

BIOENERGETICS

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Metabolism: Sum total of all the chemical reactions that are taking place in the body.

- Greek word, *metabellein*- “to change”

Includes:

- The process by which cells use food material to obtain energy
- Store excess calories for future use
- Build up various substances

Also includes degradation and excretion of unnecessary compounds.

Metabolism, is therefore, sum total of all those processes that turn food into flesh.

- **Bioenergetics**- Field of Biochemistry that deals with transformation and utilization of energy in biological systems.
- Concerns only with the initial and the final energy states of reaction components and predicts the energetic feasibility of chemical reaction.

After attending these lectures, you should be able to understand:

- Fundamental design of metabolic network; interdependence of metabolic reactions.
- Principles of bioenergetics: laws of thermodynamics; free energy, entropy and enthalpy; equilibrium constant.
- Role of ATP as energy carrier; concept of high-energy and super high-energy compounds and substrate level phosphorylation; other energy rich nucleoside triphosphates

1. Overview of Metabolism

- **General considerations**

Metabolism serves two important purposes:

- To release energy from the ingested food material through catabolic degradation, and to convert this energy into a form that can be used for cellular work.
- To transform small organic compounds into macromolecules-aspect of metabolism also includes transformation of one group of organic compounds into another.
- During catabolic degradation, the energy inherent in the organic molecules (particularly carbohydrates and lipids) is released. It is then trapped and stored as **adenosine triphosphate (ATP)**. The stored energy can be released from ATP when needed and used to perform cellular work (Fig.)

Major works:

- **Transport** of organic molecules and inorganic ions across the cell membrane.
- **Mechanical work**, such as muscle contraction
- **Electrical work** (e.g. nerve conduction)
- Ensure fidelity of **information transfer**

Second major purpose of metabolism-synthesize a vast array of macromolecules, which include carbohydrates, proteins, lipids and nucleic acids. It is amazing that so many diverse biomolecules are intracellularly synthesized from a limited number of organic compounds. Evidently, thousands of reactions are involved in the processes which split, join and rearrange the atoms of organic compounds, thus resulting in generation of complex biomolecules. **Each of these reactions are catalyzed by a specific enzyme.**

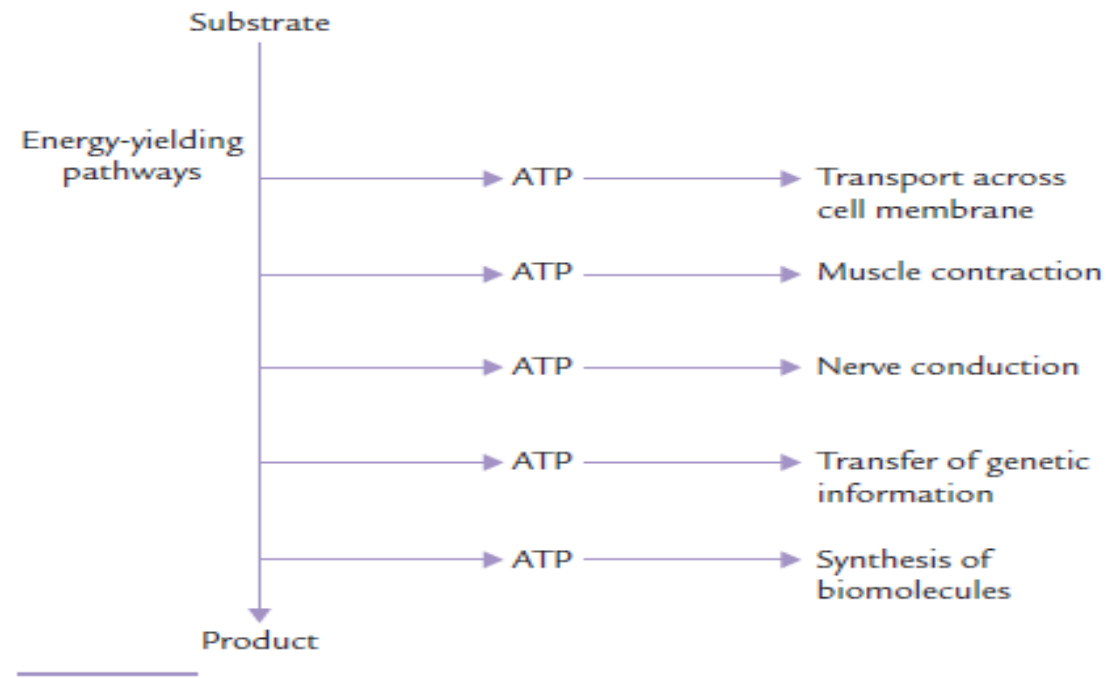


Fig. The major cellular works.

