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Progress in Biochemistry since 1949

PROGRESS IN

BIOCHEMISTRY

A report on biochemical problems and on biochemical research

since 1949

By

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FOREWORD

This book is the fifth in a series of reports on the progress in Biochemistry since 1914. The first three editions were published in the years 1924, 1931, and 1938, while the author was a member of the Medical Faculty at the German University in Prague. A fourth German edition, published in 1948 in Switzerland, was written in Turkey while the author was head of the Department of Biological Chemistry at the University of Istanbul. It was followed in 1950 by an English edition. Although this last edition has been out of print for several years, the author was hesitant to prepare a new progress report. During the last decade biochemistry has undergone a far greater rate of expansion than at any time prior to 1950. Thermodynamics, statistics, physical chemistry, genetics, bacteriology and other physical and biological sciences are being increasingly applied in biochemical research. Limits of time and of human endurance make it more and more difficult to grasp, let alone to master, the entire field of biochemistry.

In view of these difficulties it would have been reasonable to seek the cooperation of experts in different fields of biochemistry and to share with them the task of writing a competent progress report. The author was discouraged in such an appeal to others by his own frustrating experiences as a co-author of various books. In each of these cooperative books some of the authors were late in turning in their contributions. Invariably, the chapter written by the author was returned to him, sometimes after several years (!), with the request to bring the chapter up to date. It is obvious that similar delays in a progress report would defeat its purpose. Therefore the author decided to avoid this risk by writing again the entire book by himself.

The question may be raised whether a progress report of this type is not made obsolete by publication of the Annual Review of Biochemistry and by numerous series of Advances in Enzymology, Protein Chemistry and other branches of Biochemistry. The ans-

wer is that one of the reasons for writing this book is the author's desire to keep abreast of the state of Biochemistry. As a teacher, he must frequently answer the questions of students who are interested in aspects of Biochemistry which are far from the author's field of research. It is the hope of the author that this progress report, like its predecessors, will be of use to others who need a concise survey on advances in Biochemistry, and that it will help them to gain a picture of the present state of this rapidly developing area of science.

Although the author has attempted to discuss advances in the entire field of general biochemistry, he may have overlooked some of the more important papers. In selecting material for this report, preference was given to papers on the fundamental aspects of Biochemistry rather than to those describing methods or applications. It appears to be impossible to satisfy everybody in this respect. Some of the previous editions were criticized for containing reports on alkaloids, terpenes and other plant products. Yet the biosynthesis of terpenes, alkaloids, antibiotics and other products of plants and molds or bacteria is just as important to our understanding of the biosynthetic processes as is the intermediary metabolism of animals.

In writing this book an attempt was made to correlate new advances with older well known facts and to avoid mere descriptions of new observations. For obvious reasons, emphasis is placed on the most recent state of biochemical problems. This may occasionally involve unduly brief treatment of results which were obtained in the earlier years of the decade 1949-1958, and which were later modified.

The author has kept this report as short as possible. Indeed, it is slightly smaller than the preceding volume. This has been accomplished by using smaller print for the description of progress in side lines of biochemical research and by condensation of the references at the end of each subchapter. As in some of the other textbooks of Biochemistry, the references list, at the most, the names of two authors. Most of the references cited refer to original papers. However, some outstanding books and review articles are also quoted. The complicated molecular structures of the organic body constituents frequently necessitate the use of lengthy names which interfere with easy reading. Biochemists

have met this difficulty by introducing an increasing number of abbreviations. A list of these is given on the pages which follow the Table of Contents. Some of the listed abbreviations are not used in this book, but may be encountered in other texts or articles. The author has taken the liberty to abbreviate the names of four journals which are most frequently quoted in the lists of references. These abbreviations are: ABB for the Archives of Biochemistry and Biophysics, BBA for the Biochimica et Biophysica Acta, JBC for the Journal of Biological Chemistry, and JACS for the Journal of the American Chemical Society. Considerable space has been gained by the different procedures described in this paragraph. It is hoped that these changes will facilitate the use of the book.

