active transport (in the sense of energy transfer being required) is apparently indicated whenever steady-state concentration gradients are maintained between compartments of the body and irrespective of whether the transport mechanism involves some special characteristic of the membrane separating the compartments.

Some associated mathematical development [37] is used to illustrate how the mole fractions** of various charged and

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*In plants or lower forms of animals, a significant portion of the energy used for active transport may come more directly from the sun. It is conceivable that in certain unusual circumstances, energy may be transmitted to the animal from external sources such as from radiation or from changes in electromagnetic or gravitational fields.

**The advantages of using the mole fraction scale of relative composition (moles per total moles in the phase) with biochemical models are discussed in later sections.
uncharged species are related to one another across a membrane when active processes of various kinds are present. We define $g_i$ as the ratio of the mole fractions of the $i^{th}$ species across the membrane, that is,

$$g_i = \frac{\hat{x}_{i1}}{\hat{x}_{i2}}.$$  

Here, $\hat{x}_{i1}$ is the mole fraction of the $i^{th}$ substance on one side of the membrane (Compartment 1) and $\hat{x}_{i2}$ is the mole fraction of the $i^{th}$ substance on the other side of the membrane (Compartment 2). Various relationships among these gradients will be demonstrated.

It can be shown for systems in equilibrium that the gradient for any permeable species satisfies

$$g_i = K_i e^{\frac{\lambda z_i}{R T}}.$$  

where $K_i = \exp(-\frac{\Delta F^O_i}{RT})$; $z_i$ is the charge per mole on the $i^{th}$ substance; $\lambda$ is the factor arising from charge potential and is related to the so-called membrane or Donnan potential; $R$ is the gas constant; $T$ is the absolute temperature (which we assume to be equal in both compartments); and $\Delta F^O_i$ is the standard free energy parameter difference, if any, between the same substance in each compartment. The

**Note that $z_i$ is positive, negative, or zero depending on whether the $i^{th}$ substance is a cation, an anion, or uncharged, respectively.**

**$\lambda$ will be cancelled in all uses made of this equation in this section. Note that $\lambda$ does not depend on $i$.**
K_i are closely related to the mass action constants that reflect the net effect of reactions that cause specific active transport of a species from one compartment to the other, or the effects of pressure, temperature, or activity coefficient differences in the two compartments.

From Eq. (1) it follows that for any two permeable substances

\[ g_i^{z_j} g_j^{-z_i} = K_i^{z_j} K_j^{-z_i}. \]  

Thus in the previous example involving sodium chloride, sodium proteinate, and water on both sides of the membrane, Eq. (2) becomes

\[ g_{Na}^{+} g_{Cl^{-}} = K_{Na}^{+} K_{Cl^{-}}. \]  

If there are no pressure or temperature differences across the membrane and if there are no other active processes, each K_i = 1, and Eq. (2) becomes

\[ g_i^{z_j} = g_j^{-z_i} = 1, \]

and, for example, Eq. (3) becomes

\[ g_{Na}^{+} g_{Cl^{-}} = 1. \]

Assume, however, that there is a hydrostatic pressure difference across the membrane but there are no other
active processes. Then we may assume that each $K_i = K_i$, and Eq. (2) becomes

$2''\quad g_i^{z_i} g_j^{z_j} = K_i^{z_i} J_i^{z_j}$

and, for example, Eq. (3) becomes

$3''\quad g_{Na^+} g_{Cl^-} = K^2$.

Notice that in this case the reciprocal of the concentration ratio of a singly charged anion is not equal to the gradient of a singly charged cation, but proportional to it by the term $K^2$. In most mammalian systems the hydrostatic pressure gradients across membranes are relatively small (perhaps 45 mm Hg maximum or 0.059 atmosphere) so that the resulting $K$ and $K^2$ are close to 1. This means that the reciprocals of the anion gradients and the cation gradients are so nearly equal they possibly cannot be distinguished within the limits of laboratory measurement techniques.

For situations in which the only active process is a hydrostatic pressure difference, Eq. (2'') has a number of important consequences. For example, if $z_i = z_j$, it follows from Eq. (2'') that $g_i = g_j$. In other words, substances having the same charge have the same gradient. To consider one more example, suppose that $z_i = -1$ and $z_j = +2$; it then follows that $g_i^2 / g_j = K^3$. Eqs. (2), (2'), or (2'') may be used to deduce consequences for the relationship between the gradients of a variety of combinations of anions,
cations, and uncharged substances under a variety of conditions.

Suppose now in our example that there is no hydrostatic pressure gradient but that two permeable cations—sodium ions and potassium ions—are present and that potassium ions but not sodium ions are actively transported from Compartment 1 to Compartment 2. In this situation, $K_{Na^+} = 1$ and $K_{K^+} > 1$, and it follows from Eq. (2) that

$$\frac{g_{K^+}}{g_{Na^+}} = K_{K^+}.$$  

The relations of the gradients of sodium ion with anions are the same as in the previous example except that because there is no hydrostatic pressure (and no "pumping" of anions), these $K$'s are all 1. The relations of the "pumped" potassium ions and the anions are not the same as for sodium, however. Here

$$g_{K^+} \cdot g_{Cl^-} = K_{K^+},$$  

and so on for multivalent anions. The constant $K_{K^+}$ may be thought of as the equilibrium constant for the reaction

$$K_{2^+} + Cl_- = K_{1^+} + Cl_{1^-}.$$  

The effect of activity coefficient differences in determining mole fraction gradients of species across membranes can be illustrated by the case of bicarbonate ion, where, in the case of red cells and plasma and in
certain other systems too, the gradient of bicarbonate ion is different from that of chloride ion. In the case of blood, at least, this different bicarbonate ion gradient can be computed on the basis of the activity coefficients associated with all of the reactions represented by the so-called apparent first ionization constant of carbonic acid,

\[ H_2CO_3 = H^+ + HCO^-. \]

The equilibrium constants \( K \) and \( K' \) for this reaction in red cells and in plasma, respectively, are given by

\[ \begin{align*}
(4) \quad K &= 10^{-6.03}, \\
(5) \quad K' &= 10^{-6.09}.
\end{align*} \]

If we divide the mass action equation for this reaction in red cells by the mass action equation for this reaction in plasma we get (letting the gradients be red cell mole fractions divided by plasma mole fractions)

\[ \begin{align*}
\frac{\gamma_{H^+} \gamma_{HCO^-}}{\gamma_{H_2CO_3}} &= \frac{K}{K'} \\
\gamma_{H^+} \gamma_{Cl^-} &= K' \gamma_{H^+} \gamma_{Cl^-} = 1.
\end{align*} \]

We now assume that \( K_{H^+} = K_{H_2CO_3} = K_{Cl^-} = 1 \).

It then follows from Eq. (2) that

\[ \begin{align*}
\gamma_{H^+} \gamma_{Cl^-} &= K_{H^+} K_{Cl^-} = 1,
\end{align*} \]

*See [38], p. 272.
and from Eq. (1) that

$\frac{g_{H_2CO_3}}{g_{H_2CO_3}} = K_{H_2CO_3} = 1.$

If we combine Eqs. (4) through (7) we get

$\frac{g_{HCO^-}}{g_{CT^-}} = \frac{K}{K^*} = 1.148.$

In average viable young adult male blood, the mole fraction ratios of chloride and bicarbonate ions have about this relation [8, 39].

6.6. Activity Coefficients and Distribution of Uncharged Substances Between Compartments

Activity coefficient effects may possibly play a role in establishing differing mole fraction gradients for uncharged substances across membranes. This phenomenon might especially be suspected when two such substances have a widely differing effect on the dielectric constant of aqueous solutions, where difference of ionic strength occurs on the two sides of the membrane, and where more usual transport mechanisms cannot be found that explain the gradients [24]. Possible candidates for the activity-coefficient effect are the reciprocal concentration gradients of urea and glucose occurring finally between plasma and the tubular filtrate as the result of kidney function. Some actions of hormones and drugs (for example, certain diuretics) may influence activity coefficients either through an important effect on dielectric constants or through other means.
In this context, consider a simple two-phase system such as the previous example. Assume that in this system, at equilibrium, there is a "reaction" between an uncharged species \( x_{j1} \) in Compartment 1 and \( x_{j2} \) in Compartment 2. Thus

\[
x_{j1} = x_{j2},
\]

and at equilibrium the activities of the two species are equal. Observe that

\[
\frac{f_{j1} x_{j1}}{\bar{x}_1} = \frac{f_{j2} x_{j2}}{\bar{x}_2}.
\]

where \( f_{j1} \) and \( f_{j2} \) are dissimilar activity coefficients for the species in the two compartments, and \( \bar{x}_1 \) and \( \bar{x}_2 \) are the totals of the number of moles of all species in each compartment.

To illustrate how an increment of such a substance would distribute between Compartments 1 and 2, let us assume that the phases are of equal size and that the added substance is a solute (not water) having a concentration range such as occurs in biological systems. Under these circumstances, if \( f_{j2} \) is, say, one-third as large as \( f_{j1} \), and if we add small amounts of substance \( x_j \) to the system, approximately three times as much of the added substance will appear in Compartment 2 as will be distributed to Compartment

*Such a reaction might be between, say, dissolved urea in the two compartments. The relations we wish to illustrate apply as well for charged species, but the explanation becomes somewhat more complex and is unnecessary for our immediate purpose.
1. Conversely, the mole fraction $\hat{x}_{j2}$ is about three times as large as $\hat{x}_{j1}$. It is as if the chemical potency of the species in Compartment 2 were somehow degraded so that it takes about three times as many molecules in Compartment 2 to equal each molecule in Compartment 1. On the other hand, the size or amount of Compartment 2 will be increasing locally about three times that of Compartment 1.

If, in the above illustration, the compartment sizes are not equal or if we are concerned with the distribution of water among dilute solutions the quantitative relations suggested above are not correct. It is still qualitatively true, however, that the net change in the size of Compartment 2 will be greater than the net change in Compartment 1 upon the addition of substances, given the activity coefficient gradients indicated. Under both the above circumstances, the added substance appears to move toward the compartment in which its mole fraction is highest.

For very complex models that include charged species and many compartments, the Jacobian matrices mentioned earlier are a useful device for determining how added substances will distribute themselves among compartments.

The simple closed systems we have been discussing tell us nothing about the flow law by which substances are redistributed in the situations described above. However, the open models that provide for the addition of metabolites and excretion, as described briefly here and more completely in
[11], are consistent with the well known concept of "maximal" renal clearance and the form of Fick's law stating that net flow is proportional to the difference in chemical activity between two sites.

6.7. Some Influences of Large Molecular Species

In the illustrative problems used so far, the impermeable species have always had one negative charge per molecule and have been completely ionized. If these species on either side of a membrane in our examples have different valences or are differently ionized, then again gradients of the diffusible charged species will occur and a typical Gibbs-Donnan equilibrium will obtain. This situation may appear to violate the proviso that the Gibbs-Donnan phenomenon implies the transfer of energy. Consider, however, that energy must be expended in some related system to originally "package" the different macromolecules on the two sides of the membrane. A change in ionization status of these macromolecules in respect to cations or anions also implies a transfer of energy from within or without the system. Thus a change in pressure on one side of a membrane can alter the ionization status of macromolecules by altering the distribution of ionized permeable species. A change in metabolic activity within the system or an imposed electrol-

*In the case of red cells and plasma, for example, the energy for the differential "packaging" of hemoglobin in red cells may be supplied by the hematopoietic processes in bone marrow.
motive force [25] can also alter the ionization by causing a similar redistribution of permeable ionized species.

The addition of a salt in solution to the system (for example, by an infusion) can affect the ionization of the macromolecules in numerous ways, depending on whether the solution is acid or alkaline, whether common ions are present in the system, or whether a new foreign ion is introduced. Finally, an alteration in the number of moles of the macromolecules on either side of the membrane (for example, by selective denaturation) can cause a redistribution of all charged and uncharged species between the two sides of the membrane.

When one considers the wide spectrum of types, quantities, and chemical characteristics of macromolecules that can be present in biological systems, the prospects of adequately incorporating their influence in a mathematical model may appear hopeless. Fortunately, there are some ameliorating circumstances:

1. There is rather extensive knowledge of the chemical characteristics of the more widely prevalent macromolecules, especially of the important proteins.

2. Models of the type we shall describe later are capable of handling hundreds of independent and interdependent reactions, including the ionization characteristics of macromolecules and their influence on the distribution of other species in a system.

3. In specific circumstances assumptions can be made
about the characteristics of macromolecules which can simplify the model.

4. As described in Sec. 6.3, the influence of macromolecules in establishing the total distribution of substance across membranes is not as important as it was once considered to be.

