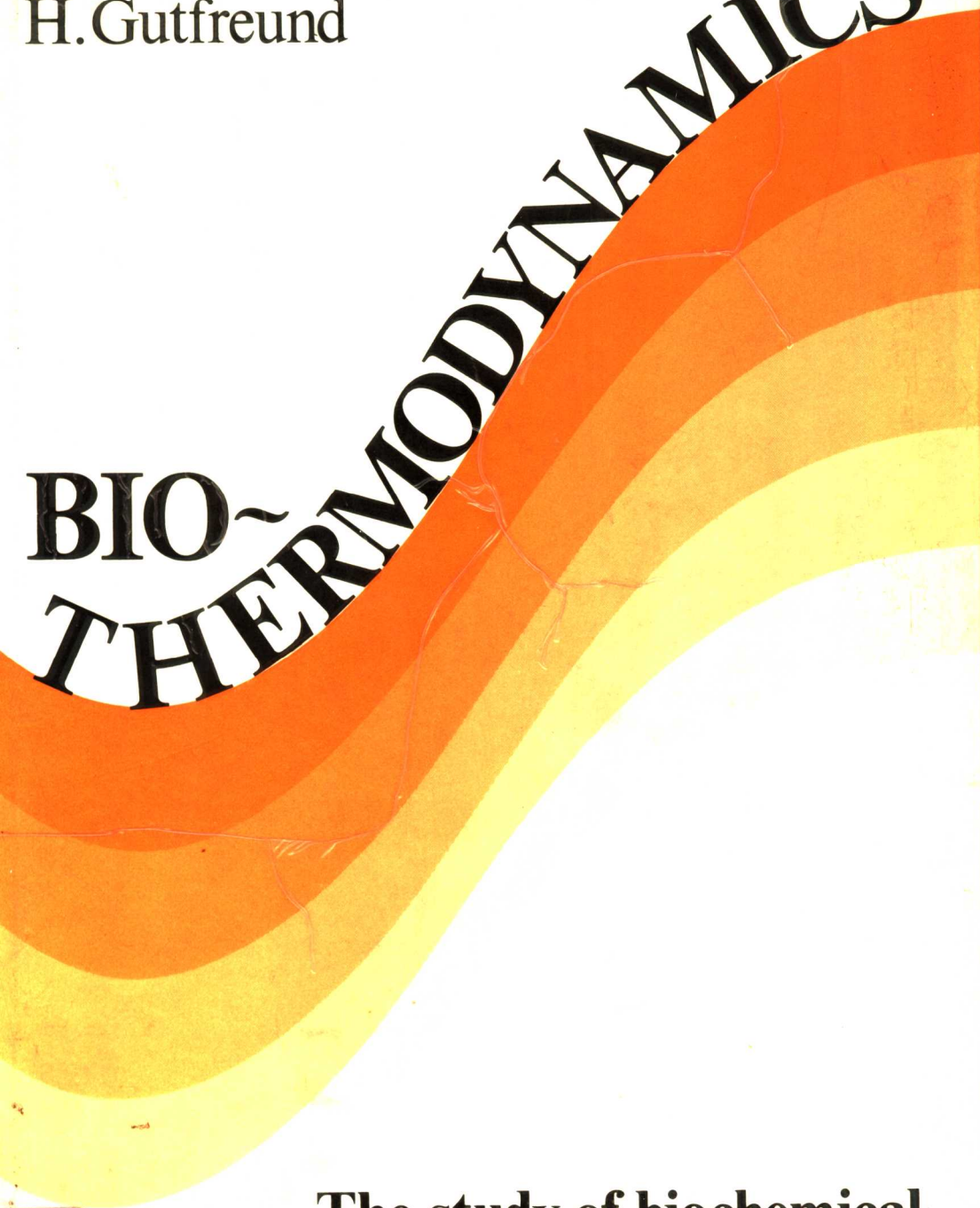


J.T. Edsall and
H. Gutfreund

BIO~THERMODYNAMICS



The study of biochemical
processes at equilibrium

BIOTHERMODYNAMICS

The Study of Biochemical Processes
at Equilibrium

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Preface

The principles of thermodynamics, and their experimental applications, have been fundamental in biochemistry, physiology, and biophysics for many years, and their importance is growing. Our aim in this short volume is to present the basic principles of thermodynamics, and to illustrate them by discussion of numerous characteristic examples of studies related to biochemical and biophysical problems. The use of certain mathematical techniques, especially partial differentiation, is of course essential for the treatment. We have endeavored to develop the mathematical treatment in a manner that is sufficiently rigorous, but with the endeavor to provide clear indications of intermediate steps in derivations. The omission of such steps, as we are aware, can cause much trouble to many students.

