ERYTHROCYTE DEFORMABILITY AS A BIOCHEMICAL INDICATOR OF ZINC STATUS

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ABSTRACT

It was hypothesized that zinc deficiency exerts an effect on RBC deformability through its influence on the plasma membrane and that this would form the basis for a biochemical indicator of zinc status. Weanling, male Sprague-Dawley rats were fed *ad libitum* modified AIN-93G diets containing 3 mg zinc/kg diet (-Zn; n=10) for six weeks. Control rats were pair-fed (+ZnPF; n=10) or fed *ad libitum* (+ZnAL; n=9) diets with 50 mg zinc/kg diet. The mean (±SEM) weight gain (g) for the three groups was -Zn=161.6 ± 8.7, +ZnPF=203.0 ± 12.8 and +ZnAL=346.5 ± 11.5. Zinc deficiency and depressed food intake significantly decreased plasma and tibia zinc concentration ($P<0.05$). Experimental treatments had small but statistically significant effects on some parts of the hematological profile. RBC deformability was measured on whole blood as a function of shear stress in the ektacytometer. Elongation index (EI), the ratio of length to width of the diffraction pattern of deforming cells, was measured. As analyzed by one-factor ANOVA, maximum elongation index (EImax), a measure of average deformability of the cell population, was not altered by zinc deficiency. The mean (±SEM) values of EImax for the three groups were -Zn=0.55 ± 0.01, +ZnPF=0.56 ± 0.01 and +ZnAL=0.56±0.01. EI was plotted against shear stress and the initial slope of the curve, a measure of membrane deformability, was not changed by zinc deficiency. The mean (±SEM) values of slope for the three groups were -Zn=0.023± 0.002, +ZnPF =0.021 ± 0.001 and +ZnAL=0.051± 0.027. No effect of zinc deficiency was found for deformability of RBC suspensions heated at 48°C for 6 minutes. The results of this study suggest that RBC deformability can not be used as a functional indicator of zinc status.

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1. INTRODUCTION

1.1 Rationale

The detection of marginal zinc deficiency has been difficult due to a lack of a specific and sensitive biochemical indicator of zinc status (Lee & Nieman 1993). There are two types of biochemical indicators for nutrients: static indicators, which are direct measurements of a nutrient or its metabolite in blood, urine or body tissue and functional indicators, which are indirect measurements of the consequence of a nutrient deficiency (Lee & Nieman, 1993). It would be beneficial to develop an indicator of zinc status as zinc deficiency does occur in the human population (Prasad, 1991). In the study to be described, it was proposed that erythrocyte deformability would be a sensitive functional indicator of zinc status.

Erythrocyte deformability is defined as the ability of erythrocytes to undergo marked changes in shape. Erythrocytes must deform to pass through the microcirculation for optimal delivery of oxygen as they have a larger diameter than that of the capillaries (Mohandas & Chasis, 1993). There are three main determinants of erythrocyte deformability: internal viscosity, surface area to volume ratio and membrane viscoelastic properties (Johnson, 1994). Bettger and O'Dell (1993) have proposed a physiological role for zinc in the plasma membrane of mammalian cells. Abnormalities reported in the erythrocyte membrane in zinc deficiency include increases in fluidity of

the lipid bilayer (Jay et al., 1987), mobility of spin probes in protein and sialic acid residues on the cell surface (Jay et al., 1987) and dephosphorylation of the membrane proteins spectrin and actin (Paterson et al., 1987). These changes could alter erythrocyte deformability by influencing the viscoelastic properties of the membrane and therefore membrane flexibility.

1.2 Hypothesis and Objectives

The hypothesis of this study was that zinc deficiency would affect erythrocyte deformability by altering plasma membrane function and that this would form the basis for a functional indicator of zinc status. Although previous studies have demonstrated minimal effects of zinc status on red blood cell deformability (Paterson & Card 1993), the techniques used have been limited in sensitivity. The objective of the current study was to investigate the influence of marginal zinc deficiency on erythrocyte deformability measured as a function of increasing shear stress in the ektacytometer. It was anticipated that this methodology would be more sensitive to changes in membrane flexibility than techniques previously employed.

