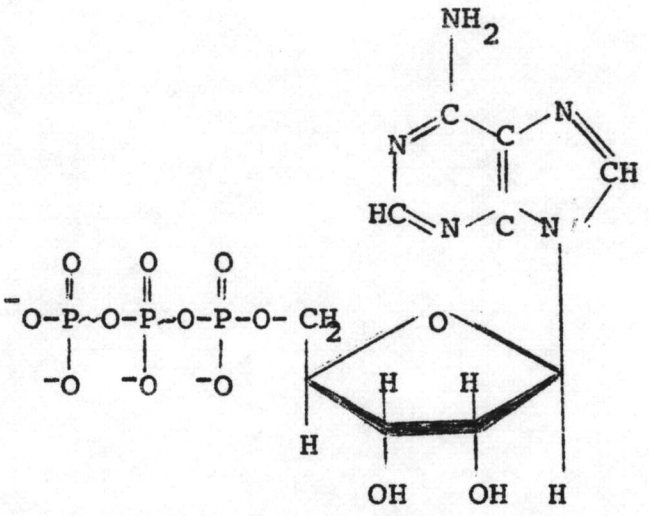
CHAPTER I

INTRODUCTION

ADENOSINE-5′-TRIPHOSPHATE (ATP)

ATP is a high energy compound with a formula structure:



Adenosine triphosphate (ATP)

ATP is a primary carrier of chemical energy in the cell, serving to transfer high energy phosphate groups from energy-yielding to energy-requiring process. It plays important roles in the conservation of energy liberated in the cell during oxidation reactions.

ATP is present not only in the soluble cytoplasm but also within organelles such as mitochondria and nuclei. The intracellular compartmentation of the ATP system is an important feature in cellular regulation of metabolism.

The concentration of ATP usually exceeds the sum of the concentration of ADP and AMP.

At pH 7.0 both ATP and ADP are highly charged anions. ATP has four ionizable protons in its triphosphoric acid group. The high concentration of negative charges around the triphosphate group of ATP is an important factor in its high-energy nature.

In the intact cell, very little ATP and ADP exist as free anions, they are largely present as the 1:1 $MgATP^{2-}$ and $MgADP^{-}$ complexes, because of the high affinity of the pyrophosphate groups for binding divalent cations and the high concentration of Mg^{2+} in intracellular fluid. The affinity of ATP for Mg^{2+} is about 10 times as great as that of ADP.

FUNCTION OF ATP

The function of ATP is the major carrier of chemical energy in the living cells of all species. Its important function is in metabolism of the cells. Metabolism is divided into two parts, catabolism and anabolism.

Catabolism is the enzymatic degradation, largely by oxidation reaction of relatively large nutrient molecules, i.e. carbohydrate, lipid, and proteins, coming either from the environment of the cell or from its own nutrient storage depots into a series of smaller, simpler molecules, e.g.,

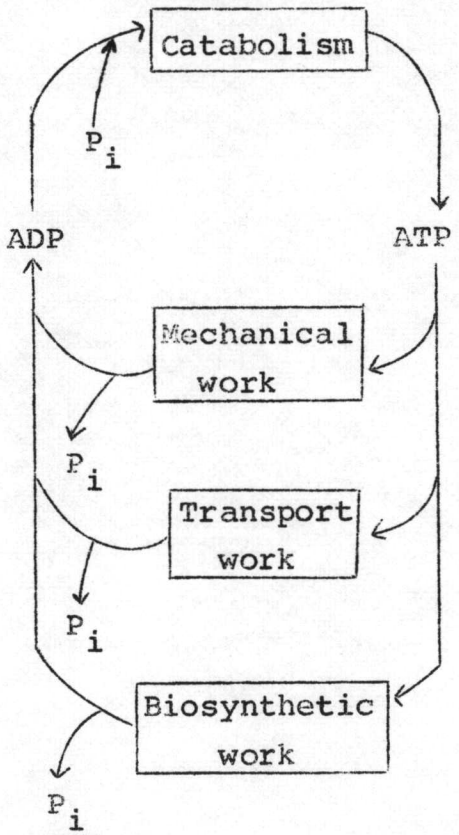
lactic acid, acetic acid, CO_2, ammonia, or urea. Catabolism is accompanied by release of the free energy inherent in conservation in the form of the phosphate bond energy of ATP.

