The other vitamins[Thiamin B_1,Niacin B_5 and Pyridoxine B_6] have the same effect on erythrocytes at different concentrations .The obtained data found that increasing concentration for all vitamins leads to the acceleration of the oxidation process (hemolysis) until it reaches the higher concentration ,at this value the oxidation process takes short time to be completed.

The effect of different vitamins [C, B_6, B_5, B_1] on erythrocytes suspension at concentration 2mM only are used for comparison between the used vitamins. It was found that vitamin[C] is the strongest hemolytic agent in comparison with the other vitamins, in which the hemolysis is fulfilled at about 20 mins. from adding the used concentration (2mM),while vitamin B_1 is the weakest one.

The variations in electrical conductivity of erythrocytes suspension with temperature was also studied for the control sample and hemolysated erythrocytes due to the effect of different vitamins (C, B_6, B_5 and B_1) at concentration 2mM.

From results it is clear that the conductivity of the treated samples by vitamins is lower than that for control .Also for all curves the conductivity increases by increasing temperature and the conductivity of samples treated with vitamin C is the higher one in comparison with other vitamins.

The dielectric measurements showed an accurate method for studying the structure and function states of erythrocytes suspensions with vitamins. The permittivity ($\acute{\varepsilon}$), the dielectric loss (ε''), conductivity (σ), relaxation time τ and dipole moment were found to change with different vitamins.

The relation between the permittivity ($\acute{\varepsilon}$) and logarithmic values of frequency, in range from 50 Hz to 100 KHz for suspensions before and after adding the used vitamins [C,B_6,B_5,B_1] at concentration 2mM was done and from which it is clear the value of the permittivity ($\acute{\varepsilon}$) at low frequency for samples after incubation is less than that for the control value.

Also, the relation between dielectric loss(ε'') and logarithmic frequency; in the range from 50 Hz to 100 KHz was studied for suspensions before and after the addition the used vitamins [C, B_6, B_5, B_1] at concentration 2mM. From figure it is clear that the higher value is that control over all other vitamins after incubation.

Also, the position of relaxation frequency was shifted f_s towards higher frequencies for treated samples together with a decrease in the peak height.

A set of semicircles were drawn to demonstrate the relation between ($\acute{\varepsilon}$) and (ε'') of suspension ,in the frequency range from 50 Hz to 100 KHz for suspensions before and after the addition the used vitamins [C,B_6,B_5,B_1] at concentration 2Mm. At which the higher semicircle is that representing control.

The relation between the conductivity of the suspension (σ) and the logarithmic values of frequency was also studied at the range from 50 Hz to 100 KHz for suspensions before and after the addition the used vitamins [C,B_6,B_5,B_1] at a concentration of 2 mM. The values of conductivity for control are higher than that for all vitamins after incubation.

The obtained results in the present work using the different biophysical techniques were very useful in estimating the blood hemolysis also to limit the amount of vitamins in blood stream.

Chapter One

BASIC CONSIDERATIONS AND REVIEW OF LITERATURE

misconception. Recent studies have shown that certain vitamins can cause toxicity when ingested in large doses [4].They have side effects as they play as oxidants for erythrocytes' hemoglobin which appears to be the main site of damage when various oxidative drugs are used[2].

1.2 Blood:

Blood is the main transport system of the body, and by means of it ,nourishment arrives at the cells. It is continuously circulating in blood vessels through out the cardio vascular system by the pumping action of the heart .It acts as a link between the various tissues and organs of the body.

The blood has general properties ;its volume is about 5 liters in average adult male (70 Kg),viscosity is about 4 times as that of water, its pH is strictly maintained at 7.4 and its osmotic pressure, which is equal to 25 mmHg, is very important in regulation of fluid exchange between blood and tissues fluids as there are three cases:

a- Fluids having osmotic pressure which is equal to that of plasma are called ***isotonic*** (ex. Nacl 0.9% and glucose 5%), these fluids can be added to the blood safely.

b- Fluids having osmotic pressure which is higher than plasma is called ***hypertonic***, if this fluid is added to the blood ,it will absorb water from the red blood cells leading to their shrinkage.

c- Fluids having osmotic pressure which is lower than plasma is called ***hypotonic***, if a hypotonic fluid is added to blood the water in fluid will be absorbed by the RBCs ,so they swell and may rupture.