No attempt was made to resolve differences in nomenclature. The author does not feel competent to make a decision between *enzymic* and *enzymatic*, or between *oxalacetate* and *oxaloacetate*. Both types of terms have been used in this text.

In view of the difficulty of writing a progress report of this type, errors in reporting and interpreting can hardly be avoided. Some of these have been detected by colleagues who were kind enough to read one or more chapters of the manuscript or parts of the proofs. The author wishes to express his gratitude for this help to Dr. Harry G. Day, Dr. John D. Hawkins, Dr. Henry R. Mahler, Dr. Becca C. Patras, Dr. Alice H. Sievert, Dr. H. William Sievert, Dr. James E. Turner, and Dr. George Vidaver. The author is particularly indebted to Dr. Maxwell Richter, who read large parts of the manuscript, and to Donald R. Montgomery who read the proofs of the entire book.

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Felix Haurowitz.

LIST OF ABBREVIATIONS

A = Ångström unit = 10^{-8} cm
ABB = Archives of Biochemistry and Biophysics (previously Archives of Biochemistry)
AcCoA = Acetylcoenzyme A
ACTH = Adrenocorticotropical hormone
ADP = Adenosinediphosphate
Ala = Alanine
AMP = Adenosinemonophosphate
APS = Adenosinephosphosulfate
Arg = Arginine
Asp = Aspartic acid
AspN or $AspNH_2$ = Asparagine
ATP = Adenosinetriphosphate
BAL = British Antilewisite = 2,3-Dimercaptopropanol
BBA = Biochimica et Biophysica Acta (previously Biochimica Acta)
BGG = Bovine γ-globulin
BMR = Basal metabolic rate
BSA = Bovine serum albumin
C = Curie = 3.7×10^{10} disintegrations per second
C* = Isotopically labeled carbon (C^{13} or C^{14})
C′ = Complement = Alexin (C'_1, C'_2, C'_3, and C'_4 are components of C′)
cal = calorie
Cal = kilocalorie = 1000 calories
CF = Citrovorum factor
CoA = Coenzyme A
CPM = Counts per minute
CMP, CDP, CTP = Cytidine mono-, di- and triphosphate
Cys = Cysteine or one half of cystine residue
d = dextrorotatory
D = derivative of dextrorotatory serine or glyceraldehyde
DFP = Diisopropylfluorophosphate, diisopropylphosphofluoridate
DNA = Deoxyribonucleic acid
DNase = Deoxyribonuclease
DNP = Dinitrophenol, dinitrophenyl-
DOC = Deoxycorticosterone
DOPA = Dihydroxyphenylalanine
DPN = Diphosphopyridine nucleotide
DPNH = reduced form of DPN
EDTA = Ethylenediamine tetraacetate
ETP = Electron transferring particle
e.u. = Entropy unit = 1 cal · degree^{-1} · mole^{-1}
ΔF = Change in free energy
FAD = Flavin adenine nucleotide
ff = and following pages
FMN = Flavin mononucleotide
F-6-P or Fru-6-P = Fructose-6-phosphate
FSH = Follicle stimulating hormone
ΔG = British symbol for ΔF
Gal-1-P = Galactose-1-phosphate
Glu-1-P = Glucose-1-phosphate
Glu = Glutamic acid
GluN or $GluNH_2$ = Glutamine
Gly = Glycine
GMP, GDP, GTP = Guanosine mono-, di- and triphosphate
ΔH = Heat of reaction
His = Histidine
Hypro = Hydroxyproline
ICSH = Interstitial cell stimulating hormone
Ileu = Isoleucine
IMP, IDP, ITP = Inosine mono-, di- and triphosphate
JACS = Journal of the American Chemical Society