6.8. Starling's Hypothesis of Capillary Equilibrium

In 1896 E. H. Starling [40] described the mechanical factors related to the transfer of fluid from the vasculature to the interstitial space, concluding that the predominant factor influencing the production of lymph was simply the gradient of hydrostatic pressure existing across the capillary membrane. It was noted that the capillary membrane, although largely impermeable to the large species such as protein, readily permitted the filtration of water, inorganic ions, and certain uncharged substances such as sucrose and urea. The existence of a hydrostatic pressure difference between the arterioles and the venules was also well known in 1896, and it was surmised that this pressure gradient was continuous along the length of the capillary intervening between the arteriole and the venule. These points summarize the basis of the Starling equilibrium hypothesis.

The capillary hydrostatic pressure at the arteriolar end is normally approximately 35 mm Hg. This pressure drops to about 15 mm Hg at the venular end of the capillary
as a result of frictional and viscous forces and resistances applied by the musculature of the arterioles. Acting in an opposite direction from interstitial fluid to capillary is the so-called oncotic pressure due to a difference in concentration of nondiffusible proteins and other colloids between plasma and interstitial fluid. This concentration gradient is equivalent to a pressure difference of approximately 25 mm Hg. There then exists at the arteriolar end of the capillary a net gradient outward from the capillary of approximately 10 mm Hg. As the hydrostatic pressure drops along the length of the capillary, these pressure gradients are reversed, the colloid osmotic pressure accounting for a greater directional trend into the capillary than is afforded by the now diminished hydrostatic pressure outward from the capillary. The Starling hypothesis also described a cycle of fluid flow. First the fluid from the arteriolar end of the capillary is virtually forced into the interstitial spaces by hydrostatic pressure. According to the Starling hypothesis, with a decrease in hydrostatic pressure along the length of the capillary, a flow of interstitial fluid is effected back into the capillary by virtue of the osmotic pressure caused by nondiffusible protein and other colloids.

These factors have been continuously studied since the time of Starling. It has been found that almost every organ has its own characteristic capillary hydrostatic pressure.
As an example, the capillaries within the kidney have a higher hydrostatic pressure than those within the liver. There seems to be more protein within the plasma of the kidney than there is within the lymph in the kidney. In the liver, however, the concentration of protein within the lymphatics almost directly equals that within the plasma. Direct photomicrographs have been made of the ends of lymphatics, which appear to terminate in a blind bulb within the interstitial space. These end lymphatics seem to possess organizational characteristics such that when a protein molecule is accepted, it is kept within the lymphatic and not permitted to transmigrate back to the venous end of the capillary. Numerous isotope tagging studies have shown that albumin, one of the more prevalent protein molecules within the vascular system, is continuously lost from the plasma. This albumin also continuously reenters the vasculature system at the large connections of the lymphatic system and the venous system in the neck. It is also now known that the protein molecule is electrically charged, a fact not known to Starling.

We have seen how disparate concentrations of charged, nondiffusible species on opposite sides of a membrane can influence the distribution of charged diffusible species. Conversely, we have noted how hydrostatic pressure changes can alter the charges of large molecules by altering distribution of the charged diffusible species. In addition, transport occurring in the vascular system [25] can alter
the distribution of chemical species between plasma and interstitial fluid in a fashion not explainable by the Starling hypothesis. Obviously, if we want to describe the intercompartmental transfer between the vascular system and the interstitial fluid, a much more complex system must be postulated than that provided by the relatively simple Starling hypothesis. References [41, 42] describe in detail how the above features can be incorporated in a mathematical model of the system.

Perhaps the greatest error in reasoning in this area results from the application of components of Starling's hypothesis to situations for which it is completely inappropriate, particularly to extracellular-intracellular fluid balance relations. It is not infrequently assumed that if the hydrostatic pressure component is not greatly different within and outside the cells, the major determinants of the balance of fluids between cells and extracellular media are the relative concentrations of the large nondiffusible molecules, especially the proteins. As demonstrated in [8], this concept ignores the important role played by metabolic activity expressed through active transport in determining this total compositional distribution between cells and extracellular fluid.
7. INFLUENCE OF ENZYMES AND HORMONES

Enzymes are organic catalysts, protein in nature, elaborated by living cells, and capable of altering the velocity of specific chemical reactions without being destroyed or permanently altered in the process ([21], p. 909). A hormone is a distinct chemical substance formed by one organ and acting in a specific manner on the function of another organ or organs of the body ([43], p. 821).

Although the distinction between enzymes and hormones may not be clear, the significant fact is that a change in the amount of either substance in a subsystem, beyond certain narrow limits, can change the characteristics of the subsystem. But if these substances, many of whose reactions and even structures are unknown, are so important in determining the characteristics of body functions, how can we hope to build biochemical models of such functions without knowing more about the actions of enzymes and hormones?

Again, this problem is not unique to the mathematical model builder, but also confronts experimental physiologists and clinical researchers at every turn. We can hope to gain by their experience in coping with the problem. Two examples will illustrate ways in which the problems presented by these extrinsic control factors may be skirted, if not attacked head-on.

We mentioned earlier that the enzyme carbonic anhydrase in the blood, operating catalytically, speeds up the usually slow hydration of carbon dioxide to carbonic acid, thus
providing for rapid absorption in the blood of carbon dioxide and the rapid expulsion of this gas from the blood at the lung surfaces. Without the presence of this enzyme in the blood, the human external respiratory system would be drastically reduced in capacity, probably below the level necessary to support life. The specific chemical mechanisms by which this catalyst operates to speed the hydration and dehydration of carbon dioxide are not known, at least in sufficient detail to incorporate the reactions explicitly in a biochemical model of the subsystem. It appears, however, that except in cases of diseases or poisoning when the normal amount of carbonic anhydrase may be markedly reduced, an excellent simulation of the subsystem can be obtained without explicitly representing the reactions of the enzyme. The presence of the enzyme within the normal range speeds up the carbon dioxide reactions so that they are no longer the important time-rate determinants for the system, and the entire set of associated reactions can be handled for many purposes as if they were instantaneous [8, 13].

Our initial investigation of urine formation [11] illustrates how at least certain aspects of a function can be studied when the characteristics of the system may be importantly altered by a change in the amount of a hormone present—in this case, the antidiuretic hormone secreted by the pituitary gland. Little is known about how this hormone acts, and no sensitive, direct, chemical laboratory
methods are available to measure changes in its concentration. It is known, however, that various changes in body conditions, such as those resulting from the ingestion of rather large amounts of water or dilute salt solutions cause a reduction in the amount of this hormone, with a resulting alteration in urinary flow and chemical composition.

For approximately the past thirty years physiologists have traditionally studied the characteristics of the urinary function with the antidiuretic hormone secretion depressed [44]. This mode of clinical investigation attempts to skirt the complicating transients introduced by the changing influence of the hormone by pushing the subject to a synthetic, steady, disease state in which the hormone is no longer present.

Using models, we have chosen another way to bypass the complicating influence of changing amounts of this hormone. We study the urinary function under conditions where the hormone is present in steady, normal amounts and the subsystem's response to moderate stress is determined by inherent control characteristics not hormonal control. We can thus investigate this intrinsic control phenomenon that appears to be influential under normal day-to-day situations [11].

As more becomes known about the mechanisms of hormonal actions, use of the above expedients will not be necessary. Models are currently under investigation, for example, in
which the rate of antidiuretic hormone formation is related to changes in plasma osmolarity while urine concentration and flow are dictated by plasma ADH levels. Such models yield the well known and characteristic renal responses to the rapid ingestion of large amounts of water.
Each of the physiological subsystems we have modeled has purely physical as well as chemical aspects, but the mathematical methods we use to represent the chemistry of the system are inappropriate for representing physical phenomena—mechanical motion, peristaltic action, etc. To specifically incorporate the appropriate physical features of each subsystem would in most cases have required a new, separate mathematical development, major additions to the computer programs, and importantly different types of laboratory validation. To incorporate any one of these physical phenomena would have involved a major research effort in fields generally outside our competence, so we naturally bypassed specific simulation of mechanical features if at all possible. Because many of these physical aspects of the body apparently function to protect the chemical stability of the system, we could examine chemical features without explicitly representing this physical buffering action. Several examples may help clarify this point.

In models of the human external respiratory system, we explicitly examined the chemical changes that occur in venous blood as it passes through the lungs to become arterial blood [8, 13]. But we made no attempt to represent the complex physical phenomena occurring between the nostrils and the lungs and within the lungs during the inspiration-expiration cycle. Here there are complex gaseous flow
patterns, mixing and varying concentration gradients during the cycle. Fortunately for our purposes, however, the total capacity of the lungs (approximately 5 liters) is very large with respect to the tidal volume (0.5 liter), and the air ducts leading to the pulmonary alveoli are very small in diameter. These physical characteristics permit the lungs to damp out the large fluctuations of alveolar gas composition that otherwise, during the intermittent inspiration-expiration cycle, would subject the flowing blood to large surges of oxygen (and alternately of carbon dioxide). To a good approximation, this lung characteristic plus the large surface area of the alveoli permit the breathing mechanisms to be represented as an appropriate volume of air of alveolar composition in equilibrium with the blood.

The physical characteristics of other input and output pathways of the body appear to have similar damping features that tend to buffer against rapid or large changes in the concentration or mass rates of flow of chemicals into or out of the body. For example, in the gastrointestinal tract the stomach appears to feed fluid chyme into the duodenum at a fairly constant rate, and the intestines continue the buffering action by regulating the rate at which nutrients reach the blood stream [45]. The combined physical and chemical regulatory features of this system appear to be so effective under normal conditions that we could simulate the net chemical output of the system as an
input to a model of the urinary function without specifically incorporating the gastrointestinal tract in the model [11].

Another buffering system includes the urinary bladder and the ureters connecting it with the kidney. This system permits collection and storage of urine over rather wide ranges of time without adversely affecting the steady-state output of this important excretory fluid.
9. NUMERICAL PREREQUISITES FOR BUILDING A CHEMICAL MODEL

Previous sections have dealt largely with concepts of models and their physiological backgrounds. We now consider the practical arithmetic of constructing models, using real values for the parameters and solving to obtain numerical answers that make sense.

The reader is warned that consideration of the subject matter below has been known to transform normal, phlegmatic mathematicians into quivering wrecks spouting gibberish and threatening to annihilate personally all experimenters in the biological field. Although slightly exaggerated here, this response merely affirms the fact that the model builder and the experimenter live in different scientific environments. For example, most experimenters have in their laboratory a pH meter that measures the "acidity" of solutions, including biological fluids. This device has the property that if properly calibrated, an experimenter anywhere in the world will obtain the same numerical dial reading if the sensing element is dipped into an identical solution. Thus experimenters can state some of the results of their experiments in numerical terms which can be checked or duplicated by any other competent investigator now or ten years hence.

A real problem arises for the model builder, however, when he attempts to use the numerical values from the "acidity" meter as parameters in a model of this same...
solution that is based on theoretical constructs. For this purpose, he must know exactly what these numerical values represent: What are the dimensions of these numbers and how do they correspond with the dimensions of other parameters necessary for the adequate representation of the state of the solution?

9.1. The Mole as the Unit of Measure of Chemical Substance

The mole, an important unit of chemical measure, is defined by Guggenheim [46] as "the amount of substance containing the same number of molecules (or atoms or radicals or ions or electrons as the case may be) as there are atoms in 12 grams of $^{12}$C." This number of molecules, etc., per unit amount of substance is called Avogadro's constant, $N_A = 6.02278 \times 10^{23}$ mole$^{-1}$.

There is no English word for the parameter for which the mole is a unit of measure. It is not a measure of mass or weight as these are usually defined, but of a third quantity different from mass and weight but proportional to both. This quantity is called Stoffmenge in German, translated to "amount of substance" in Guggenheim's definition quoted above.

An amount of pure gold weighing 1 kilogram can be characterized chemically as being 5.076 moles of substance or financially as representing 1125.28 dollars of value (at this writing). We choose the particular parameter defining the characteristics of gold and the units that are suited
to the type of computation at hand: weight and kilograms for physical computation, amount of substance and moles for chemical computation, and value and dollars for financial computation.

