ADVANCED BIOPHYSICS

Dr. S.K. Agarwal

Principal

Modi Institute of Management & Technology
Modi Education Complex,
Dadabari Extn.,
Kota 324009

A P H PUBLISHING CORPORATION
5 ANSARI ROAD, DARYA GANJ
NEW DELHI-110 002

Published by
S.B. Nangia
A P H Publishing Corporation
5 Ansari Road, Darya Ganj
New Delhi-110002
☎ 23274050
Email : aphbooks@vsnl.net

ISBN 81-7648-987-5

2005

Typesetting at
Paragon Computers
B-36, Chanakya Place
New Delhi-110 059
☎ 25509417

Printed in India at
Efficient Offset Printers
New Delhi-110 035

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PREFACE

Biophysics is a interdisciplinary science which involves the study of biological organisms at all levels from molecules and cells to the biosphere and ecosphere as a whole, from the perspective of the physical sciences. This is the field of science that explains how physical phenomena affect/account for the structure and function of biological systems. In the last two decades biophysics has made great advances in explaining and interpreting life processes.

The recognition of biophysics as a separate field is relatively recent, having been brought about by the inventions of physical tools, which greatly facilitate biological research. These tools are peculiarly adapted to the study of problems of great current importance to medicine, and problems related to disease.

The book discusses various aspects of biophysics, giving equal emphasis to each of the major aspect. The text has been written, as far as possible, in a easy, simple and understandable language, which is self contained, fully derived and critically discussed, it is supported by a large number of diagrams, which the author felt necessary.

All chapters have been carefully updated to account for the advances in biophysics. Chapter first describes Bioenergetics, followed by Photobiology, Molecular interactions. Sensory receptors, Photo-regulatory signal regulation. Biophysics of sonic vibrations, Membrane conductivity. X-ray imaging. X-ray diffraction imaging. Ultrasound imaging, Computerized axial tomography, Electrocardiography, Electroencephalography, Radiocarbon dating, DNA fingerprinting, and Chemical fingerprinting of plants, Amazing facts have been described in each chapter, which introduce the readers to the wonders of biophysics and biomolecular structures.

I am indebted to my students for their direct and indirect role in the preparation of this book, I am thankful to University Grants Commission, New Delhi for helping me to get acquainted with people working in the field of Biotechnology. Thanks are also due to Prof., R.R. Das, Jiwaji University, Gwalior, Prof. K. Dharmalingam, Madurai Kamraj University, Madurai; Prof. Gopinathan, Institute of Basic Medical Science, Madras; Prof. B.A. Ravishankar, CFIRI, Bangalore; Prof. P.S. Dubey, Vikram University, Ujjain; and Prof. L.N. Vyas, MLS University, Udaipur for their constant help in literature survey and critical discussions.

In the preparation of this book, I have relied heavily on many excellent treatises on different aspects, most of which are mentioned in the references. My attempt was to put them in unambiguous words and to find certain correlations which were not yet explored, I am greatly indebted to all the authors for their diagrams and tables that has been incorporated in this book.

It is hoped that this book would serve the needs of teachers as well as undergraduate and postgraduate students,

CONTENTS

Chapter 1

BIOENERGETICS

All life is based on energy. How we get it, and how we use it are very important. Bioenergetics is the study of energy transformations in biological systems. While this may appear to be a very dry (and boring) topic, a basic understanding of energy and its use in living organisms is invaluable. Living organisms must perform work to stay alive, grow, and reproduce. All living organisms must possess the ability to obtain energy and be able to transform that energy into a form that can be used by its cells. Practically speaking, knowing the fundamentals of bioenergetics aids in the understanding of cell functions and allows us to understand why and how the cells are able to harness energy.

The study of energy changes accompanying biochemical reactions is termed as bioenergetics or biochemical thermodynamics. The living organism is a highly organised arrangement of matter. The synthesis and maintenance of the system is possible only by the availability of certain reactions. These reactions include degradation of complex molecules of food material to simple molecules, and the synthesis of complex molecules from. the simple compounds. In the living systems, heat is stored in the form of energy rich or high energy compounds which are mainly phosphates containing molecules. Whenever the energy is needed by metabolic processes, these high energy compounds undergo decomposition and the energy liberated is utilised. Further, the synthesis of these compounds take place as a result of various metabolic activities. Such spontaneous reactions produce useful energy for the performance of various vital activities of the organisms. The raw materials are used by the organisms as forces of energy and as building blocks for its tissues.