Blood is the fluid associated in many functions for the living body which is represented in some processes: The respiratory movements of the lung when the oxygen from air is absorbed by the blood and is carried from the lung to different tissues. Blood also carries CO_2 from different tissues to the lung. Blood is the fluid of growth ,transporting nourishment from digestion and hormones from glands through the body. Also blood used as the control system for the functions of the organs of the body, when it transport hormones for sending chemical messages as occur in hemostasis and homeostasis.

Blood contains living cells, red blood cells and white blood cells which are responsible for nourishing and cleaning the body. These cells also need nourishment. Vitamins keep the blood cells healthy. Vitamins are used as antioxidants for blood cells but at certain conditions as high concentrations they have side effects as they play as oxidants. This oxidant load has been found to correlate with clinical severity of the disease and entity of the airway obstructions[5,6].

The properties and function of blood depend on its composition. When a specimen of blood, in which coagulation has been prevented, is centrifuged it separates into two main layers: a clear, normally straw colored upper layer of plasma (approximately 55% of the volume), and a lower layer of packed erythrocytes with a small amount of trapped plasma between them. Between these two layers is a narrow band (puffy coat) comprising about 1% of the volume and consisting of an upper creamy -white layer of platelets and a lower grey - white layer of white cells or leukocytes.

1.2.1 Erythrocytes

Erythrocytes (red blood cells) are highly specialized cells as shown in fig (1.1.a). Human and mammalian erythrocytes are devoid of nucleus and have a homogeneous protoplasm. Normal human erythrocytes are circular biconcave disks, the thickness of the surrounding membrane is 70 to 80 A^{o}. They are 7.5 - 8.5 μm in diameter, 2 - 2.5 μm thick at the periphery and 1 - 2 μm thick in center as shown in fig. (1.1.b). The mean surface area is 163 μm^2 and mean corpuscular volume (MCV) is 78 - 97 μm^3.

In healthy adults, there are about 5×10^{12} red cell per liter of circulating blood, the average count in male being $5 \pm 1 \times 10^{12}$, and in female $4.8 \pm 1 \times 10^{12}$ cell per liter; a newborn has up to 6×10^{12} erythrocytes per liter of blood, while an aged individual has less than 4×10^{12} erythrocytes per liter of blood.

The number of erythrocytes is altered by the external and internal factors, such as diurnal or seasonal changes, physical work, emotional stress, high altitudes, fluid loss, etc. An increase in the number of erythrocytes in the blood is called erythrocytosis, while its reduction is termed erythropenia.

The red blood cell shape may be altered by varying different chemical and physical conditions which affect the properties of the membrane and the volume of the cell[7,8,9]. The slight variation in red blood cell size is just appreciable on examination of a normal blood film. A more marked variation is called anisocytosis; although this may occur alone, it is often accompanied by changes in shape. In megaloblastic anemia anisocytosis is often marked and is associated with cells of a pear shape (poikilocytes). In severe anemia of many types - for instance in cases of diffuse marrow fibrosis or infiltration - anisocytosis is usually very striking and there may be elliptical, triangular, or bizarre - shaped red cells. Remarkable abnormalities of shape are often seen in patients with severe uraemia, carcinomatosis, and some types of chemical poisoning . That red cell changes are frequent in uraemia and carcinomatosis has just been mention. One abnormality quite common in these cases is the "burr cell" which is characterized by the presence of several short spiky projections from its surface; it must be distinguished from crenation due to artifact.

