

Viral infections and trace elements: A complex interaction

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Several trace elements are essential micronutrients and are required for various body functions and well being of the immune system. The deficiencies of trace elements and infectious diseases often coexist and exhibit complex interactions. Several trace elements such as selenium, zinc, copper, manganese, etc. have immunomodulatory functions and thus influence the susceptibility to the course and the outcome of a variety of viral infections. Some trace elements inhibit virus replication in the host cells, thus showing antiviral activity. Many trace elements act as antioxidants or help such functions that not only regulate immune responses of the host, but also may alter the genome of the viruses. The grave consequences of this may be the emergence of new infections. The trace elements, viruses and immune system interactions have been briefly reviewed in this article to highlight the importance of trace element nutrition of host in not only optimizing immune response to infections, but also in preventing viral mutations which could increase viral pathogenicity.

VIRUSES are the smallest infectious agents consisting of a single nucleic acid (RNA or DNA) encased in a protein shell, which may be covered with a lipid-containing membrane. More than 300 viruses are known to be pathogenic in humans and animals producing a variety of syndromes. Viruses are still the most common agents of all human ailments. In a large majority of the cases, viral infections are not apparent or subclinical; only in some of them is a clinical disease produced. Clinical illness following a virus infection depends upon factors both in the virus and the host. The most important factor in a virus is genomic alterations. The factors in the host mostly depend upon the nutritional status and the optimum functioning of the immune system. The life cycle of a virus starts with its entry into a host; it reaches the susceptible target cell, enters it, replicates and causes cell injury, and may be cell death. At any of these steps the life cycle of the virus can be aborted by various body mechanisms, mainly by the immune response¹.

Malnutrition has long been associated with increased susceptibility to infectious diseases. The increase in severity of infectious diseases and susceptibility in malnourished hosts is thought to be the result of an impaired immune response. For example, malnutrition could influence the

immune response by inducing a less effective ability to manage the challenge of an infectious disease. It has been demonstrated that not only is the host affected by the nutritional deficiency, but also the invading pathogen². Nutritional deficiency could be of major food components or trace elements. The minerals that are important for body functions are divided into two groups—macro elements and trace elements. The trace elements comprise of metals in biological fluids at concentrations less than 1 µg/g wet weight. They combine with vitamins, form enzymes and are necessary for almost every physiological process. Even if one mineral is lacking, the body cannot function properly. Most of the trace metals are essential nutrients for humans and animals and include selenium, zinc, copper, cobalt, manganese, molybdenum, chromium, nickel and iron. Trace elements are found in a broad range of plant and animal foods, as well as in drinking water. The functions of trace elements are determined by their charges, mobilities and binding constants to biological ligands. Some of them are used as charge carriers to conduct electric impulses along nerves, etc. while others form moderately stable complexes with enzymes, nucleic acids and other ligands. They act as triggers/activators controlling biological functions. Another group of trace elements form strong static complexes and become the integral part of proteins and enzymes. Several biological systems depend upon dietary micronutrients (e.g. copper, zinc and manganese superoxide dismutase). Oxidative stress may be an important factor in infection if micronutrients are deficient³. Some of the features of trace elements discussed here have been summarized in Table 1. Trace elements and some of their compounds show anti-viral activity by combining with cellular proteins and inactivating them. On the other hand, some trace elements enhance severity of various viral infections. Thus, trace elements may play an important role in diseases caused by viruses. This article gives an overview of the role of trace elements (micronutrients) on viral infections. Iron has not been included in the present discussion because a number of reviews have appeared recently on this topic⁴⁻⁷.