BASIC BIOENERGETICS

Bioenergetics is the study of energy supply, utilization, and dissipation in living organisms. Energy is the capacity for performing work. Nutrients contain *chemical energy* which is yielded upon chemical breakdown and can be used in the body to perform chemical, mechanical, electrical, or osmotic work. The efficiency of conversion of chemical energy to work energy is less than 25 percent - the remaining 75 percent is converted to thermal energy.

The internationally agreed *unit of energy* is the Joule (J). J= $kg/(m^2S^2)$. The older unit, still used in the united States is the calorie (cal). 1 calorie = heat required to increase the temperature of 1 gram of water from 14.5 to 15.5 °C. 1 cal = 4.184 J.

CELLULAR BIOENERGETICS;

Some properties of ATP are: ATP = ADP + Pi + 7400 cal. ATP is highly liable and is not stored in cells. It therefore needs continuous regeneration. ATP is synthesized mainly in mitochondria by the process of oxidative phosphorylation. For ATP synthesis the cells need (a) nutrients and their metabolites derived from carbohydrates (e.g., glucose), lipids (e.g., fatty acids), and protein (amino acids); (b) the initial steps are specific to the chemical nature of these components and can give a modest yield of ATP (e.g., glycolytic breakdown of glucose to pyruvate or its reversible intermediate (lactate) gives a net yield of 2 moles of ATP per mole of glucose; (c) much more ATP is generated via complete oxidation of carbohydrates, fatty acids or amino acids to Carbon dioxide and water. This involves two major associated processes within the mitochondria: (i) Tricarboxylic acid (TCA) cycle (also called Krebs cycle or Citric acid cycle), and (ii) Oxidative phosphorylation.

Oxidizable substances enter the TCA cycle after conversion to the two carbon compound, acetyl-coenzyme-A (acetyl.CoA). Their catabolism yields Carbon dioxide, a small amount of *ATP* and most important, reduced coenzyme (NADH, FADH2). NAD and PAD are derived from vitamin B, nicotinamide (niacin) and reboflavin respectively.

Most ATP is produced by the process of oxidative phosphorylation, in which hydrogen carried by reduced co-enzymes are finally oxidized to water after reaction with a series of enzymes and cofactors called the cytochrome chain (also called the respiratory or electron transport chain). The energy released by removal of hydrogen from reduced co-enzymes is used to drive the synthesis of ATP from ADP.

$$ADP + Pi + 7400 \text{ cal} = ATP.$$

WHOLE-BODY BIOENERGETICS

Energy can be neither created nor destroyed. This applies to all living organisms of the universe. Energy can be interconverted between different forms, but thermal energy (heat) can not be converted to other forms in the body. All biochemical reactions involve net exchange of free energy (ΔG). The reactions which release free energy are termed *exergonic* where as the reactions which use free energy are termed *endergonic.* It is possible, with difficulty to measure ΔG for individual reactions within cells, but impossible to measure ΔG for multiple reactions, let alone all of the reactions which aggregate to represent whole-body energy exchange. Fortunately, for all common biochemical reactions, the heat of reaction (ΔH) = ΔG (e.g., for glucose oxidation ΔG= –688 kcal/mole; ΔH = –671 kcal/mole). This means that whole-body, organ or cellular free energy exchange can be estimated from heat production of the whole-body, organ or cell, which is much easier to measure than ΔG.

The sources of energy loss as arbitrary approximations for ruminant and non-ruminant animals in terms of gross energy (GE), *digestible energy* (DE), *metabolizable energy* (ME), and *net energy* (NE) (Table 1), show that the gross energy is the total energy content of a given weight of feed. The digestible energy is faecal energy. The metabolic energy is urine and/or gas energy. The net energy is heat increment of feeding. The net energy is used first to meet the animal's maintenance energy requirement. Any surplus which is deposited in the body tissues (growth), milk, eggs etc. is defined as *retained energy* (RE):

$$RE = ME - H$$

Table 1: Sources of energy loss in ruminants and non-ruminants.