Chapter 1 presents a brief historical introduction and Chapter 2 is intended as a descriptive survey of the principles of equilibrium thermodynamics, with a brief indication of the possible approaches when non-equilibrium systems are being considered. This treatment should give the student a basic idea of the scope of the subject and of its applications to biochemical problems. A significant change in style will be noted when the more rigorous treatment of equilibria in terms of chemical potentials is presented in Chapter 3.

Biochemical systems contain many components, and the magnitude of the interactions between them is commonly far greater than in most of the systems studied in pure chemistry. Thus the thermodynamic linkage relations between components, as developed particularly by Jeffries Wyman, are of major importance. We introduce them briefly in Chapter 3, and extend the treatment in Chapter 5, as an essential part of our discussion of ligand binding, and of cooperative and anticooperative interactions. We have presented much of our discussion in this chapter in terms of hemoglobin and its interactions, which furnish a rich variety of illustrations of fundamental principles.

In Chapter 3 we have emphasized the thermodynamic properties of water and aqueous solutions, since water is of such central importance in biochemistry. This chapter also emphasizes the thermodynamics of hydrophobic interactions, including the properties of gases in aqueous solution, which are fundamental for many biochemical processes.

We are concerned with equilibrium thermodynamics, and have only referred briefly to thermodynamics of irreversible processes. However, especially in Chapter 4, we have discussed the thermodynamics of some enzyme-catalyzed reactions, in cases where an intermediate is effectively in equilibrium with the reactants during the course of the reaction. This also

includes systems of biological importance where the enzyme is present at a concentration comparable to that of the substrates that are undergoing reaction.

Calorimetric studies of biochemical systems are rapidly increasing in importance, and readily available microcalorimetric equipment is now highly accurate and convenient. The use of calorimetry in the study of biochemical processes will surely grow, and will prove an increasingly powerful method of obtaining answers to many biochemical problems. Our final chapter is devoted to calorimetry.

We have aimed to develop concepts and illustrate their use by discussion of experimental results, but have made no attempt to describe experimental techniques or procedures.

The idea of such a book as this originated about 1975, when both of us were members of the Interunion Commission on Biothermodynamics, which included representatives of the International Unions of Chemistry, Biochemistry and Biophysics. We received helpful advice and encouragement from our fellow members on the Commission, especially from Ingemar Wadso', William P. Jencks, Rodney L. Biltonen, and the late George T. Armstrong. This book, however, is an independent enterprise, and is not in any way the responsibility of the Commission. We also thank Hilary Muirhead for a critical reading of Chapter 5. It has undergone a slow incubation, with many exchanges and revisions of drafts of chapters, and with meetings twice a year in Bristol or in Cambridge, Massachusetts. We have aimed for continuity and coherence in presenting the subject matter, but have not attempted to obscure the stylistic individuality of the two authors.

We hope that the book will promote increased use of thermodynamic reasoning among biochemists and biophysicists, and will enable them to realize the power of thermodynamics in providing a deeper insight into many biochemical problems.

The authors wish to thank their respective secretaries Judith Guidotti and Audrey Harvey for their patience in repeated typing of revised versions of some of the sections. Mary Gutfreund gave considerable help in the preparation of the index and in proof reading.

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J.T. Edsall
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Historical introduction and the aims of biothermodynamics

Thermodynamics is a subject of interest to practitioners of many disciplines.

Astronomers, physicists, engineers, geologists, chemists, and biologists all have this one common grammar to their scientific language: the laws of energy utilization and interconversion. However, from a few basic laws and relationships the subject branches out into quite diverse applications. Nonetheless, if the basic principles are well understood the common problems of quite different subjects can often be seen through thermodynamics. In this way this interdisciplinary subject serves the laudable purpose of helping scientists to have a common interest.