Biochemical systems are not made up of pure substances, however, but consist of many phases, each containing hundreds or thousands of different chemical substances. In any one phase the amount of substance is

\[ x_1 + x_2 + \ldots + x_n = \sum_{j=1}^{n} x_j = \bar{x}, \]

where \( x_j \) are the mole numbers of the various species in the phase and \( \bar{x} \) is the total number of moles. The above equation contains information analogous to some of the data in a bill of lading for a freight car of mixed produce—so many dollars worth of peaches, apples, pears, etc. Just as one can sum apples and pears to total value in dollars, so one may sum glucose, urea, water, etc., to total substance in moles. We call the chemical bill of lading the composition of the system. For chemical purposes the most useful unit of measure of composition is the mole, although, as we shall see, other units involving weight or volume are frequently used in presenting laboratory data.

For certain practical and theoretical reasons in both finance and chemistry, one is often interested in the relative composition of a system. Thus the nature of an investment portfolio may be characterized by a listing of various
specific stock and bonds in terms of their fractional value of the total portfolio (dollars per total dollars). So the nature of a chemical system may be characterized by a listing of the specific chemical species in terms of their fractional substance in moles in relation to the total moles of substance in the system. This is the basis of the mole fraction scale of relative chemical composition.

Frequently one is interested only in the specific fraction of a single species or a class of species in the system. Thus one speaks of the concentration of glucose in the urine or of the concentration of all solutes in blood plasma. In the literature the "concentration" of specific species (or of groups of species) may be stated in terms of any of numerous measures of relative amount, although the mole is the appropriate unit of measure of relative chemical composition. In a mathematical sense, the terms composition and relative composition as we have used them are vectors that define the absolute and relative amount of substance in a chemical system. The mole number and concentration are, respectively, components of these vectors.

In common chemical usage in the United States, the term concentration does not have so general a meaning as we have defined [47]. It appears to be used to designate the specific fraction of a species in a homogeneous mixture, usually when the mixtures are liquid solutions, usually when the solvent is water, and when the denominator of the
fraction has the units of weight or volume of the water or of the solution. Many chemists would object to a statement like "... the concentration of urea in the solution is $6.60 \times 10^{-5}$ (moles per total moles)." A more commonly accepted statement would be "... the mole fraction of urea in the solution is $6.60 \times 10^{-5}$ (moles per total moles)." Even so, the mole fraction scale has little common usage in aqueous chemistry in spite of its many advantages.

We are thus without a general term for the specific fraction of a species in a chemical system. Rather than coin a term for this purpose we will use the word concentration where the general concept is intended, trusting that the context will always make the meaning clear.

If the mole is the unit of chemical substance, the denominator of the fraction that characterizes concentration may be any parameter that is an extensive property of the phase. An extensive property is one that is proportioned to the size of the phase, such as volume, mass, or total moles [48]. An intensive property is one that is independent of the size of the phase, such as temperature, pressure and density ([49], pp. 10-11). In choosing a scale of concentration for computational purposes, we must rely on more or less practical considerations. From our experience, we find the following practical criteria to be important:

- Equilibrium constants on the scale chosen should be dimensionless.
- The scale should be convenient for the necessarily complex mathematical derivations.
The answers obtained should be readily comparable with experimental results.

The scale chosen should result in free energy parameters varying slowly with changes in composition.*

9.2. Molar and Molal Scales of Relative Composition and Concentration

An ever-present source of arithmetic difficulty in using laboratory data to construct models arises from uncertainty about the dimensions of many data reported in the literature. In numerous cases, for example, the dimensions of equilibrium constants derived from experimental data involving concentrations are not stated and cannot be easily established from the content of the study. The following definition and discussion of the various scales of relative composition may help clarify the importance of this deficiency and illustrate the weaknesses of certain of these scales, especially when applied to biological systems.

Concentration of a chemical species on the molar scale is defined as its number of moles per liter of solution. Because gross volume of a solution is relatively easy to measure in the laboratory, this scale is the one most widely employed in the literature. On the other hand, it is inappropriate for many computational purposes. One of its more

*In our mathematical derivations the free energy parameters are $c_j = \frac{\Delta F_j^0}{RT}$ [18].
minor faults when applied to biological systems is the one mentioned in most physical chemical texts: The basis volume of the solution will change with temperature so that a correction factor must be introduced to relate molarities at different temperatures. There are much more serious objections, however, to the use of molarity in connection with biological systems. As an example consider the problem of establishing the relations that exist between various electrolytes in red cells and in blood plasma. If the molar scale is used, the number of moles* of, say, sodium ion in red cells is conventionally related to 1 liter of red cells and the moles of sodium ion in plasma to 1 liter of plasma. We know, especially for red cells, that a significant portion of the volume of the cells consists of stroma and other structure that is not homogeneous with the fluid content of the cells. In fact 1 liter of normal cells contains only about 740 ml of water, and 1 liter of plasma about 940 ml of water. Structure and other nonhomogeneous substance appear to make up about 26 percent of the cells and about 6 percent of plasma. Important computational relations that involve ratios of concentrations of species like sodium ion in plasma and red cells can be in error by a minimum factor of some 27 percent if molarity is used. For example, none of the Gibbs-Donnan relations developed in earlier sections are apparent between

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*Recall that one mole of each substance contains the same number of molecules, ions, etc., that is, $6.02278 \times 10^{23}$. 
plasma and cells if the molar scale is employed in the arithmetic computation (by hand or by electronic computer).

The molar scale has another disadvantage, shared by the molal scale discussed below. Equilibrium constants on the molar scale are not usually dimensionless as they are on some of the other scales. Thus on the molar scale the equilibrium constant for the reaction \(aA \rightleftharpoons bB + cC + dD\) will have the dimensions mole\(^2\) l\(^{-2}\) when the stoichiometric coefficients \(a, \ldots, d = 1\), or

\[
\frac{[B]^b[C]^c[D]^d}{[A]^a} = K_c,
\]

where the square brackets indicate moles per liter of solution for each species \(A, \ldots, D\), and \(K_c\) is the equilibrium constant on the molar scale. Molar (and molal) equilibrium constants will be dimensionless only when the sum of the stoichiometric coefficients of the reactants is equal to the sum of those for the products. In all other cases, the equilibrium constants will have different dimensions varying from reaction to reaction, so that absolute values of these constants (and of the standard free energy parameters calculated from the constants) cannot be conveniently compared. For all of these reasons it is strongly recommended that the molar scale not be used in biochemical work for other than perhaps the most routine purposes where the laboratory convenience of the volumetric measurement may be dominant.
Peters [20], Van Slyke, et al., [50] and others recommended the molal scale many years ago. Molality is moles of solute per kilogram of water in the solution. Investigators in the field of whole-body fluid and electrolyte distribution appear to be the most consistent in the use of the molal scale. Perhaps they are influenced by Peters who was a pioneer in the field, or they may have been led to the scale naturally by the laboratory difficulties of measuring the volume of muscle tissue. When used properly, the molal scale will not lead to the gross errors inherent in the molar scale.

The reader is warned that chemical convention, usually unstated, is such that neither the molar or molal scales are used in a consistent fashion in respect to water. For example, the previous chemical expression that we wrote using symbols $aA \neq bB + cC + dD$ was taken from Ref. [47] where it was written

$$\text{Br}_2 + \text{H}_2\text{O} \neq \text{HOBr} + \text{H}^+ + \text{Br}^- \quad \frac{[\text{HOBr}][\text{H}^+][\text{Br}^-]}{[\text{Br}_2]} = K_c,$$

$$K_c \text{ (at } 25^\circ\text{C)} = 6 \times 10^{-9} \text{ mole}^2\text{L}^{-2}.$$

What happened to the water in the above mass action\* equation for this reaction? By unstated convention in dilute solutions the molarity of water is taken to be 1. The only scales in which water approaches 1 as it reaches very

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\*The terms "mass action" and "mass balance" are firmly entrenched by tradition so we use them throughout with the understanding that they involve units of measure of chemical substance and not of mass as it is usually defined.
high concentration are the mole fraction or mole ratio scales (mole ratio equals moles per moles of water in the solution). Obviously $K_c$ is not a true molar equilibrium constant. The moles per liter of water at 25° C is 55.38 so that if the water that is part of the reaction were incorporated in the mass action expression, the true molar equilibrium constant $K'_c$ would have the following absolute value and dimensions:

$$\frac{[\text{HBr}][\text{H}^+][\text{Br}^-]}{[\text{H}_2\text{O}]^2} = K'_c,$$

$$K'_c \text{ (at 25°C)} = 1.083 \times 10^{-10} \text{ mole}^1 \text{ l}^{-1}.$$

One might argue that $K'_c$ differs only by a constant factor from $K_c$ and thus that an investigator should be free to define an equilibrium constant in any convenient way he chooses. This is true for many laboratory purposes as long as the dimensions of the constant are clearly apparent. For the purpose of constructing models, however, where hundreds of reactions interplay, great care must be exercised to insure that the scale for all constants is on a comparable basis. In models, at least of the type we shall describe, water is treated similar to all other substances and not just as an inert filler. Because water is by far the dominant substance present in biological systems, small changes in its chemical characteristics have major effects on the state of the system. This sensitivity is particularly apparent in the case of the ionization reaction of water, $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$. Typically, the mass
action expression for this reaction is written

$$[H^+][OH^-] = K_w,$$

where $K_w$ is the ionization constant for water at a particular temperature. Notice that the parameter $[H_2O]$ is again treated as approaching 1 at infinite dilution, which means that $K_w$ has the dimensions $m^2l^{-2}$. When the value for $K_w$ is being converted to a dimensionless scale like the mole fraction, this dimension must be recognized or serious error will be introduced into the model results.

9.3. The Mole Fraction, Mole Ratio, and Miscellaneous Scales of Relative Composition and "Concentration"

In our experience the mole fraction scale (moles per total moles in the phase) meets the criteria of practicability listed in Sec. 9.1 more satisfactorily than the other scales. This conclusion is not unique with us; other investigators have arrived at a similar conclusion. For example, according to Lewis and Randall ([49], p. 248), "For nearly every purpose the mole fraction furnishes the most advantageous method of measuring composition, and the employment of this measure in aqueous as well as in nonaqueous solutions is to be encouraged."

The mole ratio (moles per moles of water in the system) is as satisfactory in many respects as mole fraction. The sum of mole fractions in a phase is 1, however, a matter

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*Ref. [51], Vol. VI, p. 152.
of considerable mathematical convenience, while this does not hold for mole ratio. The use of mole fraction for aqueous solutions also provides for compatibility with gaseous phases where mole fraction is the usual scale employed. The mole fraction scale is used, therefore, in constructing all of our models and all data from the literature are converted to this scale.

Grams percent, or more frequently milligrams percent, is a scale that expresses grams (or milligrams) of a substance dissolved in 100 cubic centimeters of water. This scale has all of the disadvantages of the molar scale and more, and cannot be used to make even the simplest chemical computations without conversion. Fortunately it is passing out of use in the literature although it still appears widely in tables of data.

Volume percent expresses the volume of a gaseous substance at 0°C, 1 atmosphere, dissolved in 100 cc of the medium. Most frequently this scale is used to characterize the content of a gas dissolved in body fluids. In such cases the volume may refer only to the physically dissolved gas, or to the total of the gaseous component dissolved and combined, or to that portion combined as a specific species.

Equivalent weights and normal solutions are concepts likely to cause confusion in meaning. The value of the equivalent or combining weight of a substance can differ
depending on the type of substance and reaction involved. The same substance can even have several combining or equivalent weights depending on the type of reaction under consideration. As defined in *Lange's Handbook of Chemistry* [52],

An equivalent weight of an acid or of a base is the quantity that would furnish one gram-ionic weight of hydrogen ion (1.008 grams), or hydroxyl ion (17.008 grams) respectively if the substance were completely ionized. Thus, an equivalent of $\text{H}_2\text{SO}_4$ is one-half the molecular weight; an equivalent of $\text{Fe(OH)}_3$ (as a base) is one-third the molecular weight. An equivalent weight of salt is the gram-molecular weight divided by the total positive or total negative valence. Thus, an equivalent of $\text{Al}_2(\text{SO}_4)_3$ is one-sixth of the molecular weight. An equivalent weight of an oxidizing or reducing agent is the gram-molecular weight divided by the total change in valence of all atoms which change valence.

This latter meaning is not often encountered in biology.

A normal solution is one that contains one gram-equivalent of a substance per liter. Thus the normality of a solution can vary depending on whether the solute is an acid, base or salt, or is an oxidizing or reducing agent. Usually the meaning of normality is defined or is clear from its context, but occasionally a physiologist uses equivalents (or millequivalents) and "normal" in a way that obscures their meaning. For example, he may write, "The concentration of the infusing saline solution, $\text{NaCl}$ is 150 meq/L," meaning that the solution contains 150 meq/L of $\text{Na}^+$ (and 150 meq/L of $\text{Cl}^-$). The concentration is therefore 0.150 mole of $\text{NaCl}$ per liter; or 0.150 normal in respect
to Na\(^+\); or 0.300 osmole/L; or a mole fraction of NaCl of 2.72 \(\times\) 10\(^{-3}\). These latter statements are all less confusing about the amounts of NaCl and water in the solution, if not all equally desirable from other views.