Sources	*Ruminants*	*Non-ruminants*
Gross energy (GE)	100	100
Digestible energy (DE)	70	90
Metabolizable energy (ME)	<60	>85
Net energy (NE)	<40	>60

ENERGY AND ITS VARIOUS FORMS

Energy of a system may be defined as the capacity to do work. This capacity may be bound in the molecules. Energy exists in many forms, such as heat, light, chemical energy, and electrical energy. In living organisms the main source of energy is the *solar energy* which gets fixed up in the form of *chemical energy* of carbohydrate molecules during photosynthesis in green plants. Other organisms have to depend directly or indirectly upon this process (photosynthesis) for a constant supply of energy to maintain their structures and to perform their numerous functions. Energy is not only required for mechanical work, maintenance of body temperature and osmotic work but also to drive the numerous reactions. Various forms of energy are as follows:

(a) ***kinetic energy*** - the first type of energy is called kinetic energy (energy of motion). It includes light, heat, and the movement of molecules. It is the energy contained within a boundary by virtue of the motion of the parts contained therein.

(b) ***potential energy*** - the second type of energy is called potential energy (energy that is stored). An example is a rock rolling down a hill. The energy that was in the rock at the top of the hill is potential energy. This energy is inside the rock, due to gravity pulling down on the rock. As the rock rolls down the hill, this potential energy is the rock is. converted to kinetic energy. Other examples of potential energy include water behind a dam, and energy stored in a molecule of sugar.

Energy can be converted from potential to kinetic and back to potential, and so on. If you push the rock back up to the hill, the potential energy in your body is converted to kinetic energy as you push on the rock, and this kinetic energy is converted to potential energy within the rock which can later be converted to kinetic energy.

(c) ***Heat energy*** - in terms of kinetic theory, identically equal to the kinetic energy of motion (rotations, vibrations, translocations) of the component molecules.

(d) ***Specific heat*** - the heat energy required to raise gram of a substance one degree in temperature. A particularly important heat is that of water, by which the unit of heat energy is defined: one calorie is the amount of heat energy required to raise 1 gram of pure water 1 °C from 3.5 - 4.5 °C (where it is the most dense) at 1 atmospheric pressure.

(e) ***Heat capacity*** - the heat energy required to raise 1 molecular weight of a substance 1 °C. The unit of specific heat are cal per °C and of heat capacity are cal per °C mole.

POTENTIAL VS KINETIC ENERGY

Potential energy, as the name implies, is energy that is not yet been used, thus the term potential. Kinetic energy is the energy in use. A tank of gasoline has a certain potential energy that is converted into kinetic energy by the engine. Batteries, when new or recharged, have a certain potential. When placed into a tape recorder and played at a loud volume, the potential in the batteries is transformed into kinetic energy to drive the speakers. When the potential is all used up, the batteries are dead. In case of rechargeable batteries, their potential is re-elevated or restored. In the hydrologic cycle, the sun is the ultimate source of energy, evaporating water (in a fashion raising its potential above water in the ocean). When the water falls as rain (or snow) its potential energy is decreased. Without the sun, the water would eventually still reach sea-level, but never be evaporated to recharge the cycle.

Chemicals may also be considered from a potential energy standpoint. One kilogram of sugar has a certain potential energy. If that kilogram of sugar is burned the energy is released all at once. The energy released is kinetic energy (heat). So much is released that organisms would burn up if all the energy was released at once, organisms must release the energy a little bit at a time.

Cells convert potential energy, usually in the form of C-C covalent bonds or ATP molecules, into kinetic energy to accomplish cell division, growth, biosynthesis, and active transport, among other things.