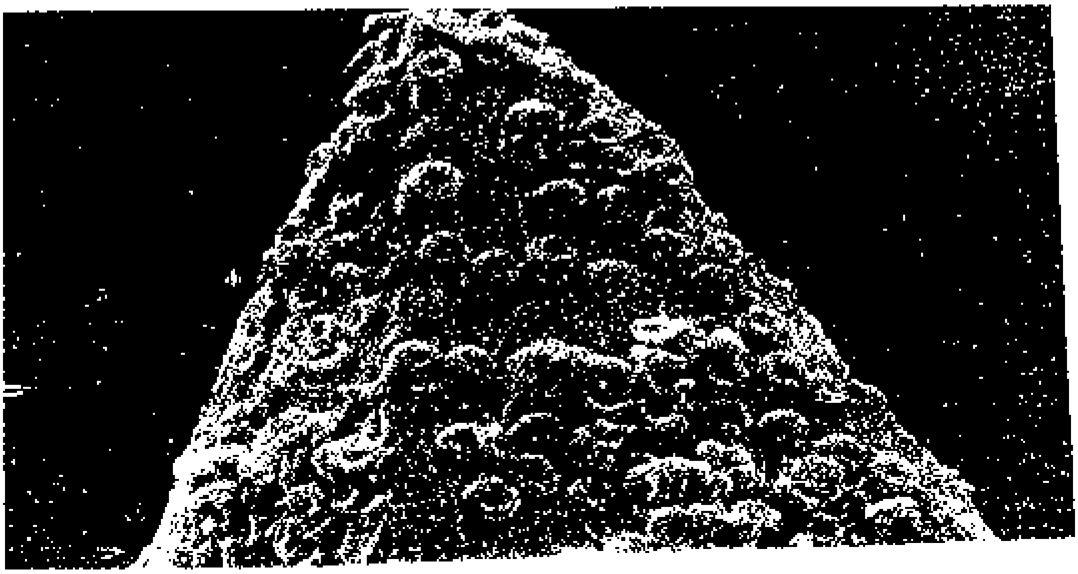

Fig. (1.1.a): A scanning electron micrograph of red blood cells clinging to a hypodermic needle.

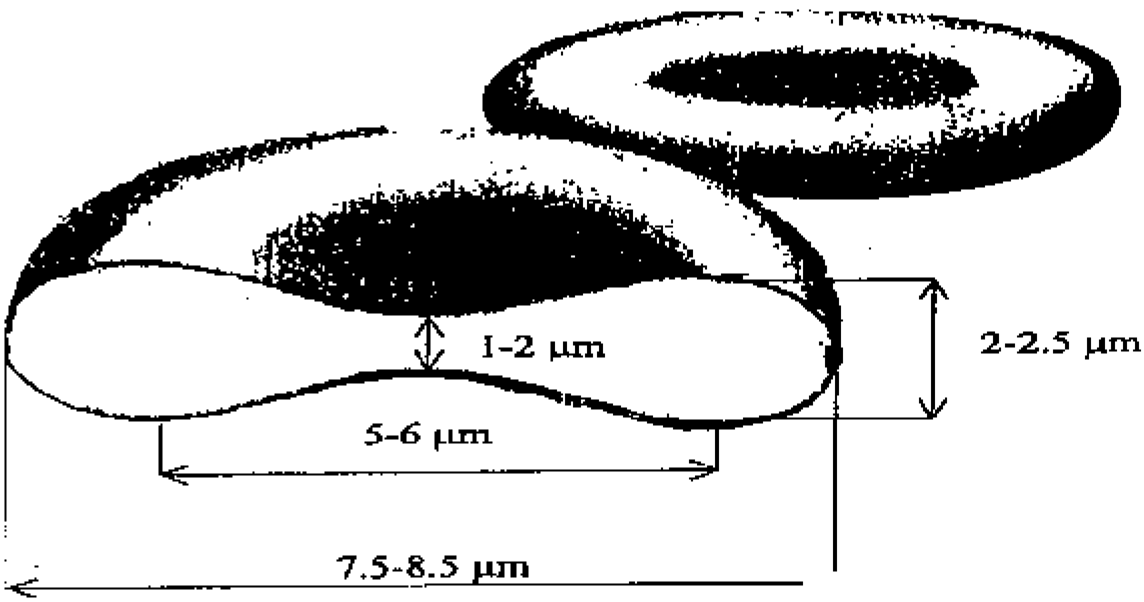

Fig. (1.1.b): Illustration for erythrocyte dimensions.

A rare disorder, several examples of which have been described in the children of consanguineous marriages, is due to the absence of betalipoprotein. One feature of the condition is a red cell abnormality known as acanthrocytosis; over 50 % of the cells may be affected, they stain like spherocytes and have sticking projections like spines. Other features of the condition are steatorrhoea, retarded growth, retinal and neurological degeneration, and ataxia [10].