The early history of thermodynamics is, of course, closely connected to the development of calorimetry. Heat measurements long preceded optical measurements on the progress of chemical and biological processes. Quantitative records of changes in the transmission of light at a specified wavelength, the most common analytical tool in a modern biochemical laboratory, are a very recent development compared with heat measurements. There was notable progress in the eighteenth century in the development of thermometry and measurements of heat capacities and other physical constants of well defined substances. The history of the development of calorimetry in chemistry is well described by Armstrong (1964), who lists the interpretive achievements side by side with those in the construction of ingenious instruments.

The intake of energy in the form of food, its storage in the organism, and its release in metabolic activity are fundamental to biochemistry and biophysics. Among the vast array of biochemical reactions, many proceed spontaneously if catalysed by suitable enzymes. Others of central importance, such as the biosynthesis of nucleic acids, proteins, and many other substances, cannot proceed spontaneously, but must be coupled to other spontaneous reactions that can provide the necessary free energy to make the synthetic process go. Living organisms transform chemical into mechanical energy, as in the contraction of muscles or the beating of cilia, and thermodynamic relations are vital to the understanding of such processes. Processes that are potentially spontaneous often do not proceed at any appreciable rate; in biochemistry this means generally that a specific enzyme must be present to catalyse the process. Such restrictions are of course necessary for the economy of the organism, to prevent wasteful release of energy when it is not needed.

Equilibrium thermodynamics, which is what we shall discuss in this book, cannot predict how fast a process will go, but can tell whether it can go at all, and how far it can go before the process comes to equilibrium.

Much of our information in such studies comes from the direct measurement of chemical equilibria, if necessary in the presence of a suitable enzyme or other catalyst to hasten the attainment of equilibrium. We also want to know how such equilibria are shifted by changes of temperature, pressure, and other variables, or by changes in the solvent medium in which the process takes place. Accurate calorimetric studies of the quantity of heat absorbed or released in the various processes are also fundamental for our knowledge of chemical equilibria. Indeed such thermal measurements, including determination of heat capacities of the substances involved, carried down to temperatures approaching absolute zero, can permit the calculation of chemical equilibria even when they have not been actually measured.

Thermodynamics, as it has developed in the nineteenth and twentieth centuries, grew out of earlier developments that occurred mainly in the eighteenth century. On the other hand, there was the early development of calorimetry, by such men as Joseph Black and Lavoisier. Indeed Lavoisier and Laplace inaugurated the study of biochemical calorimetry in 1784 by measuring the heat evolved by a small mammal when metabolizing carbohydrate, and showing that it was the same, within experimental error, as the heat of combustion of the same substance in a calorimeter.

However, the major practical development that later led to the science of thermodynamics was the invention of heat engines that transformed the heat of steam, under pressure, into mechanical work. The heat engines of Thomas Savery, Denis Papin, and Thomas Newcomen, in the early eighteenth century, were used for such purposes as pumping water out of mines. By modern standards they were grossly inefficient; nevertheless they found use. The important improvements made by James Watt, from 1763 on, made these devices much more efficient, and brought them into wider use; but all these developments occurred on a purely empirical basis, without a theoretical foundation (see for instance Cardwell, 1971).

The first major theoretical advance came in 1824 from the French engineer Sadi Carnot, who in his great memoir on the motive power of heat (Carnot, 1824) formulated the ideal conditions for the operation of an engine that transformed heat into mechanical work and calculated the maximum possible efficiency that such an engine might attain. He pictured the engine as receiving heat from a boiler at temperature T_1 and discharging it to a condenser at a lower temperature T_2 , the process involving two cycles of expansion and compression in a cylinder in which the working fluid (usually, but not necessarily, steam) drove the motion of a piston. We need not discuss the details of the Carnot cycle here; they are given in most text-books of thermodynamics. Carnot's general conclusion, however, was of immense general importance. The maximum possible efficiency of the engine – that is, the ratio of the work produced to the input of heat – depended on the