2. LITERATURE REVIEW

2.1 Zinc Metabolism

Zinc was established as an essential nutrient in 1934 (Groff et al., 1995). It is considered a trace element as the average adult body contains only 1.4 - 2.5 g of zinc (Linder, 1991). The recommended nutrient intake (RNI) of zinc for adult males is 12 mg and 9 mg for adult females (Health and Welfare Canada, 1990). It is assumed for this calculation that 35 % of zinc is absorbed but absorption may vary from 20 - 50 % (Health and Welfare Canada, 1990). The proportion of zinc absorbed is increased by zinc deficiency and by citrate (Linder, 1991). Phytate and fibre both lower the availability of zinc (Linder, 1991). Zinc absorption is influenced by mineral-mineral interactions (Groff et al., 1995). For example, copper and zinc compete for absorption and large zinc supplementation may result in copper deficiency (Linder, 1991). Muscle/organ meats provide the most zinc in the diet followed by eggs, whole grains, seeds, nuts and root and leafy vegetables (Linder, 1991). Available zinc also is derived from pancreatic proteolytic enzymes in the digestive tract (Linder, 1991).

The proximal small intestine is the site of most zinc absorption (Groff et al., 1995). Zinc is absorbed by passive carrier-mediated diffusion into mucosal cells (Linder, 1991). Absorption across the basolateral membrane is completed by active transport (Linder, 1991). Excess zinc induces the production of metallothionein, a metal binding

protein in the intestinal epithelium. Metallothionein is thought to trap zinc and prevent it from being absorbed (Linder, 1991). This zinc is lost when the mucosal cell is sloughed off (Linder, 1991).

Zinc is transported in the blood mainly bound to albumin although some is transported by α-2 macroglobulin (Linder, 1991). Histidine and cysteine also loosely bind a small percentage of the zinc in blood (Groff et al., 1995). Zinc is taken up by tissues as needed and is not stored (Groff et al., 1995). It has been proposed that zinc is taken up by tissues via multiple passive transport systems but the mechanism of zinc uptake remains unknown (Groff et al., 1995).

Zinc is excreted from the body mainly through the gastrointestinal tract (Linder, 1991). This includes unabsorbed dietary zinc and zinc from sloughed off mucosal cells (Linder, 1991). Some zinc is lost in urine, sweat, hair, skin, menstruation and semen (Linder, 1991).

There are several functions of zinc. Zinc is a component of many metalloenzymes such as carbonic anhydrase, alkaline phosphatase and alcohol dehydrogenase (Groff et al., 1995). The role of zinc is to provide structural integrity to the enzyme or to be involved at the catalytic site (Groff et al., 1995). Zinc also functions in gene expression, cell replication, membrane and cytoskeletal stabilization and as a component in some hormones (Groff et al., 1995).

2.2 Assessment of Zinc Status

Zinc deficiency does occur in the human population although a severe zinc

deficiency is rare. Severe zinc deficiency has been seen in patients receiving total parenteral nutrition without zinc supplementation and in patients with acrodermatitis enteropathica, an inborn error of zinc metabolism in which zinc absorption is impaired (Prasad, 1991). A less severe form of zinc deficiency arises as a result of several diseases such as sickle cell anemia, chronic renal and liver diseases and alcoholism (Prasad, 1991). Marginal zinc deficiency has been reported in infants and children and results in decreased growth, poor appetite and hypogeusia (Smit-Vanderkooy & Gibson, 1987; Walravens et al., 1983). As well, marginal zinc deficiency has been found in older adults, resulting in anergy, hypogeusia and decreased T cell immune function (Prasad, 1988). Detection of zinc deficiency has been difficult due to a lack of a specific and sensitive static or functional indicator of zinc status (Lee & Nieman, 1993). As marginal zinc deficiency is much more prevalent than severe zinc deficiency, it would be desirable to develop an indicator of zinc status that is specific and sensitive to a marginal zinc deficiency. Approximately 30% of the world's children are stunted and that number rises to 80% in some countries (Sandstead, 1994). There is a strong association between low birth weight, stunting and marginal zinc deficiency (Sandstead, 1994). Marginal zinc deficiency in most of these children is likely caused by consumption of a cereal-based diet which is high in phytate and therefore decreases the absorption of zinc (Linder, 1991; Sandstead, 1994).