Sometimes physiologists use the expression "half-normal saline solution," meaning that the concentration of NaCl in the solution is one-half that of a solution "isotonic" to plasma. Such a "normal" solution has the same number of moles of NaCl per liter of water as there are total moles of solute per liter of plasma. More precisely the freezing point of such a solution is close to the freezing point of plasma, indicating similar activities of water (near 0°C). A "normal" isotonic saline solution is often referred to as a 0.9 percent sodium chloride solution. This is a use of the grams percent scale and means that the solution contains 0.9 gram of sodium chloride per 100 cc of water. Such a solution has a molarity of 0.154 mole of NaCl per liter of solution (that is, 0.9 gm NaCl per 100 cc \(\times\) 10 = 9 gms NaCl per liter; 9 gms/L/58.438 mol. wt NaCl = 0.154 moles NaCl/L. The use of the word "normal" in the sense described above for characterizing solutions can cause confusion with the usual chemical definition of normal solutions.

As mentioned earlier, biologists make wide use of the term osmolarity (and osmolality) in defining the concentration of solutions and biological fluids. It is frequently not clear from the context in which they are employed how
these measures of concentration relate to the several chemical scales of concentration. Solutions (or fluids) of equal osmolarity supposedly would have equal relative osmotic pressures. Thus a solution containing 0.154 mole per liter of NaCl and 0.308 mole per liter of glucose should ideally exert the same osmotic pressure in relation to, say, pure water (assuming the NaCl is completely ionized). Both solutions would by this convention be 0.308 osmolar. Because of the practical difficulties of measuring osmotic pressure directly, it is usually determined from the depression of the freezing point of the solution in relation to pure water. Note that this procedure yields a measure of the activity of water near 0°C.

In previous sections we discussed some of the theoretical problems associated with the concept of osmotic pressure. Here we will mention only several practical problems related to the use of the concept as the basis of a measure of concentration. Supposedly, one osmole is a measure of that amount of any substance which when dissolved in water to yield a liter of solution will produce the same relative osmotic pressure as produced by 6.0228 \times 10^{23} (Avogadro's number) molecules and/or ions dissolved in the same amount of water. The measure of amount is in moles if the substance does not ionize or dissociate in water, and in moles times the number of particles produced per molecule if the substance dissociates in solution.
Consider now the practical laboratory problems of making a solution of NaCl in water having a concentration of 0.308 osmoles per liter. You are led to this concentration by having measured the freezing point of blood plasma and for some reason desire to make a NaCl solution with water having the same relative osmotic pressure as the blood plasma, that is "isotonic" to plasma. The number 0.308 was computed from observing the freezing point of the plasma to be \(-0.573^\circ C\) and from knowing that in ideal situations 1 mole of solute \((6.0228 \times 10^{23} \text{ particles in } 1 \text{ liter (more correctly } 1000 \text{ gm of water)})\) will lower the freezing point of water by \(1.86^\circ C\). The easy way to approach this problem is to assume that NaCl is completely ionized and that salt solutions of this concentration are ideal. One might, therefore, dissolve 9 gms of NaCl in 1 kg of water (or in enough water to total 1 liter if the osmolar scale is used). This solution, however, will not have the proper freezing point nor relative osmotic pressure because solutions so concentrated deviate from ideality.

The next step in sophistication is to determine from physicochemical tables or by experiment what weight of NaCl is required to be dissolved in 1 kg of water so that the freezing point of the solution is depressed by \(0.573^\circ C\) in respect to pure water. Having established this amount, we have a salt solution "isotonic" to plasma near \(0^\circ C\). But, will it also be "isotonic" at \(37^\circ C\), the body temperature? It is quite unlikely that the activity of water,
which is what is determined by these procedures, will have the same temperature coefficients in salt solution and in plasma. One is forced, therefore, to determine the NaCl concentration that yields the same water activity in saline solution and in plasma at 37°C. Difficult experimental procedures involving the measurement of partial pressures of water over both media or other equally special methods for measuring water activity at 37°C will be necessary, then, to establish true "isotonicity" between the salt solution and the plasma. Assuming this true "isotonic" saline solution has finally been determined, we may well ask, What is its biological significance? The solution is in no practical sense a substitute for plasma. Only one of the many criteria for chemical equality—the equality of the chemical potentials of water in solution and in plasma—has been satisfied. As mentioned earlier this is a necessary but not sufficient criterion of chemical similarity. If this "isotonic" saline solution were placed next to the plasma but separated by a semipermeable membrane, major transfers of solutes and water would occur between the two phases before equilibrium was reached. If the red cells that were separated from the plasma and supposedly were in steady-state equilibrium with it were suspended in the "isotonic" saline solution, major transfers of solutes and water would occur between the intracellular medium of the red cells and the solution. Finally, if the "isotonic"
solution were injected into the bloodstream of the subject from which the plasma was obtained, a redistribution of solutes and water among all of the body compartments would result. It is apparent, therefore, that equivalence of water activity alone is not a particularly useful concept for biological purposes.

The chemical difficulties of determining the activity of water are increased if mixtures of solutes are involved or if the solutes are only partially ionized. Consider as an example the difficulties in determining the amount of NaH₂PO₄ to add to 1 kg of water to make a solution having an osmolality of 0.308 osmole/kg. Differing amounts of four separate species including the un-ionized salt will be formed in solution. Because of such difficulties, the osmolality of solutions is usually established on an empirical basis by assuming complete ionization of electrolytes. For example, a solution of Na₂SO₄ having a concentration of 1 osmole/kg of water would be made up by adding one-third the molecular weight of Na₂SO₄ in grams to 1 kg of water.

The terms mole/l [53] or molon [54] have been proposed recently to designate a concentration scale based on moles of solute per kilogram of solution (cf., molal is moles per kilogram of solvent). This scale is convenient when weight titrations are employed in the laboratory. The scale is not widely used but is mentioned here for the sake of completeness.
Partial pressure is frequently used as a measure of relative composition or concentration of a species in gaseous mixtures. Partial pressure is proportional to mole fraction, the constant of proportionality being the total pressure. Thus \( p_j = x_j P \), where \( p_j \) is partial pressure of the \( j^{th} \) gaseous species, \( x_j \) is the mole fraction, and \( P \) the total pressure. The units of partial and total pressure are most frequently atmospheres or millimeters of mercury (760 mm Hg equals 1 atm) or centimeters of mercury. The partial pressure scale of relative composition has the disadvantage in comparison with mole fraction that the partial pressures do not sum to 1 except at a total pressure of 1 atm and when the unit of measure is the atmosphere.

It is common convention to refer to the partial pressures or tensions of gases dissolved in liquid phases. We should recognize that this use of partial pressure is just a convenient scale for expressing concentration or mole fraction of the dissolved gaseous species without having to make arithmetic use of the solubilities of the various gases in the liquid to compute their relative concentrations or mole fractions. There is no basic difference between, say, oxygen and urea or glucose dissolved in water. There is no more requirement that the partial pressures of water vapor and oxygen must sum to the total hydrostatic pressure on the system than there is
that the sum of the partial pressures of water vapor, urea, and glucose must sum to the total pressure. Thus in condensed phases the sum of the "partial pressures" of dissolved gases may be less than or equal to the total pressure on the system. If the sum of the "partial pressures" of the volatile constituents exceeds the total system pressure, a gaseous phase will appear and increase in size until the "partial pressures" (or better, the chemical potentials) of all species become equal in both phases.

One mole of an ideal gas occupies 22.4 liters at 0°C. The volume of such a gas is proportional to temperature in absolute degrees Kelvin. Absolute zero is \(-273.18°C\) (\(-459.72°F\)) so 0°C is 273.18°C, and 37°C, normal body temperature, is 310.18 K (Kelvin). Thus, the mole volume of an ideal gas at 37°C is \(22.4 \times \frac{310.18 K}{273.18 K} = 25.43 \text{L}\). Fortunately the gases normally present in and about biological systems at their usual pressure and temperatures approach very close to ideal behavior. We are therefore not obliged to employ the rather complex correction factors that would be necessary to properly characterize the gas state for nonideal conditions. Carbon dioxide is the only one of these gases that deviates significantly from ideal. Thus the mole volume of carbon dioxide is 22.26 L at 0°C, 25.27 L at 37°C.

The relations described above are important in the quantification of gaseous-liquid relations in biological systems, as will become more apparent in the following pages.
9.4. Solubility Coefficients and Henry's Law Constants

Henry's law states that the amount of gas dissolved in a liquid is proportional to the partial pressure. In terms of the present discussion, this becomes

\[(9) \frac{\text{mole fraction of a gas in liquid phase}}{\text{mole fraction of gas in gaseous phase}} = \text{constant.}\]

This expression is strongly suggestive of a typical mass action expression for a chemical reaction, say,

\[O_2(\text{gas phase}) = O_2(\text{liquid phase}), K_H,\]

where \(K_H\) is the equilibrium constant for a reaction between two chemical species, one in the gas phase and one in the liquid phase. Indeed one cannot distinguish mathematically between the equilibrium that exists between gas and liquid and other reactions that occur within a phase.

Henry's law constants as usually encountered in physiology are called solubility coefficients. Here again the dimensions of solubility coefficients are designed to maximize ease of laboratory manipulation rather than to clarify their meaning or aid in computation. The solubility coefficient "a" of gas in a liquid is usually defined as follows:

\[(10) \frac{\text{ml gas at STP}}{\text{ml liquid at } T'} = a \times p \text{ gas (atmospheres)}\]
where STP means standard pressure and temperature (that is 760 mm Hg, 0°C), T' is temperature of the experiment in degrees Centigrade, and p gas is the partial pressure of the gas. On this basis the solubility coefficient of a liquid for a gas at a given temperature is the number of milliliters of gas at standard conditions taken up by the liquid at the experimental temperature when the partial pressure of the specific gas in the gas phase is one atmosphere. This form of Henry's law is too cumbersome for efficient use in model building. Equation (9) in terms of mole fraction is much more straightforward. Conversion of Eq. (10) into Eq. (9) using oxygen and water at 37°C as specific examples will illustrate the arithmetic procedures involved.

The numerator of Eq. (10) mℓ O2 STP, may be converted to moles of gas by dividing by 22,400 mℓ/mole. The denominator, mℓ water at 37°C, may be converted to moles by dividing by 1000 mℓ/l to obtain liters and multiplying by 55.14 moles/liter of water at 37°C.* Partial pressure of O2 in atmospheres on the right of Eq. (10) is equivalent to mole fraction and is dimensionless. Dimensionally the conversion of Eq. (10) into Eq. (9) involves the following parameters:

---

*Moles per liter of water is 1000/18.016 x 0.99333 where 1000 is mℓ per liter, 18.016 is the gram molecular weight of water, and 0.99333 is the absolute density of water at 37°C.
(mt × moles/mt)/(mt × moles/mt × t/m) =

(a × moles)/mt/(moles/mt × t/m) = moles/moles = $K_H$.

The absolute value of "a" for oxygen and water at body temperature is 0.02386. Multiplying "a" by the absolute value of (moles/mt)gas/moles/mt × t/mt)water =

(1/22,400)/0.05514 = 8.096 × 10^{-4} as the conversion factor, we obtain $K_H$, the dimensionless equilibrium constant for the mass action equation

mole fraction $O_2$ in water/mole fraction $O_2$ in gas =

$K_H = 1.9317 \times 10^{-5}$.

In the previous section we pointed out that the important free energy parameters $c_j$ are derivable from equilibrium constants. Recall that $c_j = \Delta F_j^0/RT$ where $\Delta F_j^0$ is the standard free energy parameter for a particular reaction at a specific temperature, T is degrees Kelvin at which the model operates, and R is the universal gas constant with dimensions of calories per degree Kelvin per mole. To relate equilibrium constants to $\Delta F^0$ recall that $\Delta F^0 = -RT\ln K$, from which we obtain $c_j = -\ln K$. The $c$ value relating oxygen in gas to oxygen dissolved in water is thus

$c_{O_2} = -\ln(\hat{x}_{O_2}^{\text{water}}/\hat{x}_{O_2}^{\text{gas}}) = -\ln K_H = 10.85$.

The values for Henry's law constant relating other gases

*R may be converted to other dimensions. The dimensions given above yields $\Delta F^0$ in the conventional scale of calories per mole.*
and water in mole fractions are derivable in a similar fashion from the appropriate solubility coefficients.

