SCIENCE

VOL. 95 FRIDAY, JANUARY 9, 1942 No. 2454

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

Lancaster, Pa. Garrison, N. Y.
New York City: Grand Central Terminal

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary in the Smithsonian Institution Building, Washington, D. C.

EQUILIBRIUM THERMODYNAMICS AND BIOLOGICAL CHEMISTRY[1, 2]

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SINCE science progresses by building block upon block, it is important to examine the structure from time to time to make sure that there are no badly fitted blocks, none which are being made to carry more than their proper capacity and none which might be made more useful. This is particularly true when thermodynamics, which is a vast and variegated structure erected upon the simple bases of the two laws of thermodynamics, is applied in biology and biological chemistry to systems so much more complicated than those in which its results have been tested by physical chemists. Yet such an examination is discouraged because we are so certain of the foundation on the two laws of thermodynamics as laid out by J. Willard Gibbs.

I see no reason to question the validity of the two laws of thermodynamics, even for vital processes, and the only mistake I know in Gibbs's great paper is a trivial typographical error. However, a close examination shows that the foundation we are using is not exactly the one laid out by Gibbs. A discussion

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[1] Address of the retiring vice-president and chairman of the Section on Chemistry, American Association for the Advancement of Science, Dallas, Texas, December 29, 1941.

[2] Contribution from the Research Laboratory of Physical Chemistry, Massachusetts Institute of Technology, No. 498.

of the main ideas, without details and without mathematics, may bring out the differences and show how to minimize any difficulties which may arise from them.

From the fact that a system with constant energy which approaches equilibrium must increase in entropy, Gibbs derives two criteria of equilibrium. The first is that in a system in equilibrium there is no possible change which will increase the entropy at constant energy. The second and more useful one is that in a system in equilibrium there is no possible change which will decrease the energy at constant entropy. He then introduces a new function which he calls the potential or, more specifically, the intrinsic or chemical potential. He defines the chemical potential of a substance in a system as the derivative of the energy of the system with respect to the quantity of that substance when the entropy and volume of the system and the quantities of other components are kept constant.

The two chief characteristics of the Gibbs method are the consideration of the state of a system rather than a process, and the splitting of the consideration of the state of a complex system into the study of the chemical potentials of its individual components. The chemical potential has the characteristic properties of potentials such as the gravitational, electrical or magnetic potential. However, many of us are more familiar with it under other names or with related quantities which emphasize other characteristics. The partial molal free energy of a substance is its chemical potential per mole. The pH is the chemical potential per mole of the hydrogen ion divided by minus 2.3 RT. The osmotic coefficient of a solution is the chemical potential of the solvent divided by minus RT times the moles of solutes per mole of solvent. The activity of a substance is e with the chemical potential per mole divided by RT as exponent, and the activity coefficient is the activity divided by the concentration or by the mole fraction.

Gibbs's next step is to study equilibrium in an idealized system. I quote his own words:

> In order to arrive as directly as possible at the most characteristic and essential laws of chemical equilibrium, we will first give our attention to a case of the simplest kind. We will examine the conditions of equilibrium of a mass of matter of various kinds enclosed in a rigid and fixed envelope, which is impermeable to and unalterable by any of the substances enclosed, and perfectly non-conducting to heat. We will suppose that the case is not complicated by the action of gravity, or by any electrical influences, and that in the solid portions of the mass the pressure is the same in every direction. We will farther simplify the problem by supposing that the variations of the parts of the energy and entropy which depend upon the surfaces separating heterogeneous masses are so small in comparison with the variations of the parts of the energy and entropy which depend upon the quantities of these masses, that the former may be neglected by the side of the latter; in other words we will exclude the considerations which belong to the theory of capillarity.
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> It will be observed that the supposition of a rigid and non-conducting envelope enclosing the mass under discussion involves no real loss of generality. . . . As for the other suppositions which have been made, all the circumstances and considerations which are here excluded will afterward be made the subject of special discussion.

Most of the important results associated with Gibbs's name are reached before the return to any of the excluded considerations, and the others are obtained while all but one of these considerations are still excluded. All his results are based on the assumption that there is equilibrium with respect to every possible change, and upon the count of the number of quantities necessary to describe the thermodynamic state of the system. At equilibrium and with the restrictions given by Gibbs, the energy of any phase may be expressed as a function of its entropy, volume and the quantities of its components. The partial derivatives of the energy with respect to these other functions, which are the temperature, minus the pressure, and the chemical potentials of the components, are independent of the quantity of the phase.