Effect of trace elements on immune system

The immune system contributes to the maintenance of physiological integrity of the body mainly by eliminating foreign material and infectious microbes. This is mediated through

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Table 1. Function of trace metals and effect of their deficiency in humans¹⁵⁷

Trace metal	Biochemical function	Enzyme and protein		Effect of deficiency
		Class	Example	
Selenium	Antioxidant	Oxidoreductases, transferases	Glutathione peroxidase	Cardiomyopathy, striated muscle degeneration
Zinc	Nucleic acid and protein synthesis and degradation, alcohol metabolism	Transferases, hydrolases, lyases, isomerases, ligases, oxidoreductases, transcription factor	RNA polymerases, alcohol dehydrogenases, glucocorticoid receptor	Growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal atrophy, impaired spermatogenesis, congenital malformations
Copper	Haemoglobin synthesis, connective tissue metabolism, bone development	Oxidoreductases	Superoxide dismutase, ferroxidase ceruloplasmin	Anaemia, growth retardation, defective keratinization and pigmentation of hair, hypothermia, scurvy-like changes in skeleton
Cobalt	Methionine metabolism	Transferases	Haemocysteine methyl-transferases	Anaemia (B ₁₂) deficiency
Manganese	Oxidative phosphorylation, fatty acid, mucopolysaccharide and cholesterol metabolism	Oxidoreductases, hydrolases, ligases	Diamine oxidase, pyruvate carboxylase	Bleeding disorder (increased prothrombin time)
Molybdenum	Xanthine metabolism	Oxidoreductases	Xanthine oxidase	?Oesophageal cancer
Chromium	Binding of insulin to cells, glucose metabolism	?	?	Impairment of glucose tolerance
Nickel	?Stabilizing RNA structure	Oxidoreductases, hydrolases	Urease	?

?, Not known.

nonspecific or specific acquired immunity, which is a complicated process involving coordinated efforts of several types of cells and their secretory products; for example, various antigen presenting cells, including macrophages and T- and B-lymphocytes. Macrophages are among the cells of first line of defence due to their phagocytic, cytotoxic and secretory activities. Any foreign material that enters the body is phagocytosed and digested by macrophages. In this process the macrophage may also be damaged, thereby affecting its functions.

Micronutrients such as zinc, selenium, iron, copper, etc. can influence several components of innate immunity. Select micronutrients play an important role in alteration of oxidant-mediated tissue injury, and phagocytic cells produce reactive oxidants as part of the defence against infectious agents. Adequate micronutrients are required to prevent damage of cells participating in innate immunity. Deficiencies in zinc may reduce natural killer cell function, whereas supplemental zinc may enhance their activity. The specific effects of micronutrients on neutrophil functions are not clear⁸.

T- and B-lymphocytes are the effector cells of the immune system. B-lymphocytes produce specific antibodies in response to antigen, while T-lymphocytes help B-cells in antibody production besides mediating the cellular immune response. Cytokines are soluble glycoproteins released by cells of the immune system, which act non-enzymatically through specific receptors to regulate immune responses. They include a vast array of relatively low molecular weight, pharmacologically active proteins that are

secreted by one cell for the purpose of altering either its own functions (autocrine effect) or those of adjacent cells (paracrine effect). Cytokines resemble hormones in that they act at low concentrations, bound with high affinity to a specific receptor. Cytokine secretion profiles correlate well with the distinctive functions of helper T (Th)1 and Th2 cells, which are the major subsets of fully differentiated CD4⁺ Th cells. Th1 cells secrete interferon-gamma (IFN- γ), interleukin (IL)-2 and tumour necrosis factor-beta (TNF- β) and are responsible for cell-mediated inflammatory reactions, delayed-type hypersensitivity and tissue injury in infections and autoimmune diseases. Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13 and are associated with B-cell antibody production. Cross-regulation of the two clones is mediated by IL-10 and IFN- γ . Furthermore, TNF- α and IL-10 form an autoregulatory loop, in which TNF- α is an inducer of IL-10, and IL-10 is a down-regulator of TNF- α . Infections⁹⁻¹³ eliciting a dominant humoral immune response induce a higher expression of Th2-related cytokines and are associated with low levels of IFN- γ and IL-2, whereas those characterized by delayed-type hypersensitivity response show a higher expression of Th1 cytokines IFN- γ and IL-2 and low levels of IL-4. In a number of viral infections such as human immunodeficiency virus (HIV), herpes simplex, dengue and influenza viruses, a Th1-type response is linked to recovery from infection, while a Th2-type response tends to lead to severe pathology and exacerbation of the disease^{11,14-16}. Animal viruses have evolved strategies to survive in harmony with

