Review

Physiological roles of zinc in the plasma membrane of mammalian cells

William J. Bettger and Boyd L. O'Dell

Department of Nutritional Sciences, College of Biological Science, University of Guelph, Guelph, Ontario, Canada; and Department of Biochemistry, Animal Science Research Center, University of Missouri-Columbia, Columbia, MO USA

The precise role of zinc (Zn) is unknown and the lesions of the Zn deficient animal have been imperfectly related to the levels of total Zn, or of Zn-dependent enzymes, in the relevant tissues.[1]

E.J. Underwood, 1962

Introduction

Ten years ago[2] we proposed that Zn exerts a critical physiological role in the structure and function of biomembranes. We described a possible sequence of events that leads to the development of Zn deficiency pathology in experimental animals and hypothesized that loss of Zn ions from critical components in cell plasma membranes triggers a multitude of biochemical abnormalities. Since 1981 there has been a dramatic increase in research on Zn in many disciplines. It is now widely accepted that Zn has a variety of essential functions that are not readily explained by its role as a prosthetic group of metalloenzymes or as an effector of water-soluble, allosteric enzymes.[3-6] The purpose of this review is to describe new research that supports the hypothesis that there is a critical physiological role of Zn in cell plasma membranes and to propose how recently discovered biochemical functions of Zn in cell plasma membranes may explain the pathology of dietary Zn deficiency.

Biochemistry and metabolism of zinc

The biochemistry of Zn has been reviewed extensively.[4,7] Zn^{2+} is a IIb metal ion that, in physiological salt solutions at pH 7.4, tends to form phosphates, carbonates, and hydroxides of low solubility. In biological fluids Zn is solubilized by complexation with the oxygen, nitrogen, and sulfur ligands of hydrophilic organic molecules. Zn is a "borderline" Lewis acid under the Hard-Soft, Acid-Base Principle.[4,8] It preferentially forms coordination complexes with four ligands in a tetrahedral array, but readily accepts other coordination numbers and geometries. Zn complexes, while capable of a high level of thermodynamic stability, are generally characterized by kinetic lability and, thus, readily undergo ^{65}Zn-Zn exchange. In both blood plasma and intracellular fluid Zn is largely bound to protein. In metalloproteins Zn is bound with high affinity via three or four cysteine, histidine, glutamate, and/or aspartate residues.[5] It should be recognized that posttranslational modification of protein can create or largely eliminate a potential Zn binding ligand; for example, the phosphorylation of a serine or threonine residue effectively creates a potential Zn binding residue while the fatty acylation of a cysteine residue considerably reduces its Zn binding potential.

The metabolism of Zn in mammals is dominated by the lack of a highly selective carrier for Zn in blood and by the existence of metallothioneins, a family of intracellular Zn-binding proteins.[9-11] The normal Zn content of blood plasma is 16–20 μmol/L in rats and 12–16 μmol/L in humans. Zn in plasma is bound predominantly to α_2-macroglobulin and albumin; a small percentage (≈0.2–2.0%) of the Zn in human plasma is bound to low molecular weight substances.[12-15] A large percentage of the low molecular weight Zn fraction of plasma is believed to be bound to free amino acids, principally as ternary complexes with cysteine and histidine.[16] The concentration of Zn as the free aqueous ion, as a soluble hydroxide, or as a complex with low molecular weight monodentate ligands is believed to be very low (10^{-10}–10^{-9} M).[17,18] In most cell types, a portion of intracellular Zn exists in the form of a Zn metallothionein complex. Metallothionein gene expression is induced by Zn, glucocorticoids, glucagon, interleukin 1, and other substances;[11] metallothionein has been proposed to have a role in the control of whole body Zn metabolism via its action in the liver and intestine.[9,19-21] Zn binds tightly to metallothionein, but the half-life of Zn metallothionein is relatively short (hrs), suggesting that Zn metallothionein may

Address reprint requests to Dr. William Bettger at the Department of Nutritional Sciences, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1
Received May 21, 1992; accepted July 30, 1992.

form a reactive pool of intracellular Zn. Zn metallothionein has been shown to donate Zn to apometalloenzymes[22]; conversely, apometallothionein has been shown to remove Zn from Zn metalloproteins.[23,24] However, the intracellular "labile pool" of Zn, the pool used to support essential physiological functions, is undefined. The location, size and speciation of Zn in this pool are unknown.

Experimentally determined biochemical functions of Zn are listed in *Table 1*. Several investigators[4,5,25] have pointed out that there may be additional, as yet undiscovered, biochemical functions of Zn; we concur with this assessment.

Zinc deficiency pathology

The pathological signs of dietary Zn deficiency in humans and animals depend on the length and severity of the deficiency, the age, sex, and species of the animal, the environmental conditions and the presence or absence of iatrogenic factors.[26] The most widely utilized model to investigate the biochemical basis for Zn deficiency pathology is the short-term, severely deficient rat. The dietary regimen and the description of the resultant pathological signs of Zn deficiency were described clearly by Swenerton and Hurley[27] in 1968.

Table 1 Biochemical functions of zinc

I. Catalytic functions:
a) Zn as a co-factor in Zn metalloenzymes
1) Oxidoreductase
2) Transferase
3) Hydrolase
4) Lyase
5) Isomerase
6) Ligase
b) Zn as an effector of enzyme, transporter and membrane channel activity
1) Allosteric
2) Protein translocation
c) Zn as an effector of gene expression
1) Zn-modulated trans acting factors in DNA
2) Direct Zn binding to DNA, RNA, ribosome
d) Zn as a non-enzymatic catalyst
1) Lewis acid
2) Protein aggregating agent and precipitant
3) Site-specific antioxidant
II. Structural functions:
a) Structural roles of Zn in metalloproteins
1) Zn fingers
2) Zn clusters
3) Zn twists
4) Zn acidic clusters
5) Zn in histidine-rich domains
6) Conformation-dependent sites
b) Structural role in peptide hormones
1) Prohormone storage and release
2) Function and metabolism of hormones
c) Stabilizer of supramolecular structures
1) Membranes/membrane skeleton/organelles
2) Cytoskeleton
3) Ribosomes
4) Chromatin

Weanling rats fed ad libitum otherwise nutritionally complete, semi-purified diets containing less than 1 mg Zn/kg diet exhibit a precipitous fall in plasma Zn concentration within the first 12–24 hours (16.8 to 6.12 μmol/L). Remarkably, though there is some cycling of plasma Zn levels depending on the pattern of food intake, plasma Zn remains depressed for the duration of a 3-week experimental period. The cardinal feature of short-term, severe Zn deficiency in young rats is that the fall in plasma Zn concentration does not lead to a decreased Zn concentration in tissues, except bone, pancreas, and intestinal mucosa, compared to pair-fed and/or ad libitum-fed control rats.[28–30] Zn deprivation is characterized by rapid development of pathological signs and rapid reversibility of the signs by Zn supplementation. After consuming a Zn deficient diet for 2–4 days, young rats voluntarily decrease their food intake. Shortly thereafter, there are functional abnormalities in platelets, t-lymphocytes, and keratinocytes. The implicit basis of dietary Zn deficiency pathology is that most, if not all, cell types are rapidly and adversely affected by the decreased extracellular Zn concentration. A list of some of the pathological signs of Zn deficiency are given in *Table 2* along with a time scale for the development and reversal of each sign. A full description of Zn deficiency pathology has been presented in several reviews.[31–33]

There are three prominent theories for the biochemical basis of Zn deficiency pathology. They are: (1) a decrease in Zn metalloenzyme activity, (2) altered gene expression, and (3) altered structure and function of cell plasma membranes. The diversity of Zn deficiency signs and the pattern of temporal development suggest that no single theory adequately explains all signs of Zn deficiency. Thus, it may prove useful to explore the potential "essential biochemical functions" of Zn from the perspective of each theory.

The effect of dietary Zn deficiency on the activities of known Zn metalloenzymes has been studied in some depth with variable results.[34–36] Short-term, severely Zn-deficient rats exhibit decreased activity of a few Zn metalloenzymes in some tissues. The variable effect of dietary Zn deficiency on the activity of Zn metalloenzymes has been explained by (1) different Zn metalloenzymes have different Zn binding affinities, (2) the size and the rate of depletion of the "labile pool of Zn" differs with each tissue, (3) Zn metalloenzymes turnover at different rates in different tissues, and (4) other metals can substitute for Zn in some Zn metalloenzymes without loss of enzymatic activity.[34,35] However, Zn metalloenzymes by definition[7] have tightly bound Zn, and one would expect that they would be the last molecules to lose Zn during dietary Zn deprivation.[7,37] Moreover, some Zn metalloenzyme activities (alkaline phosphatases, DNA polymerase, RNA polymerase, poly ADP-ribose polymerase) are decreased by other physiological conditions (i.e., feed

Table 2 Rates of development and remission of zinc deficiency signs in young rodents

Sign*	Development after dietary deprivation	Remission after dietary repletion	Reference no.
Low plasma Zn (R)†	12 hr	1 day or less; 2 hr‡	52, 79, 243
Decreased food intake (R)	3–4 days	4–5 hr	244
Slowed weight gain (R)	4 days	1 day	52, 245
Depressed hemostasis (R)	4 days	4 hr‡	79
Impaired platelet aggregation (R)	7 days	>3, <7 days	80, 81
Hyperkeratotic skin lesions (R)	10–14 days	Improvement 2–3 days	27
Depressed immune function (M)	12 days	4 days	246, 247
Erythrocyte osmotic fragility (R)	21 days	1 day	52, 54, 244
Peripheral neuropathy and hyperalgesia (GP)	25 days	4 days	104, 265

*The time periods are the first reported appearance or disappearance of the deficiency sign but not necessarily its first occurrence.
†The species in which the sign was observed is indicated in parenthesis, R for rat, M for mouse, and GP for guinea pig.
‡The remission was the result of intragastric Zn therapy rather than dietary Zn.

restriction, stress, infection) that do not result in Zn deficiency pathology.[31] The search continues for metalloenzymes whose changes in activity parallel the loss of Zn in discrete intracellular compartments and directly cause observable pathology.

The role of Zn in gene expression is undeniable. Zn is a cofactor for a variety of Zn metalloenzymes that are involved with nucleic acid metabolism and Zn is a structural component of DNA-binding proteins that contain Zn fingers, clusters, and twists.[38–40] Zn deprivation, in vivo and in vitro, blocks and/or alters the cell cycle, impairs cellular differentiation, and disrupts normal patterns of protein synthesis.[31,41] Zn has been reported to be recompartmentalized after signal reception, and it has been postulated to function as a second messenger, activating such intracellular signal transducers as protein kinase C[42] and adenosine(5′)tetraphospho(5′)-adenosine hydrolase[43] and permitting the biosynthesis of enzymes required for DNA synthesis.[44] However, it remains to be proven that the altered gene expression observed in Zn-deficient rats is due to a lack of Zn in Zn metalloproteins involved with transcription and/or translation.