9.5. Activity Coefficients and Activity in Relation to Gas Solubility

When dealing with biological fluids instead of water in constructing models, we find that the measured solubility coefficients and thereby the Henry's law constants for each gas will differ from those obtained in water. The solubility of, say, oxygen is different in plasma than in water and again different in red cells. The measured equilibrium constants of other types of reactions also vary from one fluid to another. Typically this variation is considered to result from a change in effective concentration for at least some of the species involved in the reaction as the medium is altered. The concept of activity helps to explain and quantify these changes. Activity of a species on any scale of concentration approaches proportionality with concentration, on the same scale, as the concentration of the species approaches zero. As described earlier, the activity coefficient is the ratio of activity to ideal concentration for a particular species in a particular type of solution at a particular temperature and pressure (and gravitational and electromagnetic fields). In most work with solutions, a reference state is selected such that the activity coefficient for a solute approaches 1 at infinite dilution of solute and at all temperatures and all pressures.
The symbol $f$ has been used to designate activity coefficient for mole fraction; $Y$ for molality, and $y$ for molarity.

Returning to the case of oxygen dissolved in plasma, we find that the solubility coefficient at $37^\circ$C is 0.0214 instead of 0.02386 as in pure water. The comparable equilibrium constant is $1.774 \times 10^{-5}$ in contrast with $1.9317 \times 10^{-5}$ for pure water ($\ln K = 10.94$ vs 10.85).

Using activity coefficients, we could write the mass action expression for oxygen dissolved in water as

$$a_{O_2}(\text{water})/a_{O_2}(\text{gas}) = \frac{\hat{x}_{O_2}(\text{water})}{\hat{x}_{O_2}(\text{gas})} \cdot \frac{f_{O_2}(\text{water})}{f_{O_2}(\text{gas})} = K_H = 1.9317 \times 10^{-5},$$

where we may assume $f_{O_2}(\text{water})/f_{O_2}(\text{gas})$ has a value close to 1 because of the very dilute solution. A similar expression for oxygen dissolved in plasma is

$$\frac{\hat{x}_{O_2}(P_t)}{\hat{x}_{O_2}(\text{gas})} \cdot \frac{f_{O_2}(P_t)}{f_{O_2}(\text{gas})} = K'_H,$$

or

$$\frac{\hat{x}_{O_2}(P_t)}{\hat{x}_{O_2}(\text{gas})} = K_H \cdot \frac{f_{O_2}(\text{gas})}{f_{O_2}(P_t)} = K'_H = 1.774 \times 10^{-5}.$$

The activity coefficient ratio $f_{O_2}(P_t)/f_{O_2}(\text{gas})$ therefore has the value 1.089.

Activity coefficients or activities are not mere "fudge" factors but they can be derived in many cases from

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The conventional symbol used both for activity and solubility coefficient is "a." In the context each is used in the text there should be no confusion between the two.
theoretical constructs such as the Debye-Hückel theory of solutions [12, 49, 55], and the derived values correspond well with those determined experimentally. Wherever possible in constructing models of specific biological fluids, we used values for equilibrium constants measured experimentally at 37°C in the actual fluids being simulated. In such cases the appropriate activity coefficient ratios are incorporated in the equilibrium constant. When models so constructed are challenged and a redistribution of water and solute occurs between phases, the previous activity coefficient ratios may no longer obtain. For relatively small changes from normal—such as the change from venous to arterial blood where the characteristics of the plasma and red cell solutions are not greatly altered—not much error should be introduced by possible small changes in activity-coefficient ratios. However, for drastic changes from normal conditions, such as might occur in induced hypothermia where the relative volumes and concentrations of the two media may be greatly changed, significant error may be introduced by failing to account for possible changes in the appropriate activity-coefficient ratios.

9.6. Additive Qualities of Chemical Stoichiometric Expressions

In the way chemical reactions are conventionally expressed, they are not equations in the mathematical sense. Rather, they are shorthand ways of writing quantitative stoichiometric relations that exist among the reacting species and their
products. However, when these expressions are used properly they do not lead to error. Because data from the literature are universally expressed in these terms and because a great deal of manipulation of these expressions is often required to obtain input data in a form suitable for a model, we will illustrate their use for reactions of immediate interest.

When we subsequently construct a model of soda water in equilibrium with gas we shall be interested in the following reactions, among others:

(11) \[ \text{CO}_2(\text{g}) + \text{H}_2\text{O}(\ell) = \text{H}_2\text{CO}_3(\text{aq}), \quad K_3(37^\circ\text{C}) \]

(12) \[ \text{CO}_2(\text{g}) = \text{CO}_2(\text{aq}), \quad K_4(37^\circ\text{C}) \]

(13) \[ \text{CO}_2(\text{aq}) + \text{H}_2\text{O}(\ell) = \text{H}_2\text{CO}_3(\text{aq}), \quad K_3/K_4 = K_5(37^\circ\text{C}). \]

Expression (11) relates the reaction of gaseous carbon dioxide with liquid water to form carbonic acid in aqueous solution. Expression (12) describes the solubility of gaseous carbon dioxide in aqueous solution. Expression (13), the reaction of carbon dioxide in solution with water to form carbonic acid in aqueous solution, may be derived by subtracting (12) from (11). In this example the values of \( K_3, K_4, \) and \( K_5 \) are for 37°C and \( K_5 \) is obtained by dividing \( K_3 \) by \( K_4 \). The relation between the equilibrium constants is more readily apparent if the mass action form for these reactions is used as follows:

(14) \[ \frac{[\text{H}_2\text{CO}_3(\text{aq})]}{[\text{CO}_2(\text{g})][\text{H}_2\text{O}(\ell)]} = K_3, \]
Square brackets indicate concentration in moles per liter of solution. By convention the product species always appear in the numerator in mass action equations.

The Henderson-Hasselbalch equation, a modified form of the mass action equation, is widely used by physiologists to express acid-base relations in biological fluids. It may be derived as follows. The ionization of a weak acid may be written

$$HA = H^+ + A^- \quad K_A$$

In mass action form this becomes

$$\frac{[H^+][A^-]}{[HA]} = K_A$$

Taking the logarithm base 10 of both sides,

$$\log_{10} \frac{[H^+][A^-]}{[HA]} = \log_{10} K_A$$

We now arbitrarily define $pH = - \log_{10}[H^+]$ and $pK_A = -\log_{10}K_A$ and rearrange to

$$pH = pK_A + \log_{10} \frac{[A^-]}{[HA]}$$

which is the conventional form of the Henderson-Hasselbalch
equation. Some useful hand computations that provide insights about the compositional states of the fluid may be made using this equation and the fact that the sum of the amounts of the ionized and un-ionized acid is very nearly equal to the amount of the original acid in the system.*

*The amount of the ionized hydrogen is very small.
10. CONSTRUCTING A MODEL OF SODA WATER AND GASES IN EQUILIBRIUM

We can now consider practical matters relating to the construction of a model of a real chemical system. The first step is to select and carefully define the system we wish to model. For reasons that we will stipulate shortly, we have chosen to model a chemical system consisting of gases in equilibrium with water at constant and uniform temperature. The gas phase has the composition of that existing in the normal human lung, which has a relatively high percentage of carbon dioxide. The relative volumes of gas and water (approximately 125:1) are similar to those often encountered in laboratory devices called tonometers that are used for studying such systems. The system is isolated from the environment in the sense that no chemical substance flows in or out; however, heat is exchanged with the environment as in a thermostat so that constant temperature is maintained. Total pressure in the system is constant and uniform throughout at 1 atm. We have thus defined what has been called a two-phase reversible isothermal system ([48], p. 36).

The composition of the system selected is basic to biological phenomena. To quote Henderson [56], "Two chemical individuals stand alone in importance for the great biological cycle upon the earth. The one is water, the other carbon dioxide." As demonstrated by the references dealing with specific physiological functions, the model
we construct here is an important part of each of the more complex models. Because of its relative simplicity, however, this model provides an excellent example of the methods used and the practical problems encountered in building a model of a chemical problem for solution on an electronic computer.

10.1. Selecting the Significant Chemical Species

An important step in constructing the model is to stipulate the chemical species and their formulas that constitute the system and that are important in determining its characteristics. As we mentioned earlier, selection of the appropriate species to include in the system is often a matter of judgment and trial. In the present system we do not include the rare gases—helium, krypton, xenon, etc.—because in our judgment their presence is not essential to establish the system characteristics of present interest. We know, however, that the respiratory gases oxygen (O\textsubscript{2}), nitrogen (N\textsubscript{2}), carbon dioxide (CO\textsubscript{2}), and water (H\textsubscript{2}O) play a significant role. We also know that when these gases (primarily CO\textsubscript{2} in the present example) are mixed with liquid water, quite a few additional chemical species are formed through reaction.* These chemical species, the chemical reactions relating them, and the equilibrium constants on the conventional scale used in the literature and as converted to mole fraction scale are given in Table I.

*An excellent discussion of these reactions is presented by Edsall and Wyman ([55], pp. 550-590).
Table I

CONSTANTS FOR REACTIONS BETWEEN LUNG GASES AND WATER AT 37°C

<table>
<thead>
<tr>
<th>Reactions</th>
<th>( K ) (From Literature)</th>
<th>( \ln K ) (Mole Fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) ( O_2 ) (gas) = ( O_2 ) (aq)</td>
<td>0.02386(^a)</td>
<td>-10.85</td>
</tr>
<tr>
<td>(2) ( CO_2 ) (gas) = ( CO_2 ) (aq)</td>
<td>0.567(^a)</td>
<td>-7.69</td>
</tr>
<tr>
<td>(3) ( N_2 ) (gas) = ( N_2 ) (aq)</td>
<td>0.01227(^a)</td>
<td>-11.52</td>
</tr>
<tr>
<td>(4) ( H_2O ) (gas) = ( H_2O ) (liq)</td>
<td>16.15(^b)</td>
<td>2.78</td>
</tr>
<tr>
<td>(5) ( H_2O ) (liq) = ( H^+ ) (aq) + ( OH^- ) (aq)</td>
<td>( 2.419 \times 10^{-14} )(^d)</td>
<td>-39.39</td>
</tr>
<tr>
<td>(6) ( H_2O ) (gas) = ( H^+ ) (aq) + ( OH^- ) (aq)</td>
<td>( K_4 \times K_5 )</td>
<td>-36.61</td>
</tr>
<tr>
<td>(7) ( H_2CO_3 ) (aq) = ( CO_2 ) (aq) + ( H_2O ) (liq)</td>
<td>325(^e)</td>
<td>5.78</td>
</tr>
<tr>
<td>(8) ( CO_2 ) (aq) + ( H_2O ) (liq) = ( H^+ ) (aq) + ( HCO_3^- ) (aq)</td>
<td>( 1 \times 10^{-6} )(^f)</td>
<td>-18.53</td>
</tr>
<tr>
<td>(9) ( H_2CO_3 ) (aq) = ( H^+ ) (aq) + ( HCO_3^- ) (aq)</td>
<td>( 1.616 \times 10^{-4} )(^f)</td>
<td>-12.75</td>
</tr>
<tr>
<td>(10) ( HCO_3^- ) (aq) = ( CO_2 ) (aq) + ( OH^- ) (aq)</td>
<td>( K_5 \times K_7/K_9 )</td>
<td>-20.86</td>
</tr>
<tr>
<td>(11) ( HCO_3^- ) (aq) = ( H^+ ) (aq) + ( CO_3^{2-} ) (aq)</td>
<td>( 5.78 \times 10^{-11} )(^g)</td>
<td>-27.59</td>
</tr>
<tr>
<td>(12) ( CO_2 ) (aq) + ( OH^- ) (aq) = ( H^+ ) (aq) + ( CO_3^{2-} ) (aq)</td>
<td>( K_{11}/K_{10} )</td>
<td>-6.73</td>
</tr>
<tr>
<td>(13) ( H_2CO_3 ) (aq) = ( CO_2 ) (aq) + ( H^+ ) (aq) + ( OH^- ) (aq)</td>
<td>( K_9 \times K_{10} )</td>
<td>-33.61(^h)</td>
</tr>
</tbody>
</table>

\(^a\)Ref. [57], p. 8. These are solubility coefficients as defined in the text.
\(^b\)Ref. [52], p. 1460. Derived from equilibrium vapor pressure of water. The constant is dimensionless.
\(^c\)Ref. [12], p. 638. Dimension is moles\(^2\)kg\(^{-2}\).
\(^d\)Moles per kg of water = 1000/18.016 = 55.506, \( \ln = 4.0164 \).
\(^e\)Ref. [12], p. 693. Constant is dimensionless.
\(^f\)Ref. [12], p. 693. Dimension is moles\(^1\)kg\(^{-1}\)
\(^g\)Ref. [12], p. 755. Dimension is moles\(^1\)kg\(^{-1}\)
\(^h\)All constants in the last column are on the mole-fraction scale and are dimensionless.
The equilibrium constants shown are those determined in dilute water solutions at 37°C. Adjustments must be made in these constants when they are used for biological fluids, as previously discussed in connection with activity-coefficient effects. Several reactions listed are redundant (for example, numbers 8 or 9) for purposes of defining the equilibrium system.* As we shall see, however, it is not always most convenient to use reactions in the model in the same form as they are given in the literature. Reaction 9 is derived from others as indicated in the table for such convenient use in the model. From the reactions in Table I we can determine the chemical species necessary to characterize the system.