the host in which they replicate. Viruses have the ability to subvert or impair the immune response to varying degrees. Impairment of immune system often results in increased susceptibility towards pathogenic virus.

There are a large numbers of biologically active compounds which may have direct, primary or secondary effect on the immune system. The effect of chemicals, including drugs, pesticides, hydrocarbons, heavy and trace elements and many other organic and inorganic substances on the human immune system is of interest to pathologists, immunologists and toxicologists.

Various trace elements are responsible for many biochemical, immunological and physiological essential activities of the body as micronutrients. Nutrient status is an important factor contributing to immune competence. Under-nutrition impairs the immune system, suppressing immune functions that are fundamental to host protection. Under-nutrition can be due to insufficient intake of energy and macronutrients and/or due to deficiencies in specific micronutrients. Often these occur in combination. Trace elements that have been demonstrated to be required for the immune system to function efficiently include zinc, copper, iron and selenium. Animal and human studies have demonstrated that adding the deficient nutrient back to the diet can restore immune function and resistance to infection. Among the trace elements studied the most in this regard are zinc and selenium. Increasing intake of some nutrients above habitual and recommended levels can enhance some aspects of the immune function. However, excess amounts of some nutrients also impair the immune function. A summary of the effects of the deficiency of various trace elements on

the functions of different components of the immune system is presented in Table 2.

Effect of trace elements as antioxidants on virus infections

Free oxygen radicals may protect against virus attack and can produce tissue damage during this protection by triggering inflammation¹⁷. Free radicals interact with neighbouring entities and can damage molecules, cells, tissues, DNA and finally the entire organs. The body has evolved mechanisms for neutralizing free radicals, including enzymes like superoxide dismutase, catalase and glutathione peroxidase. Oxidative stress is implicated in the pathogenesis of atherosclerosis, and of viral infections caused by Sendai virus, influenza and HIV. Infection with cytomegalovirus (CMV) causes generation of intracellular reactive oxygen species, which activate NF-kappa B, a cellular transcription factor. NF-kappa B mediates expression of the CMV promoter and of genes involved in the immune and inflammatory responses. Antioxidants and aspirin inhibit intracellular reactive oxygen species, NF-kappa B and CMV¹⁸. Speir *et al.*¹⁹ reported that in the case of CMV, free radicals induce a viral promoter gene as well as turning on transcription of immediate-early and late proteins, and a number of virions. Antioxidant viral defence mechanisms become overwhelmed in chronic viral diseases. Virtually every known antioxidant, including glutathione and its precursor cysteine, is depleted by viral infection. The effects of deficient antioxidant status are

Table 2. Effect of deficiency of trace metals on the immune system

Trace metal	Components immune system							
	Killing of bacteria by neutrophils	NK cells non-specific immunity	Macrophage phagocytosis	T-lymphocyte	B-lymphocyte	Cytokine	Humoral response	Cell-mediated response
Selenium ^{46,47}	Suppressed	Suppressed	Suppressed	CD8- and CD2-T cell decreased	Suppressed	Reduced leucocyte migration inhibitory factor	Suppressed	Suppressed
Zinc ^{66,74-78}	Suppressed	NK cell activity decreased	Suppressed	No effect on mitogen stimulation	Suppressed	IL-1, -6, -2R and INF- α decreased	Abnormal proportion of Ig, or no effect	Suppressed
Copper ¹¹⁴⁻¹¹⁷	?	Suppressed	Suppressed		Suppressed	TNF, IFN decreased	Suppressed	No effect
Cobalt	Suppressed	Suppressed	Suppressed	?	?	?	?	?
Manganese ^{158,159}	?	NK cell activity decreased.	Suppressed					
Molybdenum*	?	?	?	?	?	?	?	?
Chromium ¹³		Suppressed or stimulated	Suppressed or stimulated	Suppressed or stimulated	Suppressed or stimulated	Suppressed or stimulated	Suppressed or stimulated	Suppressed or stimulated
Nickel ¹⁵⁸	?	NK cell activity decreased	Suppressed	No change	No change	?	?	?