B. Metabolic Reactions:

Interdependent and Interconnected

- Metabolic reactions do not occur in isolation, or in a random or haphazard manner. Rather they are organized into multi-step sequences called metabolic pathways.
- Each reaction forms just one step in a metabolic pathway and is part of a larger scheme that involves several other interrelated reactions.
- In metabolic pathways, the product of one reaction serves as a substrate for the next one; the product of second reaction is substrate for the third reaction, and so on.



- Such series of consecutive reactions allows the cell to carry out highly complex molecular conversions intracellularly

Conversions occur in mild conditions of **temperature and pH that prevail within the cell.**

Example: Sequence of reactions that converts glucose to pyruvate (i.e. glycolytic sequence).

As soon as the glucose enters the cell, a phosphate group from ATP is added to it to form glucose 6-phosphate. Glucose 6-phosphate becomes the substrate for the next reaction, in which an *isomerase* converts it into fructose 6-phosphate.

The latter then serves as substrate for another enzyme-catalyzed reaction, and the sequence continues through six more reactions until glucose is converted to pyruvate. Reactions of this metabolic pathway are summarized in Figure on the next slide.

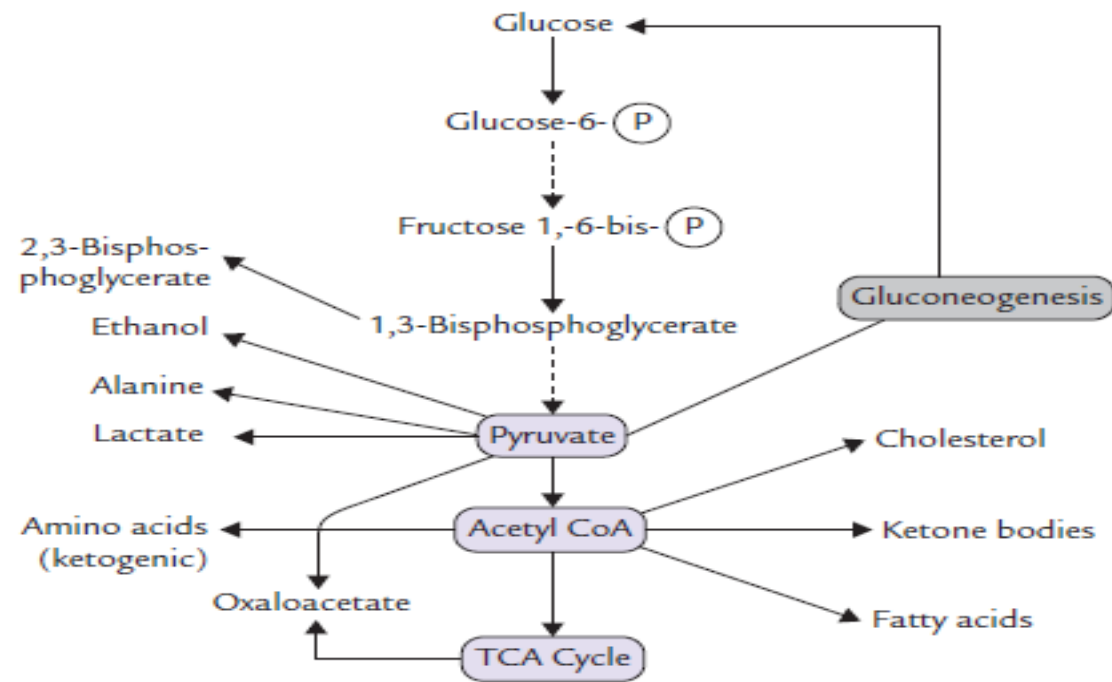


Fig. Metabolic pathways connected through branch point compounds, pyruvate and acetyl CoA.

