CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

Apoptosis

Apoptosis or programmed cell death plays a physiological process that contributes to the homeostasis of multi-cellular organisms balanced in a physiological context. Apoptosis is innate mechanism, energy dependent at respond to changes of environment both inside and outside cell. Although the signals capable of inducing apoptosis are very different such as cell membrane blebbing, cell shrinkage, nuclear condensation and formation of apoptotic bodies [25].

Apoptosis is multistage process that the activation of processes are activation networks and the last process of apoptosis include packaging of content into apoptosis bodies and phagocytosis [25, 27]. There are two overlapping signaling pathways leading to apoptosis, termed the intrinsic and extrinsic pathways [27].

1. Extrinsic pathways

The extrinsic pathway is initiated through the stimulation of the transmembrane death receptors, such as the Fas receptors, located on the cell membrane begins outside the cell through the activation of specific pro-apoptotic receptors on the cell surface. These are activated by specific molecules known as pro-apoptotic ligands (Figure 1). These ligands include Apo2L/TRAIL and CD95L/FasL and bind their cognate receptors DR4/DR5 and CD95/Fas, respectively [2, 17, 20, 41].

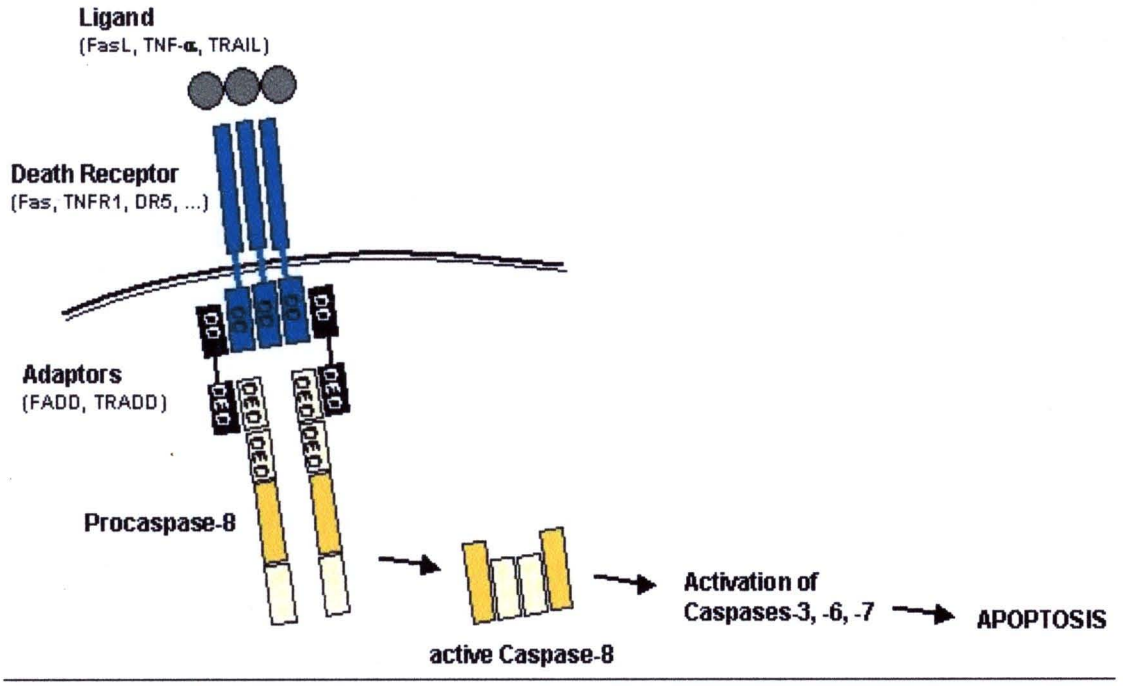


Figure 1 Receptor-mediated caspase activation at the DISC [19]

Binding of ligand induces receptor clustering and recruitment of the adaptor protein Fas-associated death domain (FADD) and the initiator caspases 8 or 10 as procaspases, forming a death-inducing signaling complex (DISC) [8, 14, 28, 47]. DISC formation brings procaspase molecules into close proximity of one another, facilitating their autocatalytic processing and release into the cytoplasm where they activate effector caspases 3, 6, and/or 7, thereby converging on the intrinsic pathway (Figure 2) [2, 5, 21, 35]. Dimerization may be crucial for caspase 8 activation, and clustering of the receptors and the associated DISC may enhance this activation [5]. DISC formation is modulated by several inhibitory mechanisms, including c-FLICE inhibitory protein (c-FLIP), which exerts its effects on the DISC by interacting with FADD to block initiator caspase activation; and decoy receptors, which can block ligand binding or directly abrogate pro-apoptotic receptor stimulation [3]. Upon DISC activation, the extrinsic pathway adopts the same effector caspase machinery as the intrinsic pathway.