The previously stated hypothesis,[2] that dietary Zn deficiency pathology is caused principally by a loss of Zn from the cell plasma membrane, follows. Cellular structures that are in physical contact with the extracellular Zn pool will be the first to lose Zn in dietary Zn deficiency. The loss of Zn from specific proteins in cell plasma membranes leads to altered membrane structure and function. The biochemical abnormalities of cells from Zn-deficient animals are a cellular response to the decreased integrity of the plasma membrane. Substantial depletion of the intracellular concentration of "functional Zn" in mammalian cells would rapidly lead to cell death; note the abnormal metabolism seen in *Euglena gracilis* cultured in Zn-deficient media,[45] the apoptosis and necrosis observed in mammalian cells cultured in media treated with pyridine-2,6-dicarboxylic acid,[46] and the necrotic lesions in fetuses from Zn-deficient dams.[47]

Experiments on the role of zinc in cell plasma membranes

The effect of Zn deficiency on cell plasma membranes

Two models exist to study the effect of low extracellular Zn concentration on the function of mammalian cells. The first model involves the production of short-term, severe dietary Zn deficiency in an appropriate experimental animal. This model is described in the section that follows on the effect of added Zn on biomembranes. The second model involves the incubation of mammalian cells in vitro with chelating agents to remove and/or withhold Zn from cells. This latter model has been used to describe an essential role of Zn in cell division.[48,49] However, the low selectivity of chelators for Zn and their potential for direct chemical interactions with the cell plasma membrane, the cell surface, and/or the interior of the cell (i.e., by receptor-mediated endocytosis) are major drawbacks to interpretation of such in vitro research. Chelators remove Zn from mammalian cells to a variable degree,[50] and the in vitro production of Zn deficiency by the use of chelating agents has not, to date, proven useful in defining the role of Zn in cell plasma membranes.[51]

Erythrocytes. Dietary Zn deficiency causes an abnormality in the erythrocyte membrane. Erythrocytes from Zn-deficient rats have increased osmotic fragility that is rapidly (1 day) reversed by dietary Zn supplementation, but not by in vitro addition of Zn.[52] This defect has been reported to be sensitive to diet composition, including sulfur amino acid concentration, protein source, lipid content, and lipid type.[52–54] Erythrocytes from Zn-deficient rats also have increased sensitivity to hemolysis by sodium dodecyl sulfate, sodium dodecyl n-sarcosine, and melittin, and decreased sensitivity to hemolysis by dimethyl sulfoxide.[55] However, they have a normal response to many hemolysins.[55] Erythrocytes from Zn-deficient rats have normal rates

of filterability in the presence and absence of diamide, compared with those of pair-fed controls,[56] but have decreased levels of maximal deformability as measured by ektacytometry.[57] Erythrocytes from Zn-deficient rats have a similar size and shape compared with those from Zn-adequate, pair-fed controls.[58,59] Erythrocytes from rats deficient in both Zn and vitamin E have increased peroxidative fragility compared with vitamin E-deficient controls.[60,61] However, erythrocytes from Zn-deficient rats have a normal lifespan when injected into the blood of Zn-adequate rats,[58] and have normal mechanical fragility and heat-induced fragility.[62] Erythrocytes from Zn-deficient rats have been reported to have increased fluidity of the lipid bilayer as measured by the mobility of 5-doxyl stearate spin probe and increased mobility of a spin probe covalently bound to sialic acid residues on the cell surface.[63] There are conflicting reports that the mobility of a covalently bound maleimide spin probe is changed; one states that probe mobility is increased[62] and another that it is unaltered[64] in erythrocyte membranes from Zn-deficient rats.

The biochemical changes responsible for the altered functional and physical properties of the erythrocyte membrane in Zn-deficient rats are unknown; however, it is clear that the erythrocyte membrane, but not the erythrocyte cytosol, has a depressed Zn concentration.[65] In addition, erythrocytes from Zn-deficient rats have significantly decreased concentrations of ATP and 2,3-diphosphoglycerate,[66] spermidine,[67] basic amino acids,[68] and phosphatidylinositol-bis-phosphate[69] relative to Zn adequate ad libitum-fed, but not pair-fed, controls. The erythrocyte membranes[70,71] and erythrocyte membrane Triton-shells[72] have changes in lipid composition that are characterized by altered cholesterol:phospholipid ratio, phospholipid composition, and/or phospholipid fatty acid composition. There is a greater extent of dephosphorylation of spectrin and actin in isolated erythrocyte membranes from Zn-deficient rats than in controls,[56] and there is an altered protein composition of the membrane skeleton extracted in a low ionic strength buffer.[73] Ca^{2+} ATPase and 5'-nucleotidase activities in erythrocytes from both Zn-deficient rats and pigs are lower than controls.[74] Erythrocytes from Zn-deficient rats have enhanced uptake of ^{65}Zn in vitro.[75] There is increased ^{65}Zn-Zn exchange in vivo in erythrocytes from human patients with Zn deficiency[76] and decreased alkaline phosphatase activity in erythrocyte membranes from young men fed marginally deficient diets.[77]

Platelets. Both breeder females and immature male rats fed low zinc diets exhibit prolonged bleeding times.[78,79] Dietary Zn deprivation causes impairment of platelet aggregation in rats,[80,81] guinea pigs[82] and humans.[83] Platelets in platelet-rich plasma show an impaired secondary phase of aggregation with a reduced response to such aggregating agents as ADP, collagen, and arachidonic acid.[80,81] Washed platelets are also less responsive to ADP.[81] Their uptake of external Ca^{2+} in response to ADP is impaired, while the release of internal Ca^{2+} is unchanged.[84] Normal platelets also show impairment of the secondary phase of aggregation when they are treated with the calcium channel blocker, verapamil.[85] Based on these observations, it has been proposed that the platelet pathology in Zn deficiency involves a defective calcium channel or signal transduction mechanism.[84] Platelets from Zn-deficient rats have a decreased number of PGE_1 receptors, but the receptors have an enhanced affinity for PGE_1.[86]

Leukocytes and other blood cells. Dietary Zn deficiency has detrimental effects on other blood cells that may be related to alterations in the properties of plasma membranes. Zn deficiency in rodents causes an involution of the thymus, a reduction in the mass of the spleen, and a decrease in circulating lymphocyte number, suggesting impaired proliferation of lymphocytes.[87] Whether there is an alteration in the T lymphocyte subsets in vivo, secondary to decreased total lymphocyte number, is currently being debated.[88–90] The proliferative response of lymphocytes isolated from spleen appears to depend on the age of the experimental animal, the length and severity of Zn deficiency, and the mitogen used to stimulate proliferation.[91–94] However, incubation of isolated lymphocytes with chelators, EDTA, or o-phenanthroline suppresses the proliferative response to mitogens, an effect that appears reversible by incubation with Zn in vitro.[95,96] Macrophage function is depressed in Zn-deficient animals[93,97] and there is impaired neutrophil chemotaxis in Zn-deficient rats,[98] rhesus monkeys,[99] and in human patients with acrodermatitis enteropathica.[100,101] Zn deficiency in rats leads to a reduced vasodilation in response to bradykinin and prostacyclin.[102] Monolayers of capillary endothelial cells, made Zn deficient in vitro, show decreased barrier function as regards albumin movement.[103] For each cell type there is an apparent reduced responsiveness to external signals.

Neurons. There are altered functions of brain and the peripheral nervous system in dietary Zn deficiency. Zn-deficient guinea pigs[104] and chicks[105] have decreased rates of action potential propagation in the sciatic nerve. In Zn-deficient rats there is an increased binding of naloxone to opiate receptors, due to an increased number of binding sites; however, the binding of glutamate and aspartate to their receptors in hippocampal synaptosomes is unaltered.[106,107] Abnormal synaptic field potentials occur in response to low frequency stimulation of hippocampal mossy fiber axons in Zn-deficient rats,[108] and in Zn deficient guinea pigs there is impaired glutamate-dependent Ca^{2+} uptake in isolated brain synaptosomes.[109] There is a decreased in vitro polymerization rate of brain tubulin in Zn-deficient rats[110–113] and pigs,[110] but the effect in rats is largely the result of depressed food intake.[112,113] In chicks and guinea pigs, species that are most susceptible to Zn deficiency neuropathy, Zn status has no effect on the rate of tubulin polymerization.[113]

Lipoproteins. Dietary Zn deficiency has been associated with altered lipid metabolism and altered susceptibility to lipid peroxidation in many tissues and in lipoproteins. These topics have been reviewed recently[25,114,115] and will not be updated here. The contribution of compositional changes in plasma membrane lipids and of oxidative and peroxidative stress to the etiology of Zn deficiency pathology is unknown.

Zinc in cell plasma membranes

Zinc is found at remarkably high concentrations in many cell membrane fractions; some concentrations are given in *Table 3*. Unfortunately, few plasma membranes have been analyzed for Zn concentration. In spite of obvious concerns for redistribution of physiological levels of Zn during tissue disruption and the possibility of net Zn loss or gain during the isolation process, it appears that the relative Zn concentration in various cell fractions is of biological significance. High concentrations of Zn in cell plasma membranes suggest critical physiological functions for Zn in this organelle.[6,116,117]

More important than the question of the total Zn concentration in cell plasma membranes is the location of Zn within the membrane in vivo. The precise location and speciation of the labile pool of Zn in cell plasma membranes are unknown, but are critical to our understanding of function. To aid in understanding location and speciation of Zn in cell plasma membranes, consider the Zn concentration in four zones of the cell plasma membrane: (1) the outer cell surface, (2) the aqueous domain of pores and channels within the membranes, (3) the inner surface of the cell plasma membrane, and (4) specialized membrane structures. A diagram of Zn binding zones 1–3, based on the structure of the human erythrocyte membrane, is shown in *Figure 1*. No Zn is considered to be present within the lipid phase of the membrane bilayer unless it is bound to a lipid-soluble Zn chelator such as protoporphyrin IX. Zone 3 includes all proteins of the membrane skeleton. Not shown in *Figure 1* are specialized membrane structures that include tight junctions, gap junctions, membrane caps, and endocytotic coated pits.

Some of the Zn in cell plasma membranes is present as Zn metalloproteins. These proteins may include the following enzymes: alkaline phosphatase,[118] ecto 5′-nucleotidase,[119] Zn endopeptidases,[120] carbonic anhydrase,[121] Cu,Zn superoxide dismutase,[122] and possibly, some phospholipases.[123]

In addition to Zn in metalloenzymes, some Zn in cell plasma membranes in vivo is "in transit" across cell plasma membrane. The precise mechanisms by which Zn crosses cell plasma membranes of mammalian cells is unknown; however, multiple passive transport systems appear to be involved.[124,125] The rate and the route of Zn transport is largely dependent on the chemical forms of Zn presented to the cell.[126–128] It is generally believed that the pores, channels, and carriers involved in Zn transport are not highly selective. The Zn that is continuously present in these structures may be significant in terms of Zn mass and regulatory function in ion transport systems.[125,129–131]

Table 3 The concentration of zinc in membrane fractions of eucaryotic cells

Membrane or fraction	Species	Zn concentration μg/g protein	Reference no.
Erythrocyte plasma membrane	rat	61 ± 4	65
		31 ± 2	71
	humans	20 ± 2	249
		32 ± 4	250
		195 ± 126	232, 251
	pig	56 ± 2	252
	dog	163 ± 57	253
	trout	105 ± 8	254
Taste bud mixed membranes	bovine	826 ± 22	255
Kidney microsomes	bovine	107 ± 12	256
Testis microsomes	monkey	4,360 ± 1430	257
Pancreas β-cell microsomes	mouse	235 ± 209	258
Brain myelin	rat	930 ± 460	259
Cerebellum myelin	rat	53 ± 8	260
		140 ± 10	259
Hippocampus myelin	rat	85 ± 3	260
Cerebral cortex myelin	rat	53 ± 3	260
		290 ± 40	259
Uterus microsomes	rat	700 ± 130	261
Myometrium microsomes	rat	770 ± 50	261
Liver microsomes	rat	92 ± 3	262
		224 ± 45	116
		104 ± 14	263
Lung microsomes	rat	139 ± 7	262
Skeletal muscle (red) microsomes	rat	70 ± 2	264
Skeletal muscle (white) microsomes	rat	39 ± 2	264