?, Not known; *Effects described are via creating deficiency of copper by molybdenum supplements.

visible at the cellular level. Products of lipid peroxidation can be toxic. When membranes are degraded, these toxins are released into the body. Glutathione affects at least four fundamental processes: liver detoxification, lymphocyte activation, viral transactivation and mitochondrial function. Patients with chronic hepatitis C have significant glutathione deficiency. Clinical and *in vitro* studies strongly support the use of antioxidants in persistent viral infection. Currently, the most important antioxidant that needs to be restored and maintained in AIDS patients is glutathione. It is possible that the basic mechanisms of oxidant/antioxidant viral activation/deactivation apply to other viruses also. The trace elements copper, manganese, selenium and zinc act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress. It is necessary to maintain a balance between the harmful pro-oxidant components produced and the antioxidant compounds that counter these effects²⁰⁻²². A delicate balance also exists for the redox trace elements such as copper, which can initiate free radical reactions but is also a cofactor of copper/zinc-superoxide dismutase, a free radical scavenging enzyme. Metal chelators, such as ceruloplasmin play an important role to contain the reactive copper ion. Selenium is most severely deficient in traumatized patients who need adequate supplementation during parenteral micronutrition to assist the free radical scavenging activity of glutathione peroxidase and the immune system. Mice deficient in selenium are more susceptible to infection with coxsackievirus, as well as with influenza virus. By linking HCV replication and pathogenesis to the selenium status and dietary oxidant/antioxidant balance of the host, the existence of a viral glutathione peroxidase (GPx) gene could help explain why HCV disease progression is accelerated by oxidant stresses such as alcoholism and iron overload²³. GPx is a prototypical eukaryotic selenoprotein, with the rare amino acid selenocysteine (Sec) at the enzyme active site, encoded by the UGA codon in RNA. Selenium-dependent GPx modules are encoded in a number of RNA viruses, including HIV-1, HIV-2, HCV, coxsackievirus B3 (B3V), and measles virus. Analysis of the sequences of multiple viral isolates reveals conservation of the putative GPx-related features, at least within viral subtypes or genotypes, supporting the hypothesis that these are functional GPx modules²⁴.

The immune system is altered in selenium-deficient animals, as is the viral pathogen itself. Sequencing of viral isolates recovered from selenium-deficient mice demonstrates mutations in the viral genome of both coxsackievirus and influenza virus. These changes in the viral genome are associated with increased pathogenesis of the virus. The antioxidant selenoenzyme, glutathione peroxidase-1, is found to be critically important, as glutathione peroxidase knockout mice develop myocarditis, similar to the Se-deficient mice, when infected with the benign strain of myocarditis²⁵. Abundant evidence demonstrates the antioxidant role of zinc.

Two antioxidant mechanisms have been proposed for zinc: zinc ions may replace redox active molecules, such as iron and copper at critical sites in cell membranes and proteins; alternatively, zinc ions may induce the synthesis of metallothionein, sulfhydryl-rich proteins that protect against free radicals²⁶. Selenium and copper concentrations in erythrocytes can improve the trace element dependent antioxidative status²⁰. Some unknown interactions between the essential micronutrients zinc and selenium on the one hand and zinc and redox metabolism on the other are key features of the cellular homeostatic zinc system²².