Anabolism is the enzymatic synthesis of relatively large molecular components of cells, e.g., polysaccharides, nucleic acids, protein, and lipid, from simple precursor molecules. Since the synthetic process results in increased size and complexity of structure and thus a decrease in entropy, it requires input of free energy, which is furnished by the phosphate bond energy of ATP. Catabolism and anabolism take place concurrently in cells.

THE ENERGY CYCLE IN CELLS

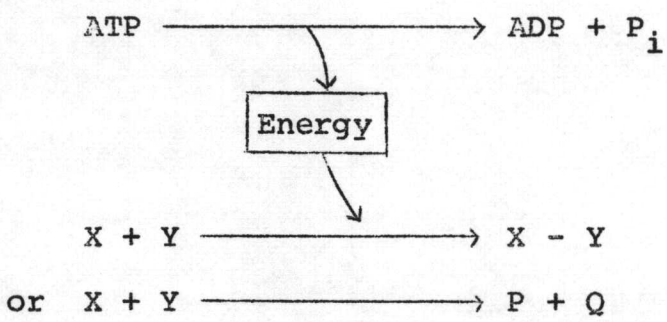
Consecutive chemical reaction make possible the specific biological function of the ATP - ADP energy cycle as the connecting link between two large networks of enzyme - catalysed reaction in the cell. One of these networks conserves chemical energy derived from the environment by causing the phosphorylation of the energy - poor ADP to the energy-rich ATP during catabolism. Since the ATP can diffuse to those sites in the cells when its energy is required, it is thus also a transport form of energy. The chemical energy of ATP is then released during transfer of its terminal phosphate groups to certain specific acceptor molecules which become energized and can do work. The other

network utilizes the energy of ATP to carry out the biosynthesis of cell components from simple precursors, with simultaneous break down its terminal phosphate group of the ATP to ADP. Like the building block biomolecules, these consecutively linked networks of enzyme-catalyzed reactions are essentially identical in all living species. The ATP-ADP energy cycle is shown in a diagram below:



This figure was taken from Lehninger (1970).

The energy from catabolism is conserved in the form of ATP. The energy from ATP degradation is then used for synthesis of new bond from two small molecules (X and Y) into large molecules (X - Y, or P + Q). These reaction is found in anabolism which is called energy coupling, illustrated below:



The role of ATP in the metabolism may be divided into 2 parts:

1. ATP in metabolic reaction, and
2. ATP acted as a regulator in metabolism.

1. <u>ATP in metabolic reaction</u>

ATP obtained from catabolism is used in the synthesis of new bond in anabolism.

2. <u>ATP acted as a regulator in metabolism</u>

ATP may control the catabolism and anabolism. It has been shown that ATP and the degradation products of ATP, i.e. ,ADP and AMP, can stimulate or inhibit allosteric enzymes activity. ATP inhibits the activity of the enzymes

for catabolism or energy regenerating system and stimulates the activity of the enzymes for anabolism or energy utilizing. ADP or AMP have the opposite activity. Therefore, the high level of ATP in the cell will increase the rate of anabolism while the low level of ATP will decrease the rate of catabolism. ATP can activate some enzymes from inactive form to active form, by the phosphorylation or adenylylation the enzymes.

ATP AND RED BLOOD CELLS

As it does in all living cells, ATP plays a vital role in erythrocyte metabolism (Brewer, 1969). An oxidative phosphorylation system is not present in mature mammalian red cell and they must rely on glycolysis for regeneration of ATP. A reasonably normal level of ATP is necessary for the survival of the erythrocyte during its *in vivo* life span. The three known major functions of ATP in the erythrocyte include (1) the priming of glycolysis at the hexokinase and phosphofructokinase steps, (2) maintenance of red cell shape, and (3) provision of energy for cation pumping.