The phase rule needs nothing further, for it depends upon the number of independent variables and not upon the value of any of them. The application of the "Gibbs-Duhem equation," by which the chemical potential of one component can be determined if those of all the others are known, requires a knowledge of the variation of the chemical potentials with composition at constant temperature and pressure. The treatment of chemical equilibrium and of the distribution of substances between phases needs also a knowledge of the variation of the potentials with temperature and pressure at constant composition.

Equilibrium in these restricted systems is conveniently treated in terms of the Gibbs free energy, which may be defined as the sum for all the components of the products of the chemical potential by the quantity of the corresponding component. Then the conditions of equilibrium may be expressed as follows: the temperature must be the same throughout the system; the pressure must be the same except on opposite sides of a rigid membrane impermeable to at least one component; and the change in free energy must be zero for any possible change at constant temperature and pressure.

From statistical mechanics or from experimental observation, we know that the chemical potential of a substance at very low concentrations varies at constant temperature and pressure as RT times the logarithm of its concentration. Combined with the conditions for equilibrium and the thermodynamic relations for the variation of the chemical potential with temperature and pressure, this leads to all the classical expressions for chemical or physical equilibrium. For many purposes it is sufficient to know these relations and the fact that the deviations have not become large at the lowest concentrations studied experimentally. Statistical mechanics, again confirmed by experiment, gives us this assurance and at the same time furnishes the tools for a quantitative study of the deviations. It tells us that the forces between molecules cause a variation in the chemical potential of an ion which starts out proportional to the square root of the ionic strength with a coefficient determined by theory, and a variation in the chemical potential of any substance which starts out proportional to the concentration of the added substance with a coefficient which is proportional to the interaction between a molecule of the added substance with one of the substance under consideration, corrected for the interaction of the displaced solvent. When the two substances are the same, the ratios of these effects to the ideal solution term are proportional to the square root of the concentration or to the concentration, and are also proportional to the ratio of the interaction energy to RT. In aqueous solutions, which are of primary interest in biological chemistry, much of this interaction is due to the displacement of water, and is thus directly proportional to the volume of the solute molecule. Other interactions depend upon the number of active groups and are also usually larger for large molecules.

The restriction that electrical influences must be absent is not a serious one, for we are able to control the external electrical field and to eliminate it when we wish. The gravitational field must be taken into account for precision work. This effect is also proportional to the volume of the solute molecule and to the difference between its density and that of the medium. For a protein in water, with densities 1.33 and 1.00, the relative change in concentration per centimeter is 10^{-8} times the molecular weight. For most soluble proteins the effect is small. For viruses it is very large. The extension of this treatment to centrifugal fields, which behave like very large gravitational fields, is one of the most powerful methods for the study of large molecules.

The difficulties with large molecules increase for heterogeneous equilibrium. Since the interfacial area can be decreased by the formation of a large particle from several small ones, the solubility of a dispersed phase depends upon the size of the particles. If the shape of the particles remains constant, the effect on the chemical potential of the solute is proportional to the molal volume of the solute, and to the interfacial tension, and inversely proportional to a linear dimension of the particle. The vapor pressure of water is ten times as great from a drop 10^{-7} cm in diameter as from a plane surface. The effect of particle size on the solubility of salts indicates that the interfacial tension is ten to fifty times as great as the surface tension of water. Protein molecules are so large that the interfacial tension of a protein-solution surface must be very small indeed if the effect of particle size does not persist to particles of the size usually used for solubility measurements. The concentration at an interface of a substance which reduces the interfacial tension is proportional to the change in surface tension per mole of solute, and thus proportional to the number of surface active groups per molecule which can reach the surface. Surface denaturation is apparently due to the disruptive flattening of a globular molecule so that more groups can reach the interface. The importance of absorption at interfaces in biological systems must be extremely great.

If one of the phases is solid, the effect of strain, which causes the pressure to be different in different directions, is usually greater for large molecules than for small because the strain per molecule is greater and also because there is a smaller probability that the larger molecules can adjust themselves to relieve the strain.