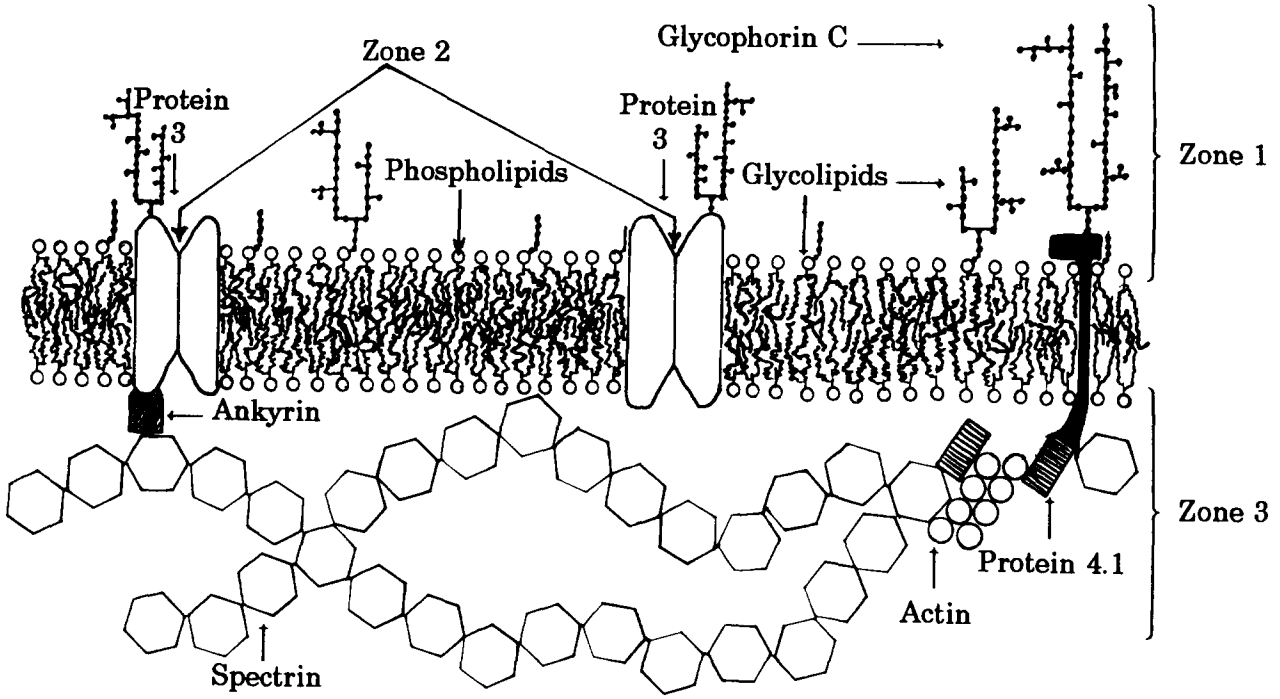


Figure 1 This diagram of a cross-section of the human erythrocyte membrane illustrates the types of structures that are present typically in zinc binding zones 1, 2, and 3.

Effect of added Zn on biomembranes

Since Warren et al.[132] described the use of Zn in vitro to isolate plasma membranes, Zn has been considered to be a potential stabilizer of cell plasma membranes and other intracellular, membrane-encapsulated organelles. Zn has been used to stabilize nuclei,[133] lysosomes,[134-136] cell cytoskeletons,[137-140] myelin membranes,[141,142] brush border membrane vesicles,[143] erythrocyte membranes,[144,145] sperm outer membranes,[146] and the plasma membranes of a variety of cells.[145,147-149]

Explanation of the molecular basis for the "membrane-stabilizing" effect of Zn has focused on five major areas: (1) promotion of membrane skeletal and cytoskeletal protein associations,[150-165] (2) blockage of membrane channels caused by viruses, microbial toxins, and amphipathic molecules,[145,147,166-173] (3) antioxidation and protection against the disrupting effects of lipid peroxidation and protein oxidation,[25,174] (4) antagonism of the adverse effects of Ca^{2+},[145,175-182] and (5) direct alteration of the physical state of membrane lipid.[183-188] Though there are reports that Zn does not act as a stabilizer of cell plasma membranes in some experimental systems,[189-192] most data suggest that Zn, if present at sufficient concentration, is a highly effective stabilizer of cells and cell membranes.

In addition to its role as a membrane stabilizer, Zn has been described as a modulator of cell signaling.[193] Mechanisms suggested for this modulation fall into three categories: (1) blockage of receptor-gated and voltage-gated ion channels,[194-207] (2) regulation of protein and phosphatidylinositol phosphorylation and dephosphorylation,[37,208-222] and (3) regulation of hormone binding to cell surface receptors.[223-235]

Do studies that define pharmacological actions of Zn on cell plasma membranes provide information about the physiological functions of Zn? A lack of Zn in cell plasma membranes, whether achieved by dietary Zn deficiency in vivo or by Zn chelators in vitro, does not necessarily lead to biochemical abnormalities that are the opposite of those seen with added Zn. For example, Zn has been shown to protect erythrocyte membranes against lipid peroxidation and peroxidative damage[236]; however, dietary Zn deficiency does not cause increased lipid peroxidation or increased peroxidative fragility in erythrocytes.[61] Similarly, dietary Zn deficiency causes an increased osmotic fragility in erythrocytes,[52] but added Zn does not affect erythrocyte osmotic fragility except at concentrations that cause the precipitation of intracellular hemoglobin.[189] The primary value of in vitro studies related to the biochemical mechanisms by which Zn exerts its effect on cell plasma membranes is that they describe events that are sensitive to changes in Zn concentration. In addition, pharmacological studies can identify sites on specific proteins that bind Zn and alter membrane function. However, each in vitro effect of Zn on the function of a membrane protein must be examined for its sensitivity to alterations of physiological Zn concentrations in vivo.

Mechanisms for the physiological roles of zinc in the cell plasma membrane

The multitude of effects of added Zn on cell plasma membranes reflects the diverse biochemical functions of the Zn atom and the chemical complexity and dy-

namic nature of cell plasma membranes. As defined earlier and illustrated in *Figure 1,* molecular mechanisms exist for Zn function in cell plasma membrane zones 1, 2, and 3. On the outer cell surface (zone 1, *Figure 1*), Zn promotes the binding of the water-soluble peptide growth hormone to the cell surface prolactin receptor.[227] In the plane of the lipid bilayer (zone 2), Zn has been shown to bind deep within the pores of a voltage-gated Na channel in the brain[197] and within a Ca channel in myotubes.[198] Zinc has been reported to induce aggregation of band 3, the anion channel, in the erythrocyte membrane.[237] On the inner surface of the cell plasma membrane (zone 3), Zn promotes the binding of protein kinase C to receptor sequences on membrane skeleton proteins.[210] In spite of our rapidly expanding knowledge of the effects of added Zn on cell membranes in vitro, the biochemical roles of Zn in cell plasma membranes in vivo are unknown. We suggest that the critical physiological roles of Zn in cell plasma membranes involve the same mechanisms that have been elucidated by in vitro addition of Zn to membrane proteins: (1) direct allosteric-like effect on the conformation of individual proteins and (2) direct effect on protein-protein interactions (ternary complexes of Zn and two separate proteins). The identity of proteins that are sensitive to a decrease in physiological Zn concentrations is unknown, but the proteins are likely to include cell surface receptors to hormone-like substances, ion and water channels, and enzyme/protein "receptors" on the inner surface of cell plasma membrane.

The original Zn-membrane hypothesis[2] considered Zn primarily as a stabilizer of biomembranes and postulated that its absence causes a loss of plasma membrane integrity. Many of the biochemical changes found in cells from Zn-deficient animals were proposed to be compensatory to membrane destabilization.[2] Now the effect of dietary Zn deficiency on cell plasma membranes can be described in functional terms: (1) a slight increase in sensitivity to non-specific stressors of the intact membrane, including: (a) low osmotic pressure, (b) peroxidative stress, (c) membrane-expanding hemolysins, (d) temperature, and/or (e) mechanical/shear stress; (2) and a pronounced decrease in sensitivity to a large subset of normal physiological signals, as defined earlier. We now suggest that Zn at physiological concentrations is a permissive factor that is essential for normal signal transduction by cell plasma membranes. A lack of Zn in cell plasma membranes desensitizes the membranes to signal molecules and causes the cell to be anesthetized to normal external stimuli rather than to be destabilized. In terms of Zn deficiency pathology at the cellular level, an insensitivity of the membrane to external stimuli may be the basis for decreased platelet Ca^{2+} uptake and aggregation (impaired response to ADP, collagen, and arachidonic acid), decreased lymphocyte mitogenicity (impaired response to some lectins), decreased neutrophil chemotaxis (impaired response to FMLP), decreased macrophage phagocytosis (impaired response to opsonins), decreased capillary endothelial cell control of vasodilation (impaired response to bradykinin, angiotensin), and decreased Ca^{2+} uptake by synaptosomes (impaired responsiveness to glutamate). A lack of Zn in cell plasma membranes apparently causes a heterologous desensitization[238–241] of receptors to signal molecules that is uniquely expressed by each cell type. It is important to note that the membrane defect characteristic of short-term, severe dietary Zn deficiency is principally one of dose/response to external signalling molecules; the cell from a Zn-deficient animal has a diminished response to a given dose of agonist.

Possible sequence of events during the development of zinc deficiency pathology

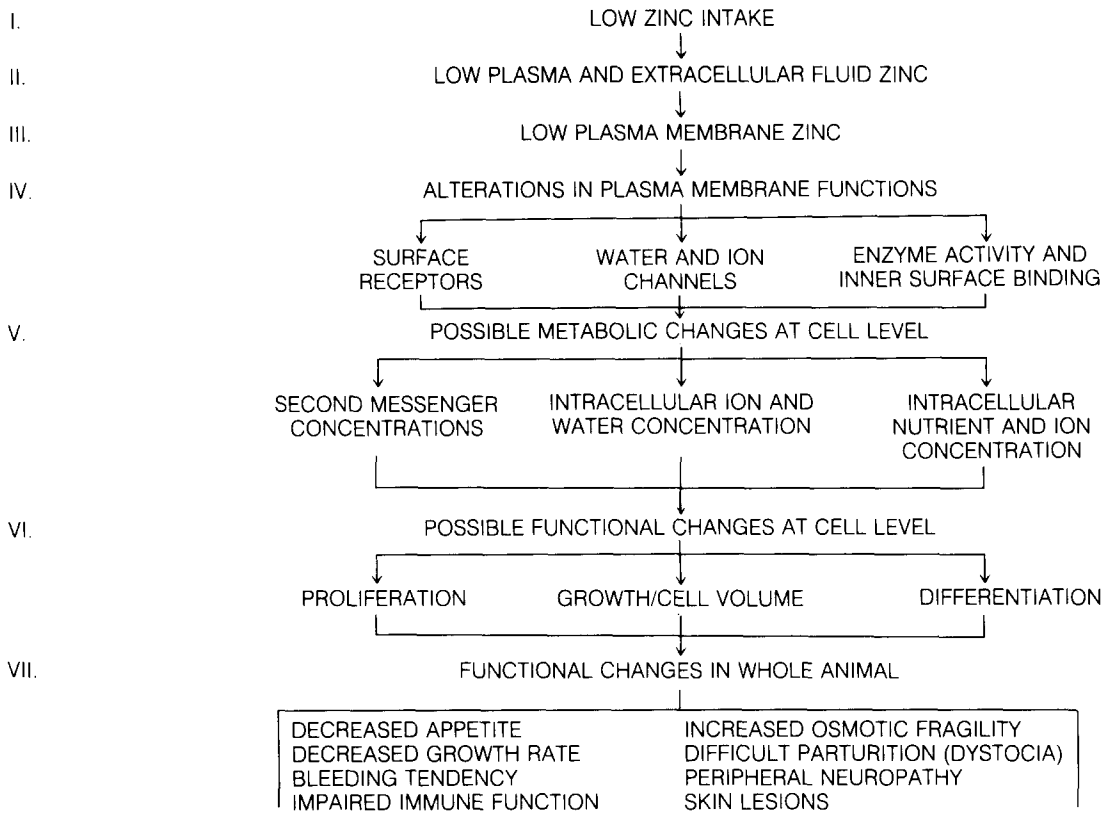
Speculation as to the mechanism by which Zn deficiency produces pathology in animals seems appropriate. Dietary deprivation of Zn rapidly leads to a low concentration of Zn in plasma and other extracellular fluids. This is followed by a decreased Zn concentration in the cell plasma membrane although no change in the total cell Zn is detectable. The specific locations of the Zn binding sites in the plasma membrane are unknown, but binding may occur on the outer surface, within aqueous channels through the lipid bilayer, or on the inner surface of the membrane. Other cations, such as Ca^{2+}, Mg^{2+}, Cu^{2+}, and Fe^{2+}, may compete for these sites and further decrease Zn concentration. Transition metals may also catalyze the oxidation of sulfhydryl groups leading to decreased numbers of Zn binding sites.