10.2. The Components and "Mass" Constraints for the System: The Problem in Tableau Form

Next we need to establish the type and amount of the components by which the constituent chemical species are to be defined and constrained in our model. We have a wide choice possible in selecting these components, but they must satisfy the molecular formulas of the species, and it is convenient if this can be accomplished by simple, whole-number combinations. Most important of all, the

*If we were constructing a kinetic model, this redundancy would not exist. If expressions 8 and 9 each represented a kinetic path for the formation and destruction of HCO₃⁻ both sets of reactions would have to be used as reflecting different mechanisms with different time rates. In equilibrium models, however, the amount of a species is independent of the pathway by which it is formed.
components and the constraints they represent must not be redundant. As an example, we should not use \( \text{CO}_2, \text{HCO}_3^- \), and \( \text{OH}^- \) as components because the components \( \text{CO}_2 \) and \( \text{OH}^- \) are sufficient to define the component \( \text{HCO}_3^- \). The components we have selected for our soda-water model having the species given in Table I are \( \text{O}_2, \text{CO}_2, \text{N}_2, \text{H}^+, \) and \( \text{OH}^- \).

The tableau constructed to represent this two-phase gas-aqueous system is shown in Fig. 1. Note that the five components and the constraints they represent provide for the definition of thirteen chemical species in the two phases. We can determine that the five components are the minimum and correct number required by noting that there are the same number of restricting equations as there are linearly independent columns. This latter phrase means that we can choose some five columns and by properly adding or subtracting them obtain the remaining eight columns.

The five component-constraint rows and thirteen species columns shown in the upper portion of Fig. 1 constitute a \( 5 \times 13 \) matrix. We call the form of Fig. 1 a tableau instead of a matrix because it does not contain the zero entries. The mass balance relations shown at the bottom of Fig. 1 make clear the constraints represented by the component rows.

We must now establish the absolute values of these

*Such tableaux are described in detail in [10, 13, 18]. In addition, [18] contains more complete definitions of the notions of component, substance, species and phase than has been presented here.*
### Output Composition By Chemical Species

<table>
<thead>
<tr>
<th>Components (Moles)</th>
<th>Source</th>
<th>Gas Phase</th>
<th>Aqueous Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Designation</td>
<td></td>
<td>0₂</td>
<td>0₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO₂</td>
<td>CO₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N₂</td>
<td>N₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H₂O</td>
<td>H₂O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OH⁻</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H₂O₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H₂CO₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCO₃⁻</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CO₃⁻</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b₁</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b₂</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b₃</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b₄</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b₅</td>
<td>1</td>
</tr>
<tr>
<td>Free Energy Parameters</td>
<td>c₁</td>
<td>c₂</td>
<td>c₃</td>
</tr>
</tbody>
</table>

### MASS BALANCE RELATIONS

- Input $O_2 = x_1 + x_5$
- Input $CO_2 = x_2 + x_6$
- Input $N_2 = x_3 + x_7$
- Input $H^+ = x_4 + x_8 + x_10 + x_{12} - x_{13}$
- Input $OH^- = x_4 + x_9 + x_{10} + x_{11} + x_{12} + x_{13}$

**Fig. 1 Tableau for the Illustrative Soda-Water Model.**
constraints from the definition of the system. Recall that we are mixing 1 kilogram of pure water and 125 liters of gas of typical lung composition at 37°C and 1 atmosphere total pressure. The problem is to compute the number of moles, \( x_j \), and mole fraction, \( \hat{x}_j \), of each of the thirteen chemical species in the system after equilibrium is reached. The composition in millimeters of mercury, mole fraction, and the amount of the gas in moles is given in Table II. The moles in 1 kilogram of water equals 1000 gm/18.016 gm mol. wt \( \text{H}_2\text{O} \) equals 55.506 moles. We use these data to establish the constraints for the system shown in Table III.

10.3. Establishing Appropriate Standard Free Energy Parameters

From Fig. 1 we must determine what \( c_j \) values are appropriate to choose from Table I, in what columns they may be placed, and what their proper sign and absolute values shall be. We will demonstrate two of several algebraic techniques for establishing these matters. The first is a short-cut method; the second involves more steps but is less likely to cause error when used with complex models.

For an \( m(\text{rows}) \times n(\text{columns}) \) matrix, it is sufficient that \( n - m \) of the \( c_j \) are determined. The remaining \( c_j \) may be set to arbitrary values \([13, 18, 19]\) (but values having the proper algebraic relation to those determined from the literature, as we presently demonstrate). In

*Using 1 kilogram of water instead of 1 liter will closely approximate our simulated tonometer ratio of 125:1, gas to water, and avoid the requirement to correct for the changing density of water with temperature.*
Table II

COMPOSITION OF 125 LITERS OF GAS, ONE ATM, 37°C

<table>
<thead>
<tr>
<th>Species</th>
<th>Partial Pressure</th>
<th>Mole Fraction</th>
<th>Moles&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen, O₂</td>
<td>100.0 mm Hg</td>
<td>0.1316</td>
<td>0.6469</td>
</tr>
<tr>
<td>Carbon Dioxide, CO₂</td>
<td>40.0</td>
<td>0.0526</td>
<td>0.2602</td>
</tr>
<tr>
<td>Nitrogen, N₂</td>
<td>572.96 mm Hg</td>
<td>0.7539</td>
<td>3.7058</td>
</tr>
<tr>
<td>Water, H₂O</td>
<td>47.04</td>
<td>0.0619</td>
<td>0.3043</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>760</strong></td>
<td><strong>1.0000</strong></td>
<td><strong>4.9172</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup>The mole volume of a perfect gas at 37°C = 25.43; of CO₂ = 25.27.

Table III

CONSTRAINTS FOR MODEL

<table>
<thead>
<tr>
<th>Component</th>
<th>From Gas, g_i (Moles per 125 L, 1 atm, 37°C)</th>
<th>From Water, w_i (Moles per Kilogram)</th>
<th>Total b_i (Moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂</td>
<td>g₁ = 0.6469</td>
<td>w₁ = 0.0</td>
<td>0.6469</td>
</tr>
<tr>
<td>CO₂</td>
<td>g₂ = 0.2602</td>
<td>w₂ = 0.0</td>
<td>0.2602</td>
</tr>
<tr>
<td>N₂</td>
<td>g₃ = 3.7058</td>
<td>w₃ = 0.0</td>
<td>3.7058</td>
</tr>
<tr>
<td>H⁺</td>
<td>g₄ = 0.3043</td>
<td>w₄ = 55.506</td>
<td>55.8103</td>
</tr>
<tr>
<td>OH⁻</td>
<td>g₅ = 0.3043</td>
<td>w₅ = 55.506</td>
<td>55.8103</td>
</tr>
</tbody>
</table>
the present problem, \( n - m = 13 - 5 = 8 \), the number of free energy parameters that must be selected from Table I. As a matter of arithmetic convenience, the remaining five parameters may be set equal to zero. Not just any five free energy parameters, \( c_i \), may be arbitrarily set to zero—only those for columns that are linearly independent. In chemical terms, this means that the \( c \) values may be arbitrarily set for only those sets of five species whose members do not define a complete, valid reaction. For example, if in Fig. 1 we choose \( O_2 \) in the aqueous phase among the set of five whose \( c \) value shall be zero, we cannot include \( O_2 \) in gas as a member of this set because there is an explicit reaction between these two species \( (O_2(\text{gas}) = O_2(\text{aq})) \). Similarly, we cannot choose \( CO_2 \), \( OH^- \), and \( HCO_3^- \) as common members of the set of five because there is a reaction involving these three species. Within these limitations, we arbitrarily choose zero values for the free energy parameters for the first five species listed in the aqueous phase of Fig. 1, that is, \( c_5 \) through \( c_9 \). The remaining \( c_j \) may be selected from among the experimentally determined values listed in Table I.

The values for \( c_1 \), \( c_2 \), and \( c_3 \) are the Henry's law constants for the solubility of \( O_2 \), \( CO_2 \), and \( N_2 \) (reactions 1, 2, and 3 from Table I). The value for \( c_4 \) relates the partial pressure of water vapor in the gas phase to \( H^+ \) and \( OH^- \) in the aqueous phase (reaction 6, Table I).
Reaction 6 is derived from reactions 4 and 5 in the table. The values for \( c_{10}, c_{12}, \) and \( c_{13} \) are derived from reactions 5, 10, 13, and 12 of Table I in a straightforward manner. Two algebraic methods for checking proper placement of these \( c_j \), their proper signs, and the relations among the arbitrary and experimentally determined values are described below.

For relatively simple models the short-cut method for properly placing free energy parameters involves writing or visualizing the conventional stoichiometric expression for the valid reaction involved. For each species involved in the reaction, substitute the proper \( c_j \) multiplied by the stoichiometric coefficient preceded by a plus sign when the species conventionally occurs on the left of the arrows and a minus sign when the species is to the right of the arrows, all equated to \( \ln K \). Thus for \( \text{CO}_3^- \), column 13, the appropriate expression is 12 from Table I:

\[
\text{CO}_2(\text{aq}) + \text{OH}^- (\text{aq}) = \text{H}^+ (\text{aq}) + \text{CO}_3^- (\text{aq}), \quad \ln K = -6.73.
\]

From this expression one obtains,

\[
1 \, c_6 + 1 \, c_9 - 1 \, c_8 - 1 \, c_{13} = -6.73,
\]

or, since \( c_6 = c_9 = c_8 = 0 \),

\[
c_{13} = 6.73.
\]

The second method for selecting and placing the free
energy parameters involves the algebraic elimination of the appropriate Lagrange multipliers, \( \pi_i \), associated with each row constraint of the matrix. References \([13, 18, 19, 58]\) show that the minimization of the free energy function is equivalent to the existence of numbers \( \pi_1, \pi_2, \ldots, \pi_m \), the Lagrange multipliers, which satisfy:

\[
\sum_{i=1}^{m} \pi_i a_{ij} = c_j + \ln \hat{x}_j, \quad j = 1, 2, 3, \ldots, n.
\]

The left-hand side of the above equation can be eliminated by addition or subtraction in those specific situations in which the species \( x_j \) of the right-hand side are involved in a valid reaction. With the elimination of \( \pi_1 \) an equation is obtained relating \( c_j \) and \( \hat{x}_j \). The following illustrations should help clarify this procedure.

Consider the determination of \( c_1 \) and \( c_5 \), the parameters associated with \( O_2 \) in the gas phase and \( O_2 \) in the aqueous phase. We write

\[(17) \quad \pi_{O_2} = c_1 + \ln \hat{x}_{O_2}^{(\text{gas})}, \]
\[(18) \quad \pi_{O_2} = c_5 + \ln \hat{x}_{O_2}^{(\text{aq})}. \]

Subtracting (18) from (17) we get

\[(19) \quad 0 = c_1 - c_5 + \ln \frac{\hat{x}_{O_2}^{(\text{gas})}}{\hat{x}_{O_2}^{(\text{aq})}}, \]

or rearranging, we get

\[(20) \quad c_1 - c_5 = \ln \frac{\hat{x}_{O_2}^{(\text{aq})}}{\hat{x}_{O_2}^{(\text{gas})}}. \]
Inasmuch as we arbitrarily set $c_5 = 0$,\(^*\)

\[(21) \quad c_1 = \ln \frac{\dot{x}_{O_2}^{aq}}{\dot{x}_{O_2}^{gas}}.\]

Observe that the right side of (21) is the logarithm of the mass action form of reaction 1, Table I, and that the value of $\ln K = \ln \frac{\dot{x}_{O_2}^{aq}}{\dot{x}_{O_2}^{gas}} = -10.85$. This, then, is the absolute value and proper sign to use for the free energy parameter $c_1$ of column 1 in the model if $c_5 = 0$.