- *The energy inherent in the substrate, glucose, is released in small packets in a stepwise fashion and is effectively captured.*

Principles of Bioenergetics

- Energy: Capacity to do work- required for performing various activities. Living cell needs energy for performing various activities
- Energy transformations, both by the living systems as well as by the artificial devices, take place according to the laws of thermodynamics.
Thermodynamics-branch of physical science that deals with energy changes

LAWS OF THERMODYNAMICS

The First Law

Also referred to as the *law of conservation of energy*, states that energy can neither be created nor destroyed. In course of any physical or chemical reaction, one form of energy may change to some other form, but the total amount of energy in the universe always remains constant.

- For example, electric energy changes to heat energy in room heater, heat energy changes to mechanical energy in rail engine and mechanical energy changes to electric energy in hydroelectric plants.

These examples show that various forms of energy are interconvertible. However, none of these transformations brings about any net generation or loss of energy.

The Second Law

- *All physical and chemical reactions tend to proceed in such a direction that useful energy of the reacting system is irreversibly converted to a randomized and useless form, known as **entropy**.* The reactions proceed in this direction till entropy reaches maximum possible under the prevailing circumstances. At this point, called the equilibrium point, no further progress of the spontaneous reaction is possible.
- Thus in all reacting systems, disorder or randomness is favoured at the cost of orderliness. To be more explicit, fall in useful energy content of the system occurs with a concomitant rise in the randomized energy of the universe.
- The reacting system implies collection of matter undergoing a reaction, and the universe includes both, the system and its surroundings. This literally includes the whole of earth, or even the outer space.

Bioenergetics: Field of biochemistry that deals with the study of energy changes in metabolic reactions.

- All living organisms conform to the laws of thermodynamics.

B. Free Energy, Entropy and Enthalpy

- Useful energy is the form of energy that is capable of performing work.

Broadly classified in two major types:

1. Heat energy-capable of performing work through change of temperature.

2. Free energy-capable of performing work at a constant temperature.

- Since human body maintains a constant temperature (i.e. isothermic), it cannot utilize the heat energy. **Free energy** is the useful form of energy in humans because of its ability to function at constant temperature. It performs various functions and, in the process, gets converted to the randomized form, i.e. **entropy**.
- Relations can be expressed in the equation form on the next slide:

$$\Delta G = \Delta H - T\Delta S$$

- ΔG is change in the free energy content of the reacting system. It equals the difference in energy level between the substrates and products.
- ΔS is change in entropy of the universe.
- T is absolute temperature measured in Kelvin.
- ΔH implies change in heat content of the system, which is termed as enthalpy (the word means “warming within”).

- In course of any ongoing reaction, entropy of the universe (system surroundings) always increases. *The value of S , therefore, has a positive sign.* Since rise in entropy is accompanied by a corresponding fall in free energy of the system, *the value of G is negative.* The enthalpy change may be positive or negative, depending on whether the system absorbs or releases heat. It is positive when the heat absorption occurs and is negative when heat release occurs. However, in the isothermic state prevailing in the body, its value is zero.
- Finally, it must be emphasized that entropy or disorder is not an entirely useless activity. Since increase in entropy is an irreversible process, it gives direction to all biological activities.

C. Standard Free Energy Change

- The free energy change, ΔG is the most important thermodynamic function because it is the driving force of the reactions. Like ΔH , it is measured in kilocalories per mole (kcal/mol) or kilojoules per mole (KJ/mol). A negative sign of ΔG indicates an **exergonic reaction**, the one that proceeds spontaneously and forms product from substrate. A positive sign of ΔG indicates an **endergonic reaction** which can proceed only in the backward direction.
- *At equilibrium, ΔG equals zero.* ΔG is not a property of the reaction as such but is affected by relative reactant concentrations. To obtain a convenient energetic expression that predicts the equilibrium of the reaction, we have to define the **standard free energy change** (ΔG^0).

The standard conditions are as below:

- Temperature of 25°C (298°K).
- Pressure of 1.0 atm (760 mmHg).
- pH 7.0.
- The reactants and products are all present in the concentration of 1.0 M

- *The standard free energy change of a given reaction is an **immutable constant** and it equals the difference in energy level between the substrates and products, under standard conditions, and is shown as Table on slide number 26.*
- It predicts equilibrium of a reaction.