Static indicators of a nutrient are direct measurements of that nutrient or its metabolite in blood, urine or body tissue (Lee & Nieman, 1993). Though many of these tests are suitable for human studies, they frequently do not reflect the overall nutrient

status of the individual (Lee & Nieman, 1993). The indirect measurement of the consequence of a nutrient deficiency such as the failure of a physiological process that relies on that nutrient is a functional indicator (Gibson, 1990; Lee & Nieman, 1993). These tests are useful because they relate the nutrient to its function in the body but they tend to be nonspecific because they may respond to other factors (Lee & Nieman, 1993).

2.2.1 Static Indicators of Zinc Status

2.2.1.1 Plasma or Serum Zinc Concentration

Plasma or serum zinc concentration is the most commonly used index of zinc status (Gibson, 1990). These indicators are decreased in experimentally controlled zinc deficiency studies (Baer & King, 1984; Ruz et al., 1991; Solomons, 1983) but in less controlled conditions, the response is not consistent because of confounding variables (Ruz et al., 1991). Plasma or serum zinc concentration is modified by a variety of stresses that induce hepatic uptake of zinc such as exercise, infection, chronic disease, oral contraceptive use and pregnancy (Aggett, 1991). Plasma or serum zinc concentration is increased during fasting and may be decreased after feeding (Elia et al., 1984; King, 1990). These factors plus others lead to diurnal variation in plasma or serum zinc level (Aggett & Favier, 1991). Plasma or serum zinc is decreased by rapid tissue synthesis because it reflects the primary extracellular source of zinc used for tissue synthesis (Aggett & Favier, 1991). As well, plasma or serum zinc concentration may not be decreased in marginal zinc deficiency because zinc homeostasis is reestablished (Gibson et al, 1989). Therefore, plasma or serum zinc concentration is neither a specific

nor a sensitive indicator of zinc status.

2.2.1.2 Urinary Zinc Concentration

Urinary zinc concentration decreases with zinc deficiency (Baer & King, 1984; Ruz et al., 1991; Solomons, 1983) but this static test is not specific because hyperzincuria occurs in cirrhosis, diabetes mellitus, sickle cell disease, injury, burns, acute starvation and certain renal diseases (Gibson, 1990; Solomons, 1979; Thompson, 1991). As well, it is difficult to obtain a 24-hour urinary collection and to prevent contamination (Solomons, 1979). It is possible to use smaller urine collections if the zinc:creatinine ratio is calculated (Gibson, 1990).

2.2.1.3 Hair Zinc Concentration

Hair zinc concentration is limited in detecting severe zinc deficiency because hair zinc concentration increases with severe zinc deprivation in rats (Aggett & Favier, 1991). This is most likely because of decreased hair synthesis due to zinc deficiency which allows the zinc to accumulate at a normal rate (Aggett & Favier, 1991). Hair zinc concentration may reflect chronic marginal zinc status in children (Gibson et al., 1989; Hambidge et al., 1972) as low hair zinc concentrations have been associated with growth retardation, a clinical feature of marginal zinc status (Aggett, 1991; Gibson, 1990). Gibson et al. (1989) identified a group of boys in Southern Ontario with low height percentiles and low hair zinc concentrations. These boys showed a significant positive growth response to zinc supplementation. Many confounding factors such as

contamination of zinc from the environment and washing procedure, as well as effects of hair treatment, hair colour, hair location on the body, sex and age on hair zinc concentration reduce its reliability as an indicator of zinc status, especially in adults (Klevay et al., 1987). For example, low serum zinc concentration of lacto-ovo-vegetarian female adolescents was not associated with low hair zinc concentration (Donovan & Gibson, 1995).

2.2.1.4 Leukocyte Zinc Concentration

Total leukocyte zinc concentration was examined as an indicator of zinc status because leukocytes are nucleated and would possibly reflect the zinc content of other nucleated tissues (Aggett & Favier, 1991). The leukocyte population is heterogenous and the subsets have different half-lives and zinc content (Aggett & Favier, 1991). If a shift occurs in the number of cells in the leukocyte subpopulations as a result of disease, then significant changes could occur in the zinc content of the total population independent of a change in zinc status (Aggett & Favier, 1991). Neutrophil zinc concentration has been investigated as an index of zinc status to eliminate this problem (Gibson, 1990). As well, neutrophils have a short half-life (approximately 6-8 hours) and high zinc content (Gibson, 1990; Lee et al., 1993). Total leukocyte and neutrophil zinc concentrations have been shown to decrease in zinc deficiency but these results have been inconsistent possibly because separation and analysis is difficult and lengthy (Prasad & Cossack, 1982; Ruz et al., 1992). Ruz et al. (1992) fed a zinc-deficient diet to young men for seven weeks and found no changes in neutrophil zinc concentration whereas in