JBC = Journal of Biological Chemistry
k = Velocity constant
K = Equilibrium constant
kcal = Kilocalorie = 1000 cal
l = levorotatory
L = Derivative of levorotatory serine or glyceraldehyde
Leu = Leucine
Lys = Lysine
mC = Millicurie = 10^{-3} Curie
μl = Microliter = 10^{-6} liter
μg = Microgram = 10^{-6} gram
Met = Methionine
NMN = Nicotinamide mononucleotide
NPN = Nonprotein nitrogen
OYE = Old yellow enzyme
Ⓟ = phosphate residue in organic compounds
PABA = p-Aminobenzoic acid
P* = Isotopically labeled phosphorus
PAM = Pyridine aldoxime methiodide
PAPS = 3'-Phosphoadenosine-5'-phosphosulfate
PAS = Periodic acid Schiff reaction
PASA = *p*-Aminosalicylic acid
PBI = Protein-bound iodine
PCMB = *p*-Chloromercuribenzoate
PGA = Pteroylglutamic acid
Phe = Phenylalanine
P_i = Inorganic phosphate
PNA = Pentose nucleic acid
PP = Pyrophosphate
Pro = Proline
PRPP = 1-Phosphoribo-5-pyrophosphate
Q_x = Quantity of substance x produced or consumed per mg of dry tissue per hour
R = Gas constant = 1.98 cal · mole^{-1} · degree^{-1} = 8.315 joules · mole^{-1} · degree^{-1}
RDE = Receptor destroying enzyme
R_f = Rate of flow
RNA = Ribonucleic acid
RNase = Ribonuclease
R-5-P = Ribose-5-phosphate
R.Q. = Respiratory quotient
Ru-5-P = Ribulose-5-phosphate
ΔS = Entropy change of reaction
S* = Isotopically labeled sulfur
SC factor = Factor which links succinic dehydrogenase to cytochromes
SDC = Succinic dehydrogenase complex
Ser = Serine
S_f = Flotation constant
T = Absolute temperature in degree Kelvin
TAME = Tosyl-arginine methyl ester
TCA = Trichloroacetic acid
TEPP = Tetraethylpyrophosphate
THFA = Tetrahydrofolic acid
Thr = Threonine
TPN = Triphosphopyridine nucleotide
TPNH = reduced form of TPN
Tris = Tris(hydroxymethyl)amino-[methane
Try = Tryptophan
Tyr = Tyrosine
UDPG, UDPGal = Uridyldiphosphoglucose, Uridyldiphosphogalactose
UMP, UDP, UTP = Uridine mono-, di- and triphosphate
Val = Valine

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I. Thermodynamics in Biochemistry.

A. Introduction. Thermodynamic calculations and considerations occupy an increasing space in biochemical publications. They are an important tool in unravelling the complicated pathways of intermediary metabolism. Although this approach to biochemical problems has been treated extensively in several books on physical biochemistry (A-1 to A-5) and in review articles (A-6), it is discussed only briefly in most textbooks of biochemistry. For this reason, and in view of recent corrections of some of the fundamental thermodynamic data, it may be useful to discuss in this first chapter the principles of the thermodynamic approach to biochemical problems.

A-1. *H. G. Bray* and *K. White,* Kinetics and Thermodynamics in Biochemistry (1957). – A-2. *E. A. Dawes,* Quantitative Problems in Biochemistry, Edinburgh (1956). – A-3. *H. A. Krebs* and *H. L. Kornberg,* Energy Transformations in Living Matter, Berlin (1957). – A-4. *H. Netter,* Biologische Physikochemie, Potsdam (1950). – A-5. *H. Bull,* Physical Biochemistry, New York (1951). – A-6. *A. B. Pardee,* in *D. Greenberg,* Chemical Pathways of Metabolism, page 1, New York (1954).

B. ΔH and ΔF. According to the well-known first law of thermodynamics the energy content of a system is constant; it can neither increase nor decrease without transfer of energy to or from the environment. However, we can convert one form of energy into another. Thus, the energy stored in the nutrients and introduced with these into the animal organism is converted into heat, mechanical work and, occasionally, also into other forms of energy. It is customary to measure all these forms of energy in calories (cal) or kilocalories (1 kcal = 1000 cal). The selection of calories as units of energy is arbitrary, but is logical since it is possible to convert all forms of energy into heat. This principle is utilized in the bomb calorimeter and in the classical animal calorimeters.