Interaction of trace elements with virion

Trace elements bind to the proteins that serve essential functions in a virion. The NS3 region of the hepatitis C virus encodes for a serine protease activity, which is necessary for processing of the nonstructural region of the viral polyprotein. The minimal domain with proteolytic activity resides in the N-terminus, where a structural tetradentate zinc-binding site is located. The ligands have been identified by X-ray crystallography as three cysteines (Cys97, Cys99, and Cys145) and one histidine residue (His149), which are postulated to coordinate the metal through a water molecule. Evidence for rearrangements of the metal coordination geometry induced by complex formation with an NS4A peptide cofactor have been reported²⁷. A unique structural feature of the HCV NS3 protein N-terminal domain has been reported to be the presence of a zinc-binding site exposed on the surface, subject to a slow conformational exchange process^{28,29}. A survey of isosteric replacements of the phosphonoalanine side chain coupled with a process of conformational constraint of a bisbenzimidazole-based, Zn(2+)-dependent inhibitor of HCV NS3 serine protease results in the identification of novel series of active compounds with extended side chains. However, Zn(2+)-dependent HCV NS3 inhibition is relatively insensitive to structural variations, but dependent on the presence of negatively charged functionality. This result is interpreted in the context of an initial electrostatic interaction between protease and inhibitor that is subsequently consolidated by Zn(2+), with binding facilitated by the featureless active site and proximal regions of the HCV NS3 protein³⁰. The matrix protein M1 of influenza virus has a peptide linker (M1Lnk). The pH-dependent conformational transition of M1Lnk strongly suggests that the inter domain linker region of M1 also undergoes a pH-dependent unfolding-refolding transition in the presence of Zn(2+). A small but significant portion of the M1 protein is bound to Zn(2+) in the virion, and the Zn(2+)-bound M1 molecule may play a special role in virus uncoating by changing the disposition of the N- and C-terminal domains upon acidification of the virion interior³¹.

Zinc binds to several other viruses, for example, Ebola and human papillomavirus type 16. VP30 is an essential activator of Ebola virus transcription. A conspicuous structural

feature of VP30 is an unconventional zinc-binding Cys (3)-His motif that stoichiometrically binds zinc ions in a one-to-one relationship. Substitution of the conserved cysteines and histidine within the motif leads to a complete loss of the capacity for zinc binding³². The E6 protein of human papillomavirus 16 has two putative zinc ion binding sites crucial for its function. Degenkolbe *et al.*³³ reported that a specific chelating agent, which functionally mimics a metallochaperone, stabilized the soluble monomeric form of E6 and inhibited multimerization *in vitro*. They have proposed that chelating agents of appropriate strength could assist zinc delivery to recombinant metalloproteins *in vitro* and may even destabilize existing agglomerates. Specific zinc-finger architecture is required for the nucleic acid chaperone function of HIV-1 nucleocapsid protein³⁴, while Wu³⁵ has reported that ZAS: C₂H₂ zinc finger proteins are involved in growth and development. Studies by Bergstrom *et al.*³⁶ have shown that polysulfonates derived from metal thiolate complexes are inhibitors of HIV-1 and various other enveloped viruses *in vitro*. HIV-1 nucleocapsid Zn (2+) fingers are required for efficient reverse transcription, initial integration processes and protection of newly synthesized viral DNA³⁷. HIV-1 Tat protein directly activates neuronal N-methyl-D-aspartate receptors at an allosteric zinc-sensitive site³⁸. McGrath *et al.*³⁹ have reported that human cellular nucleic acid-binding protein Zn²⁺ fingers support replication of HIV-1 when they are substituted in the nucleocapsid protein. Lee *et al.*⁴⁰ have described zinc-finger-dependent HIV-1 nucleocapsid protein-TAR RNA interactions.