SCHEME OF BIOENERGETICS

The molecules in motion possess capacity to carry out work, the energy possessed by a molecule or a body by virtue of its motion is termed as "kinetic energy. This type of energy depends upon the mass of the body and its velocity of motion in accordance to the following reaction:

$$K.E. = 1/2mv^2$$

Where KE represents the kinetic energy, m the mass of the body and v the velocity of the body,

The degradation of the foodstuffs of high potential energy to products of low energy coupled to a mechanism for collection and storage energy (Figure 1), This mechanism consists of the conversion of inorganic phosphate compound into high energy phosphate compound. This high energy phosphate compound can in turn be degraded back into inorganic phosphate after releasing energy, Another coupling mechanism ensures that this energy is made into useful energy,

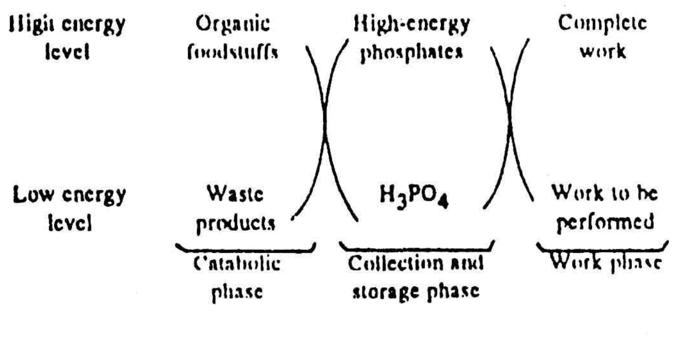

Figure 1: Scheme of bioenergetics.

Catabolism of food stuffs may be divided into three phases: *Firstly,* food material of high molecular weight (polysaccharides, lipids) are digested to products more easily absorbed. These reactions are hydrolytic and the small amount of energy liberated is not recoverable but is lost as heat. Products of this stage of catabolism include mainly common food sources like glycerol, fatty acids and many amino acids.

Second phase of catabolism converts this diverse collection of compounds into small number of intermediates for final oxidation in the next stage. Hexose and fatty acids are firstly activated by the formation of esters with phosphate and co-enzyme-A respectively. At this stage some free energy is liberated. In addition to the anaerobically producing lactic acid or alcohol depending upon the organism, lactic acid formation (glycolysis) makes available about 50,000 calorie and alcohol formation (fermentation) about 62,000 calorie and the rest of the energy is available during later oxidation. Higher animals in general can oxidise glucose completely to Carbon dioxide and water, and liberate much free energy. Under aerobic conditions pyruvic acid rather than lactic acid is the main product and this is further oxidised to acetyl coenzyme-A. Acetyl coenzyme-a, is also produced from fatty acids, the glycerol of fat joins the carbohydrate path at the triose state.

Certain amino acids are catabolized via special pathway, but initially deaminated to form the corresponding keto acids. The nitrogen of amino group follows its own pathway to eventually appear in the urine has urea and ammonia. Sulphur contained in the amino acid (for example methionine) is oxidized to inorganic sulphate, the carbon skeleton of a few amino acids are converted to ketone bodies or acetyl coenzyme-A, But many, amino acids either yield pyruvic acid, α-keto glutaric acid, or oxalacetic acid directly on deamination or form products readily convertible to those α-ketoacids. During this second phase a small amount of recoverable free energy is liberated.

During the *third* phase, all of the products of the second phase are channelled into a single mechanism known as tricarboxylic acid or Krebs cycle (Figure 2), is a sequence of reactions which releases the carbon dioxide, while it feeds pair of their hydrogen into oxidative chain to form water.

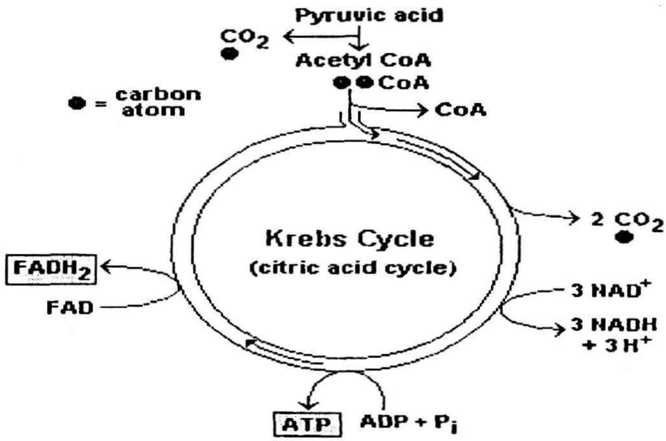

Figure 2: A summary diagram of the Krebs cycle.