temperature difference ($T_1 - T_2$) between the boiler and the condenser, and only a fraction of the thermal energy could be converted into work. However, the reverse process – the complete dissipation of mechanical energy into heat – was all too easy. Thus Carnot's work pointed to a fundamental irreversibility in natural processes; and in this respect he anticipated what later came to be known as the second law of thermodynamics. When he wrote his great memoir Carnot, like many of his contemporaries, still thought of heat as a caloric fluid which could pass from one body to another; the concept of the conservation of energy and the absolute scale of temperature had not yet been established, so that Carnot's analysis was still incomplete. There is evidence from later notes, unpublished until long after his death, that he was working toward the concept of conservation of energy; but he died in a cholera epidemic in 1832, at the age of 36. A recent article by Wilson (1981) gives a valuable portrayal of Carnot and his work and the book by Cardwell (1971) covers the whole development from the early steam engines to the enunciation of the first and second laws of thermodynamics.

The conception of heat as a form of energy, and the quantitative conversion of one form of energy into another, became definitely established in the decade after 1840. The idea of conservation of energy was put forward by a German physician, Julius Robert Mayer, a man fertile in ideas and interpretations. It was James Prescott Joule, however, whose careful experimental studies of the conversion of electrical and mechanical energy into heat established quantitatively the interrelation between different forms of energy (see particularly Joule, 1849). It was the great memoir on conservation of energy by Hermann von Helmholtz (1847), however, which provided a systematic formulation of the concept that was decisive for the general acceptance of the first law of thermodynamics. (It is interesting to note that Poggendorf, editor of the *Annalen*, rejected the paper when Helmholtz submitted it to him.) Helmholtz was almost certainly the most productive biophysicist of all time (Konigsberger, 1965). He demonstrated, while still a young army surgeon, that metabolic energy was quantitatively transformed into work and heat in muscle. He made immense contributions to physiological optics and auditory physiology, and was the first to measure the speed of conduction in nerve, in addition to his work on thermodynamics. He ended his career as Professor of Physics in Berlin, having earlier held chairs of Anatomy and Physiology at several universities.

Since that time studies of the mechanism of energy transduction have become a central theme in biophysics. The energy which becomes available during the metabolic breakdown is utilized for such diverse processes as the synthesis of nucleic acids (information storage) and proteins (functional and structural) as well as for the transmission of information in nerve conduction, the synthesis and transmission of messengers, and many mechanical processes related to muscle contraction. The compounds which undergo this metabolic breakdown for the provision of energy are, in turn, synthesized in the photosynthetic processes, which utilize the energy of the electromagnetic

radiation received from the sun. An interesting discussion of the efficiency of the food chain, from an ecological point of view, is contained in a recent popular book (Colinvaux, 1980).

The fundamental importance of Carnot's work took some time to be appreciated. Its recognition came from about 1850 on, thanks largely to William Thomson (Lord Kelvin) in Great Britain and Rudolf Clausius in Germany. It was Kelvin who first established, on a rigorous basis, the thermodynamic (absolute) scale of temperature – a concept that indeed had been under discussion by various authors from about the beginning of the nineteenth century. Making use of this concept, Kelvin was able to formulate more exactly Carnot's equations for the maximum efficiency of an ideal heat engine, working between the temperature (T_1) of the boiler and that (T_2) of the condenser. The maximum ratio of the work done to the heat input was given by the simple expression

$$\text{work/heat energy} = (T_1 - T_2)/T_1.$$

Thus the possible yield of work depended on the temperature difference and was always less than the input of heat energy, except in the impossible case of a condenser held at a temperature of absolute zero. It is to Kelvin, and especially to Clausius, that we owe the development of the concept of entropy (from the Greek word *τροπή*: transformation), which is central to the second law of thermodynamics. Entropy, unlike energy, is not a quantity that remains constant, independent of time. Instead, for any closed system that cannot exchange matter or energy with its surroundings, any spontaneous process that may occur must involve an *increase* of entropy. Entropy is a measure of the irreversibility of natural processes. We deal more fully with this powerful and subtle concept in Chapter 2, and shall make constant use of it in the rest of this book.