GPx is the prototypical eukaryotic selenoprotein, with the rare amino acid selenocysteine at the enzyme active site, encoded by the UGA codon in RNA. *Molluscum contagiosum*, a DNA virus, has been shown to encode a functional selenium-dependent GPx enzyme. Using modifications of conventional sequence database searching techniques to locate potential viral GPx modules, combined with structurally guided comparative sequence analysis provides compelling evidence that selenium-dependent GPx modules are encoded in a number of RNA viruses, including potentially serious human pathogens like HIV-1, HIV-2, HCV, CB3V and measles virus. Analysis of the sequences of multiple viral isolates reveals conservation of the putative GPx-related features, at least within viral subtypes or genotypes, indicating that these may be functional GPx modules²³. By linking HCV replication and pathogenesis to the selenium status and dietary oxidant/antioxidant balance of the host, the existence of a viral GPx gene could help explain why HCV disease progression is accelerated by oxidant stresses such as alcoholism and iron overload²⁴.

Effect of trace elements on virus infections

During most viral infections the plasma levels of trace elements change, but it is not clear if this reflects changes

in the infected tissues also. Influence of trace elements have been studied in a large number of viruses belonging to different groups. Table 3 presents a list of viruses that have been studied with each trace element. The trace elements that have predominantly toxic effects on the body have been excluded from the present discussion.

Effect of selenium

Selenium is a trace element which is also essential for normal functioning of the immune system. Plant foods are the major dietary sources of selenium and also some meats and seafood. The amount of selenium in the soil determines its amount in the plant foods that are grown in that soil and the animals that feed on those grains or plants. Soils in some parts of the US have very high levels of selenium, while very low amounts are seen in some parts of China and Russia⁴¹⁻⁴⁴. Selenium deficiency is linked to Keshan disease, which is associated with an enlarged heart and poor heart function⁴⁵.

Selenium is an essential component of selenocysteine-containing protein. It is involved in most aspects of cell biochemistry and function and influences the immune system. It is an important part of antioxidant enzymes that protect cells against the effects of free radicals, which are produced during normal oxygen metabolism. The antioxidant GPx protects neutrophils from oxygen-derived radicals that are produced to kill ingested foreign organisms⁴⁶. As a constituent of selenoproteins, selenium is required for the functioning of neutrophils, macrophages, NK cells and T-lymphocytes. Selenium has also been associated with reduced apoptosis in animal models⁴⁶. In addition, adequate selenium may enhance resistance to infections through modulation of interleukin production and subsequently the Th1/Th2 response. Selenium supplementation up-regulates IL-2 and increases activation, proliferation, differentiation, and apoptosis of T helper cells. Elevated selenium intake may reduce cancer risk and may alleviate other pathological conditions, including oxidative stress and inflammation. Selenium appears to be a key nutrient in counteracting the development of virulence and inhibition of HIV progression to AIDS. It is required for sperm motility and may reduce the risk of miscarriage. Selenium deficiency has been linked to adverse mood states and some findings suggest that selenium deficiency may be a risk factor in cardiovascular diseases⁴⁷.

Effect of selenium on coxsackie B virus infection: The classical example of relationships between nutrition and viral infection is juvenile cardiomyopathy known as Keshan disease. It has been shown that selenium-deficient mice develop myocarditis when infected with a normally benign strain of coxsackievirus. Thus, a non-myocarditic strain of Coxsackie B virus (CBV) is converted to virulence when inoculated into selenium-deficient mice^{2,25}.