It has been shown that activation of the extrinsic pathway through the binding of CD95L/FasL to CD95/Fas can result in 2 apoptotic programs, termed type I and type II. Type I cells are able to overcome the need for mitochondrial amplification of the death signal in CD95-mediated apoptosis by producing sufficient amounts of caspase 8 at the DISC to directly cleave and activate effector caspases and execute cell death [15]. Because type I cells bypass mitochondrial involvement in CD95-mediated apoptosis, expression of Bcl-2 or Bcl-X_L has no inhibitory effect on their apoptotic program. Conversely, type II cells produce minimal amounts of active caspase 8 at the DISC and require the mitochondrial amplification of the CD95 signal [6]. This signal is probably through the pro-apoptotic BH3 domain, which only contains the Bcl-2 family member, Bid [6]. The cleavage of Bid by caspase 8 results in its translocation to the mitochondria where it initiates the release of mitochondrial factors, which in turn augment cell death. Because type II cells rely on the apoptotic function of mitochondria, expression of Bcl-2/Bcl-X_L does confer protection from CD95-mediated apoptosis [6]. An explanation for the differences between type I and type II cells remains unclear, although differential expression of inhibitors of the death receptor signaling cascade, such as c-FLIP or X-linked inhibitor of apoptosis protein (XIAP),has been suggested to play a role [46].

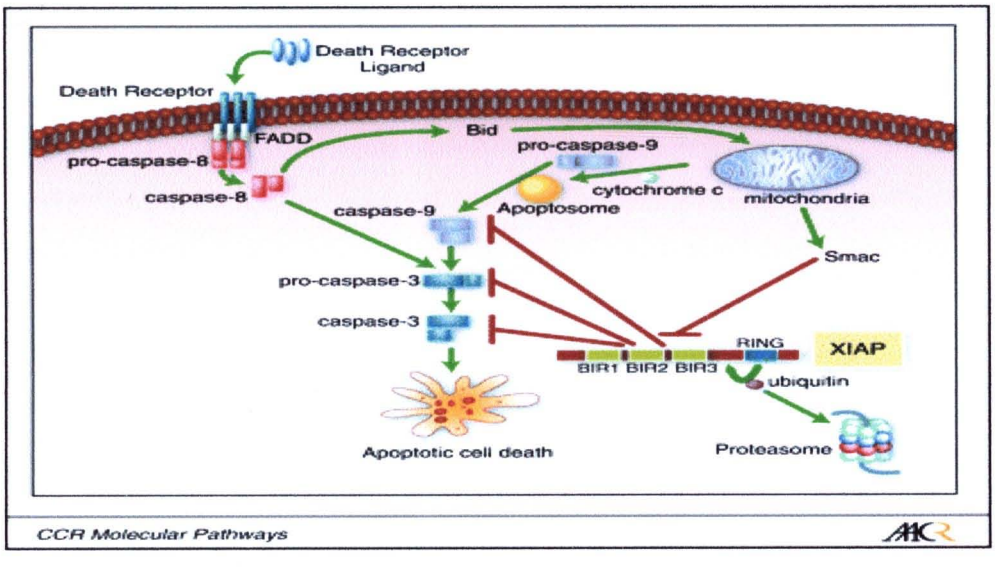


Figure 2 Apoptosis signaling pathway [24]