Snow (1959) states, in his provocative essay on 'the two cultures' that it should be just as much part of a prerequisite for an educated man to be able to talk about the second law of thermodynamics as it is to have read the plays of Shakespeare. There are many very deep analyses of the second law still debated in the current literature, but then the same is true in expert interpretations of Shakespeare.

It is a characteristic feature of biological processes that energy interconversion occurs under conditions of negligible or very small temperature gradients – they are essentially isothermal throughout. Needham (1971) describes how, towards the end of the nineteenth century, it became clear that the maximum temperature gradients which could be encountered in muscle could not result in the efficiency realized in terms of a heat engine. Fick's (1893) comments on the origin of muscle power are of interest in this connection. The mechanism of muscle contraction must depend on direct coupling between chemical and mechanical energy. While the complete molecular details of this process are not yet understood, the combined results of investigations into the structure of muscle fibres, as well as their physiological and molecular

responses to stimulation by a nerve impulse, have provided plausible models for the mechanism of energy transduction in this system (see for instance Keynes and Aidley, 1981). Several different aspects of this important problem in biothermodynamics will be discussed in various sections throughout this volume.

The man who laid the foundations of chemical thermodynamics in the late nineteenth century was J. Willard Gibbs of Yale University (see Gibbs, 1876, 1931). Gibbs considered the relations involved in the establishment of equilibrium, for systems composed of any number of chemical components and phases, as functions of temperature, pressure, and chemical composition. He also showed how the analysis could be extended to include other forms of energy, such as surface energy or electrical energy, when these might become important. He also defined new thermodynamic functions, such as the enthalpy (heat content) and the functions now known as the Helmholtz energy and the Gibbs energy (the latter has commonly been called the free energy by many chemists). These functions are of the utmost importance in thermodynamics today, and we shall make constant use in this book of the enthalpy and the Gibbs energy.

Gibbs wrote in a highly condensed and mathematical style, and few chemists of his generation could understand or make use of the powerful methods he developed. He did, however, greatly influence the group of physical chemists led by J. H. van't Hoff, Svante Arrhenius, and Wilhelm Ostwald, who from about 1885 on developed the physical chemistry of solutions and the concept of electrolytic dissociation. It was primarily G. N. Lewis and his school in California, in the early twentieth century, who showed how to apply the methods of Gibbs to experimental data in the study of chemistry, and the book on thermodynamics by Lewis and Randall (1923) had a profound influence on a whole generation of chemists and biochemists.

Another great landmark in the development of the subject was the 'heat theorem' of Walther Nernst (1906) which became the basis of the third law of thermodynamics. This made possible, at least for many systems, the calculation of chemical equilibria from purely thermal measurements: determinations of heat capacities, and of the heat evolved or absorbed in phase transitions. This we discuss further in Chapter 2.

As more and more specialized interests have developed in the study of energy relationships in biology, a division has occurred between those who are primarily interested in the storage of oxidative and photosynthetic energy (the bioenergeticists) and those who are interested in the utilization of such stored energy for biosyntheses, muscle contraction, transport, and the transmission and transduction of information. The subject of bioenergetics is now largely concerned with the mechanisms of oxidative phosphorylation and photosynthesis. These are the two main processes for the production of ATP (adenosine triphosphate). Many objections have been raised to the terminology used in relation to ATP synthesis and utilization. The terms 'energy store' and 'high energy phosphate bonds' have been much criticized. Such semantics has contributed nothing to the progress of science. It is hoped that the definitions

and explanations given in this volume will be unambiguous and a reasonable approach to the correct ones.

ATP can be called the energy store of biological systems because of its special role at the junction between its formation during oxidative and anaerobic metabolism as well as photosynthesis and its utilization by energy-requiring processes. The principal energy consumption is due to the biosynthesis of macromolecules, muscular and other movement, the transport of ions against concentration gradients, and a great variety of control processes. This function is due to its characteristic physicochemical properties, especially of its phosphate ester bonds. These properties and their thermodynamic consequences are discussed in some detail in Chapter 4.

Edsall (1974) and other contributors to a symposium on the history of bioenergetics describe how the different aspects of this subject have developed during the last two centuries. In that collection of papers it is clearly seen that an understanding of the energy relationships went side by side with the exploration of chemical pathways.