Table 3. Studies on the effect of trace metals on different virus infections

Virus	Trace metal*							
	Selenium	Zinc	Copper	Cobalt	Manganese	Molybdenum	Chromium	Nickel
Adeno					134, 135			
AMV			113					
CoxBV	2, 25, 48–52	49, 106		106				154, 155
EBV					139			
EMV				126, 127	138			
ECHO	63							
Ebola		32						
HAV		88, 89	64					
HBV	64	64, 73, 90–93	64	128	138			
HCV	64	23, 24, 27–30, 94–97	64, 117–119					
HIV	57–62	23, 34–40, 76, 78, 98–105	120			144		
HPV		30						
HSV	50, 63			122–125	140	144, 145		
HSV Bovine			114, 115			114	151	
Influenza	2, 25, 53–56					145		
IBRV			116			118	152, 153	
IPNV		107						
JEV					132, 133			
MCMV								156
Measles					129			
Polio	63	108	118		108, 130, 131			
Pox	50				136	145		
Rhino V		81–87						
SFV			121		138			
Sindbis		109–111						
Sandfly fever							150	
VEE					138			
WN			118					

*Reference number; AMV, Avian myeloblastosis virus; IBRV, Infectious bovine rhinotracheitis virus; CoxBV, Coxsackie B virus; IPNV, Infectious pancreatic necrosis virus; HPV, Human papilloma virus.

This conversion is accompanied by changes in the genetic structure of the virus so that its genome closely resembles that of other known virulent CBV strains. Similar alterations in virulence and genomic composition of CBV could be observed in mice fed with normal diet, but genetically deprived (knockout mice) of the antioxidant selenoenzyme GPx^{2,25,48}. Ilback *et al.*⁴⁹ have reported that concentrations of a number of trace elements in the serum and pancreas change preceding the development of pancreatitis during the early phase of CBV infection in female Balb/c mice.

Cermelli *et al.*⁵⁰ studied the antiviral effects of selenium compounds on coxsackievirus B5 replication. The inhibitory activity of selenite on viral replication is due to its toxicity following its interaction with thiols. Zinc is another inhibitor of selenite toxicity, which also counteracts the antiviral effect of selenite. A direct inhibitory effect of selenite on coxsackievirus replication may explain the efficacy demonstrated by this compound in the prophylaxis of Keshan disease. Mercury, a selenium antagonist is known to aggravate CBV virus infection⁵¹.

Beck and Matthews⁵² have reported that infection with myocarditic strains of CBV induces an inflammatory res-

ponse in the cardiac tissue. Heart damage is induced by immune response and not by the direct viral effects on the heart tissue. Chemokines are secreted during an infection in order to attract immune cells to the site of injury, and have been found to be important for the development of CBV-induced myocarditis. Further, a deficiency in selenium influences the expression of mRNA for the chemokine, monocyte chemo-attractant protein-1, which may have implications for the development of myocarditis in the selenium-deficient host. Expression of mRNA for IFN- γ is also greatly decreased in selenium-deficient animals⁵². Thus, a deficiency in selenium can have profound effects on the host as well as on the virus itself. How the alteration of immune response of selenium-deficient animals affects the development of the virulent genotype remains to be answered.

Effect of selenium on influenza virus infection: Selenocystamine inhibits influenza virus-associated RNA transcriptase, thus inhibiting the virus replication in eggs even when added up to 4 h after virus infection^{53,54}. It was suggested to be due to chelation of zinc by selenocystamine. Se-

lenium-deficient mice develop severe pneumonitis when infected with a mild strain of influenza virus A/Bangkok/1/79. The increased virulence observed in the selenium-deficient mice is due to mutations in the influenza virus genome, resulting in a more virulent genotype. The mutations occur mostly in the gene for the M1 matrix protein, an internal protein that is thought to be relatively stable. A total of 29 nucleotide changes are observed in this gene, and all 29 changes are identical in three separate isolates taken from three different selenium-deficient mice. In contrast, only one to three mutations are seen in the genes for the haemagglutinin or neuraminidase proteins, surface antigens that are known to be highly variable. Once the mutations have occurred, even hosts with normal nutritional status are susceptible to the newly virulent strain⁵⁵. The immune system is altered in selenium-deficient animals, as is the viral pathogen itself. These changes in the viral genome are associated with the increased pathogenesis of the virus^{2,47,56}.