Although mechanical, chemical and other forms of energy can be converted quantitatively into heat, the reverse reaction, conversion of heat into other forms of energy, is never quantitative. Some

of the energy is responsible for the thermal motion of atoms and molecules and cannot be released. We designate it as "internal energy"; that portion of energy which can be converted into other energy forms is called "free energy" and denoted by the symbol F. The total heat content of a system, called its enthalpy and designated by the symbol H, is the sum of free energy and internal energy; the latter is proportional to the absolute temperature, T, and is therefore denoted by the symbol TS. Hence, we can write the equation: $H = F + TS$.

In biochemistry we are not concerned with the absolute energy content of biochemical systems, but with changes in their energy content during metabolic or other reactions. The changes in H, F, and S are designated by the symbols ΔH, ΔF, and ΔS, respectively. If the thermodynamic values of a system in state 1 are H_1, F_1, and S_1, and in state 2 are H_2, F_2, and S_2, then $\Delta H = H_2 - H_1$, $\Delta F = F_2 - F_1$, and $\Delta S = S_2 - S_1$. Accordingly, we can write for the reaction which leads from state 1 to state 2:

$$\Delta H = \Delta F + T\Delta S \tag{1.1}$$

We designate ΔH as the heat change, ΔF as the change in free energy, and ΔS as change in entropy of the system. We can consider the entropy as a measure of the randomness of the system. Thus, when ice of 0°C is heated and converted into water of 0°C, all of the heat is used up to break up the regular arrangement of the water molecules in the ice crystal so that they can flow freely. In this reaction all of the heat is consumed for the increase in entropy; ΔH and ΔS are positive, whereas ΔF is zero since no utilizable energy is gained.

C. Determination of ΔH and Calorimetry. In calorimetry a similar principle is applied. All or nearly all of the energy of a chemical reaction is converted into heat without release of utilizable free energy. A small correction for RT (about 0.6 kcal, see p. 6) per mole of gas formed during combustion is necessary to obtain ΔH. Under the usual conditions of calorimetry this correction is negligibly small. The heat measured in a calorimeter is, therefore, a measure of the total heat change during a chemical reaction. It does not give any information on ΔF, the change in free energy. Anticipating later sections of this chapter, it may be stated

that we are much more interested in ΔF than in ΔH because ΔF permits us to gain much more insight into the mechanism of a reaction.

In the past it may have appeared that the only reactions which would take place spontaneously were those in which heat was given off whereas reactions in which heat was absorbed could not take place unless energy was supplied from outside. This older view is easily disproved. A very common reaction of this type occurs when ice is mixed with solid sodium chloride; as is well known, a reaction takes place quite spontaneously in which the temperature of the system drops from 0°C to less than −10°C. It is clear, therefore, that ΔH does not allow any prediction in this respect.

We have mentioned before that entropy increases when ice is converted into water; for similar reasons entropy also increases when crystalline sodium chloride is dissolved in water. If we place the ice-NaCl mixture into a calorimeter, it absorbs heat from the calorimeter and the temperature decreases. Since heat is absorbed by the system, ΔH has a positive value. Quite generally, we call reactions in which heat is absorbed, *endothermic* reactions, and those in which heat is given off, *exothermic* reactions. In endothermic reactions ΔH is a positive value; in exothermic reactions, in which the heat content of the system decreases, ΔH has a negative sign. According to equation I.1, the sign of ΔH depends on that of ΔF and/or ΔS. In an endothermic reaction at least one of these values must be positive, in exothermic reactions at least one of them negative.