A somewhat more complex situation exists for the selection of $c_4$ for $H_2O$ in the gas phase. Here we write

\[(22) \quad \pi_{H^+} + \pi_{OH^-} = c_4 + \ln \dot{x}_{H_2O}^{gas},\]

\[(23) \quad \pi_{H^+} = c_8 + \ln \dot{x}_{H^+}^{aq},\]

\[(24) \quad \pi_{OH^-} = c_9 + \ln \dot{x}_{OH^-}^{aq}.\]

Subtracting (23) and (24) from (22), we get

\[(25) \quad 0 = c_4 - c_8 - c_9 + \ln \frac{\dot{x}_{H_2O}^{gas}}{\dot{x}_{H^+}^{aq} \cdot \dot{x}_{OH^-}^{aq}},\]

and rearranging, we get

\[(26) \quad c_4 - c_8 - c_9 = \ln \frac{\dot{x}_{H^+}^{aq} \cdot \dot{x}_{OH^-}^{aq}}{\dot{x}_{H_2O}^{gas}}.\]

The right side of (26) is the logarithm of the conventional mass action form for reaction 6, Table I, with $\ln K = -36.61$.

Column 13, $CO_3^{2-}$, contains a negative coefficient. In this case the elimination of the Lagrange multipliers

\(^*\)If $c_5$ were set at some other arbitrary value than zero, say 12, then $c_1 - c_5 = -22.85$ is the proper parameter for column 1, with 12 the value for column 5.
proceeds as follows:

\[(27)\quad \pi_{\text{CO}_2} - \pi_{\text{H}^+} + \pi_{\text{OH}^-} = c_{13} + \ln \hat{x}_{\text{CO}_3},\]
\[(28)\quad \pi_{\text{CO}_2} = c_6 + \ln \hat{x}_{\text{CO}_2},\]
\[(29)\quad \pi_{\text{H}^+} = c_8 + \ln \hat{x}_{\text{H}^+},\]
\[(30)\quad \pi_{\text{OH}^-} = c_9 + \ln \hat{x}_{\text{OH}^-}.\]

Subtracting (28) from (27), adding (29), and then subtracting (30) gives (27) - (28) + (29) - (30) =

\[0 = c_{13} - c_6 + c_8 - c_9 = \ln \hat{x}_{\text{H}^+} \cdot \hat{x}_{\text{CO}_3} / \hat{x}_{\text{CO}_2} \cdot \hat{x}_{\text{OH}^-}.\]

But \(c_6 = c_8 = c_9 = 0\), so from reaction 12, Table I,

\[-c_{13} = \ln \hat{x}_{\text{H}^+} \cdot \hat{x}_{\text{CO}_3} / \hat{x}_{\text{CO}_2} \cdot \hat{x}_{\text{OH}^-} = -6.73,\]

or \(c_{13} = 6.73\).

Observe that in this case the sign of \(\ln K\) as conventionally given in Table I is changed when \(c_{13}\) is incorporated in the model. In the present soda-water model all coefficients of the matrix are either 0, 1, or -1. More complex models may have coefficients greater than 1. In such cases, both sides of the equations involving the \(\pi_i\) are multiplied by the appropriate coefficient before the algebraic elimination of the \(\pi_i\).

10.4. Output Format and Solution of Soda-Water Problem

Having now established all parameters necessary to obtain a solution for the equilibrium composition of all species assumed important in characterizing the soda-water model, we insert the parameters into the program for a
digital computer. Clasen [58] describes the mathematical procedures used in the program to obtain the solution by minimizing the free energy of the system subject to the constraints. These need not concern us for the present purpose. Here we will discuss the format of the computer output, and demonstrate how the results may be compared with answers from other sources.

Table IV is a reproduction of the page from a computer printer that lists the input parameters we inserted and states the problem in matrix form. This printout of data used by the computer provides a means for checking these parameters to make sure that no errors were made in inserting the data into the program.

Under ROWS in the table is a listing of the components and the amounts, $b_i$, in moles, of these components. Note that the machine uses floating decimal point notation. Thus, row 1 says that the sum of the $O_2$ component in all species in both gas and aqueous phases is $6.4690000E - 01$. This means that the machine read $6.4690000 \times 10^{-1}$ for this value. Compare this with the value we computed for oxygen in the last column of Table III. The values for the remaining constraining rows are similarly printed and may also be compared with the values established in Table III. Note that we have chosen to introduce water in terms of $H^+$ and $OH^-$. Under the next heading, MATRIX, the species in each
Table IV
PRINT-OUT FROM AN ELECTRONIC COMPUTER LISTING
INPUT PARAMETERS FOR THE SODA-WATER PROBLEM

SODA WATER MODEL

| ROWS |  
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| O2  | 6.4690000E-01 |
| CO2 | 2.6020000E-01 |
| N2  | 5.7058000E 00 |
| OH- | 5.5810300E 01 |
| H+  | 5.5810300E 01 |

| MATRIX |  
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GAS PHASE |  
| O2  | -10.850000 | 1.000 O2 |
| CO2 | -7.590000  | 1.000 CO2 |
| N2  | -11.520000 | 1.000 N2 |
| H2O | -36.610000 | 1.000 H+ |
|     |             | 1.000 OH- |

| AQUEOUS PHASE |  
| O2  | 0.       | 1.000 O2 |
| CO2 | 0.       | 1.000 CO2|
| N2  | 0.       | 1.000 N2 |
| H+  | 0.       | 1.000 H+ |
|     | 0.       | 1.000 OH-|
| H2O | -39.390000| 1.000 H+ |
| HCO3-| -20.860000| 1.000 CO2 |
| H2CO3| -33.610000| 1.000 H+ |
| CO3- | 6.730000  | 1.000 CO2 |
|     |           | -1.000 H+ |
|     |           | 1.000 OH- |

| MATRIX 1 2 3 4 5 6 7 8 9 10 11 12 13 |  
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1  | 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| 2  | 0 1 0 0 1 0 0 0 0 0 0 1 1 1 1 1 |
| 3  | 0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 |
| 4  | 0 0 0 1 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1 |
| 5  | 0 0 0 1 0 0 0 1 0 1 0 1 0 1 0 1 1 1 1 |

SIMPLEX
SOLVE
OUTPUT
phase are listed, together with their standard free energy parameters and the matrix coefficients for the rows (components) associated with each of these species (columns). As a double check in this case, we asked the machine to print the tableau form of the matrix, which appears next. Note that this tableau is the same form that we constructed and showed in Fig. 1. The five rows of the tableau correspond to the components listed under the ROWS heading at the top of the table, and the columns correspond to the species listed in order under the MATRIX heading just preceding. The appropriate coefficients for each column also appear in the printed tableau. The values given for the free energy parameters may be checked against those previously established and listed in Table I. The positioning of these parameters in the appropriate column and their sign may be checked by using the Lagrange multiplier technique or the short-cut method described above.

SIMPLEX is an instruction to the machine to use the linear programming subroutine described in [58] for establishing initial values for \( x_j \), the moles of all species. Next, SOLVE instructs the machine to proceed with the computation of the equilibrium composition, and OUTPUT calls for printing the answers after a predetermined degree of precision is reached.

Table V is a reproduction of the format of the computer output showing the answers obtained to the problem. We
### Table V

PRINT-OUT FROM AN ELECTRONIC COMPUTER GIVING THE SOLUTION TO THE SODA-WATER PROBLEM AT 40-MM CARBON DIOXIDE PARTIAL PRESSURE

**SODA WATER MODEL**

RMS MASS BALANCE ERROR = 2.076E-07 MAX. ERRORK = 3.076E-07 ON ROW H+
RMS EQUILIBRIUM ERROR = 2.398E-07 MAX. ERkur = 3.347E-07 IN OH- OF AQUEOUS PHAS

**OPTIMAL SOLUTION**

<table>
<thead>
<tr>
<th>GAS PHASE</th>
<th>AQUEOUS PHAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-BAR</td>
<td>4.51594E 00</td>
</tr>
<tr>
<td>PH</td>
<td>-0.9</td>
</tr>
<tr>
<td>O2</td>
<td>MOLES 6.46758E-01</td>
</tr>
<tr>
<td></td>
<td>MFRAC 1.31563E-01</td>
</tr>
<tr>
<td>CO2</td>
<td>MOLES 2.58833E-01</td>
</tr>
<tr>
<td></td>
<td>MFRAC 5.26515E-02</td>
</tr>
<tr>
<td>N2</td>
<td>MOLES 4.70538E 00</td>
</tr>
<tr>
<td></td>
<td>MFRAC 7.53748E-01</td>
</tr>
<tr>
<td>H2O</td>
<td>MOLES 3.04967E-01</td>
</tr>
<tr>
<td></td>
<td>MFRAC 6.20364E-02</td>
</tr>
<tr>
<td>H+</td>
<td>MOLES -0.8</td>
</tr>
<tr>
<td></td>
<td>MFRAC -0.5</td>
</tr>
<tr>
<td>OH-</td>
<td>MOLES -0.9</td>
</tr>
<tr>
<td></td>
<td>MFRAC -0.1</td>
</tr>
<tr>
<td>HCU3-</td>
<td>MOLES -0.5</td>
</tr>
<tr>
<td></td>
<td>MFRAC -0.2</td>
</tr>
<tr>
<td>H2CO3</td>
<td>MOLES -0.6</td>
</tr>
<tr>
<td></td>
<td>MFRAC -0.6</td>
</tr>
<tr>
<td>CO3-</td>
<td>MOLES -0.9</td>
</tr>
<tr>
<td></td>
<td>MFRAC -0.4</td>
</tr>
</tbody>
</table>

* This title is not a misprint. The program provides a maximum field of twelve spaces for compartment names.
are told first what errors exist in the final solution. These errors in the mass balance constraints and similar errors in mass action relations in the optimum solution are largely the result of round-off errors in the machine. Under the heading for each phase, GAS and AQUEOUS, the following information about the solution is listed: X-BAR (\( \bar{x} \)) or total number of moles in the phase, the pH where appropriate, and the number of moles and the mole fraction of each species in both phases representing the computed equilibrium composition.

A special routine in the program directs the machine to calculate pH from the number of moles of \( H^+ \) and \( H_2O \) in

---

*The exact definitions of these errors are complex and unnecessary for our present purposes.

**The criticism has been made that showing five to seven digits to the right of the decimal point (see Table V) indicates more significant figures than the accuracy of the input warrants. There are several answers to this criticism. First, by photographically reproducing the output of a computer as we have done in Table V we avoid the human errors that inevitably occur if the numbers are rounded to a significant figure by hand and recopied. Second, the machine we use normally prints the number of digits shown in the table (it uses many more places in the computation). To instruct the machine to change its normal ways and to round to some computed or empirical significant place would require reprogramming and more importantly the use of additional computer capacity. In addition, the degree of accuracy of many of the parameters we must use from the literature are not indicated so that rounding must be largely empirical in any event. One past incentive for the use of the significant figure convention was that the hand computation of additional places was costly in time and effort. With the use of a computer, the additional places are essentially free. In view of all the above circumstances, we maintain that it is not less appropriate to print, say, 1.32341 \( \times 10^{-3} \) than round to 1.30 \( \times 10^{-3} \).
the aqueous phase at equilibrium. The moles of water are converted to liters of water using an appropriate conversion factor. The negative \( \log_{10} \) of the ratio of moles of \( \text{H}^+ \) to liters of \( \text{H}_2\text{O} \) is then determined as pH.\(^*\) The value of the computed pH, 4.59, may appear low to those accustomed to 7.39 for normal arterial plasma at the same carbon dioxide partial pressure of 40 mm Hg. We have checked this value of 4.59 in the laboratory and find it nicely in line for this gas-aqueous system. As we will demonstrate shortly, the higher pH in plasma results from additional constituents present in that system.

The consistency of the solution to the problem as given in Table V may be checked by applying the

\(^*\)It would probably be more appropriate to convert moles of water to kilograms of water for this purpose so that we would have a true molal scale. However, at 37°C the difference would only be 0.003 pH units, within the limits of accuracy of most laboratory determinations of pH. In addition, this procedure for computing pH is an empirical matter in which we have attempted to find the method that gave best fit to laboratory pH measurements under known conditions. In recent years conclusive arguments have been presented, based on both theoretical [48] and practical electrolytic cell considerations [59], that pH values are not direct measures of hydrogen-ion concentration or activity. Rather they are a reliable measure of an empirical "acidity" of the unknown solution in relation to a standard solution having the same general characteristics, including temperature. Conventional pH measurements are indeed most useful for control and comparison purposes, but are not suited for thermodynamic calculations or mass action law use. An important problem in the validation of chemical models is to relate the "acidity" determined by a biophysical model to the "acidity" so widely measured in the laboratory with pH meters. This relationship can only be established by empirical trial for the particular types of solutions that are dealt with.
Henderson-Hasselbalch equation to the several acid-base reactions represented in the model.