The following scenario can be visualized. Loss of Zn from specific proteins in the plasma membrane results in alterations of surface receptors, water and ion channels, and the binding of enzymes/proteins to the inner surface of the membrane. Defects in these critical structures lead to a desensitization of the cell to external signal molecules and to functional changes at the cellular level. Consequently, the concentrations of second messengers and the activities of ion pumps change. In this regard, it is known that the number of prostaglandin binding sites on platelets is decreased, calcium uptake by platelets and cortical synaptosomes is decreased, Na,K-ATPase activity in sciatic nerve is decreased, and Ca-ATPase activity in erythrocyte membranes is decreased. Following malfunction of the plasma membrane components, one would expect a decreased rate of cell proliferation, growth, and differentiation, including lymphocyte maturation, spermatogenesis, and keratinocyte differentiation. Intra- and extra-cellular water distribution and intracellular ion concentrations change. There is a loss of appetite. Without the stimulation of growth factors, nutrient uptake by cells is impaired. These alterations of cell function may explain much of the gross pathology associated with Zn deficiency in animals.

Summary and future research directions

The diversity of the functions of Zn in biological systems is gradually being elucidated. A new appreciation

Table 4 Sequence of events during dietary zinc deprivation



of the vital functions of Zn in the structure and function of non-enzymic proteins has attracted scientists from many disciplines to study Zn metabolism and function. In spite of recent discoveries, the biochemical basis of Zn deficiency pathology in animals remains unelucidated. This review describes one hypothesis to explain the mechanism of Zn deficiency pathology. Cells in Zn-deficient animals lose Zn from their plasma membranes, which results in a desensitization of the membrane to signal transduction. Ultimately, overt signs of dietary Zn deficiency may be attributable to this abnormality. Though Zn may have pharmacological use as a membrane stabilizer, Zn should no longer be considered to exert a critical physiological role in the stabilization of cell plasma membranes. Alternatively, Zn should be considered a permissive factor for normal cell signalling. Zn exerts its permissive role by binding to and maintaining appropriate tertiary and/or quaternary structure in multiple cell plasma membrane proteins.

Future research on the critical physiological roles of Zn in cell plasma membranes should focus on three areas. First, the plasma membranes from many cell types should be analyzed for Zn under conditions of variable extracellular Zn. Speciation of Zn in the plasma membrane under these conditions should be examined. Second, when the identities of the plasma membrane proteins that are sensitive to a loss of Zn are established, the roles of Zn in the structure and function of these proteins need to be defined. Finally, the effect of dietary Zn deficiency on cell plasma membranes should be compared with the effect of stress and/or infection on cell plasma membranes, because both of these conditions cause a decrease in extracellular Zn concentration.[242]

References

1 Underwood, E.J. (1962). *Trace Elements in Human and Animal Nutrition*, p. 173, Academic Press, New York, NY USA
2 Bettger, W.J. and O'Dell, B.L. (1981). Critical physiological role of zinc in the structure and function of biomembranes. *Life Sci.* **28,** 1425–1438
3 Vallee, B.L. (1991). Zinc and fundamental life processes. *J. Inorgan. Biochem.* **43,** 84
4 Williams, R.J.P. (1989). An introduction to the biochemistry of zinc. In *Zinc in Human Biology*, (C.F. Mills, ed.), p. 15–31, Springer-Verlag, London, UK
5 Vallee, B.L. (1990). Zinc co-ordination, function, and structure of zinc enzymes and other proteins. *Biochem.* **29,** 5647–5659
6 Williams, R.J.P. (1984). Zinc: what is its role in biology? *Endeavour* **8,** 65–70
7 Vallee, B.L. and Galdes, A. (1984). The metallobiochemistry of zinc enzymes. *Adv. in Enzymol.* **56,** 283–430
8 Cotton, F.A., Wilkinson, G., and Gaus, P.L. (1987). *Basic Inorganic Chemistry*, p. 206–225, John Wiley and Sons, New York, NY USA
9 Cousins, R.J. (1985). Absorption, transport and hepatic me-

tabolism of copper and zinc: special reference to metallothionein and ceruloplasm. *Physiol. Rev.* **65,** 238–309
10 Bremmer, I. and May, P.M. (1989). Systemic interactions of zinc. In *Zinc in Human Biology,* (C.F. Mills, ed.), p. 95–108, Springer-Verlag, London, UK
11 Bremmer, I. and Beattie, J.H. (1990). Metallothionein and the trace minerals. *Ann. Rev. Nutr.* **10,** 63–83
12 Whitehouse, R.C., Prasad, A.S., and Cossack, Z.T. (1983). Determination of ultrafilterable zinc in plasma by flameless atomic absorption spectrophotometry. *Clin. Chem.* **29,** 1974–1977
13 Bloxam, P.C., Tan, J.C.Y., and Parkinson, C.E. (1984). Non-protein bound zinc concentration in human plasma and amniotic fluid measured by ultrafiltration. *Clin. Chem. Acta* **144,** 81–93
14 Harris, W.R. and Keen, C.L. (1989). Calculations of the distribution of zinc in a computer model of human serum. *J. Nutr.* **119,** 1677–1682
15 Faure, H., Favier, A., Tripier, M., and Arnaud, J. (1990). Determination of the major zinc fractions in human serum by ultrafiltration. *Biol. Trace Elem. Res.* **24,** 25–37
16 Berthon, G., May, P.M., and Williams, D.R. (1978). Computer simulation of metal-ion equilibria in biofluids. 2. Formation constants for Zn(II)-binary and ternary complexes and improved models for low molecular-weight zinc species in blood plasma. *J. Chem. Soc. Dalton Trans.*, 1433–1438
17 Magneson, G.R., Pwathingal, J.M., and Ray, W.J. (1987). The concentrations of free Mg^{2+} and free Zn^{2+} in equine blood plasma. *J. Biol. Chem.* **262,** 11140–11148
18 Simons, T.J.B. (1991). Intracellular free zinc and zinc buffering in human red blood cells. *J. Membrane Biol.* **123,** 63–71
19 King, J.C. (1990). Assessment of zinc status. *J. Nutr.* **120,** 1474–1479
20 Hempe, J.M., Carlson, J.M., and Cousins, R.J. (1991). Intestinal metallothionein gene expression and zinc absorption in rats are zinc-responsive but refractory to dexamethasone and interleukin 1α. *J. Nutr.* **121,** 1389–1396
21 Lowe, N.M., Bremmer, I., and Jackson, M.J. (1991). Plasma ^{65}Zn kinetics in the rat. *Br. J. Nutr.* **65,** 445–455
22 Udom, A.O. and Brady, F.O. (1980). Reactivation in vitro of zinc-requiring apoenzymes by rat liver zinc-thionein. *J. Biochem.* **187,** 329–335
23 Zeng, J., Heuchel, R., Schaffiner, W., and Kagi, J.H.R. (1991). Thioneine (apometallothionein) can modulate DNA binding and transcription activation by zinc finger containing factor Sp1. *FEBS Lett.* **279,** 310–312
24 Zeng, J., Vallee, B.L., and Kagi, J.H.R. (1991). Zinc transfer from transcription factor IIA fingers to thionein clusters. *Proc. Natl. Acad. Sci. USA* **88,** 9984–9988
25 Bray, T.M. and Bettger, W.J. (1990). The physiological role of zinc as an antioxidant. *Free Rad. Biol. Med.* **8,** 281–291
26 Hambridge, K.M., Casey, C.E. and Krebs, N.F. (1986). Zinc. In *Trace Elements in Human and Animal Nutrition,* (W. Mertz, ed.), 5th ed., p. 1–137, Academic Press, New York, NY USA
27 Swenerton, H. and Hurley, L.S. (1968). Severe zinc deficiency in male and female rats. *J. Nutr.* **95,** 8–18
28 Huber, A.M. and Gershoff, S.N. (1970). Effects of dietary zinc and calcium on the retention and distribution of zinc in rats fed semi-purified diets. *J. Nutr.* **100,** 949–954
29 O'Dell, B.C., Reeves, P.G., and Morgan, R.F. (1976). Interrelationships of tissue copper and zinc concentrations in rats nutritionally deficient in one or the other of these elements. In *Trace Substances in Environmental Health,* (D.D. Hemphill, ed.), Vol. 10, p. 411–421, U. of Missouri-Columbia, Columbia, MO USA
30 Milne, D.B., Ralston, N.V.L., and Wallwork, J.C. (1985). Zinc content of blood cellular components and lymph node and spleen lymphocytes in severely zinc-deficient rats. *J. Nutr.* **115,** 1073–1078
31 Clegg, M.S., Keen, C.L., and Hurley, L.S. (1989). Biochemical pathologies of zinc deficiency. In *Zinc in Human Biology,* (C.F. Mills, ed.), p. 129–145, Springer-Verlag, New York, NY USA
32 McClain, C.J., Kasarskis, E.J., and Allen, J.J. (1985). Functional consequences of zinc deficiency. *Prog. Food Nutr. Sci.* **9,** 185–226
33 Prasad, A.S. (1985). Clinical manifestations of zinc deficiency. *Ann. Rev. Nutr.* **5,** 341–363
34 Roth, H.P. and Kirchgessner, M. (1980). Zn metalloenzyme activities. Changes and biochemical aspects in Zn deficiency. *World Rev. Nutr. Diet.* **34,** 144–160
35 Kirchgessner, M. and Roth, H.P. (1983). Nutritional influence of zinc on the activity of enzymes and hormones. In *Metal Ions in Biological Systems,* (H. Sigel, ed.), Vol. 15, p. 363–414, Marcel Dekker, New York, NY USA
36 Baer, M.T., King, J.C., Tamura, T., Margen, S., Bradfield, R.B., Weston, W.L., and Daugherty, N.A. (1985). Nitrogen utilization, enzyme activity, glucose intolerance and leukocyte chemotaxis in human experimental zinc depletion. *Am. J. Clin. Nutr.* **41,** 1220–1235
37 Hubbard, S.R., Bishop, W.R., Kirschmeier, P., George, S.J., Cramer, S.P., and Hendrickson, W.A. (1991). Identification and characterization of zinc binding sites in protein kinase C. *Science* **254,** 1776–1779
38 Vallee, B.L. and Falchuk, K.H. (1981). Zinc and gene expression. *Philos. Trans. R. Soc. London Ser. B.* **294,** 185–197
39 Wu, F.Y.H. and Wu, C.W. (1987). Zinc in DNA replication and transcription. *Ann. Rev. Nutr.* **7,** 251–272
40 Vallee, B.L., Coleman, J.E., and Auld, D.S. (1991). Zn fingers, Zn clusters and Zn twists in DNA-binding protein domains. *Proc. Natl. Acad. Sci. USA* **88,** 999–1003
41 Chesters, J.K. (1991). Trace element-gene interactions with particular reference to zinc. *Proc. Nutr. Soc.* **50,** 123–129
42 Forbes, I.J., Zalewski, P.O., Giannakis, C., Petkoff, H.S., and Cowled, P.A. (1990). Interaction between protein kinase C and regulatory ligand is enhanced by a chelatable pool of cellular zinc. *Biochim. Biophys. Acta* **1053,** 113–117
43 Grummt, F., Weinmann-Dorsch, C., Schneider-Schaulies, J., and Lux, A. (1986). Zinc as a second messenger of mitogenic induction. *Exp. Cell Res.* **163,** 191–200
44 Chesters, J.K., Petrie, L., and Travis, A.J. (1990). A requirement for Zn^{2+} for the induction of thymidine kinase but not ornithine decarboxylase in 3T3 cells stimulated from quiescence. *Biochem. J.* **272,** 525–527
45 Falchuk, K.H., Hardy, C., Ulpino, L., and Vallee, B.L. (1978). RNA metabolism, manganese, and RNA polymerases of Zn-sufficient and Zn-deficient Euglena gracilis. *Proc. Natl. Acad. Sci. USA* **75,** 4175–4179
46 Martin, S.J., Mazdai, G., Strain, J.J., Cotter, T.G., and Hannigan, B.M. (1991). Programmed cell death (apoptosis) in lymphoid and myeloid cell lines during zinc deficiency. *Clin. Exp. Immunol.* **83,** 338–343
47 Keen, C.L. and Hurley, L.S. (1989). Zinc and reproduction: Effects of deficiency of foetal and postnatal development. In *Zinc in Human Biology,* (C.F. Mills, ed.), p. 183–220, Springer-Verlag, London, UK
48 Chesters, J.K., Petrie, L., and Vint, H. (1989). Specificity and timing of the Zn^{2+} requirement for DNA synthesis by 3T3 cells. *Exp. Cell Res.* **184,** 499–508
49 Chesters, J.K. and Boyne, R. (1991). Nature of the Zn^{2+} requirement for DNA synthesis by 3T3 cells. *Exp. Cell Res.* **192,** 631–634
50 Fischer, P.F. and Bettger, W.J. (1992). The relationship between the rate of chelator-induced zinc efflux from erythrocytes and zinc status. *Biol. Trace Elem. Res.* **34,** 287–297
51 Schroeder, J.J. and Cousins, R.J. (1991). Maintenance of zinc-dependent hepatic functions in rat hepatocytes cultured in medium without added zinc. *J. Nutr.* **121,** 844–853
52 O'Dell, B.L., Browning, J.D., and Reeves, P.G. (1987). Zinc deficiency increases the osmotic fragility of rat erythrocytes. *J. Nutr.* **117,** 1883–1889
53 O'Dell, B.L., Browning, J.D., and Reeves, P.G. (1985). Interaction of zinc and major nutrients in the stability of rat erythrocytes. In *Trace Elements in Man and Animals,* (C.F. Mills, I. Bremner, and J.K. Chesters, eds.), p. 75–79, Proc. of Fifth International Symposium on Trace Elements in Man and Animals, Commonwealth Agricultural Bureaux, Slough, UK