another human zinc deficiency study, Prasad & Cossack (1982) found decreased neutrophil zinc levels after only four weeks on a zinc-deficient diet. Relatively large amounts of blood are needed for these assays which limits their use for infants and children (Gibson, 1990).

2.2.1.5 Erythrocyte Zinc Concentration

The response of erythrocyte zinc concentration to experimentally induced zinc depletion-repletion has been inconsistent (Baer & King, 1984; Prasad et al., 1978; Ruz et al., 1992). Baer & King (1984) and Ruz et al. (1992) found no change in erythrocyte zinc concentration in young men fed a zinc-deficient diet whereas Prasad et al. (1978) found a decrease in erythrocyte zinc concentration in a similar study. This assay will not reflect recent changes in body zinc stores because the lifespan of human erythrocytes is long (120 days) (Gibson, 1990).

2.2.1.6 Salivary Zinc Concentration

Salivary zinc concentration was investigated as an index of zinc status because zinc appears to be part of the protein gustin (Gibson, 1990). Gustin is involved in taste acuity which is a parameter impaired by marginal zinc deficiency (Gibson, 1990). The response of salivary zinc concentration to zinc deficiency has not been consistent possibly because flow rate and stimulation of saliva and contamination by cells in the mouth are difficult to control (Aggett & Favier, 1991; Gibson, 1990). Greger & Sickles (1979) found decreased salivary zinc concentration in young females fed a zinc-deficient

diet whereas Baer & King (1984) found no change in salivary zinc concentration in young men fed a zinc-deficient diet.

2.2.2 Functional Indicators of Zinc Status

2.2.2.1 Plasma, Neutrophil and Erythrocyte Membrane Alkaline Phosphatase Activity

Plasma alkaline phosphatase activity has been shown to decrease in some zinc deficiency studies (Baer et al., 1985; Heinen et al., 1995; Papadopoulou et al., 1996; Naber et al., 1996; Taylor et al., 1992) but not in others (Milne et al., 1987; Ruz et al., 1991; Weisman & Hoyer, 1985). For example, the activity of plasma alkaline phosphatase decreased in young men fed a zinc-deficient diet (Baer et al., 1985) but was not significantly changed in a similar study (Ruz et al., 1991). Decreased serum alkaline phosphatase activity associated with decreased serum zinc concentration was found in severely zinc-deficient rats (Naber et al., 1996). The specificity of plasma alkaline phosphatase as an index of zinc status is limited because this enzyme is dependent on other factors such as bone disease and liver dysfunction (Bales et al., 1994). Neutrophil alkaline phosphatase activity has not proved to be a useful index of zinc status as its activity has not been changed in some zinc deficiency studies (Baer et al., 1985; Ruz et al., 1991). Decreased activity of alkaline phosphatase in erythrocyte membrane was shown during zinc deficiency in young men (Ruz et al., 1992). Healthy males supplemented with 50 mg of zinc per day for four weeks had increased activity of this enzyme along with slightly increased plasma zinc concentration (Samman et al., 1996). Erythrocyte membrane alkaline phosphatase activity may be a useful indicator of zinc

status.

2.2.2.2 Plasma 5'-Nucleotidase

The enzyme 5'-nucleotidase catalyzes the cleavage of phosphate from 5'-nucleotides to form nucleosides (Ruz et al., 1991). No response of plasma 5'-nucleotidase activity was found in zinc deficiency in young men (Ruz et al.,1991) but Bales et al. (1994) found that plasma 5'-nucleotidase activity dropped in response to zinc deficiency and increased with repletion in older adults. Although this enzyme may be a potential index of zinc status, activity of plasma 5'-nucleotidase is high in certain conditions such as liver cancer, which would decrease the specificity of the index (Bales et al., 1994).