The free energy change (ΔG) is the driving force for chemical reactions. In spontaneous exergonic reactions there is release of free energy (ΔG is negative), and in endergonic reactions there is requirement for free energy (positive ΔG).

- The standard free energy change ($\Delta G^{0'}$) must be differentiated from the *actual free energy change* (ΔG) of a reaction:

$\Delta G^{0'}$ is measured under standard conditions, stated above and has a constant value for a given reaction.

| Metabolite | $\Delta G^{\circ'}$ |
|--|---------------------|
| Phosphoenol pyruvate | −14.8 |
| Carbamoyl phosphate | −12.3 |
| 1,3-Bisphosphoglycerate | −11.8 |
| Acid phosphate | −11.2 |
| Creatine phosphate | −10.3 |
| Arginine phosphate | −7.6 |
| ATP to ADP + P _i | −7.3 |
| ATP to AMP + PP _i | −7.7 |
| ATP to AMP + P _i + P _i | −14.6 |
| Glucose 1-phosphate | −5.0 |
| Glucose 6-phosphate | −3.3 |
| Glycerol 1-phosphate | −2.2 |

Table: Standard free energy of hydrolysis of some common metabolic intermediates

The actual free energy change is measured at the conditions prevailing during the reaction. It is a function of the conditions of concentration, pH and temperature actually prevailing during the reaction, which are not necessarily the standard conditions.

Further, the value of the actual free energy change keeps changing as the conditions change. It has negative value in the beginning of the reaction, which becomes less and less negative as the reaction progresses towards equilibrium. At equilibrium its value falls to zero.

- G_0' has a definite relation with G and with equilibrium constant of the reaction, as illustrated in Boxes 1 and 2, respectively.

Relationship Between the Standard Free Energy Change (ΔG^0) and the Actual Free Energy Change (ΔG)

The actual free energy change (ΔG) and the standard free energy change (ΔG^0) of a chemical reaction (\rightleftharpoons) are related to each other as shown in the equation below:

$$\Delta G = \Delta G^0 + 2.303 RT \log \frac{[B]}{[A]}$$

where T is absolute temperature measured in kelvin, R is the gas constant, and [A] [B] are the actual concentrations of the reaction substrate and product respectively.

- Box 1

The Standard Free Energy Change is Related to Equilibrium Constant

In a reversible chemical reaction ($A \rightleftharpoons B$) equilibrium describes a state when rate of the forward reaction equals that of the reverse reaction. In this state no net chemical transformation occurs, so that equilibrium concentrations of both A and B are invariable. Their ratio is termed as equilibrium constant (K'_{eq} of the reaction)

$$K'_{eq} = \frac{[B]}{[A]}$$

where [A] and [B] refer to the equilibrium concentration of the reactant and the product, respectively. At equilibrium the relation between $\Delta G^{0'}$ and ΔG is as follows:

$$\Delta G = \Delta G^{0'} + 2.303 \log RT \frac{[B]}{[A]}$$

Since $\Delta G = 0$ at equilibrium, it can also be rewritten as:

$$\Delta = G^{0'} + 2.303 RT \log K'_{eq}$$

When $K'_{eq} = 1$, value of $\log K'_{eq}$ is zero. Hence, $\Delta G^{0'} = 0$, meaning that product contains the same amount of free energy as the reactant, and therefore the reaction stands at equilibrium. Likewise, if the $K_{eq} > 1.0$, its $G^{0'}$ is negative and if $K_{eq} < 1.0$, its $G^{0'}$ is positive and the reaction respectively proceeds in the forward and the reverse direction. Table 8.3 summarizes these points.

The standard free energy change of a chemical reaction is simply a different mathematical way of expressing its equilibrium constant.

- Box 2

| K_{eq} | $\Delta G^{0'}$ | Direction of reaction (starting with 1M components) |
|----------|-----------------|---|
| > 1.0 | Negative | Forward |
| 1.0 | Zero | Stands at equilibrium |
| < 1.0 | Positive | Reverse |

Table 8.3. Relationship between K_{eq} , $\Delta G^{0'}$ and the direction of chemical reactions under standard conditions

Standard Free Energy Changes of Sequential Reactions

Total standard free energy change of a reaction sequence equals the sum of the standard free energy changes of the individual reactions of the sequence.