There are several functions for erythrocytes :-

- The respiratory function of erythrocytes is carried out by the respiratory pigment hemoglobin, which is able to combine with oxygen and carbon dioxide.
- The nutritional function of erythrocytes consists in absorbing amino acids onto their surfaces and transporting them from the digestive organs to the cells of the body.
- The defense function of erythrocytes is the ability of binding toxins (poisonous harmful substances) by the erythrocyte – borne protein substances, antibodies. In addition, erythrocytes are actively involved in coagulation.
- The enzymatic function of erythrocytes consists in their transport of enzymes. They carry the following enzymes: true cholinesterase, enzyme which breaks down acetylcholine; carbonic anhydrate which, depending on the conditions, catalyzes either the formation or breakdown of carbonic acid in the capillary blood of the systemic and pulmonary circulation; methemoglobin reductase, an enzyme which maintains hemoglobin in a reduced state.
- The regulation of blood pH is performed by the erythrocytes using hemoglobin; hemoglobin is a powerful buffer, accounting for 70 to 75% of buffer capacity of the blood. The buffer characteristics of hemoglobin are determined by the fact that hemoglobin and its derivatives have the properties of weak acids and bases[11].

1.2.2 Hemoglobin

Hemoglobin molecule as shown in fig (1.2) consists of four poly peptide chains,two identical alpha chains,each with 141 amino acids, and two beta chains,each with 146 amino acids.The protein portion of each of these chains is called "globin tetramer"and non protein portion called hememoietry.

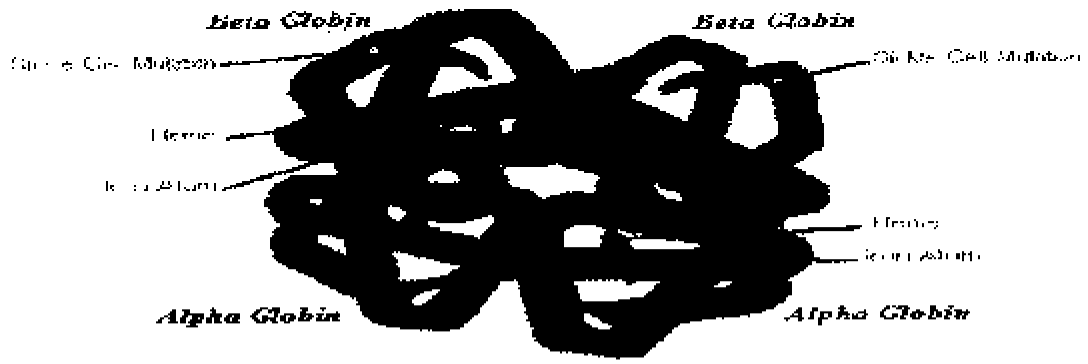

Fig. (1.2): Hemoglobin molecule

Hemoglobin represents a complex chemical compound composed of 600 amino acids and has molecular weight of 66000 ± 2000, it consists of a protein (globin) and an iron containing porphyrin ring (hem) as shown in fig (1.3).

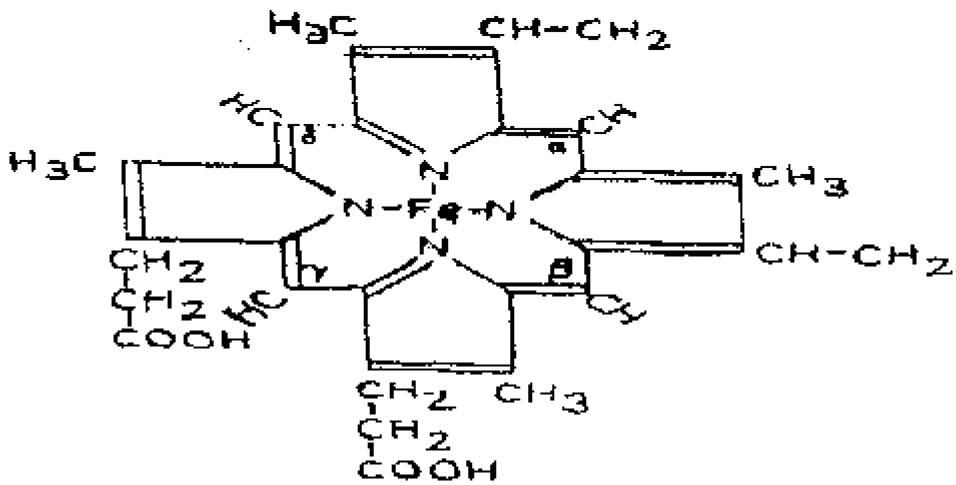

Fig. (1.3): The structure of hem molecule.

replaced by Hb A, this replacement is completely by the age of 6^{th} month.