1. The priming of glycolysis

(a) Phosphorylation of glucose by ATP

This is a priming step, in which the neutral glucose molecule is mobilized and made ready for the

subsequent steps by its phosphorylation at the expense of ATP. The phosphorylation of glucose by ATP to yield glucose-6-phosphate is catalyzed by two types of enzyme, which differ in their sugar specificity, hexokinase and glucokinase. Hexokinase is the more important and is the enzyme normally employed by most cells.

(b) Phosphorylation of fructose-6-phosphate

In this, the second of the two priming reaction of glycolysis a second molecule of ATP is invested to phosphorylate fructose-6-phosphate to fructose-1, 6-diphosphate by action of phosphofructokinase.

2. Maintenance of red cell shape

Palek et al. (1972) have demonstrated that the decrease of ATP and accumulate of calcium in stored erythrocytes induce conformational change of membrane fibrous (contractile) proteins that results in decrease in membrane elasticity and permeability. This is in turn reflected by formation of small ghosts. The formation of small ghosts reflects increased rigidity and decreased permeability of red cell membranes due to Ca^{2+}, ATP-dependent conformational changes (contraction) of membrane fibrous proteins. The alterations in shape and decreases in filterability and deformability which resembled analogous changes in metabolically depleted cells were attributed to

ATP depletion and intracellular accumulation of calcium (Haradin et al., 1969; Nakao et al., 1959; Weed et al., 1969; Nakao et al., 1961 and La Celle, 1969).

3. Provision of energy for cation pumping

It is well established that in the human red cells,ATP is essential for maintenance of cation balance and for the couple sodium-potassium active transport mechanism (Hoffman, 1962). Brewer (1969) speculated that the lower erythrocytic ATP levels in thalassemia is mechanically rather than biochemically determined. It is possibly resulted from the smaller size of the thalassemic erythrocyte and greater relative utilization of energy from ATP for cation transport. Small cells have a greater surface area to volume ratio, and hence a greater passive cation leak relative to volume. In agreement with these ideas, iron deficient cells also have low ATP levels (Brewer, 1967b).

Brewer and Powell (1964, 1966) reported that American negro with a glucose-6-phosphate dehydrogenase deficiency had approximately twice the normal level of ATP in his erythrocytes. Studies of the kindred of this individual indicated that this abnormality termed "elevated erythrocytic ATP", represented an inherited trait (Brewer, 1964; Brewer, 1965).

Determination of ATP content of erythrocytes is also of considerable interest from the view point of hereditary abnormalities of red cell metabolism, such as pyruvate kinase deficiency and certain genetic disorders characterized by increased or decreased red cell ATP level. This level is controlled by a multifactorial genetic system (Brewer, 1967a). A quantitative trait such as ATP may be the first line of defense of a population against a disease such as malaria. There is a two-fold variation in level of ATP from one individual to another in both the American Negro and Caucasian population (Brewer, 1967a; Brewer, 1965; Brewer and Powell, 1965). These authors also showed that Negroes have a significantly lower mean level of ATP in their red cells than Caucasians and suggested that a low ATP level may be one of the selective genetic factors concerned in the relative immunity of certain racial groups of P. falciparum malaria.

Several methods for the measurement of red cell ATP levels have been developed recently. These methods include:

1. Paper and column chromatography.
2. Measuring glucose-6-phosphate formation in the hexokinase reaction with glucose-6-phosphate dehydrogenase (G-6-PD) and NADP.

3. Employing the "backwards" glyceraldehyde phosphate dehydrogenase reaction to measure the oxidation of NADH + H^+.
4. The luciferin-luciferase system.

Each of these techniques has been reported to give a reproducible result and adequate recovery of ATP levels. The simplest and most reproducible of this method is the luciferin-luciferase enzyme method (Beutler and Mathai, 1967).