Some other areas of biochemistry and physiology should be mentioned in which the study of energy changes has made interesting contributions. Calorimetric measurements have been carried out on biological systems at different levels of organization: the reader is referred to the surveys by Kleiber (1961) and Ihde and Janssen (1974) for the study of mammals and to Beezer (1980) for the analysis of heat measurements of whole cells. The study of energy changes during the stimulation and function of muscle and nerve fibres is not only of interest in itself, but it has also played an important part in the history of the development of calorimetry. A. V. Hill's classical investigations of heat changes in active nerve and muscle fibres spanned nearly 60 years from 1912 (see Hill, 1965). Hill and his colleagues (see Howarth, 1970) were able to measure the progress of reactions which resulted in 5 μ K temperature changes during a time course of 50 ms after stimulation of nerve or muscle fibres. While modern advances in electronics have been applied by other investigators, it is of interest that the resolution obtained by Hill with thermopiles and galvanometer systems, designed in his laboratory, has not been surpassed.

Advances in the interpretation of experiments on muscle in terms of energy balance come from the combination of heat measurements with other methods of analysis. Modern techniques of observing rapid chemical and spectroscopic changes during muscle contraction, as well as accurate calorimetric data on the chemical changes which occur during contraction and recovery, are continuing to increase the insight into the thermodynamics and mechanism of muscular activity (for a review of work from many laboratories see Homsher and Keen, 1978). This is of particular interest since these studies are going on side by side with a more and more detailed understanding of the molecular events during the interaction of well defined proteins in a muscle.

In connection with the above application of heat measurements on complex systems it should be pointed out that the lack of specificity of heat measurements can be a major contribution to the discovery of new

phenomena. If the measured heat production or uptake of the organized system does not correspond to the sum of the heat changes during the known individual processes which take place, then one has evidence for additional previously neglected component reactions. This has turned out to be the case in muscle contraction, where an initial excess heat production has been discovered (see review by Homsher and Keen, 1978). This approach is likely to give similar useful information on other complex systems as calorimetric studies are being more widely used to monitor the progress of reactions in organized systems and in isolation. A range of calorimeters for different purposes is continuously being developed and these calorimeters are becoming commercially available. These techniques are, however, still grossly under-used, considering their potentiality.

During the period 1930–50 a considerable research effort was directed towards the energy requirements for the biosynthesis of proteins – which were at that time thought to be the only macromolecules with genetic and chemical specificity. Calorimetric and other data were collected which permitted calculations of the thermodynamic data for amino acids, peptides, and proteins (see for instance Borsook and Deasy, 1951). This is not the place to review the investigations which demonstrated during the last 25 years how – in chemical terms – the energy of hydrolysis of the phosphate ester bonds of ATP is used for the synthesis of peptide bonds. Similarly phosphate ester bond hydrolysis of several nucleotide triphosphates is coupled to the specificity and control of polypeptide chain assembly on microsomes, with the genetic pattern provided by the nucleic acid template. The replication of the nucleic acids is in turn coupled to the hydrolysis of nucleotide phosphate esters.

Conceptually it is easier to understand how calorimetry can provide information about the energy balance for a sequence or for parallel processes, as compared to other thermodynamic information, which requires a deeper understanding of the subject for conversion of experimental data into thermodynamic parameters. These other methods involve the interpretation of equilibrium measurements on a large variety of different processes. A list of phenomena of biological interest which can be observed at equilibrium includes osmotic pressure, solubility, electrode potentials, oxidation–reduction potentials, ionization involving protons and other ions, binding equilibria of substrates to macromolecules, protein–protein interaction, and, last but not least, chemical equilibria. Many research workers interested in the physico-chemical aspects of biochemistry and physiology have not only stimulated but have made major contributions to our present understanding of the behaviour of electrolytes, the properties of ionizing groups, and the energies of other non-covalent interactions in aqueous systems (see Cohn and Edsall, 1943; Edsall and Wyman, 1958; Tanford, 1961). The importance of the physical chemistry of aqueous solutions has been recognized in most text-books on general physiology. The contributions of biochemists and their interest in oxidation–reduction potentials and other equilibria for the understanding of energy relationships was surveyed in a symposium held at the New York