Reaction 1



Reaction 2



The sum of these two reactions is



It has a standard free energy change of $(-4.0) + (-3.3) = -7.3 \text{ kcal/mole}$.

Note: Value of the standard free energy of ATP hydrolysis can be thus obtained in an indirect manner only.

D. Role of ATP in Cell Bioenergetics

ATP (Adenosine triphosphate) serves as the mediator of biological energy transfers. Acts as an energy carrier in biological systems. Links the energy-yielding (i.e. exergonic) and the energy-requiring (i.e. endergonic) processes.

Composition: ATP is a nucleotide consisting of the following three components (Fig.):

1. *Purine base* which is adenine.
2. *Ribose sugar* which is a 5-carbon sugar (i.e. a pentose).

The first carbon of ribose is linked to N-9 of adenine through N-glycosidic linkage.

3. *Phosphate groups*, three in number, are designated α -, β - and γ -phosphates. The α -phosphate is linked to C-5 of the ribose.

- **Functions:** ATP serves as a link between the energy-yielding (exergonic) pathways and the energy-requiring (endergonic) processes
- Normally, the exergonic processes are thermodynamically favoured and therefore, occur spontaneously. The endergonic processes, on the other hand, are thermodynamically un-favoured, and hence, require input of energy to be driven forward. These two types of processes are coupled, so that the energy of exergonic reaction can be used to drive progression of the endergonic reaction.
- In most biological transformations, ATP is generated during exergonic reactions, using the free energy released. Thus each molecule of ATP represents stored free energy.

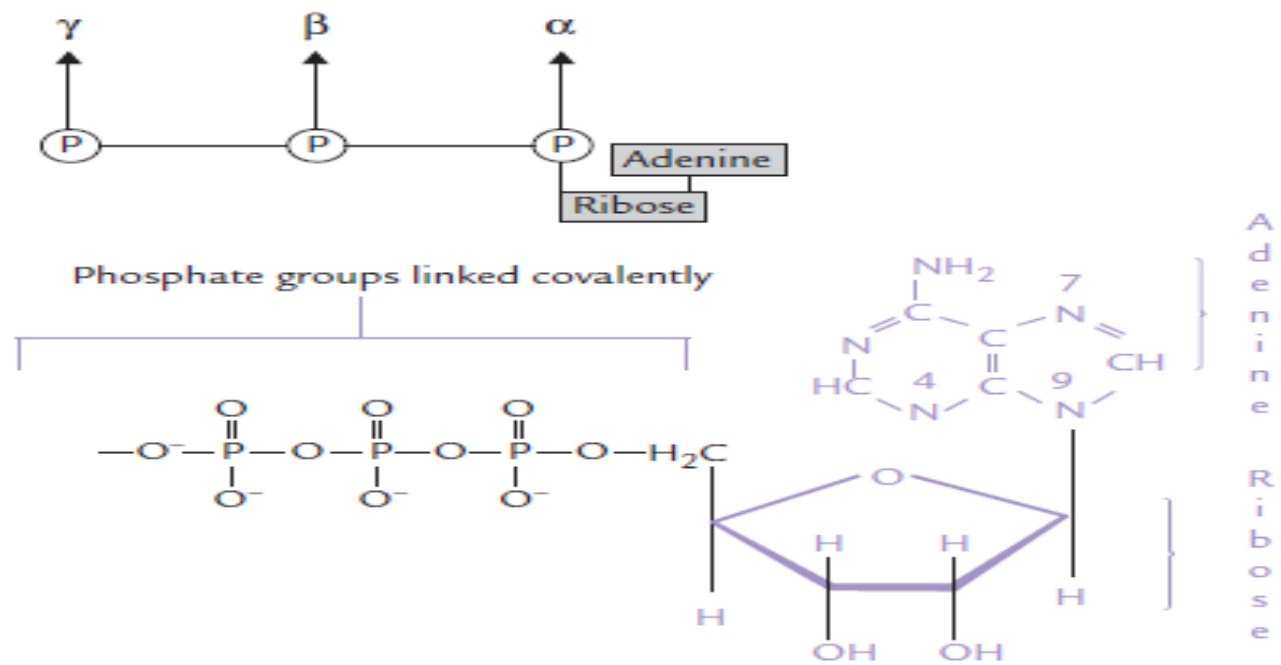


Fig. Structure of ATP.