54 Roth, H.P. and Kirchgessner, R.K. (1991). The effect of dietary fats on the hemolysis resistance of the erythrocyte membrane during alimentary zinc and calcium deficiency in rats. *Z. Ernahrungswiss* **30,** 98–108

55 Paterson, P.G. and Bettger, W.J. (1985). Effect of dietary zinc intake on the stability of the rat erythrocyte membrane. In *Trace Elements in Man and Animals,* (C.F. Mills, I. Bremner, and J.K. Chesters, eds.), p. 79–83, Proc. of Fifth International Symposium on Trace Elements in Man and Animals, Commonwealth Agricultural Bureaux, Slough, UK

56 Paterson, P.G., Allen, O.B., and Bettger, W.J. (1987). Effect of dietary zinc deficiency on the endogenous phosphorylation and dephosphorylation of rat erythrocyte membrane. *J. Nutr.* **117,** 2096–2105

57 Patterson, P.G. and Card, R.T. (1991). The effect of zinc deficiency on erythrocyte (RBC) deformability as measured by ektacytometer. *FASEB J.* **5,** A3305

58 Paterson, P.G. and Bettger, W.J. (1986). Effect of dietary Zn intake on the hematological profile of the rat. *Comp. Biochem. Physiol.* **83A,** 721–725

59 Bettger, W.J. (unpublished observations)

60 Machlin, L.J. and Gabriel, E. (1980). Interactions of vitamin E with vitamin B12 and zinc. *Ann. N.Y. Acad. Sci.* **355,** 98–108

61 Record, I.R., MacQueen, S.E. and Dreosti, I.E. (1990). Zinc, iron, vitamin E, and erythrocyte stability in the rat. *Biol. Trace Elem. Res.* **23,** 89–96

62 Kubow, S.J. and Bettger, W.J. (1988). The mobility and reactivity of maleimide-binding proteins in the rat erythrocyte membrane. Effects of dietary zinc deficiency and incubation with zinc in vitro. *Can J. Physiol. Pharmacol.* **66,** 66–71

63 Jay, M., Stuart, S.M., McClain, C.J., Palmieri, D.A., and Butterfield, D.A. (1987). Alterations in lipid membrane fluidity and the physical state of cell surface sialic acid in zinc deficient rat erythrocyte ghosts. *Biochim. Biophys. Acta* **897,** 507–511

64 Palmieri, D.A., Stuart, S.M., McClain, C.J., Jay, M., Meyer, N., and Butterfield, D.A. (1991). Zinc deficiency and the physical state of rat erythrocyte membrane proteins. *J. Trace Elem. Exp. Med.* **4,** 11–17

65 Bettger, W.J. and Taylor, C.G. (1986). Effects of copper and zinc status of rats on the concentration of copper and zinc in the erythrocyte membrane. *Nutr. Res.* **6,** 451–457

66 Avery, R.A. and Bettger, W.J. (1991). Effect of dietary Zn deficiency on 2,3-diphosphoglycerate and adenosine triphosphate concentrations in the rat erythrocyte. *J. Nutr. Biochem.* **2,** 395–398

67 Avery, R.A. and Bettger, W.J. (1988). Effect of dietary zinc deficiency and the associated drop in voluntary food intake on rat erythrocyte membrane polyamines. *J. Nutr.* **118,** 987–994

68 Bettger, W.J. (1989). The effect of dietary zinc deficiency on erythrocyte-free and membrane-bound amino acids in the rat. *Nutr. Res.* **9,** 911–919

69 Gaudette, D.C., Driscoll, E.R., and Bettger, W.J. (1992). Zinc deficiency alters the content and fatty acid composition of phosphatidylinositol bis-phosphate (PIP_2) in the rat erythrocyte membrane. *J. Nutr. Biochem.* **3,** 309–312

70 Driscoll, E.R. and Bettger, W.J. (1991). The effect of dietary zinc deficiency on the lipid composition of the rat erythrocyte membrane. *Lipids* **26,** 459–466

71 Johanning, G.L. and O'Dell, B.L. (1989). Effect of zinc deficiency and food restriction on erythrocyte membrane zinc, phospholipid and protein content. *J. Nutr.* **119,** 1654–1660

72 Driscoll, E.R. and Bettger, W.J. (1993). The effect of dietary zinc deficiency in the rat on the lipid composition of the erythrocyte membrane Triton shell. *Lipids* (in press)

73 Avery, R.A. and Bettger, W.J. (1992). Zinc deficiency alters the protein composition of the membrane skeleton but not the extractability or oligometric form of spectrin in rat erythrocyte membrane. *J. Nutr.* **122,** 428–434

74 Johanning, G.L., Browning, J.D., Bobilya, D.J., Veum, T.L., and O'Dell, B.L. (1990). Effect of zinc deficiency on enzyme activities in rat and pig erythrocyte membranes. *Proc. Soc. Exp. Biol. Med.* **195,** 224–229

75 Van Wouwe, J.P., Veldhuizen, M., De Goeij, J.J.M., and Van Den Hamer, C.J.A. (1991). Laboratory assessment of early dietary, subclinical zinc deficiency: a model study on weanling rats. *Pediatr. Res.* **29,** 391–395

76 McWilliams, P.L., Agarwal, R.P., and Henkin, R.I. (1983). Displacement of ^{65}Zn from erythrocyte (RBC) membranes of patients with Zn deficiency: a possible functional test to estimate Zn deficiency. *Am. J. Clin. Nutr.* **37,** 719

77 Ruz, M., Cavan, K.B., Bettger, W.J., and Gibson, R.S. (1992). Erythrocytes, erythrocyte membranes, neutrophils and platelets as biopsy materials for the assessment of zinc status in humans. *Br. J. Nutr.* **68,** 515–527

78 O'Dell, B.L., Reynolds, G., and Reeves, P.G. (1977). Analogous effects of zinc deficiency and aspirin toxicity in the rat. *J. Nutr.* **107,** 1222–1228

79 Gordon, P.R. and O'Dell, B.L. (1980). Short-term zinc deficiency and hemostasis in the rat. *Proc. Soc. Exp. Biol. Med.* **163,** 240–244

80 Gordon, P.R. and O'Dell, B.L. (1980). Rat platelet aggregation impaired by short-term zinc deficiency. *J. Nutr.* **110,** 2125–2129

81 Emery, M.P., Browning, J.D., and O'Dell, B.L. (1990). Impaired hemostasis and platelet function in rats fed low zinc diets based on egg white protein. *J. Nutr.* **120,** 1062–1067

82 Gordon, P.R. and O'Dell, B.L. (1983). Zinc deficiency and impaired platelet aggregation in guinea pigs. *J. Nutr.* **113,** 239–245

83 Gordon, P.R., Woodruff, C.W., Anderson, H.L., and O'Dell, B.L. (1982). Effect of acute zinc deprivation on plasma zinc and platelet aggregation in adult males. *Am. J. Clin. Nutr.* **35,** 113–119

84 O'Dell, B.L. and Emery, M. (1991). Compromised zinc status in rats adversely affects calcium metabolism in platelets. *J. Nutr.* **121,** 1763–1768

85 Emery, M. and O'Dell, B.L. (1987). Biphasic platelet aggregation in rat plasma and the effect of calcium flux modulators. *Thromb. Res.* **46,** 617–623

86 Li, E.T., and O'Dell, B.L. (1986). Effect of zinc status on the binding of prostaglandins to ovarian membranes and intact platelets of pregnant rats. *J. Nutr.* **116,** 1448

87 Keen, C.L. and Gershwin, M.E. (1990). Zinc deficiency and immune function. *Ann. Rev. Nutr.* **10,** 415–431

88 Fraker, P.J., Hildebrandt, K., and Luecke, R.W. (1984). Alteration of antibody mediated responses of suckling mice to T-cell-dependent and -independent antigens by maternal marginal zinc deficiency: restoration of responsivity by nutritional repletion. *J. Nutr.* **114,** 170–179

89 Dowd, P.S., Kelleher, J., and Guillow, P.J. (1986). T-lymphocyte subsets and interleukin-2 production in zinc deficient rats. *Br. J. Nutr.* **55,** 59–65

90 King, L.E. and Fraker, P.J. (1991). Flow cytometric analysis of the phenotype distribution of splenic lymphocytes in zinc-deficient adult mice. *J. Nutr.* **121,** 1433–1438

91 Kramer, T.R. (1984). Reevaluation of zinc deficiency on conconavalin A-induced rat spleen lymphocyte proliferation. *J. Nutr.* **114,** 953–963

92 Hildebrandt, K., Luecke, R., and Fraker, P.J. (1982). Effect of maternal dietary zinc on growth and mitogenic responsiveness in suckling mice. *J. Nutr.* **112,** 1921–1928

93 James, S.J., Swendseid, M., and Makinodan, T. (1987). Macrophage-mediated depression of t-cell proliferation in zinc deficient mice. *J. Nutr.* **117,** 1982–1988

94 Cook-Mills, J. and Fraker, P.J. (1993). Functional capacity of residual lymphocytes from zinc deficient adult mice. *Br. J. Nutr.* (in press)

95 Chesters, J.K. (1972). The role of zinc ions in the transformation of lymphocytes by phytohaemagglutinin. *Biochem. J.* **130,** 133–139

96 Falchuk, K.H. and Krishan, A. (1977). 1,10-Phenanthroline inhibition of lymphoblast cell cycle. *Cancer Res.* **37,** 2050–2056

97 Wieth, J.J., Fraker, P.J., and Kierszenbaum, F. (1989). Zinc requirement for macrophage function: effect of zinc deficiency on the uptake and killing of a protozoal parasite. *Immunology* **68,** 114–119

98 Fenton, M.R., Burke, J.P., Miller, M.L., and Tursi, F.D. (1980). The effect of a zinc deficient diet on the enzymatic activity of rat neutrophils. *Nutr. Rep. Intl.* **22,** 323–328

99 Vruwink, K.G., Fletcher, M.P., Keen, C.L., Golub, M.S., Hendrickx, A.G., and Gershwin, M.E. (1991). Moderate zinc deficiency in rhesus monkeys. An intrinsic defect of neutrophil chemotaxis corrected by zinc repletion. *J. Immunol.* **146,** 244–249

100 Weston, W.L., Huff, J.C., Humbert, J.R., Hambidge, K.M., Neldner, H., and Walravens, P.A. (1977). Zinc correction of defective chemotaxis in acrodermatitis enteropathica. *Arch. Dermatol.* **113,** 422–425