Perhaps the most important difference between real systems and the idealized ones of Gibbs is that real systems are seldom truly at equilibrium. This is obviously true of biological systems, but it is also true of most of the inanimate systems we study. Although we talk often of the absoluteness of thermodynamic equilibrium, we usually assume in practice that the equations for equilibrium hold if the system is at equilibrium with respect to the change we are studying and there is no closely related change progressing rapidly. The only excuse I can find for this assumption is that it appears to work. I quote Gibbs again, this time from his letter to Bancroft:

> The meagreness of the results obtained in my E. H. S. [Equilibrium of Heterogeneous Substances] in the matter of electrolysis has a deeper reason than the difficulty of the evaluation of potentials.
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> In the first place, cases of true equilibrium (even for open circuit) are quite exceptional. Thus the single case of unequal concentration of the electrolyte can not be one of equilibrium since the process of diffusion can not be stopped. Cases in which equilibrium does not subsist were formally excluded by my subject, and indeed could not be satisfactorily treated without the introduction of new ideas quite foreign to those necessary for the treatment of equilibrium.
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> Again, the consideration of the electrical potential in the electrolyte, and especially the consideration of the

difference of potential in electrolyte and electrode, involves the consideration of quantities of which we have no apparent means of physical measurement, while the difference of potential in ''pieces of metal of the same kind attached to the electrodes'' is exactly one of the things which we can and do measure.

The case of unequal concentration of the electrolyte, or a concentration cell with transference, is a cell in which two identical electrodes are immersed in solutions of the same electrolyte at different concentrations, the solutions meeting at a liquid junction. The passage of current effects exactly the same change of state as diffusion, or its reverse. Yet electromotive force measurements may be made very accurately, and the chemical potentials determined from them agree excellently with those by other methods. A slight effect of a very sharp concentration gradient was first noted by Buehrer and me with hydrochloric acid cells. We attributed the effect to a change in temperature due to the heat of dilution by diffusion. Our explanation was confirmed by Hamer's work with sulfuric acid, for which concentrations may be chosen to make the heat effect either very large or zero, and the magnitude of the electrical effect depends entirely on the magnitude of the heat effect. The fact that an irreversible process giving the same change of state as the reversible process affects the measurement of the chemical potential only as it affects the temperature is the best justification I know for our use of equilibrium thermodynamics in systems which are not in equilibrium with respect to all processes.

An extreme example is the quasi-thermodynamic treatment of the rates of chemical reactions, based on Brönsted's theory of the critical complex. It assumes that the rate of a reaction is proportional to the concentration of an intermediate critical complex which decomposes so rapidly that it almost always forms the products if it is made from the reactants and almost always forms the reactants if it is made from the products. It is so unstable that its concentration can not be measured, but the effect of changes in the medium on the reaction rate can be determined by calculating from the theories of statistical mechanics its activity coefficient and those of the reactants. The success of this treatment is very nearly the same as that of the application of the same theories of statistical mechanics to chemical or physical equilibrium in stable systems.

The properties of a system which is not at equilibrium depend upon more variables than those of a system at equilibrium, for they must depend also upon the extent of every change of state which is not at equilibrium. These changes include: chemical reactions, which may be either homogeneous or heterogeneous; changes of concentration within a phase, between two phases or at an interface; changes of particle size in a dispersed phase, which may be solid, liquid or gas; changes of orientation relative to an external field, relative to an interface, or relative to other molecules in a solid or in a liquid crystal; and changes of crystal systems, changes of shape or changes of strain in a solid phase. If the extent of the change can be measured it is possible to treat the system as though it were at equilibrium at any stage of the change. Otherwise we assume that the system has had time to reach equilibrium. The time necessary to reach substantially the equilibrium state will vary enormously for different changes and different systems. It is much larger for many biological systems than for most of the systems studied by physical chemists. Each extension should be tested to ensure that the times are sufficient.

If my talk should persuade you to read Gibbs, you will note that many of his expressions contain an inequality as well as the equality I have discussed. This inequality covers the case in which a component is entirely absent from a phase which is in equilibrium with another phase which contains that component. Then we can only say that the chemical potential of the substance in the phase in which it is absent is equal to or greater than its potential in the rest of the system. For substances whose behavior approaches the perfect gas law or Henry's law at zero concentration, the chemical potential is minus infinity at zero concentration, and is therefore certainly less than its potential in any phase where the concentration is finite. Either it is impossible to have a component entirely absent from a phase in equilibrium with a phase in which it is present or its potential must behave very differently in such a phase and in one in which the substance can be present. Though most of us find the first assumption more reasonable, there seems no possibility of a direct answer to this problem. We may, however, define a component as practically absent from a phase if its concentration is so small that we can not measure it or its effect on the chemical potential of any other component by the methods we are using to study the system. By this criterion we often find a component practically absent from a phase in equilibrium with another phase in which it is present.