As mentioned before, ΔH and ΔF are energy terms and are, therefore, expressed in calories. Since the term $T\Delta S$ is also an energy term, its dimensions are also calories; consequently the dimensions of ΔS are calories per degree. Obviously, the energy content of a system depends also on its weight. It is customary to express each of the energy terms, ΔH and ΔF, in calories per mole or kilocalories per mole (kcal/mole), whereas ΔS is expressed in calories per mole per degree (cal/mole/deg); sometimes the latter term is replaced by the term e.u. (entropy unit).

It is a consequence of the first law of thermodynamics that heat changes, ΔH, are additive. If we bring a system from a state 1 into state 2 and then into state 3, and if the respective heat changes are ΔH_{12} and ΔH_{23}, respectively, the total heat change, ΔH_{13}, is the sum of $\Delta H_{12} + \Delta H_{23}$. The same heat change ΔH_{13} is

found when we bring the system directly from state 1 to state 3. In other words, ΔH is independent of the pathway of the reaction. This important law is the basis of calorimetry. We need not know what reaction takes place in a calorimeter; indeed we do not know the intermediates which are formed in the combustion of proteins, carbohydrates, lipids or any other substance. Nevertheless, we always find the same heat of combustion, provided the combustion is complete and identical combustion products are formed.

The same principle can be applied to other reactions. Thus, the heat of combustion of glucose is $\Delta H = -673$ kcal/mole and that of lactic acid is $\Delta H = -327$ kcal/mole. Hence, the heat of reaction I.2:

(I.2) $C_6H_{12}O_6 \rightarrow 2\,C_3H_6O_3$
Glucose — Lactic acid

is $-673-(2\times-327) = -19$ kcal/mole. In this manner numerous ΔH values can be determined indirectly from the heats of combustion. It would be impossible to determine the heat of reaction I.2 by direct calorimetry since it proceeds only in the presence of complicated mixtures of enzymes, coenzymes, and salts, and is accompanied by changes in pH and by phosphate transfer.

Although calorimetry had been known for a long time, it was used almost exclusively for the determination of heats of combustion. Very rarely attempts were made to determine the heat changes which accompany hydrolysis, protein denaturation, and other reactions of biochemical interest. The ΔH values in these reactions are several orders of magnitude lower than the heats of combustion. Thus ΔH for the combustion of one gram of glucose is approximately -3.7 kcal; since the molecular weight of glucose is 180, $\Delta H = -3.7\times180 = -670$ kcal/mole as mentioned in the preceding paragraph.

The heat change accompanying mutarotation of glucose, i.e., conversion of α-glucose to β-glucose, is only -0.28 kcal/mole (Sturtevant, C-1) or -0.0016 kcal ($= 1.6$ cal) per gram of glucose. Similar low values are obtained for the heat changes which accompany hydrolytic or transfer reactions. They can be measured only with special, highly sensitive, calorimeters which were not available earlier. Even at the present time only very few laboratories are able to carry out determinations of such low ΔH values. The determinations are complicated by unavoidable simultaneous reactions such as ionization, pH changes, or formation of a new phase; therefore the measured ΔH values frequently in-

clude the heats of ionization, neutralization, or solution. In spite of all these difficulties extremely valuable ΔH values were determined for a number of reactions of biochemical interest, particularly by J. Sturtevant and his co-workers (C-4). Thus the heat of hydrolysis of polylysine by trypsin has been found to be −0.32 kcal per mole of peptide bond; after correction for the heat of ionization and for buffer effects, the true heat of hydrolysis is −1.24 kcal/mole (Sturtevant, C-4). Similarly, the heat for the enzymatic hydrolysis of ATP to ADP and phosphate was determined as −4.7 kcal/mole (C-2, 3). It is the same as ΔH of the hydrolysis of pyrophosphate. ΔH for the hydrolysis of sucrose in the presence of the enzyme sucrase is −3.3 kcal/mole; ΔH for the hydrolysis of urea by urease is −13.5 kcal/mole (C-5); the high ΔH value for the hydrolysis of urea is probably due to the heat of neutralization of NH_3 and CO_2 formed in this reaction. In combination with determinations of ΔF, these measurements permit us to calculate the entropy change and thereby to get more insight into the mechanism of enzyme action.