2. Intrinsic pathway

The intrinsic pathway is triggered by cellular stress. The intrinsic apoptotic process occurs in the mitochondrial caused by factors such as DNA damage oncogene activation and heat shock [1]. Upon receiving the stress signal will cause the proapoptotic proteins of Bcl2 family proteins (BID, BAX and BAK) in the cytoplasm. BAX and BID stimulate the rapture of the mitochondria to signal the release of the internal content. However, the signal of BAX and BID is not enough to trigger a full release of the mitochondria content. BAK, another proapoptotic protein that resides within the mitochondria, is also needed to fully promote the release of cytochrome c and the intramembrane content from the mitochondria [23]. Following the release, cytochrome c forms a complex in the cytoplasm with adenosine triphosphate (ATP), an energy molecule and apoptotic proteaseactivating factor-1 (Apaf-1). This complex activates caspase-9, an initiator protein. On the other hand, the activated caspase-9 works together with the complex of cytochrome c, ATP and Apaf-1 to form an apoptosome, which in turn activates caspase-3, the effector protein that initiates degradation. Apart from the release of cytochrome c from the intramembrane space, the intramembrane content released also contains apoptosis inducing factor (AIF) to facilitate DNA fragmentation, and Smac/Diablo proteins to inhibit the inhibitor of apoptosis (IAP) [23].

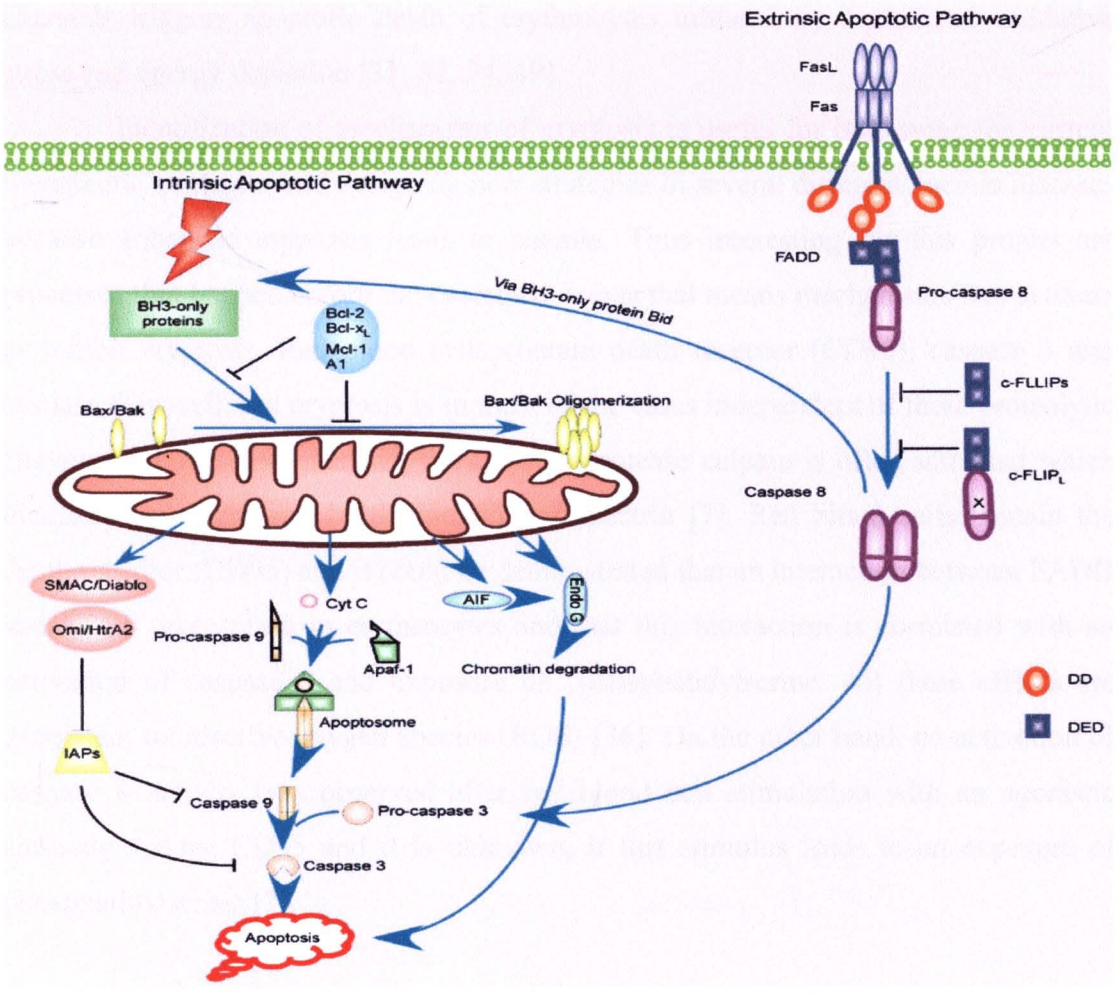


Figure 3 Intrinsic apoptotic pathway [37]