Too great insistence on thermodynamic rigor is not always helpful. I think it has hurt one important aspect of applied physical chemistry—the measurement of hydrogen-ion activity or pH. Most physical chemists have never liked the saturated potassium chloride bridge system for two reasons. They believe it is not accurate experimentally and not rigorous theoretically. It is certainly easy to make inexact measurements of pH by this method, and there must be thousands such made every day. I believe, however, that the experimental uncertainty due to the liquid junction potential can usually be reduced below the uncertainty due to the electrode just as in a simple concentration cell. The important factor is probably the heat effect at the liquid boundary, which I discussed above. The broad junction developed by Clark diminishes the disturbance, but not always enough to make up for the increase in the difference between the two solutions. Thermostating of the junction with liquid to give a large thermal capacity should greatly decrease the experimental difficulty. I think that the same amount of care would give as great precision with the salt bridge cell as with the cell with a silver chloride electrode and added chloride for the determination of acid and base dissociation constants by extrapolation to zero concentration. Effects which start out proportional to the square root of the ionic strength are only half as large for the salt bridge cell.

The theoretical difficulty lies in the fact that we can not measure the chemical potential or the activity of a single ion species. This is closely related to the fact that there is no rigorous definition of electrical potential difference between two different media as noted in the last paragraph quoted from Gibbs. Such difficulties are not new in thermodynamics. We can not determine the enthalpy or heat content of any substance, but we can measure the difference of enthalpy corresponding to a given change of state. So we have agreed on the arbitrary definition that the enthalpy shall be zero for each element and for the hydrogen ion in water, each in its standard state. The inclusion of the hydrogen ion takes care of the fact that we can not measure the properties of any single ion species. The standard free energies, or chemical potentials, are defined by a similar arbitrary definition. The standard molal electrode potentials require an arbitrary definition only for hydrogen and for the hydrogen ion in water, but not for the other elements.

We need an arbitrary definition of the variation of one single ion activity with changes in the medium. Various definitions have been proposed, but the physical chemists have not been able to come to an agreement in this matter. For simple solutions the best proposed so far is the extension of Brönsted's theory of specific ion interaction which, for electrolytes with univalent ions, states that in a pure salt the activity coefficients of the two ions are equal, and that in salt mixtures of constant total concentration the logarithm of the activity coefficient of any ion is a linear function of the concentrations of ions of the opposite sign, but is independent of the concentrations of ions of the same sign. The theory for more complex electrolytes is just as definite but not so simple to state. This definition gives a means of calculating single ion activities in simple solutions of known composition, but it does not give any means of measuring single ion activities.

A definition which is much more generally useful is that at constant temperature the electromotive force of a cell made up of a hydrogen electrode in the solution under consideration, a saturated potassium chloride bridge, and a constant electrode measures the chemical potential of the hydrogen ion in the solution under consideration. Then the replacement of the hydrogen electrode by any other electrode will give a measure of the chemical potentials of the substances reacting at that electrode. Such a definition does not contradict any measurements or theories which do not involve single ion activities. If the molal electrode potential for the cell with hydrogen electrode is so chosen that the activity of the hydrogen ion approaches its concentration in one extremely dilute solution, the same relation will hold in all dilute solutions for the hydrogen ion and also for any other ion. This definition gives no deviations from the logarithmic term proportional to the square root of the concentration. We should be able to calculate the change in potential of this cell with changing salt concentration to terms proportional to the salt concentration. Some of my early work indicates that at moderate concentrations there is a contradiction between this definition and that of Brönsted for hydrochloric acid solutions, but the contradiction is probably not serious. There is much experimental work which indicates that the definition is not a useful one for solutions very concentrated in a non-electrolyte, in which the solvent may be regarded as changed. Such solutions also offer experimental difficulties which make the application more difficult as well as less useful.