3- **Hb A** (*adult* Hb):which includes 2 subtypes:

- **Hb A_1:** It represents about 97 % of the adult Hb.It is formed of 2 alpha and 2 beta chains.
- **Hb.A_2**: It represents about 2% of the adult Hb and it is formed of 2 alpha and 2 delta chains.

b-Abnormal types of Hb:

The abnormality may be in quantity or in quality of Hb:

1- *Quantitative abnormality* :due to presence of higher concentration of one of the above types of normal Hb, but in an abnormal stage of life ,e.g *thalassemia*(persistence of fetal Hb in the adulthood).

2- *Qualitative abnormality*: due to presence of abnormal type of Hb which is not present normally at any stage of life. This may due to abnormalities in the alpha chains,e.g.*Hb H* or abnormalities in the beta chains,e.g. *Hb S*

Reaction forms of Hb:

a- Normal reactions :

1- Oxyhemoglobin (oxygenated Hb or Hbo_2): Molecular oxygen is carried on Hb by binding loosely with the ferrous iron atom of the heme molecule, so each Hb molecule can carry either 1,2,3 or 4 molecules of oxygen according to O_2 saturation of its subunits.

2- Reduced hemoglobin (Hb): Which does not carry O_2.

b- Certain other forms can be produced if Hb is acted upon by other oxidants e.g. sulphemoglobin, hemichrome and methemoglobin.

Sulphemoglobin (S-Hb) :

S-Hb contains one more sulphur atom than normal Hb and appears to be a product of the reaction of a Hb-hydrogen peroxide complex with hydrogen sulphide. The iron is in the ferrous state but will not carry or deliver oxygen although it still binds carbon ,monoxide (CO) to form carboxy sulphemoglobin[13].

S-Hb, which is an inert non-toxic substance, does not alter the red cell life span, and remains in cells until they are removed from the circulation. S-Hb only appears in the plasma after lysis of red blood cells containing the pigment. There are no genetic disorders, which result in sulphemoglobinaemia but produced by oxidative stress or certain drugs, such as the sulphonamides[14].

Carboxyhemoglobin (Hb CO) :

Hb CO is formed from Hb in the presence of CO. The affinity of Hb for CO is over 200 times its affinity for O_2. Because of this, only small quantities of CO in the inspired air can result in the formation of large amounts of **Hb CO** and hence greatly reduce the oxygen-carrying capacity of the blood[15].

Methemoglobin (met-Hb) :

In the circulation, Hb normally takes the form of oxyhemoglobin (oxy_Hb), or deoxy hemoglobin (deoxy Hb).The reaction of hem ring with oxygen is shown in fig. (1-4)