Note that the computed equilibrium mole fraction values for the gas phase in Table V are not quite the same as for the gas that was added to the original kilogram of water (Table II). This discrepancy is caused by the solubility of the gases in the water and by the reactions of carbon dioxide with water. The difference between the input composition of gas and the composition of the gas at equilibrium with water may be made as small as desired (but not zero) by increasing the volume and thereby the magnitude of the components arising from the original input gas source.

The computation of pH, although a simple procedure in itself, is representative of a feature of the program that enables one to design and execute quite complex experiments on a computer. Instead of a single computer program with a fixed function, we have found it expedient to use a system of integrated programs, each of which carries out a specific function. For example, there is a subprogram that "solves" the basic chemical equilibrium, another that prints the tableau, another that computes and prints a Jacobian matrix, etc. For a particular application these subprograms may be called on in any desired sequence and in any pattern of iteration.* Furthermore, special

*The reader interested in details of this and other programming techniques should consult [60,61].
computations related to the particular problem may be interspersed in sequencing the subprograms.

This special computational feature was used, for example, to develop oxygen dissociation curves for hemoglobin [8] and to represent the operation of a pH-stat where the acidity of any desired phase (compartment) is maintained at constant pH as components are added or subtracted from the system [8]. Perhaps more importantly, this computational feature may be used to introduce time into certain models of physiological function, as mentioned in an earlier section and as described more fully in [11].

10.5. Experimenting with the Model--The Construction of Carbon Dioxide Dissociation Curves

For the present, this feature of internal program control will be used to construct what have been called carbon dioxide dissociation curves for the soda-water model. The characteristics of these curves have classically been used to demonstrate an important feature of blood in carbon dioxide respiration ([1], pp. 40-59; [55], pp. 561-571). The carbon dioxide dissociation curves are typically constructed by plotting total carbon dioxide in the aqueous phase against partial pressure of carbon dioxide in millimeters of mercury. To construct these curves using our model, we require a device that will automatically increase the partial pressure of the carbon dioxide in the gas phase but keep the total pressure of the gas at one atmosphere. We choose to start at about 5 millimeters partial pressure
of carbon dioxide and increment its partial pressure by about 10 millimeters between each solution to the problem. The total pressure is maintained constant during this series by reducing the partial pressure of nitrogen by the exact amount of the increment in carbon dioxide. The partial pressures of oxygen and water vapor are maintained constant during this series.

Table VI is a printout from a computer showing the solution to the problem at a partial pressure of carbon dioxide of about 251 mm Hg. Changes that occur in the composition and mole fraction of all species in the system in respect to 40 mm Hg may be observed by comparing the values in Table VI with those in Table V. The internal control program was used to provide computation and printout of partial pressure of carbon dioxide in millimeters of mercury. The program was directed to look up the computed mole fraction of carbon dioxide in the gas phase at each solution and to multiply this value by 760 mm Hg per atmosphere to convert to millimeters of mercury.

The components as computed by direction of the internal program may be observed and checked as they are printed in Table VI and listed under columns B through B₄. Column B gives the sums of all components in moles from both water and gas similar to those we computed by hand and listed in Table III.* Column B₁ lists the components from the original

*The absolute values in Table III are different because of the differences in partial pressures of carbon dioxide and nitrogen.
-113-

Table VI
PRINT-OUT FROM AN ELECTRONIC COMPUTER GIVING THE INPUTS AND SOLUTION TO THE SODA-WATER PROBLEM AT 251-°K CARBON DIOXIDE PARTIAL PRESSURE

<table>
<thead>
<tr>
<th>Row Name</th>
<th>B</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2</td>
<td>6.4710352E-01</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
</tr>
<tr>
<td>CO2</td>
<td>1.6030465E-00</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
</tr>
<tr>
<td>N2</td>
<td>2.2331783E-00</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
</tr>
<tr>
<td>OH-</td>
<td>5.5810374E-01</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
</tr>
<tr>
<td>*CO2</td>
<td>-0.</td>
<td>0.</td>
<td>-0.</td>
<td>-0.</td>
<td>-0.</td>
</tr>
</tbody>
</table>

**Matrix**

<table>
<thead>
<tr>
<th>Row Name</th>
<th>B</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2</td>
<td>-10.850000</td>
<td>1.000</td>
<td>02</td>
<td>-0.</td>
<td>-0.</td>
</tr>
<tr>
<td>CO2</td>
<td>-7.690000</td>
<td>1.000</td>
<td>CO2</td>
<td>-0.</td>
<td>-0.</td>
</tr>
<tr>
<td>N2</td>
<td>-11.520000</td>
<td>1.000</td>
<td>N2</td>
<td>-0.</td>
<td>-0.</td>
</tr>
<tr>
<td>H2O</td>
<td>-36.610000</td>
<td>1.000</td>
<td>H2O</td>
<td>-0.</td>
<td>-0.</td>
</tr>
</tbody>
</table>

**Table VI**

| PRINTOUT FROM AN ELECTRONIC COMPUTER GIVING THE INPUTS AND SOLUTION TO THE SODA-WATER PROBLEM AT 251-°K CARBON DIOXIDE PARTIAL PRESSURE |
|---|---|---|---|---|---|---|
| **O2** | **CO2** | **N2** | **OH-** | **H2O** | ***CO2** |
| 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 |
| 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |

**Optimal Solution**

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water, that is, 1 kilogram converted to moles. The multiplier at the top of each column (in the case of $B_1$, it is 1) represents the number of units of the components of $B_1$ to be used as inputs. Thus if we wished to use 5 kilograms of water instead of 1, this multiplier need only be changed to 5. Column $B_2$ lists the increment of carbon dioxide and decrement of nitrogen to be added algebraically to the starting mixture. The values listed are in mole fraction so the $B_2$ multiplier is 4.9172, the number of moles in 125 liters of gas at 37°C (see Table II). The use of this multiplier converts mole fractions to moles to be added (algebraically) to the total moles of components. Column $B_3$ lists the mole fractions of the original gas mixture in the series (approximately 5 mm CO$_2$ partial pressure). These values are also multiplied by 4.9172 to convert to moles and are added to the values from the other columns to establish total $B$. Column $B_4$ is used as a matter of convenience to keep track of the mole fractions of the gases being used at each solution point. Note that $B_4$ equals $B_2$ plus $B_3$, and since the multiplier for $B_4$ is zero, no addition to total $B$ is made from this column.

Another device is used in the model to compute the total carbon dioxide in the aqueous phase. This consists of adding an additional constraining row to the matrix having a coefficient in each column of the aqueous compartment for each species containing the component carbon dioxide. We then create a new conceptual compartment or
phase that we call SUM containing a conceptual species called SUMCO₂. Because we have created a new compartment containing only one species whose c value is zero, we are free to add this additional constraining row (whose amount is zero in this case) without altering the equilibrium solution to the problem. Row 6 in the tableau shown in Table VI is this constraining row and column 14 is the conceptual compartment containing the species designated SUMCO₂. By this device we make the machine add the moles of carbon dioxide component in the species CO₂, HCO₃⁻, H₂CO₃, and CO₃²⁻ of the aqueous phase and print this sum at each solution point.* Each species containing the carbon dioxide component has only 1 mole per mole of species so that the coefficients are all 1 for this case.

The data printed in Table VI are the answers for only one of thirty-six solutions to the problem in which the carbon dioxide partial pressure is incremented by 10 millimeters between solution points. The specific data for total moles of carbon dioxide in the aqueous phase vs partial pressure of carbon dioxide were read from the printouts for each solution point and plotted as curve 1 in Fig. 2.**

---

*This technique for counting by adding conceptual compartments and constraining rows is used extensively and described in [8] and [62] where models are developed that incorporate complex molecules like hemoglobin and serum albumin. In these cases the conceptual compartments are used to keep track of the state of the numerous active sites of the large molecules that may combine with H⁺ (or other ions), O₂, or CO₂. More rigorous explanations of this efficient method for representing complex molecules and other matters are contained in [63] and [64].

**This curve may be compared with that labeled -∞ in Fig. 2, page 569, [55], which corresponds to a solution of CO₂ in pure water.
Fig. 2—Carbon dioxide dissociation curves for soda water with and without added buffer (dotted contour lines are lines of constant pH)
The curves labeled 7, 8, and 9 in our Fig. 2 were obtained from the machine for systems analogous to those used by Edsall and Wyman ([55], p. 569) and may be compared directly with their similarly labeled curves. The modified systems were obtained by adding to the water 0.04 mole of sodium hydroxide, NaOH, plus 0.06 mole of a buffer consisting of an acid HA and its conjugate base A⁻. The numbers 7, 8, and 9 refer to the pKₐ assigned as the negative log₁₀ of the dissociation constant of the weak acid HA. Table VII is the machine printout for one solution point (pCO₂ = 50.46 mm, pKₐ = 8). Note in the printed input information and in the tableau how the NaOH and HA are added as components (rows) and as species (columns). The reader is invited to check the position sign and value of the standard free energy parameter c HA for the weak acid used in the example of Table VII.

The differences in shape between curve 1 of Fig. 2 (for pure CO₂ and water) and curves 7, 8, and 9 (with added buffer) illustrate the importance of the additional buffer systems that exist in blood, notably hemoglobin. As pointed out by Edsall and Wyman ([55], p. 571), a curve for a buffer having a pKₐ lying between 7 and 8 will

---

*There is no column provided for NaOH as a species because it is supposedly completely ionized. If we wish to be somewhat more rigorous, we should add columns for the sodium salts of the other anions present as these are not completely ionized [65]. However, the ionization constants of these salts are large in respect to the acid ionization constants of interest here, and their inclusion would not alter the results significantly.
## Table VII

### SOLVE WATER MODEL CO2 INCREMENTED BY ABOUT 10 MM PARTIAL PRESSURE

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**OPTIMAL SOLUTION**

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**OPTIMAL PRESSURE**

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**Notes:**
- The table presents the results of a computer simulation for solving the SODA WATER model, considering the incrementation of CO₂ by about 10 MM partial pressure.
- The solution includes calculations for both gas and aqueous phases, along with optimal solutions for various concentrations.
- The table also outlines the optimal pressure value obtained from the simulation.
have a maximum slope when pCO₂ is near 50 mm. Such a buffer would give maximum efficiency in the uptake and release of carbon dioxide in the body as the partial pressure is varied in this range in the vascular system of the body and in the lungs.

At this point we make no claim for having solved biochemical problems on a computing machine that are impossible to solve by hand. Rather, the example of soda water with and without added buffer was selected as an illustration of how a program is constructed to solve problems closely related to physiological chemistry. It is of interest to note, however, that the some 430 data points (pCO₂, pH, and total CO₂) used in the construction of Fig. 2 were obtained in one computer run of approximately four minutes duration.* The addition of a dozen more species and other pKₐ values would not have increased the computer time required to any great degree, as a significant amount of time was used in loading the machine and translating the program language (Fortran IV) into machine language. These factors would not increase with the larger problem. In contrast it might take several days to compute by hand the points necessary to construct Fig. 2 with an equal degree of accuracy. An important additional advantage of the illustrative buffered soda-water model is that it provides a good

*The machine output at each solution also contains a complete listing of the number of moles and mole fraction for each of the species in the gas and aqueous phases as indicated in Table VII. The time mentioned above was obtained using an IBM 7044 machine and Fortran IV as the programming language.
basis for expansion to represent more precisely the bio-
physicochemical features of real biological fluids and a
physiological subsystem.
11. EXPANDING THE SODA-WATER MODEL

Various types and degrees of physiological realism may be added to the soda-water model in different ways, depending on one's particular interests. Obvious steps are to add a third compartment representing red cells, incorporate at least a simple simulation of hemoglobin, and alter the free energy parameters to reflect those measured in body fluids [13, 19]. One might next wish to add more of the important reactions of hemoglobin in the red cells [8, 66] and the chemical buffering phenomena of serum albumin in plasma [62]. An initial simulation of whole-body phenomena can be obtained by adding aggregated compartments representing the readily exchangeable interstitial and intracellular fluids and electrolytes [41, 42]. The model can be made dynamic by providing for excretion and the addition of components due to metabolism. The means by which this can be accomplished were described briefly in an earlier section and more completely in [11]. One might next wish to explore the possibilities for realistically representing the effects of certain important hormones using the enlarged model.
REFERENCES