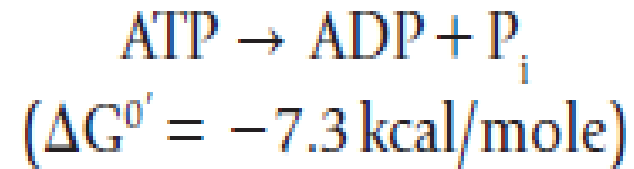
Some of the energy requiring processes include:

- Biosynthesis
- Active transport
- Transfer of genetic information and movement

ATP serves as a primary and universal carrier of free energy.

Referred to as the *currency of free energy in the body*

- **Standard free energy ($\Delta G^{0'}$):** The standard free energy of hydrolysis of ATP is **7.3 kcal/mole**. It implies that 7.3 kcal of free energy is released, under standard conditions, from 1.0 mole of ATP by the following reactions.



Reformation of ATP occurs by attachment of a terminal phosphate group to ADP. The process requires input of the same amount of free energy. The energy obtained from various catabolic pathways is used for the ATP generation: 7.3 kcal of energy is required for the synthesis of 1.0 M of ATP (from 1.0 M each of ADP and P_i). Therefore, each mole of ATP represents 7.3 kcal of stored energy. During the energy-requiring processes, hydrolysis of ATP occurs, which liberates the required energy. In this way, generation of ATP is followed by its hydrolysis, which is again followed by regeneration, and so on. Thus the γ -phosphate group of ATP continually undergoes removal and replacement in a cyclic fashion: the process is termed the **ATP cycle**. It ensures formation of ATP with energy release and transfer of this energy to processes which require it.

E. Low-Energy, High-Energy and Super High-Energy Compounds

- ATP is high-energy compound placed higher than several compounds on the thermodynamic scale. Such compounds having $\Delta G^{0'}$ less than that of ATP are termed the low-energy compounds. On the other side of the thermodynamic scale lie certain compounds, $\Delta G^{0'}$ of which is higher than that of ATP. Such compounds are referred to as the **super high-energy compounds**; phosphoenol pyruvate (PEP) and creatine phosphate having $\Delta G^{0'}$ of 14.8 kcal/mole and 10.3 kcal/mole respectively are some examples.

- ***Phosphoenol pyruvate*** (PEP): An intermediate in glycolysis. Its conversion to the next glycolytic intermediate, pyruvate, under standard conditions, yields 14.8 kcal/mole energy. This is more than sufficient for the formation of ATP, which needs 7.3 kcal/mole. Thus, ATP formation (i.e. phosphorylation) is coupled with transformation of a substrate (i.e. PEP in this case). This process is called **substrate level phosphorylation**.



- ***Creatine phosphate:*** Stored in the skeletal muscles, where it helps in quick in generation of ATP. During muscle exercise, ATP is rapidly hydrolyzed to meet the energy needs of the exercising muscle. Consequently, the ATP levels within the muscular tissue tend to get depleted. Creatine phosphate replenishes the ATP under these circumstances.
- Conversion of this compound to creatine and phosphate releases energy, which is used for ATP generation. An example of the substrate level phosphorylation



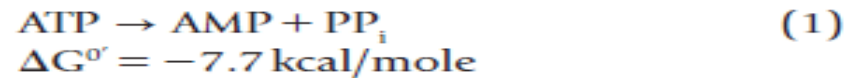
CK permits skeletal muscles to perform the intermittent strenuous work by maintaining the intracellular ATP levels normal.

Since ATP lies midway in the thermodynamic scale, i.e. between the super high energy compounds and the low energy compounds, it is a suitable compound to serve as a currency of free energy in the body.

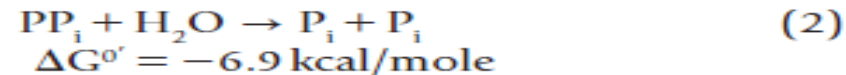
- Phosphoenol pyruvate, creatine phosphate and other such high energy compounds play a crucial role in the energy transfer of biochemical reactions.