101 Businco, L., Menghi, A.M., Rossi, P., D'Amelio, R., and Galli, E. (1980). Zinc-dependent chemotactic defect in an infant with acrodermatitis. *Arch. Dis. Child.* **55,** 966–968

102 Browning, J.D., Reeves, P.G., and O'Dell, B.L. (1987). Zinc deficiency in rats reduces the vasodilation response to bradykinin and prostacyclin. *J. Nutr.* **117,** 490–495

103 Hennig, B., Wang, Y., Ramasamy, S., and McClain, C.J. (1992). Effects of zinc deficiency on *in vitro* endothelial barrier function. *J. Nutr.* **6,** 1242–1247

104 O'Dell, B.L., Becker, J.K., Emery, M.P., and Browning, J.D. (1989). Production and reversal of the neuromuscular pathology and related signs of zinc deficiency in guinea pigs. *J. Nutr.* **119,** 196–201

105 O'Dell, B.L., Conley-Harrison, J., Browning, J.D., Besch-Williford, C., Hempe, J.M., and Savage, J.E. (1990). Zinc deficiency and peripheral neuropathy in chicks. *Proc. Soc. Exp. Biol. Med.* **194,** 1–4

106 Essatara, M.B., Morley, J.E., Levine, A.S., Elson, M.K., Shafer, R.B., and McClain, C.J. (1984). The role of endogenous opiates in zinc deficiency anorexia. *Physiol. Behav.* **32,** 475–478

107 Slevin, J.T. and Kasarskis, E.J. (1985). Effects of zinc on markers of glutamate and aspartate neurotransmission in rat hippocampus. *Brain Res.* **334,** 281–286

108 Hesse, G.W. (1979). Chronic zinc deficiency alters neuronal function of hippocampal mossy fibers. *Science* **205,** 1005–1007

109 O'Dell, B.L. and Browning, J.D. (1991). Zinc status and glutamate stimulation of calcium uptake by guinea pig cortical synaptosomes. *FASEB J.* **5,** A4110

110 Hesketh, J.E. (1981). Impaired microtubule assembly in brain from zinc-deficient pigs and rats. *Int. J. Biochem.* **13,** 921–926

111 Oteiza, P.I., Hurley, L.S., Lonnerdal, B., and Keen, C.L. (1988). Marginal zinc deficiency affects maternal brain microtubule assembly in rats. *J. Nutr.* **118,** 735–738

112 Oteiza, P.I., Cuellar, S., Lonnerdal, B., Hurley, L.S., and Keen, C.L. (1989). Influence of maternal dietary zinc intake on in vitro tubulin polymerization in fetal rat brain. *Teratology* **41,** 97–104

113 Spurlock, M.E., Browning, J.D., and O'Dell, B.L. (1992). Low zinc status in guinea pigs and chicks does not decrease reassembly rate of brain microtubules. *J. Nutr. Biochem.* **11,** 594–598

114 Cunnane, S.C. (1988). Role of zinc in lipid and fatty acid metabolism and in membranes. *Prog. Food & Nutr. Sci.* **12,** 151–188

115 Bettger, W.J. (1993). Zinc and selenium, site-specific versus general antioxidation. *Can. J. Physiol. Pharmacol.* (in press)

116 Thiers, R.E. and Vallee, B.L. (1957). Distribution of metals in subcellular reactions of rat liver. *J. Biol. Chem.* **226,** 911–920

117 Coughlin, R.T., Tonsager, S., and McGroarty, E.J. (1983). Quantitation of metal cations bound to membranes and extracted lipopolysaccharide of Escherichia coli. *Biochem.* **22,** 2002–2007

118 Chan, J.R.A. and Stinson, R.A. (1986). Dephosphorylation of phosphoproteins of human liver plasma membranes by endogenous and purified liver alkaline phosphatases. *J. Biol. Chem.* **261,** 7635–7639

119 Pilz, R.B., Willis, R.C., and Seegmiller, J.E. (1982). Regulation of human lymphoblast plasma membrane 5′-nucleotidase by zinc. *J. Biol. Chem.* **257,** 13544–13549

120 Lennarz, W.J. and Strittmatter, W.J. (1991). Cellular functions of metallo-endoproteinases. *Biochim. Biophys. Acta* **1071,** 149–158

121 Fernley, R.T. (1988). Non-cytoplasmic carbonic anhydrases. *Trends in Biochem. Sci.* **13,** 356–359

122 Petkau, A., Copps, T.P., and Kelly, K. (1981). Superoxide dismutase within the bovine erythrocyte membrane. *Biochim. Biophys. Acta* **645,** 71–80

123 Moreau, H., Pieroni, G., Jolivet-Reynaud, C., Alouf, J.E., and Verger, R. (1988). A kinetic approach for studying phospholipase C (Clostridium perfringens α toxin) activity on phospholipid monolayers. *Biochem.* **27,** 2319–2323

124 Tacnet, F., Watkins, D.W., and Ripoche, P. (1990). Studies of zinc transport into brush border membrane vesicles isolated from pig small intestine. *Biochim. Biophys. Acta* **1024,** 323–330

125 Ballatori, N. (1991). Mechanisms of metal transport across liver cell plasma membranes. *Drug Metab. Rev.* **231,** 83–132

126 Kalfakakon, V. and Simons, T.J.B. (1990). Anionic mechanisms of zinc uptake across the human red cell membrane. *J. Physiol.* **421,** 485–497

127 Ackland, M.L. and McArdle, H.J. (1990). Significance of extracellular zinc-binding ligands in the uptake of zinc by human fibroblasts. *J. Cell. Physiol.* **145,** 409–413

128 Aiken, S.P., Horn, N.M., and Saunders, N.R. (1992). Effects of amino acids on zinc transport in rat erythrocytes. *J. Physiol.* **445,** 69–80

129 Crofts, J.N. and Barrett, G.J. (1990). The liver cell plasma membrane Ca^{2+} inflow systems exhibit a broad specificity of divalent metal ions. *Biochem.* **269,** 579–587

130 Bennekou, P. and Christophersen, P. (1990). The gating of human red cell Ca^{2+}-activated K^+-channels is strongly affected by the permeant cation species. *Biochim. Biophys. Acta* **1030,** 183–187

131 Pardo, L.A., Heinemann, S.H., Terlau, H., Ludewig, U., Lorra, C., Pongo, O., and Stuhmer, W. (1992). Extracellular K^+ specifically modulates a rat brain K^+ channel. *Proc. Natl. Acad. Sci.* **89,** 2466–2470

132 Warren, L., Glick, M., and Nass. M. (1966). Membranes of animal cells. Methods of isolation of the surface membrane. *J. Cell Physiol.* **68,** 269–286

133 Doyle, M.J., Price, M.P., and Frieden, E. (1981). Stabilization of amphibian and mammalian liver nuclei by zinc and other metal ions. *Comp. Biochem. Physiol.* **68C,** 115–120

134 Chvapil, M., Ryan, J.N., and Brada, Z. (1972). Effects of selected chelating agents and metals on the stability of liver lysosomes. *Biochem. Pharmacol.* **21,** 1097–1105

135 Cho, C.H., Pfeiffer, C.J., and Cheema, A. (1980). Studies of zinc and histamine on lysosomal fragility: possible role in stress ulceration. *Pharmacol. Biochem. Behav.* **13,** 41–44

136 Pfeiffer, C.J., Bulbena, O., Esplugues, J.V., Escolur, G., Navarro, C., and Espluges, J. (1987). Anti-ulcer and membrane stabilizing actions of Zn acexamate. *Arch. Int. Pharmacodyn. Ther.* **285,** 148–157

137 Moore, P.B., Ownby, C.L., and Cornaway, K.L. (1978). Interactions of cytoskeletal elements with the plasma membrane of sarcoma 180 ascites tumour cells. *Exp. Cell Res.* **115,** 331–342

138 Avnur, Z. and Geiger, B. (1981). Substrate-attached membranes of cultured cells isolation and characterization of ventral cell membrane and the associated cytoskeleton. *J. Mol. Biol.* **153,** 361–379

139 Zobel, C.R. and Manuel, H. (1984). The platelet reticular network. *J. Ultrastruct. Res.* **89,** 89–97

140 Zobel, C.R. (1988). The platelet cytoskeleton: evidence for its structure from interactions with $ZnCl_2$. *J. Submicrosc. Cytol. Pathol.* **20,** 269–275

141 Inouye, H. and Kirschner, D.A. (1984). Effects of $ZnCl_2$ on membrane interactions in myelin of normal and shiverer mice. *Biochim. Biophys. Acta* **776,** 197–208