Free energy liberated or consumed in any reaction is the difference between the free energies of formation and reactants in the reaction.

LAWS OF THERMODYNAMICS

A quick review of the laws of thermodynamics is in order. While these laws should be familiar to you, you should make sure that you have a good understanding of what these laws mean,

The first law of thermodynamics

Energy can be changed from one form to another/ but it cannot be created or destroyed. The total amount of energy and matter in the universe remains constant, merely changing from one form to another. The first law of thermodynamics (conservation) states that energy is always conserved, it cannot be created or destroyed.

The first law of thermodynamics has been defined in various ways:

1. The total energy in an isolated system always remains constant, although there may be a change from one form to another.
2. The energy of an isolated system remains constant and whenever a quantity of some form of energy disappears, an

exactly equivalent quantity of some other form of energy must be produced.

3. Any gain or loss of energy by the system must be exactly equivalent to the loss or gain respectively by the surroundings of the system.
4. Whenever a certain quantity of energy is produced, an equivalent amount of other form of energy must be used up.
5. Energy can neither be created nor destroyed, the only change which energy can undergo is a transformation from one form to another.
6. The total energy of a system, plus its surroundings remains constant,

These definitions differ slightly from one another but the basic idea is that energy can neither be created nor destroyed.

This law is telling you that energy cannot come from nowhere. Therefore, a reaction which requires the input of energy cannot occur unless there is a source for the needed energy. Imagine that there is pen on the table next to the computer. You would never expect the pen to suddenly rise off the table and move towards the ceiling. That is because such a movement requires energy to move the pen against the force of gravity. Unless someone gives the pen this energy, it will not move. You could pick up the pen and throw it so that it hits the ceiling. In this case, you are giving energy to the pen. This energy ultimately comes from the nutrients that you eat and convert into the muscle and fuel the muscle that you used to move the pen.

Einstein has shown that matter and energy are equivalent and interrelationship is given by the famous Einstein equation:

$$E = mc^2$$

where E is the energy, m is the mass and c^2 is the velocity of light. Hence, 1 gm of matter is equal to 2.1×10^{13} calories, a stupendously large amount of energy.

Suppose we put some amount of heat in a system. Since the heat energy cannot be lost, it must remain either wholly or partly as internal energy in the system, or can wholly or partly be used up by the system in doing mechanical work. In the general case, when the heat absorbed goes both to increase the internal energy and to produce some mechanical external work, we must have Heat absorbed = Increase of internal energy + work done by the system. If the final and the initial internal energies of the above systems are E_2 and E_1 respectively, then the increase in internal energy is $E_2 - E_1 = \Delta E$ (the symbol Δ always signifies increase, that is Final - Initial, irrespective of whether it is positive or negative). If the heat absorbed is q and the work done by the system W, then by substituting these values in the foregoing equation we get:

$$E_2 - E_1 = \Delta E = q - W$$

The first law of thermodynamics includes more specific mass of constant heat sums which is of great importance in energy transformations. It states that the total amount of heat produced or absorbed if a chemical reaction is carried out in stages is equal to the total amount of heat evolved or consumed when the reaction occurs directly. A good example is the oxidation of glucose to Carbon dioxide and water.

1. $C_6 - H_{12}O_6 + 6O_2 + 6H_2O + 6CO_2 + 673$ (kcal)
2. $C_6H_{12}O_6 = 2C_2H_5OH + 18$ (kcal)

   $2C_2H_5OH + 6O_2 = 6H_2O + 4CO_2 + 655$ (kcal)

Thus, no matter what pathway a particular reaction follows, the total amount of heat evolved or absorbed is always the same.

LIMITATIONS OF FIRST LAW OF THERMODYNAMICS

There are three main limitations of the first law of thermodynamics. These are as follows:

(a) The first law of thermodynamics simply establishes equivalence between different forms of energy. However, this law does not tell us under what conditions and to what extent it is possible to bring about conversion of one form of energy into the other.

(b) The first law of thermodynamics is a quantitative statement which does not prelude the existence of either a heat engine or a refrigerator. The first law does not contradict the existence