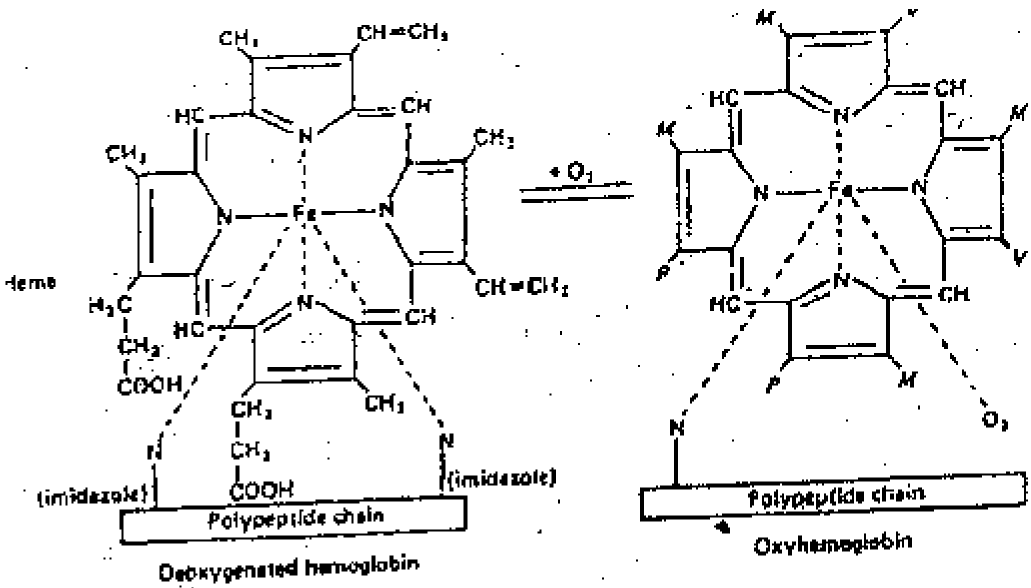

Fig (1.4): The reaction of hem ring with oxygen..

Some pathological - conditions are associated with the formation of methemoglobin, a stable combination of hemoglobin with oxygen. In this compound oxygen binds to the iron of hem, oxidizes it and makes it trivalent (Fe^{+3}), forming methemoglobin.

Methemoglobin is an oxidized Hb and incapable of carrying oxygen, it is an inert non-toxic substance and small amount of it normally is formed continuously in red cells during glycolysis but it is enzymatically reduced back to Hb. The ferric iron is continuously reduced back to ferrous state to restore normal Hb and NADH-linked methemoglobin reductase-I accounts for over 60 % of this reconversion. Methemoglobin reductase and NADH-Methemoglobin reductase-II are minor pathways, as are the non-enzymatic activities of ascorbic acid and reduced glutathione[14].Met Hb remains in erythrocytes during their lifetime, unless hemolysis releases the pigment into plasma[16].

A normal physiological function of Hb is reversible binding of dioxygen molecule (O_2), which can only occur with the iron of heme in the reduced (Fe^{+2}) state. In normal erythrocytes, Hb undergoes autoxidation to met-Hb, which contains iron in the oxidized (Fe^{+3}) state, and does not function in the body as an oxygen carrier. As much as 0.5 percent of total Hb Fe^{+2} of a red cell is converted to met-Hb Fe^{+3} each day by autoxidation[17].

The oxidation of Hb to met-Hb results in the formation of the superoxide radical (O_2^-) by the transfer of single electron. The 3 enzyme superoxide dismutase (SOD), present in erythrocytes, catalyses the conversion of the O_2^- to hydrogen peroxide (H_2O_2) and O_2[18].

$$2\,O_2 + 2\,H^+ \longrightarrow H_2O_2 + O_2 \qquad (1)$$

The H_2O_2 is then decomposed gluthione peroxidase system or by catalase which are also present in RBC's. These two systems thus protect the RBC's from any damaging effects of an accumulation of O_2 or H_2O_2.

$$H_2O_2 \xrightarrow{\text{Catalse}} H_2O + 1/2\,O_2 \qquad (2)$$

The accumulation of large amount of methemoglobin in the blood retards oxygen transport to tissue which is fatal.

The interaction of hemoglobin with the erythrocyte membrane

The interaction of hemoglobin with the erythrocyte membrane has recently received considerable attention. Two classes of binding sites have been, identified, and one of these, the higher affinity class, has been located on the cytoplasmic domain of the major erythrocyte protein, band 3[19,20,21& 22].

A structural model of the cytoplasmic domain of band 3 based on morphological considerations and also on observations described by Philip S. Low 1986 is shown in fig (1.5).