I do not believe that anything I can say about hydrogen ion activity measurements will either increase or decrease the use of such measurements or change the technique of routine work. I do hope that what I have said will reduce the sense of inferiority, which has become almost a complex, and will thus increase the precision of this type of measurement when the system and the problem justify such precision.

The definition of the hydrogen ion activity also makes the cell with inert electrode and salt bridge give a direct measure of the oxidation-reduction potential, which may be considered a measure of the chemical potential of the electron. The complications which arise when oxidation-reduction reactions will not proceed rapidly at the electrode except with catalysts have played such an important part in the study of these potentials that they need no emphasis here.

These two equilibria involve two substances, protons and electrons, which exist in ordinary solutions only at extremely minute concentrations, but which still attain equilibrium with the rest of the system rapidly. Another very important equilibrium involves water, the most abundant component of biological systems, which appears able to go from one part of the body to another so rapidly that minute changes in its chemical potential are important. The activity of water in our blood is only about half a per cent. less than in pure water and only about two thousandths of a per cent. less than in its ultrafiltrate. Yet the balance in a biological system appears to depend upon a small fraction of the latter difference, which corresponds to the effect of a pressure of twenty-five millimeters of mercury. Even the simplest system of interest to the biological chemist is a very complicated one for accurate thinking. To improve over the ideal solution treatment developed by van't Hoff and Donnan, it is necessary to keep very clear the distinction between activity coefficients and osmotic coefficient and not to forget which variables are being maintained constant. This is also one of the cases in which second approximations partially cancel each other so that the first approximation is much better than a treatment which uses the second approximation in one part but the first in another.

The distributions of many other substances throughout the body make some of the most interesting and probably the most important problems of biological chemistry. Not all can have the same chemical potential throughout, but the first step in the study must be the determination of how far the actual distribution varies from the equilibrium distribution because of impermeability, slow permeability or other cause. The rest of the problem will require knowledge outside of thermodynamics, although there will be no violation of thermodynamic laws. For two examples, it would be very valuable to know how closely two changes of state must be coupled so that a decrease in free energy in one may compensate for an increase in free energy in the other, and also what concentration gradients are produced by a temperature gradient which is maintained constant in spite of flow of heat.

The details of a review of this kind are essentially personal and subjective. The examples are mostly taken from protein chemistry, partly because I believe it important, but largely because I am less ignorant of protein chemistry than of the other branches of biological chemistry. I have called attention to those applications of thermodynamics which I believe need more careful scrutiny and to another application of which my opinion is much higher than those of most physical chemists. Yet it would be unfortunate if this question should become one of my authority against that of my colleagues. The most important part of my contribution is impersonal and objective. It is the reminder that each new application can and must be thoroughly tested without regard to the name associated with it.

THE TRAINING OF BIOLOGISTS[1]

By Professor **PAUL WEISS**

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A FEW comments to introduce our topic may seem appropriate. Why this conference on the Training of Biologists? Well, simply because by shaping future biologists, we shape the future of biology. And there seem to be some grounds for concern. The question is: Can we profitably consider the many ramifications of a *training program* in biology without having a unified concept of *Biology* itself? There is our cardinal issue: What *is* biology, and what would we want it to be like? If we accord to biology the dignity of an integrated, consistent and coherent science, rather than view it merely—if you pardon the irreverent figure of speech—as a holding company embracing numerous separate and independent enterprises, then a clear understanding about the substance, aims and limits of this science would help to establish a sort of central beacon with regard to which we could orient ourselves, when dealing with subordinate issues. Then, anybody's problem becomes everybody's concern, and the common ground for this meeting of representatives of diverse interests is satisfactorily circumscribed.

Our meeting seems timely, or even overdue, in view of world events. In this world crisis, science finds itself confronted with mounting short-range demands and an altered long-range outlook. All around we hear it being predicted, that after this crisis has come to pass—in fact, if it is to be overcome for good—this world can never be the same again; and that, if the change is to be for the better, the critical, disciplined, fact- and relation-conscious mind of the scientist will have to be accorded a major share in the improvement.

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[1] Address by the Chairman of the Conference on the Training of Biologists, held in connection with the Fiftieth Anniversary Celebration of The University of Chicago on September 18–20. A full report of the proceedings of the conference will be published at a later date. Aid by the Carnegie Corporation of New York is gratefully acknowledged.