RELATIONSHIP BETWEEN ATP LEVELS AND MALARIA

The erythrocytic ATP levels is an important genetic system affecting fitness relative to malaria. In contrast to other human genetic system postulated to play such a role (hemoglobin-S, thalassemia, glucose-6-phosphate dehydrogenase deficiency), ATP is controlled by multifactorial genetic system (Brewer, 1967a).

A clinical syndrome of malaria is characterized by fever, varying degrees of anaemia, splenic enlargement, intravascular haemolysis and sometimes sudden shock. The various syndromes resulting from the physiological and pathological involvement of certain organs including the brain, liver and kidneys (Adams and Maegraith, 1953). The pathological changes are believed to be due to the tissue anoxia caused by anoxemia, deviation in the blood flow from

the vasomotor changes and/or disseminated intravascular coagulation, alteration in the capillary endothelial cells, histotoxic anoxia and rigidity of red cell. Much recently accumulated evidence suggested that the internal change in red cell *per se* may be responsible for the outset of these processes. These red cells will loose the deformable property and will not be able to pass through the capillaries, and may lead to the obstruction of the flow of blood in vessels of various organs which have been demonstrated in autopsies (Dudgeon and Clarke, 1917; Spitz, 1946).

Erythrocyte deformability may be defined as those geometric and physical characteristics which permit a cell whose greater diameter normally exceed 8 μ to pass through 12 μ in diameter (Weed, 1970). The remarkable deformability of normal mature erythrocyte depends on at least three factors:

1. Maintenance of the biconcave shape, which in turn depends on a high ratio of surface area to volume.
2. Normal internal fluidity of the cell, which depends primarily on the properties of normal haemoglobin.

3. Intrinsic membrane deformability which is significantly affected by the relationship between intracellular ATP, calcium and magnesium levels (Weed et al., 1969; Weed, 1970).

The red cell alteration that may occur as a consequence of ATP depletion are the sphere transformation, loss of membrane lipid (Haradin et al., 1967), decreased membrane deformability, and increased viscosity of washed red cell suspension. Changes of the erythrocyte properties consequence to ATP depletion are:

Disc-sphere transformation (10-24 hr)

K^+ loss, Na^+ gain (10 hr ⟶)

Swelling (10-24 hr)

Shrinking (24 hr ⟶)

Loss of membrane lipid (24 hr ⟶)

Decreased membrane deformability (4 hr ⟶)

Decreased filterability (5 hr ⟶)

Ca^{2+} accumulation (10 hr ⟶)

Increased viscosity of washed red cell suspension (10 hr ⟶)

Increased membrane Hb and non-Hb protein (24 hr)

It is interesting to note that these alterations in red cells with ATP depletion are very similar to those in malarial infection as shown in Table 1.

A hypothesis has been put forward that the rigidity of red cells in malarial infection which obstruct the blood vessels of various organs might be due to the depletion of ATP levels in these erythrocytes (Areekul, 1973).

Another hypothesis has been put forward that the level of ATP in the erythrocytes of the host may influence the rate of increase of parasitaemia and hence the clinical severity of the infection (Brewer and Powell, 1965; Eaton and Brewer, 1969). In order to test these hypothesis, the present work was undertaken to investigate the ATP content in erythrocytes of patients with P.falciparum malaria, rhesus monkeys infected with P. knowlesi and mice infected with P. berghei.

Table 1 Comparison of the similarities in the red cell alterations in malaria and in consequence to ATP depletion*.

	Malaria	ATP depletion
Deformability of red cell		
- Viscosity	Increased	Increased
- Filterability	Decreased	Decreased
Electrolytes		
- Potassium	Decreased	Decreased
- Sodium	Increased	Increased
ATP level	Increased or Decreased	Decreased
Membrane lipids	Increased	Decreased
Osmotic fragility	Increased or Decreased	Not done
Disc-sphere transformation	Positive or Negative	Positive

*From Areekul (1973).