2.2.2.3 Serum Thymulin

Serum thymulin activity was found to be decreased in experimental human zinc deficiency (Prasad et al., 1988). Patients with acute lymphoblastic leukemia had decreased activity of serum thymulin associated with decreased plasma zinc concentration during the onset and relapse of the disease (Mocchegiani et al., 1994). Zinc appears to be required to maintain the biologically active form of thymulin (Prasad et al., 1988). Although there is a need for more evidence, serum thymulin may be a reliable index of zinc status.

2.2.2.4 Taste Acuity

Taste acuity tests have been developed as noninvasive functional indicators of zinc status because diminished taste acuity is a feature of marginal zinc deficiency (Gibson, 1990). Experimental zinc deficiency in young men (Ruz et al., 1991) and zinc deficiency in pregnant women (Mahomed et al., 1993) did not alter taste acuity. However in a different study performed using pregnant women, taste acuity was associated with depressed serum zinc and increased with zinc supplementation (Garg et al., 1993). Taste acuity does not seem to be a reliable index of zinc status possibly because taste is difficult to measure objectively (Gibson, 1990; Thompson, 1991).

2.2.2.5 Plasma and Erythrocyte Metallothionein

Plasma metallothionein, a zinc binding protein, has been suggested to be useful in interpreting plasma zinc concentration because plasma metallothionein concentration is decreased during zinc deficiency and increased in response to confounding conditions such as stress (King, 1990). It was proposed that zinc deficiency would be indicated when both plasma zinc and plasma metallothionein concentrations are low; low plasma zinc concentration accompanied by high plasma metallothionein concentration would indicate tissue redistribution of zinc as a result of non-nutritional factors (King, 1990). Unfortunately, plasma metallothionein concentrations are low and difficult to measure and concurrent zinc deficiency and non-nutritional factors that influence plasma zinc concentration have shown intermediate values for plasma metallothionein (Bremner, 1993). These problems decrease the usefulness of this indicator of zinc status.

Zinc deficiency has been proposed to decrease synthesis and/or accelerate the degradation of erythrocyte metallothionein whereas zinc supplementation would induce synthesis during erythropoiesis (Grider et al., 1990). Erythrocyte metallothionein levels were decreased by severe zinc deficiency in adult humans and increased with zinc supplementation (Grider et al., 1990; Thomas et al., 1992). These results indicate erythrocyte metallothionein may be a useful indicator of zinc status for severe zinc deficiency although erythrocyte metallothionein concentration may be influenced by hemolytic anemia (Bremner, 1993).

As shown above, there is no specific and sensitive static or functional indicator of zinc status. There is a need for the development of an indicator of zinc status for marginal zinc deficiency. At present, the best approach to estimate zinc status is to use a combination of static and functional indicators (Gibson, 1990). The following is a discussion of erythrocyte deformability as a possible indicator of zinc status.

2.3 Erythrocyte Deformability as a Proposed Indicator of Zinc Status

2.3.1 Physiological Importance

The ability of erythrocytes to undergo marked changes in shape is referred to as deformability. It is necessary for erythrocytes to deform to be able to pass through the microcirculation (Shiga et al., 1990; Smith, 1983). The average diameter of human erythrocytes is 8 μm whereas the diameter of the capillaries through which the erythrocytes pass is approximately 2 to 3 μm (Mohandas & Chasis, 1993). Erythrocytes must be able to undergo extensive passive deformation for optimal delivery of oxygen

(Mohandas & Chasis, 1993). When erythrocytes become less deformable, they can no longer traverse the endothelial slits in the spleen and may be removed from the circulation (Shiga et al., 1990; Smith, 1983). Erythrocyte membranes must be resistant enough to allow the erythrocyte to deform and avoid shear-induced cell fragmentation (Mohandas et al., 1982).

Erythrocyte deformability reduces blood viscosity in large vessels (Bull et al., 1984; Shiga et al., 1990). Under uniform shear stress the normally biconcave disc-shaped erythrocyte is deformed into a flat ellipsoid which reduces the shear-induced viscosity of the blood (Bull et al., 1984; Shiga et al., 1990). As well, tank-treading motion of the membrane in which the membrane rotates around the cytoplasm contributes to the reduction of blood viscosity (Evans, 1989).