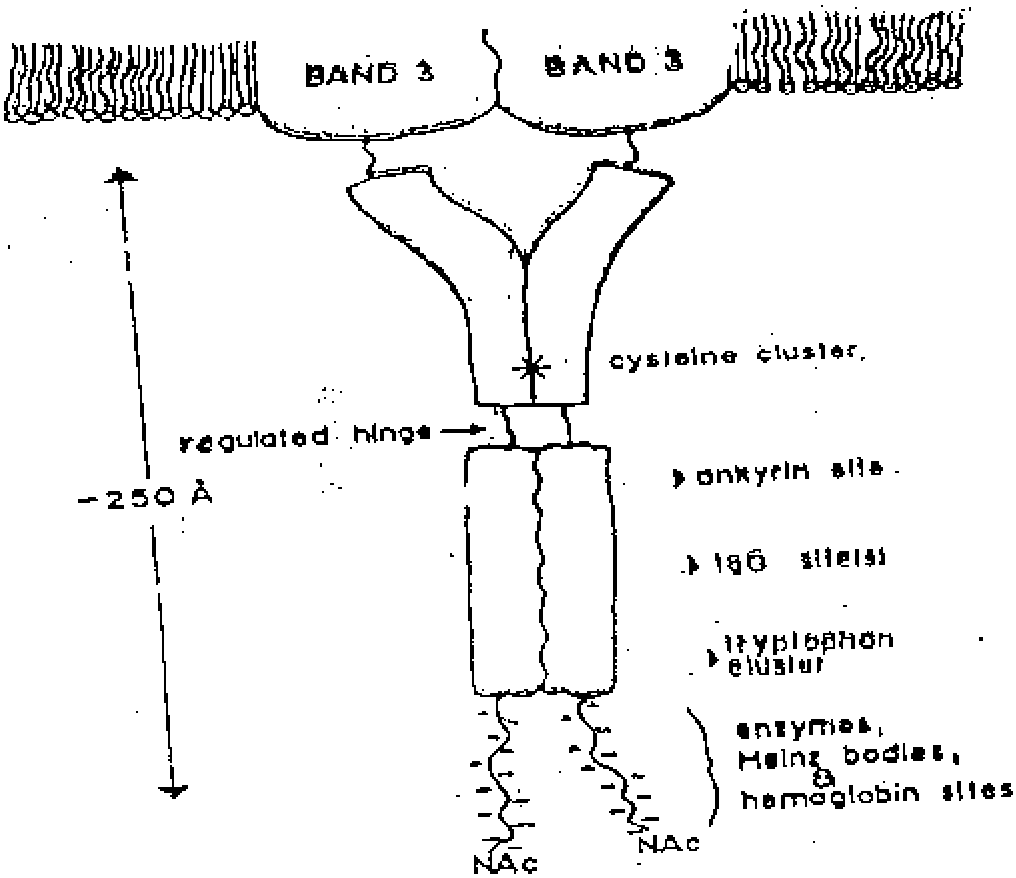

Fig. (1.5): Sketch of the major structural features of the cytoplasmic domain of band 3.

This interaction has been shown to be electrostatic in nature with the affinity of hemoglobin for the membrane increasing as pH and ionic strength decrease [19,20,23 and 24]. A similar pH dependence of hemoglobin binding to the membrane has also been reproted for intact cells[25].

The, isolated cytoplasmic domain of band 3 has also been shown to bind two molecules of hemoglobin, supporting the identification of band 3 as a maior membrane attachment site for soluble hemoglobin[26].

Denatured hemoglobins also bind tightly to the erythrocyte membrane, often forming dens aggregates on the membrane surface termed

Heinz bodies. These patches of denatured hemoglobin are frequently observed in aged cells[27], and in cells containing unstable hemoglobins[28,29]. The Heinz bodies are believed to be responsible for the hemolysis which commonly accompanies the anemia.

1.2.3 Hemolysis of erythrocytes:

Hemolysis is defined as erythrocyte destruction with the release of hemoglobin into the blood plasma. Hemolysis can occur both in the vessels (in vivo) and outside the body (in vitro).

Hemolysis outside the body can be induced by hypotonic solutions. This type of hemolysis is called. Osmotic Hemolysis. A strong shaking or mixing of blood in an ampule results in the rupture of erythrocyte membranes; this is called mechanical hemolysis. Some chemical or physical agents can destroy proteins, oxidize hemoglobin and damage erythrocyte membrane; this is oxidative hemolysis.

Injury to erythrocyte membranes with hemoglobin release may also be caused by some physical factors; for example, high temperatures cause the destructions of proteins, while freezing of blood is destructive to erythrocytes.Some hemolysis continually occurs in the body as aging erythrocytes become worn-out. This normally occurs only in the liver, spleen, bone marrow. Since hemoglobin is ingested by the cells of these organs, it is absent in the plasma of the circulating blood.In certain conditions and diseases, hemolysis is accompanied by the appearance of free hemoglobin in the plasma of the Circulating blood (hemoglobinemia) and urine (hemoglobinuria). In particular, this occurs in snake or scorpion bites, multiple bee stings, malaria, and group – mismatched blood transfusions.