Apoptosis in mature erythrocytes called "eryptosis"

Apoptosis or programmed cell death of erythrocytes called "eryptosis" may similarly contribute to cell death of red blood cells like apoptosis to the death of nucleated cells. Erythrocytes are devoid of mitochondria and nuclei. Nevertheless, they can undergo a form of programmed cell death like apoptosis. This cell death mode is called eryptosis and controls the removal of defective erythrocytes. Removal of erythrocytes is carried out by phagocytosis which is stimulated by exposure of phosphatidylserine at the outer membrane leaflet [7, 11, 36] Programmed erythrocyte death or eryptosis is affected by the phosphotidylserine scrambling by activation of the Ca^{2+} sensitive scramblase [12, 49], and the cell shrinkage mediated by Ca^{2+} sensitive "Gardos" K^{+} channels [34]. In conclusion, the opening of cation

channels triggers apoptotic death of erythrocytes initiated by osmotic or oxidative stress and energy depletion [31, 32, 34, 49].

Identification of mechanisms of eryptosis is useful for improving the current therapeutic strategies and designing new strategies in several different anemia diseases because enhanced eryptosis leads to anemia. Thus interesting for this project are processes that happen before the described events that means mechanisms that activate or inhibit eryptosis. Red blood cells contain death receptor (CD95), caspase 3 and caspase 8 as well, but eryptosis is in most of the cases independent of these proteolytic enzymes (Figure 3, 4). Instead, the cysteine protease calpain is often activated which mediates cell shrinkage by degradation of spectrin [7]. Red blood cells contain the death receptor (CD95) and it could be demonstrated that an interaction between FADD and CD95 takes place in erythrocytes and that this interaction is correlated with an activation of caspase 8 and exposure of phosphatidylserine. All these effects are dependent on reactive oxygen species (ROS) [36]. On the other hand, no activation of caspase 8 activity was observed after red blood cell stimulation with an agonistic antibody against CD95 and it is unknown, if this stimulus leads to an exposure of phosphatidylserine [7].

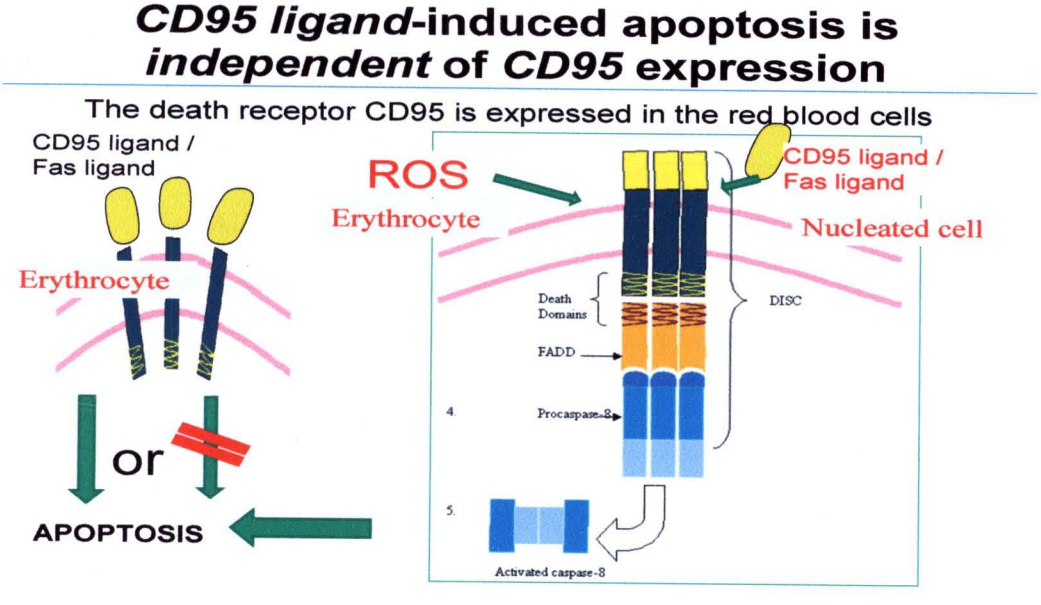


Figure 4 CD 95 ligand-induced apoptosis is independent of CD95 expression

Thalassemias

Thalassemias are a group of inherited diseases of the red blood cells leading to hemolytic anemia characterized by defective hemoglobin synthesis. This even leads to impaired erythropoeisis, hemolysis in the peripheral circulation and deposition of excess iron in the tissues [15, 16].