F. Cleavage of ATP to AMP and Pyrophosphate

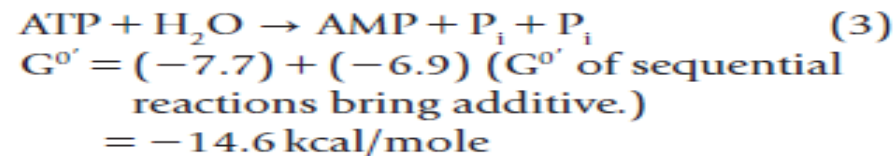
In course of most endergonic reactions, ATP is converted to ADP by removal of the terminal γ -phosphate group (orthophosphate cleavage). However, in certain other endergonic reactions, the β -and the γ -phosphate groups are simultaneously removed in one piece to form AMP and pyrophosphate (pyrophosphate cleavage).



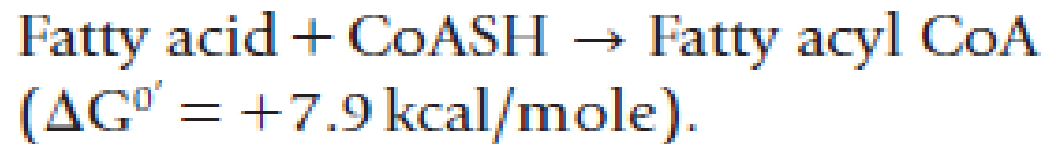
This followed by cleavage of the pyrophosphate, which yields two phosphate groups.



Sum of these two reactions and its $G^{o'}$ is



- This is double than the usual 7.3 kcal/mole yield by orthophosphate cleavage of ATP. Release of a larger amount of energy in this manner can drive certain complex biosynthetic reactions forward. For example, 7.3 kcal/mole, obtained by orthophosphate cleavage of ATP would be insufficient to drive the activation of fatty acid to completion, which requires an input of about 7.9 kcal/mole.



- Release of 14.6 kcal/mole of energy by the pyrophosphate cleavage of ATP can drive this reaction to completion.
- Amount of energy provided by pyrophosphate cleavage of ATP is evidently far in excess of that required for the fatty acid activation. The surplus (i.e. $14.6 - 7.9 = 6.7$ kcal/ mole) dissipates as entropy. However, *dissipation is not a wasteful expenditure, since it helps to drive the reaction forward and further ensures its irreversibility.*

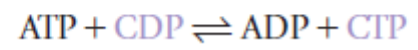
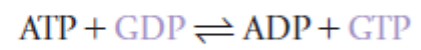
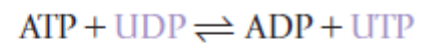
- In contrast to ADP, which returns to the ATP cycle by directly accepting a phosphate group, AMP requires participation of an additional enzyme, *adenylate kinase*, to form ATP. This enzyme catalyzes reversible



- The ADPs so formed directly accept phosphate group(s) to form ATP. Acting in the reverse direction, the *adenylate kinase* has another important role: it helps to maintain a constant intracellular ATP level by catalyzing the transfer of the β -phosphate of one ADP to another ADP molecule. Thus it supplements the ATP generation by *creatine kinase* in the contracting muscle.

G. Other Energy Rich Nucleoside Triphosphates

- There exist several other nucleoside 5'-triphosphates which are analogous in structure to ATP, and which are also energy-rich like ATP. Some examples are guanosine triphosphate (GTP), cytidine triphosphate (CTP), and uridine triphosphate (UTP). They are present in all cells but in a much lower concentration than ATP. Although ATP is the major carrier of the phosphate group, the other types of nucleoside triphosphates are specialized to serve in certain biosynthetic pathways. For example, *CTP is used in phospholipid biosynthesis and UTP in glycogen synthesis*. Synthesis of the nucleoside triphosphates is catalyzed by the enzymes called *nucleoside diphosphokinases*: ATP mostly serves as the donor of the terminal phosphate groups.
- Low concentration of corresponding 2'-deoxyribonucleoside 5'-triphosphates is also present in cells. These are 2'-deoxyadenosine 5'-triphosphate (dATP) and 2'-deoxyguanosine 5'-triphosphate (dGTP), 2'-deoxycytidine 5'-triphosphate (dCTP), and 2'-deoxythymidine 5'-triphosphate (dTTP).



- END