142 Earl, C., Chantry, A., Mohammed, N., and Glynn, P. (1988).

Zinc ions stabilize the association of basic protein with brain myelin membranes. *J. Neurochem.* **51,** 718–724
143 Tacnet, F., Ripoche, P., Roux, M., and Newmann, J.M. (1991). ^{31}P-NMR study of pig intestinal brush-border membrane structure: effect of zinc and cadmium ions. *Eur. Biophys. J.* **19,** 317–322
144 Bereza, U.L., Brewer, G.J., and Mizukami, I. (1982). Association of calmodulin inhibition, erythrocyte membrane stabilization and pharmacological effects of drugs. *Biochim. Biophys. Acta* **692,** 305–314
145 Bashford, C.L., Rodrigues, L., and Pasternak, C.A. (1989). Protection of cells against membrane damage by haemolytic agents: divalent cations and protons act at the extracellular side of the plasma membrane. *Biochim. Biophys. Acta* **983,** 56–64
146 Silvestroni, L., Menditto, A., Modesti, A., and Scarpa, S. (1989). Zinc uptake in human seminal spermatozoa: characterization and effects on cell membranes. *Arch. Androl.* **23,** 97–103
147 Mahadevan, D., Ndirika, A., Vincent, H., Bashford, L., Chambers, T., and Pasternak, C. (1990). Protection against membrane-mediated cytotoxicity by calcium and zinc. *Am. J. Pathol.* **136,** 513–520
148 Seagrave, J.C., Tobey, R.A., and Hildebrand, C.E. (1983). Zinc effects on glutathione metabolism. Relationship to zinc induced protection from alkylating agents. *Biochem. Pharmacol.* **32,** 3017–3021
149 Pasantes-Morales, H., Wright, C.E., and Gaull, G.E. (1984). Protective effect of taurine, zinc and tocopherol on retinol-induced damage in human lymphoblastoid cells. *J. Nutr.* **114,** 2256–2261
150 Hesketh, J.E. (1983). Zinc binding to tubulin. *Int. J. Biochem.* **15,** 743–746
151 Eagle, G.R., Zombola, R.R., and Himes, R.H. (1983). Tubulin-zinc interactions: binding and polymerization studies. *Biochem.* **22,** 221–228
152 Serrano, C., Dominguez, J.E., and Avila, J. (1988). Identification of zinc-binding sites of proteins: zinc binds to the amino-terminal region of tubulin. *Anal. Biochem.* **172,** 210–218
153 Ceska, T.A. and Edelstein, S.J. (1984). Three-dimensional reconstruction of tubulin in zinc-induced sheets. *J. Mol. Biol.* **175,** 349–370
154 Arevalo, M.A., Nieto, J.M., Andreu, D., and Andreu, J.M. (1990). Tubulin assembly probed with antibodies to synthetic peptides. *J. Mol. Biol.* **214,** 105–127
155 Rozychi, M.D., Stetzkowski-Marden, F., and Edelstein, S.J. (1987). Conservation of tubulin α-β differences in zinc-induced sheets with variations in pH and microtubule-associated protein content. *J. Ultrastruct. & Mol. Struct. Res.* **98,** 48–59
156 Strzelecka-Golaszewska, H., Prochniewicz, E., and Drabikowski, W. (1978). Interaction of actin with divalent cations-characterization of protein-metal complexes. *Eur. J. Biochem.* **88,** 229–237
157 Strzelecka-Golaszewska, H., Prochniewicz, E., and Drabikowski, W. (1978). Interaction of actin with divalent cations-the effects of various cations on the physical state of actin. *Eur. J. Biochem.* **88,** 219–227
158 Simchowitz, L. and Cragoe, E.J. (1989). Polyvalent cations inhibit human neutrophil chemotaxis by interfering with the polymerization of actin. *J. Biol. Chem.* **265,** 13457–13463
159 Deinum, J., Baudier, J., Briving, C., Rosengren, L., Wallin, M.I., Gerard, D., and Haglid, K. (1983). The effect of S-100a and S-100b proteins and Zn^{2+} on the assembly of brain microtubule proteins in vitro. *FEBS Lett.* **10,** 287–291
160 Fujii, T., Gochou, N., Akabane, Y., Fujii, M., Kondo, Y., Suzuki, T., and Ohki, K. (1986). Effect of zinc ions on the interaction of S-100 protein with brain microtubule proteins. *Chem. Pharm. Bull.* **34,** 5040–5044
161 Baudier, J. and Cole, R.D. (1987). Interactions between the microtubule-associated π proteins and S100b regulate π phosphorylation by the Ca^{2+}/calmodulin-dependent protein kinase II. *J. Biol. Chem.* **263,** 5876–5833
162 Baudier, J., Glasser, N., Haglid, K., and Gerard, D. (1984). Purification, characterization and ion binding properties of human brain S100b protein. *Biochim. Biophys. Acta* **790,** 164–173
163 Marx, G. and Hopmeier, P. (1986). Zinc inhibits FPA release and increases fibrin turbidity. *Am. J. Hematology* **22,** 347–353
164 Marx, G. (1987). Protofibrin clots induced by calcium and zinc. *Biopolymers* **26,** 911–920
165 Marx, G., Hopmeier, P., and Gurfel, D. (1987). Zinc alters fibrin ultrastructure. *Thromb. Hemost.* **57,** 73–76
166 Wilmsen, H.U., Pattus, F., and Buckley, J.T. (1990). Aerolysin, a hemolysin from Aeromonas hydrophila, from voltage gated channels in planar lipid bilayers. *J. Membrane Biol.* **115,** 71–81
167 Kaszuba, M. and Hunt, G. (1990). Protection against membrane damage: a ^{1}H-NMR investigation of the effect of Zn^{2+} and Ca^{2+} on the permeability of phospholipid vesicles. *J. Inorg. Biochem.* **40,** 6217–6225
168 Castranova, V. and Miles, P.R. (1977). Study of amino and sulfhydryl sites in the sodium pathway in dog red blood cell membranes. *J. Membr. Biol.* **33,** 263–279
169 Menestrina, G. (1986). Ionic channels formed by Staphylococcus aureus alpha toxin: voltage-dependent inhibition by divalent and trivalent cations. *J. Membrane Biol.* **90,** 177–190
170 Pasternak, C.A., Bashford, C.L., and Micklem, K.J. (1985). Ca^{2+} and the interaction of pore formers with membranes. *J. Biosci.* **8,** 273–291
171 Pasternak, C.A. (1987). A novel form of host defence: membrane protection by Ca^{2+} and Zn^{2+}. *Biosci. Rep.* **7,** 81–91
172 Boyle, M.D.P., Langone, J.J., and Boros, T. (1979). Studies on the terminal stages of immune hemolysis. IV. Effect of metal salts. *J. Immunol.* **122,** 1209
173 Portlock, S.H., Clague, M.J., and Cherry, R.J. (1990). Leakage of internal markers from erythrocytes and lipid vesicles by melittin, gramicidin S and alamethicin: a comparative study. *Biochim. Biophys. Acta* **1030,** 1–10
174 Wilson, R.L. (1989). Zinc and iron in free radical pathology and cellular control. In *Zinc in Human Biology,* (C.F. Mills, ed.), p. 147–172, Springer-Verlag, London, UK
175 Lynn, W., Mathews, D., Cloyd, M., Wallwork, J., Thompson, A., and Sachs, C. (1989). Intracellular Ca^{2+} and cytotoxicity. *Arch. Environ. Health* **44,** 323–330
176 Henseleit, U., Plasa, G., and Haest, C. (1990). Effects of divalent cations on lipid flip-flop in the human erythrocyte membrane. *Biochim. Biophys. Acta* **1029,** 127–135
177 Baudier, J., Haglid, K., Haiech, J., and Gerrard, D. (1983). Zinc ion binding to human brain calcium binding proteins, calmodulin and S100b protein. *Biochem. Biophys. Res. Commun.* **114,** 1138–1146
178 White, G.C. and Raynor, S.T. (1982). The effect of phenothiazine and non-phenothiazine inhibitors of calmodulin on platelet calcium fluxes. *Biochem. Biophys. Res. Commun.* **104,** 1066–1072
179 Varecka, L., Peterajova, E., and Pogady, J. (1986). Inhibition of divalent cations and sulfhydryl reagents of the passive Ca^{2+} transport in human red blood cells observed in the presence of vanadate. *Biochim. Biophys. Acta* **856,** 585–594
180 Csermely, P., Sandor, P., Radics, L., and Somogyi, J. (1989). Zinc forms complexes with higher kinetic stability than calcium. *Biochem. Biophys. Res. Commun.* **165,** 838–844
181 Nishimura, M. (1987). Zinc competitively inhibits calcium-dependent release of transmitter at the mouse neuromuscular junction. *Pflugers Arch.* **410,** 623–626
182 Brewer, G.J. (1983). The pharmacological use of zinc. In *Metal Ions in Biological Systems,* (H. Sigel, ed.) Vol. 4, p. 57–75, Marcel Dekker, New York, NY USA
183 Hauser, H. (1991). Effect of inorganic cations on phase transitions. *Chem. Phys. Lipids* **57,** 309–325
184 Deleers, M., Servais, J., and Wulfert, E. (1986). Synergistic effects of micromolar concentration of Zn^{2+} and Ca^{2+} on membrane fusion. *Biochem. Biophys. Res. Commun.* **137,** 101–107
185 Bevon, D.R., Worrell, W.J., and Barfield, K.D. (1983). The

interaction of Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} with phospholipid bilayer vesicles. *Colloids Surf.* **6,** 365–376

186 Deleers, M., Servais, J.P., and Wulfert, E. (1986). Micromolar concentrations of Zn^{2+} potentiates Ca^{2+} -induced phase separation of phosphatidyl serine containing lysosomes. *Biochem. Biophys. Res. Commun.* **136,** 476–481

187 Barfield, K.D. and Bevan, D.R. (1985). Fusion of phospholipid vesicles induced by Zn^{2+}, Cd^{2+} and Hg^{2+}. *Biochem. Biophys. Res. Commun.* **128,** 389–395

188 Gordyziel, S.A., Flanagan, D.R., and Swanbrick, J. (1982). Interaction of monomolecular films of biological significance with heavy metal ions and complexes at the air-water interface. *J. Colloid Interface Sci.* **86,** 178–184

189 Rifkind, J.M. (1983). Interaction of zinc with erythrocytes. In *Metal Ions in Biological Systems,* (H. Sigel, ed.) Vol. 15, p. 275–317, Marcel Dekker, New York, USA

190 Weismann, K. and Mikkelsen, H.I. (1980). Osmotic lysis of erythrocytes in relation to the zinc concentration of the medium. *Arch. Dermatol. Res.* **269,** 105–107

191 Deuticke, B., Lutkemeier, P., and Poser, B. (1991). Influence of phloretin and alcohols on barrier defects in the erythrocyte membrane caused by oxidative injury and electroporation. *Biochim. Biophys. Acta* **1067,** 111–122

192 Bashford, C.L., Alder, G.M., Graham, J.M., Menestrina, G., and Pasternak, C.A. (1988). Ion modulation of membrane permeability: effect of cations on intact cells and on cells and phospholipid bilayers treated with pore-forming agents. *J. Membrane Biol.* **103,** 79–94

193 Anonymous (1991). Importance of zinc for hormone binding and signal transduction: limiting mechanisms in zinc deficiency? *Nutr. Rev.* **49,** 369–370

194 Green, W.N., Weiss, L.B., and Andersen, O.S. (1987). Batrachotoxin-modified sodium channels in planar lipid bilayers. Ion permeation and block. *J. Gen. Physiol.* **89,** 841–872

195 Ravindran, A., Schild, L., and Moczydlowski, E. (1991). Divalent cation selectivity for external block of voltage-dependent Na^+ channels prolonged by batrachotoxin. Zn^{2+} induces substates in cardiac Na^+ channels. *J. Gen. Physiol.* **97,** 89–115

196 Schild, L., Ravindran, A., and Moczydlowski, E. (1991). Zn^{2+}-induced subconductance events in cardiac Na^+ channels prolonged by batrachotoxin. Current-voltage behavior and single-channel kinetics. *J. Gen. Physiol.* **97,** 117–142

197 Green, W.N., Weiss, L.B., and Andersen, O.S. (1987). Batrachotoxin-modified sodium channels in planar lipid bilayers. Characterization of saxitoxin- and tetrodotoxin-induced channel closures. *J. Gen. Physiol.* **89,** 873–903

198 Winegar, B.D. and Lansman, J.B. (1990). Voltage-dependent block by zinc of single calcium channels in mouse myotubes. *J. Physiol.* **425,** 563–578

199 Nachshen, D.A. (1984). Selectivity of the Ca binding site in synaptosome Ca channels. Inhibition of Ca influx by multivalent metal cations. *J. Gen. Physiol.* **83,** 941–967

200 Franciolini, F. and Nonner, W. (1987). Anion and cation permeability of a chloride channel in rat hippocampal neurons. *J. Gen. Physiol.* **90,** 453–478

201 Spalding, B.C., Taber, P., Swift, J.G., and Horowicz, P. (1990). Zinc inhibition of chloride efflux from skeletal muscle of *Rana pipiens* and its modification by external pH and chloride activity. *J. Membrane Biol.* **116,** 195–214

202 Spalding, B.C., Swift, J.G., and Horowicz, P. (1986). Zinc inhibition of potassium efflux in depolarized frog muscle and its modification by external hydrogen ions and diethylpyrocarbonate treatment. *J. Membrane Biol.* **93,** 157–164

203 Yeh, G-C., Bonhaus, D.W., and McNamara, J.O. (1990). Evidence that zinc inhibits N-methyl-D-aspartate receptor-gated ion channel activation by noncompetitive antagonism of glycine binding. *Mol. Pharmacol.* **38,** 14–19

204 Legendre, P. and Westbrook, G.L. (1990). The inhibition of single N-methyl-D-aspartate-activated channels by zinc ions on cultured rat neurones. *J. Physiol.* **429,** 429–449

205 Celentano, J.J., Gyenes, M., Gibbs, T.T., and Farb, D.H. (1991). Negative modulation of the γ-aminobutyric acid response by extracellular zinc. *Mol. Pharmacol.* **40,** 766–773

206 Frelin, C., Cognard, C., Vigne, P., and Lazdunski, M. (1986). Tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels differ in their sensitivity to Cd^{2+} and Zn^{2+}. *Eur. J. Pharmacol.* **122,** 245–250

207 Smart, T.G. (1992). A novel modulatory site for zinc on the $GABA_A$ receptor complex in cultured rat neurones. *J. Physiol.* **447,** 587–625

208 Murakami, K., Whiteley, M.K., and Routtenberg, A. (1987). Regulation of protein kinase C activity by cooperative interaction of Zn^{2+} and Ca^{2+}. *J. Biol. Chem.* **262,** 13902–13906

209 Csermely, P., Szamel, M., Resch, K., and Somogyi, J. (1988). Zinc can increase the activity of protein kinase C and contributes to its binding to plasma membranes in T lymphocytes. *J. Biol. Chem.* **263,** 6487–6490

210 Zalewski, P.D., Forbes, I.J., Giannakis, C., Cowled, P.A., and Betts, W.H. (1990). Synergy between zinc and phorbol ester in translocation of protein kinase C to cytoskeleton. *FEBS Lett.* **273,** 131–134

211 Csermely, P., Szamel, M., Resch, K., and Somogyi, J. (1988). Zinc increases the affinity of phorbol ester receptor in T lymphocytes. *Biochem. Biophys. Res. Commun.* **154,** 578–583

212 Forbes, I.J., Zalewski, P.D., Giannakis, C., and Betts, W.H. (1990). Zinc induces specific association of pkc with membrane cytoskeleton. *Biochem. Intl.* **22,** 741–748