Calorimetry has also been used to determine the energies of chemical bonds between different atoms. Thus, by comparing the heat of combustion of methane (CH_4) with that of the combustion of elemental carbon and hydrogen, the bond energy for the four C-H bonds of methane can be measured; one fourth of this energy, 87.3 kcal/mole, is generally accepted as the average bond energy of a C-H bond. Similarly the following bond energies have been obtained: C-C 58.6, C=C 100, C-N 48.6, C-O 142-152, and O-H 110.2 kcal/mole.

C-1. *J. Sturtevant*, J. Phys. Chem. *45*, 127 (1941). — C-2. *R. J. Podolsky* and *J. M. Sturtevant*, JBC *217*, 603 (1955). — C-3. *R. J. Podolsky* and *M. F. Morales*, JBC *218*, 945 (1956). — C-4. *J. M. Sturtevant* et al., JACS *77*, 1495 (1956); ibid. *73*, 2454 (1951). — C-5. *C. R. Bauer* and *C. L. Gemill*, ABB *35*, 110 (1952).

D. The change of free energy, ΔF. Although ΔF, like ΔH, is measured in kcal/mole, it cannot be determined calorimetrically. Direct measurement of ΔF is possible only in those biochemical reactions in which a transfer of electrons takes place and in which this transfer causes a potential difference in a system of two electrical half-cells. Although this is the most direct method for determining ΔF, it is rarely possible to apply it to biochemical reactions. Some examples of its application will be given later.

As mentioned in the preceding section, ΔF is the change in free energy when a system passes from one state to another state. Let

us assume that we are dealing with a reaction of the type A+B = C+D, where we mix arbitrary amounts of substance A and B and obtain as products certain amounts of the substances C and D. Let us further assume that *at equilibrium* the concentrations of these four substances will be a_e, b_e, c_e, and d_e. Then, according to the mass action law, the equilibrium constant is $K = c_e d_e / a_e b_e$. If at another state of the system the concentrations are a, b, c, and d, and cd/ab is different from $c_e d_e / a_e b_e$, the system cannot be at equilibrium. The difference in free energy between the two systems, ΔF, is a measure of the tendency of the system to pass into the equilibrium state. It is determined by equation I.3:

$$\Delta F = -RT \ln K + RT \ln \frac{c\,d}{a\,b} \tag{I.3}$$

where R is the gas constant (1.987 cal/mole/degree), T the absolute temperature, K the equilibrium constant of the reaction, and ln the symbol for natural logarithms; the term ln K can be replaced by 2.3 log K. Although ΔF, according to equation I.3 can be calculated for any arbitrary concentrations of the reactants, A, B, C, and D, it is customary to select as reference state the so-called standard state in which the concentration of all reactants is 1.0 molar[1]. Hence, in this state a, b, c, and d are 1.0, and $cd/ab = 1.0$; since the logarithm of 1 is 0, the second term in equation I.3 becomes zero and the free energy difference between the standard state and the equilibrium state is, according to equation I.4a:

$$\Delta F_0 = -RT \ln K \tag{I.4a}$$

At other concentrations of A, B, C, and D, the free energy change, ΔF, can be calculated from equation 4b:

$$\Delta F = \Delta F_0 + RT \ln \frac{c\,d}{a\,b} \tag{I.4b}$$

ΔF_0 is designated as the *standard* free energy change. At 25°C the absolute temperature is 298°; hence the standard free energy change at that temperature is:

$$\Delta F_0 \text{ (at } 25^0\text{ C)} = -1.987 \times 298 \times 2.3 \log K = -1360 \log K \tag{I.5}$$

If the equilibrium constant is measured at two different temperatures, ΔH can be calculated from the two values of ΔF or

[1] In precise physical chemical calculations molality is used instead of molarity and activity of the reactants instead of concentration.