2.3.2 Cellular Determinants

There are three main determinants of erythrocyte deformability: internal viscosity, surface area to volume ratio and membrane viscoelastic properties (Johnson, 1994). Hemoglobin concentration is the main contributor to internal viscosity because it is the most abundant intracellular protein in the erythrocyte (Shiga et al., 1990). When the concentration is increased, there is less movement of the cellular contents and less tank-treading motion of the membrane which decreases deformability (Smith, 1983). Hemoglobin concentration can be increased by dehydration or by loss of membrane while retaining a constant amount of hemoglobin (Smith, 1983).

Erythrocytes have a biconcave form with a large surface area to volume ratio,

allowing them to fold and bend as they pass through small vessels because of the excess membrane (Shiga et al., 1990). Spherical erythrocytes have a low surface area to volume ratio and are less deformable because the erythrocyte membrane can expand only minimally and a much greater force is needed to deform a spherical cell (Mohandas & Chasis, 1993; Shiga et al., 1990). Erythrocytes become spherical because of an abnormality in one of the cytoskeletal proteins such as in hereditary spherocytosis (Shiga et al., 1990). The surface area to volume ratio may be decreased by decreasing the surface area of the red blood cell or by increasing the volume of the cell (Smith, 1983).

It is the membrane viscoelastic properties that are relevant to the assessment of zinc status. Because the erythrocyte membrane behaves as deformable viscoelastic material, it is able to deform and return to its original shape (Smith, 1983). A material is viscoelastic if recovery after deformation is not immediate (Whorlow, 1992). Low membrane flexibility may limit cell deformability (Johnson, 1994). The erythrocyte membrane is composed of the lipid bilayer and the cytoskeletal proteins (Shiga et al., 1990). The cytoskeleton is thought to be the determining factor for membrane cellular deformability although there is some evidence that proteins located in the lipid bilayer can influence membrane flexibility (Mohandas & Chasis, 1993; Shiga et al., 1990). The cytoskeleton provides rigid support and stability to the bilayer and the capability to change shape (Mohandas & Chasis, 1993). Abnormal shape and/or increased fragility seen in elliptocytosis or spherocytosis are the result of absent or abnormal cytoskeletal proteins (Shiga et al., 1990). It has been suggested that zinc may play a role in the

regulation of structure and function of proteins in the cytoskeleton of the erythrocyte membrane (Bettger & O'Dell, 1993).

2.3.3 Physiological Role of Zinc in the Erythrocyte Membrane

The structural organization of the erythrocyte membrane is composed of a phospholipid bilayer and a cytoskeleton (Lee, 1993). The phospholipids of the bilayer are oriented with the nonpolar hydrophobic tails directed towards each other and the polar-head hydrophilic groups directed outward towards the aqueous environment (Lee, 1993). The proteins located in the membrane are classified as peripheral or transmembrane proteins (Lee, 1993). The peripheral proteins do not completely penetrate the membrane and may be exposed only at one surface whereas transmembrane proteins penetrate the membrane completely (Lee, 1993). Transmembrane proteins act as anchoring sites for the cytoskeleton (Mohandas & Chasis, 1993). The two main transmembrane proteins are glycophorin A and band 3 (Lee, 1993). The main proteins of the cytoskeleton are spectrin, actin, protein 4.1, adducin and ankyrin (Figure 2.1) (Lee, 1993). The cytoskeleton is composed of 200 nm long spectrin tetramers joined at their ends by junctional complexes (Lee, 1993). Spectrin is composed of two subunits (α spectrin and β spectrin) which intertwine to form a heterodimer (Mohandas & Chasis, 1993). Two heterodimers associate head-to-head to form $(\alpha\beta)_2$ tetramers (Mohandas & Chasis, 1993). The ends of spectrin are associated with short oligomers of actin composed of 12 monomers of actin and tropomyosin (Mohandas & Chasis, 1993). Usually 6 spectrin ends are associated with each actin oligomer to result in an