213 Mozier, N.M., Walsh, M.P. and Pearson, J.D. (1991). Characterization of a novel zinc binding site of protein kinase C inhibitor-1. *FEBS Lett.* **279,** 14–18

214 Pearson, J.D., DeWald, D.B., Mathews, W.R., Mozier, N.M., Zürcher-Neely, H.A., Heinrikson, R.L., Morris, M.A., McCubbin, W.D., McDonald, J.R., Fraser, E.D., Vogel, H.J., Kay, C.M., and Walsh, M.P. (1990). Amino acid sequence and characterization of a protein inhibitor of protein kinase C. *J. Biol. Chem.* **265,** 4583–4591

215 Fallon, R.J. (1990). Staurosporine inhibits a tyrosine protein kinase in human hepatoma cell membranes. *Biochem. Biophys. Res. Commun.* **170,** 1191–1196

216 Tonks, N.K. and Charbonneau, H. (1989). Protein tyrosine dephosphorylation and signal transduction. *TIBS* **14,** 497–500

217 Findik, D. and Presek, P. (1988). Zn^{2+} enhances protein tyrosine kinase activity of human platelet membranes. *FEBS Lett.* **235,** 51–56

218 Pernelle, J-J., Creuzet, C., Loeb, J., and Gacon, G. (1991). Phosphorylation of the lymphoid cell kinase $p56^{lck}$ is stimulated by micromolar concentrations of Zn^{2+}. *FEBS Lett.* **281,** 278–282

219 Weinberger, R.P. and Rostas, J.A.P. (1991). Effect of zinc on calmodulin-stimulated protein kinase II and protein phosphorylation in rat cerebral cortex. *J. Neurochem.* **57,** 605–614

220 Alitalo, K., Keski-Oja, J., and Bornstein, P. (1983). Effects of Zn^{2+} ions on protein phosphorylation in epithelial cell membranes. *J. Cell. Physiol.* **115,** 305–312

221 Smith, J.B., Dwyer, S.D., and Smith, L. (1989). Cadmium evokes inositol polyphosphate formation and calcium mobilization. Evidence for a cell surface receptor that cadmium stimulates and zinc antagonizes. *J. Biol. Chem.* **264,** 7115–7118

222 Ramamoorthy, S. and Balasubramanian, A.S. (1991). Zn^{2+}-dependent tyrosine phosphorylation of a 68-kDa protein and its differentiation from Mg^{2+}-dependent tyrosine phosphorylation in sheep platelets. *Arch. Biochem. Biophys.* **286,** 433–440

223 Pang, D.T. and Shafer, J.A. (1985). Inhibition of the activation and catalytic activity of insulin receptor kinase by zinc and other divalent metal ions. *J. Biol. Chem.* **260,** 5126–5130

224 Ezaki, O. (1989). IIb group metal ions (Zn^{2+}, Cd^{2+}, Hg^{2+}) stimulate glucose transport activity by post-insulin receptor kinase mechanism in rat adipocytes. *J. Biol. Chem.* **264,** 16118–16122

225 Marangos, P.J., Patel, J., Martino, A.M., Dilli, M., and Boulenger, J.P. (1983). Differential binding properties of adenosine receptor agonists and antagonists in brain. *J. Neurochem.* **41,** 367–374

226 Greengard, J.S. and Griffin, J.H. (1984). Receptors for high

molecular weight kininogen on stimulated washed human platelets. *Biochem.* **23,** 6863–6869
227 Cunningham, B.C., Bass, S., Fuh, G., and Wells, J.A. (1990). Zinc mediation of the binding of human growth hormone to the human prolactin receptor. *Science* **250,** 1709–1713
228 Cunningham, B.C., Mulkerrin, M.G., and Wells, J.A. (1991). Dimerization of human growth hormone by zinc. *Science* **253,** 545–548
229 Lowman, H.B., Cunningham, B.C., and Wells, J.A. (1991). Mutational analysis and protein engineering of receptor-binding determinants in human placental lactogen. *J. Biol. Chem.* **266,** 10982–10988
230 Shuster, T.A., Nagy, A.K., Conly, D.C., and Farber, D.B. (1992). Direct zinc binding to purified rhodopsin and disc membranes. *Biochem. J.* **282,** 123–128
231 Smith, C.P. and Huger, F.P. (1983). Effect of zinc on [^{3}H]-QNB displacement by cholinergic agonists and antagonists. *Biochem. Pharmacol.* **32,** 377–380
232 Maneva, A.I., Manev, V.V., and Sirakov, L.M. (1984). The effect of zinc on [^{59}Fe]-lactoferrin binding to blood neutrophilic leukocytes and colostral cells. *Int. J. Biochem.* **16,** 793–797
233 Nunez, D., Kumar, R., and Hanahan, D.J. (1989). Inhibition of [^{3}H]platelet activating factor (PAF) binding by Zn^{2+}: a possible explanation for its specific PAF antiaggregating effects in human platelets. *Arch. Biochem. Biophys.* **272,** 466–475
234 Heyns, A. du P., Eldor, A., Yarom, R., and Marx, G. (1985). Zinc-induced platelet aggregation is mediated by the fibrinogen receptor and is not accompanied by release or by thromboxane synthesis. *Blood* **66,** 213–219
235 Teiwani, G.A. and Hanissian, S.H. (1990). Modulation of mu, delta and kappa opioid receptors in rat brain by metal ions and histidine. *Neuropharmacol.* **29,** 445–452
236 Girotti, A.W., Thomas, J.P., and Jordan,, J.E. (1985). Inhibitory effect of zinc II on free radical lipid peroxidation in erythrocyte membranes. *J. Free Rad. Biol. Med.* **1,** 395–401
237 Clague, M.J. and Cherry, R.J. (1989). A comparative study of band 3 aggregation in erythrocyte membranes by melittin and other cationic agents. *Biochim. Biophys. Acta* **980,** 93–99
238 Green, A., Milligan,, G., and Dobias, S.B. (1992). G_i down-regulation as a mechanism for heterologous desensitization in adipocytes. *J. Biol. Chem.* **267,** 3223–3229
239 Sibley, D.R. and Lefkowitz, R.J. (1985). Molecular mechanisms of receptor desensitization using the β-adrenergic receptor-coupled adenylate cyclase system as a model. *Nature* **317,** 124–129
240 Hausdorff, W.P., Caron, M.G., and Lefkowitz, R.J. (1990). Turning off the signal: desensitization of β-adrenergic receptor function. *FASEB J.* **4,** 2881–2889
241 Hoak, J.B., Thomas, A.P., Rooney, A.P., Higaski, K., and Rubin, E. (1992). Ethanol and signal transduction in liver. *FASEB J.* **6,** 2386–2396
242 Van Vouwe, J.P., Veldhuizen, M., Van Den Hamer, C.J.A., and De Goeij, J.J.M. (1990). Discrimination between low dietary zinc and endotoxin exposure: a model study on weaning rats. *Ped. Res.* **28,** 332–335
243 Hurley, L.S., Gordon, P., Keen, C.L., and Merkhofer, L. (1982). Circadian variation in rat plasma zinc and rapid effect of dietary zinc deficiency. *Proc. Soc. Exp. Biol. Med.* **170,** 48–52
244 Chesters, J.K. and Quarterman, J. (1970). Effects of zinc deficiency on food intake and feeding patterns of rats. *Br. J. Nutr.* **24,** 1061–1069
245 Williams, R.B. and Chesters, J.K. (1970). The effects of early zinc deficiency on DNA and protein synthesis in the rat. *Br. J. Nutr.* **24,** 1053–1059
246 DePasquale-Jardieu, P. and Fraker, P.J. (1979). The role of corticosterone in the loss in immune function in the zinc-deficient A/J mouse. *J. Nutr.* **109,** 1847–1855
247 Zwicki, C. and Fraker, P. (1980). Restoration of the antibody mediated response of zinc/caloric deficient neonatal mice. *Immunological Comm.* **9,** 611–626
248 Bettger, W.J., Fish, T.J., and O'Dell, B.L. (1978). Effects of copper and zinc status of rats on the concentration of copper and zinc in the erythrocyte membrane. *Nutr. Res.* **6,** 451–457
249 Ho, S.Y., Catalanotto, F.A., Lisack, R.P., and Dore-Duffy, P. (1986). Zinc in multiple sclerosis. II. Correlation with disease activity and elevated plasma membrane-bound zinc in erythrocytes from patients with multiple sclerosis. *Ann. Neurol.* **20,** 712–715
250 McWilliams, P.L., Agarwal, R.P., and Henkin, R.I. (1983). Zinc concentration in erythrocyte membranes in normal volunteers and in patients with taste and smell dysfunction. *Biol. Trace Elem. Res.* **5,** 1–8
251 Cunnane, S.C., Ho, S.Y., Dore-Duffy, P., Ells, K.R., and Horrobin, D.F. (1989). Essential fatty acid and lipid profiles in plasma and erythrocytes in patients with multiple sclerosis. *Am. J. Clin. Nutr.* **50,** 801–806
252 Johanning, G.L., Browning, J.D., Bobilya, D.J., Veum, T.L., and O'Dell, B.L. (1990). Effect of zinc deficiency and food restriction in the pig erythrocyte fragility and plasma membrane composition. *Nutr. Res.* **10,** 1463–1471
253 Chvapil, M., Montgomery, D., Ludwig, J.C., and Zukoski, C.F. (1979). Zinc in erythrocyte ghosts. *Proc. Soc. Exp. Biol. Med.* **162,** 480–484
254 Bettger, W.J., Spry, D.J., Cockell, K.A., Cho, C.Y., and Hilton, J.W. (1987). The distribution of zinc and copper in plasma, erythrocytes and erythrocyte membranes of rainbow trout (Salmo gairdneri). *Comp. Biochem. Physiol.* **871,** 445–451
255 Law, J.S., Nelson, N., and Henkin, R.I. (1983). Zinc localization in taste bud membranes. *Biol. Trace Elem. Res.* **5,** 219–224
256 Jayawickreme, C.K. and Chatt, A. (1990). Studies on zinc and cadmium bound proteins in bovine kidney by biochemical and neutron activation techniques. *Biol. Trace Elem. Res.* **27,** 503–512
257 Srivastava, A. and Setty, B.S. (1985). The distribution of zinc in the subcellular fractions of the rhesus monkey testis. *Biol. Trace Elem. Res.* **7,** 83–87
258 Andersson, T., Berggren, P.D., and Flat, P.R. (1980). Subcellular distribution of zinc in islet β-cell fractions. *Horm. Metab. Res.* **12,** 275–276
259 Rajan, K.S., Colburn, R.W., and Davis, J.M. (1976). Distribution of metal ions in the subcellular fractions of several rat brain areas. *Life Sci.* **18,** 423–432
260 Saito, T., Itoh, T., Satoh, H., and Saito, K. (1988). Copper and zinc distribution in eight regions and subcellular fractions of rat brain. *J. Trace Elem. Exp. Med.* **1,** 33–40
261 Daniel, E.E., Fair, S., and Kidwai, A.M. (1971). Zinc and smooth muscle contractility. II. Zinc content and accumulation in rat uterus and their relation to contractility changes from zinc and ouabain. *J. Pharmacol. Exp. Ther.* **178,** 290–298
262 Hammermueller, J.D., Bray, T.M., and Bettger, W.J. (1987). Effect of zinc and copper deficiency on microsomal NADPH-dependent active oxygen generation in rat lung and liver. *J. Nutr.* **117,** 894–901
263 Edwards, C., Olson, K.B., Heggen, G., and Glenn, J. (1961). Intracellular distribution of trace elements in liver tissue. *Proc. Soc. Exp. Biol. Med.* **107,** 94–97
264 Kondo, H., Kimura, M., and Itokawa, Y. (1991). Manganese, copper, zinc and iron concentrations and subcellular distribution in two types of skeletal muscle. *Proc. Soc. Exp. Biol. Med.* **196,** 83–88
265 O'Dell, B.L., Conley-Harrison, J., Besch-Williford, C., Browning, J.D., and O'Brien, D. (1990). Zinc status and peripheral nerve function in guinea pigs. *FASEB J.* **4,** 2919–2922