Genes involved are those encoding the globin cahins building hemoglobin. Normally, hemoglobin production involves two types of genes which produce two different pairs of proteins. One type is alpha, the other is beta. Thalassemia occurs when a gene mutation leads to insufficient levels of the associated globin. There are two types of thalassemia: in alpha thalassemia, the production of the alpha globin chain is affected and in beta thalassemia that of the beta globin chain. There are a number of different forms of alpha and beta thalassemias, with symptoms ranging form mild to severe [15, 42, 43].

1. Alpha (α) thalassemias

Alpha (α) thalassemia is caused by decreased or absent in the alpha chain of the hemoglobin molecule. There are two genes loci, alpha-1(*HB1*) and alpha-2(*HB2*) loci and so four alleles. It is also connected to the deletion of the 16p chromosome. Alpha (α) thalassemia result in decreased alpha-globin production, therefore fewer alpha-globin chains are produced, resulting in an excess of β chains in adults and excess γ chains in newborns.

There are two clinically significant forms of alpha thalassemia:

1. Hb Bart (γ4) hydrops fetalis syndrome

1.1 All four alpha globin genes are deleted (−−/−−), which is so severe that death can occur in prior to birth. Clinical findings include fetal generalized edema, ascites, pleural and pericardial effusions, and severe hypochromic anemia.

2. Hemoglobin H (Hb H) disease

2.1 Three alpha chain genes are deleted (−−/−α), resulting include moderate microcytic hypochromic anemia, hemolysis with Heinz bodies, splenomegaly, rare extramedullary hematopoiesis, and propensity of acute hemolysis after oxidative stress, drug therapy, or infection.

Carrier states for alpha thalassemia include:

1. Alpha thalassemia (trait)

1.1 Loss of function of two alpha globin genes (-α/−α or−−/αα).

1.2 Mild microcytic anemia may be present; normal hemoglobin electrophoresis, often misdiagnosed as iron deficiency.

2. Beta (β) thalassemias

Beta thalassemia is caused by mutations in the beta chain of the hemoglobin molecule. There are due to mutations in the HBB gene on chromosome 11, [48] also inherited in an autosomal-recessive system. The disease severity depends on the gene of the mutation. Mutations are characterized as (β^0 or β thalassemia major) if they prevent any formation of β chains (which is the most severe form of β thalassemia); they are characterized as (β^+ or β thalassemia intermedia) if they allow some β chain formation to occur. In either case there is a relative excess of α chains, but these do not form tetramers: rather, they bind to the red blood cell membranes, producing membrane damage, and at high concentrations they form toxic aggregates.

3. Incombination with other hemoglobinopathies

Thalassemia can co-exist with other hemoglobinopathies. The most common of these are:

3.1 Hemoglobin E/thalassemia: common in Cambodia, Thailand, and parts of India; clinically similar to β thalassemia major or thalassemia intermedia.

3.2 hemoglobin S/thalassemia, common in African and Mediterranean populations; clinically similar to sickle cell anemia, with the additional feature of splenomegaly

3.3 hemoglobinC /thalassemia: common in Mediterranean and African populations, hemoglobin C/β^0 thalassemia causes a moderately severe hemolytic anemia with splenomegaly; hemoglobin C/β^+ thalassemia produces a milder disease.

The pathophysiology of thalassemias relates directly to the variation in hemoglobin levels that dependends on the extent of accumulation of excess unmatched globin chains. In thalassemia, there is an excess production of reactive oxygen intermediates leading to oxidative stress that contributes to the shortened life span of erythrocytes. The treatment of thalassemia is dependent on blood transfusion used to treat severe forms of thalassemia. Repeated blood transfusions lead to an increase of

iron in the body. Iron increases can damage the heart, liver and other organs. To help prevalent organ damage, patients receive an iron chelator that binds to irons and helps the body get rid of excess iron [9, 10, 43, 115].

The accumulation of unpaired alpha globin chains in beta thalassemia is leading to enhanced oxidative stress in the progenitor cells of the erythrocytes and in the red blood cells themselves. The excess presence of the alpha globin chains is a primary reason for the cellular oxidative damage and also iron overload. As a result of both high plasma iron and high intracellular non-hemoglobin iron in beta thalassemia, there is an enhanced generation of ROS. Thus, red blood cells of thalassemic patients exhibit an increased level of oxidative damage and are hypersensitive to the exposure to oxidative stress [16, 22, 44].