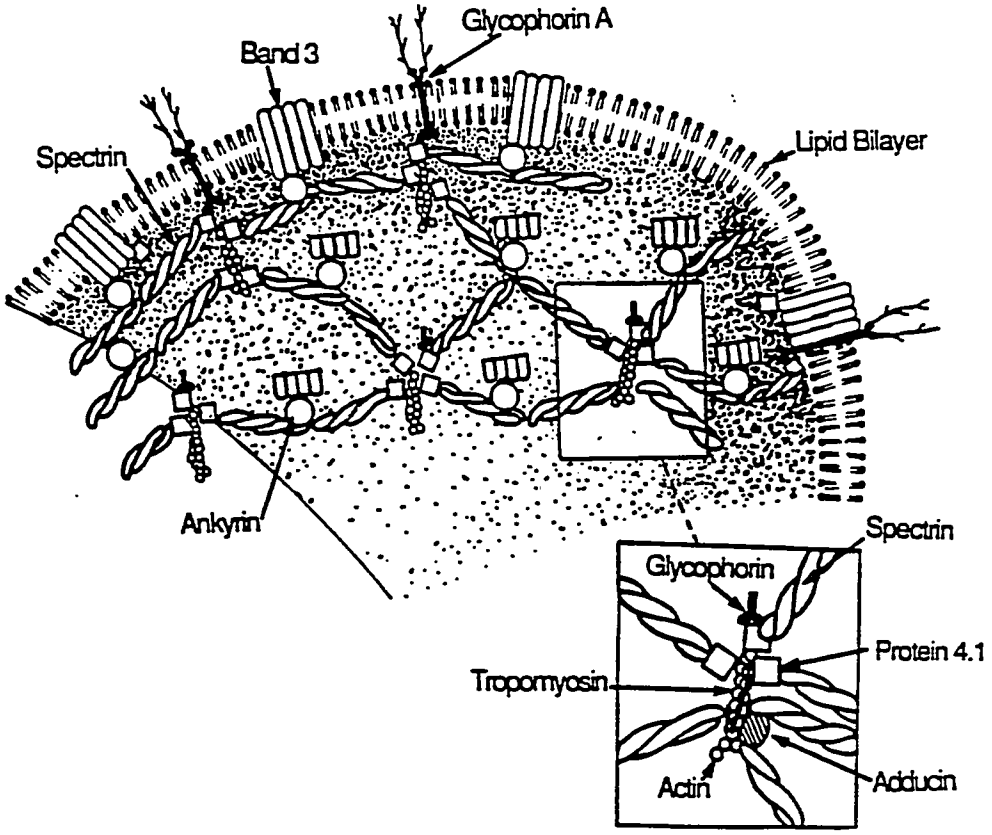

Figure 2.1. Erythrocyte membrane proteins. This figure illustrates the relationship between the cytoskeletal and the integral membrane proteins in the erythrocyte membrane. (From Lee, G. R., Bithell, T. C., Foerster, J., Athens, J. W. & Lukens, J. N. (1993). Wintrobe's Clinical Hematology. (9th ed.). Lea & Febiger, Philadelphia, PA, U.S.A., with permission.)

approximate hexagonal lattice (Mohandas & Chasis, 1993). Protein 4.1 stabilizes the actin-spectrin junction by directly interacting with spectrin (Mohandas & Chasis, 1993; Lee, 1993). Adducin increases the stability of the junction (Lee, 1993). There are two interactions that anchor the cytoskeleton to the lipid bilayer (Lee, 1993). The first is the interaction of ankyrin with spectrin and band 3 in the lipid bilayer (Mohandas & Chasis, 1993). The second is the interaction of protein 4.1 with glycophorin A (Mohandas & Chasis, 1993).

A model of how membrane deformation is regulated by the cytoskeleton has been proposed (Figure 2.2) (Mohandas & Chasis, 1993). When the erythrocyte is nondeformed, spectrin molecules are in a folded conformation. When the erythrocyte deforms, the shape of the cell is changed but constant surface area must be maintained to allow for reversible deformation. The cytoskeleton rearranges to allow for deformation by the uncoiling and extension of certain spectrin molecules and the compression of other spectrin molecules. Eventually, the spectrin molecules will become maximally extended. This is the limit of reversible deformability. To deform beyond this point would necessitate the breaking of protein-protein interactions.

Erythrocyte membrane deformability may be a sensitive functional indicator of zinc status. This is based on the hypothesis that zinc has a physiological role in the plasma membrane of mammalian cells (Bettger & O'Dell, 1981). In addition to the established role of zinc in the activity of many metalloenzymes and in gene expression, Bettger and O'Dell (1993) have recently reviewed the literature in support of additional functions for zinc in the plasma membrane. Zinc concentration is high in many cell