The enhanced oxidative stress activates the eryptosis cascade in red blood cells and is followed by an enhanced exposure of phosphatidylserine, which can be avoided by the reduction of oxidative stress in a mouse model [18, 33]. Enhanced exposure of phosphatidylserine reduces the life span of the erythrocytes and is correlated with an increased risk for thrombosis [4, 15]. An understanding which ROS-stimulated processes influence eryptosis positively or negatively can therefore also be of clinical interest.

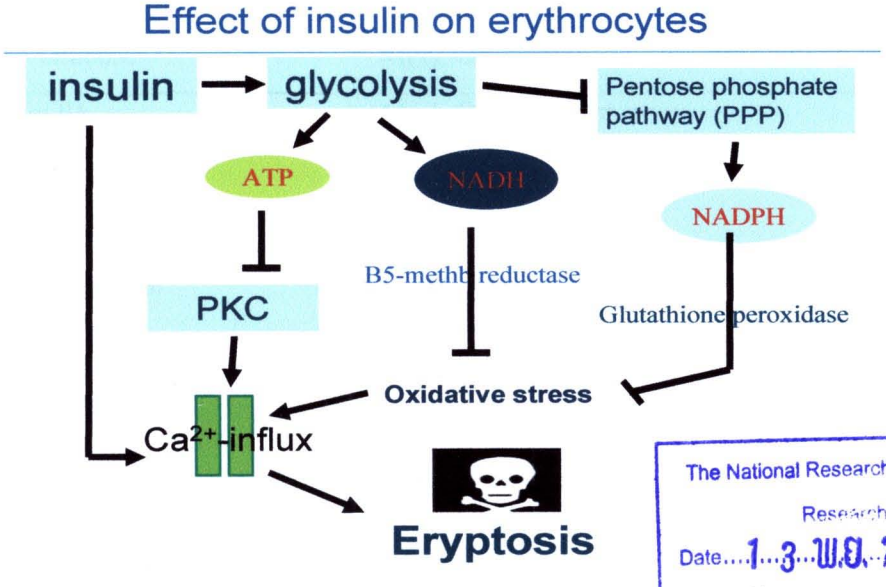
CD95-mediated role for eryptosis

The death receptor CD95 is expressed in the red blood cells but the impact of its activation on the induction of eryptosis has not been analysed. CD95 is activated by the binding of an appropriate extracellular ligand (CD95L), but can also happen independently of a ligand. CD95 activation happens via trimerization of the receptor which leads to the binding of intracellular signaling molecules such as the FADD. This binding interaction is mediated by the death domains. This complex allows the binding of caspase 8. This so-called DISC complex (death inducing signaling complex) activates caspase 8 and leads to binding of large receptor aggregates [17, 40].

THE NATIONAL RESEARCH COUNCIL OF THAILAND
RESEARCH LIBRARY

Influence of insulin on eryptosis

Insulin has well-known activities in controlling energy metabolism. Recently, several studies have suggested that insulin may protect cell from apoptosis by decreasing oxidative stress in different cell lines [26]. Human erythrocytes express a receptor for insulin respond to insulin by increasing phosphorylation of tyrosine residues in several proteins. These effects occur through activation of phosphofructokinas (PFK), the key regulator glycolytic enzyme of the glycolytic pathway [50]. Indeed, insulin stimulates glycolysis in red blood cells [50]. Enhanced glycolysis leads to an increased turnover rate of NAD/ NADH and to an increased level of ATP (Figture 5). Thus, we postulate that insulin reduces eryptosis by enhanced production of ATP which is an inhibitor of the proapoptotic protein kinase C (PKC). Furthermore, NADH generated by glycolysis can serve as an essential cofactor to reduce methemoglobin and thus reducing oxidative strees [38]. On the other hand, activation of glycolysis by insulin will reduce the amount of glucose metabolized by the pentosephosphatepathway and reduce the amount of NADP/NADPH turnover. Since NADPH is an important factor to reduce oxidative stress by maintaining the reduced glutathione pool in the cell [20], insulin might also serve as a proapoptotic factor.



The National Research Council of Thailand
Research Library
Date 13 W.C. 2555
Record No. E47352
Call No.

Figure 5 Effects of insulin on erythrocytes