Effect of selenium on HIV infection: HIV/AIDS-related malabsorption can deplete levels of many nutrients. Selenium deficiency is commonly associated with HIV/AIDS, and has been associated with a high risk of death from this disease and faster disease progression⁵⁷⁻⁵⁹. Selenium also may be needed for the replication of the HIV virus, which could deplete host levels of selenium⁵⁸.

Selenium supplementation may down-regulate the abnormally high levels of IL-8 and TNF- α observed in HIV disease, which has been associated with neurological damage, Kaposi's sarcoma, wasting syndrome, and increased viral replication⁶⁰. Taken together, these findings suggest a new mechanism through which selenium may affect HIV-1 disease progression. If one considers all nutrient factors that are associated with survival, only selenium deficiency is a significant predictor of mortality. The profound effect of selenium on disease progression may reflect its action in antioxidant defence systems as well as gene regulation^{60,61}. In combination with known cellular mechanisms involving selenium, viral selenoproteins may represent a unique mechanism by which HIV-1 monitors and exploits an essential micronutrient to optimize its replication relative to the host.

It is likely that several of the biological effects of selenium are linked with selenoprotein activity. Effects of the anti-oxidant selenoprotein GPx on the inhibition of HIV activation have been well documented. Hence, increased expression of this enzyme can stimulate viral replication and subsequent appearance of cytopathic effects associated with an acutely spreading HIV infection. The effects of GPx on both phases of the viral life cycle are likely to be mediated via its influence on signalling molecules that use reactive oxygen species. Similarly, selenium can alter mutagenesis rates in both viral genomes⁶². A comparison between the effects of selenium and selenoproteins on viral infections may yield new insights into the mechanisms of action of this element.

Effect of selenium on herpes simplex virus infection: Heart damage caused by herpes simplex virus (HSV-1) is significantly milder in selenium-deficient than in selenium-adequate mice. Therefore, the selenium status of the murine host selectively influences the degree of viral-induced myocarditic lesions⁶³. Selenium compounds show only limited activity against HSV-1 and vaccinia virus⁵⁰. This shows that selenium deficiency does not affect all viral infections to the same extent.

Effect of selenium on hepatitis virus infection: Kalkan *et al.*⁶⁴ have studied serum trace elements, including selenium in sera of patients with viral hepatitis (A, B, C, D, E) cases, and statistically compared with the controls. A significant decrease in selenium levels has been suggested in patients with HCV infection due to the defence strategies of organisms probably induced by substances like retinol or various carotenoids.

Effect of zinc

Zinc is found in a wide variety of foods including beans, nuts, certain seafoods, whole grains and dairy products. Oysters contain more zinc than any other food, but red meat and poultry provide a major part. Zinc is an essential mineral that is found in almost every cell. It stimulates the activity of approximately 100 enzymes^{65,66}. Zinc supports a healthy immune system and is needed for wound healing, the sense of taste and smell, and for DNA synthesis⁶⁶⁻⁶⁹.

Zinc deficiency most often occurs when zinc intake is inadequate or poorly absorbed, due to increased losses of zinc from the body, or when the body's requirement for zinc increases⁷⁰⁻⁷². Signs of zinc deficiency include growth retardation, hair loss, diarrhoea, delayed sexual maturation and impotence, eye and skin lesions, and loss of appetite.

Zinc mostly remains intracellular and participates in many physiological mechanisms. Liver functions like urea formation require the presence of zinc⁷³. Adequate zinc status is essential for T-cell division, maturation and differentiation, lymphocyte response to mitogens, programmed cell death of lymphoid and myeloid origins, gene transcription and biomembrane functions^{66,74}. The immune system is adversely affected by even moderate degrees of zinc deficiency. Severe zinc deficiency depresses immune function⁷⁵. Primary and secondary antibody responses are reduced in zinc deficiency and generation of splenic cytotoxic T-cells is reduced after immunization⁷⁶.

When zinc supplements are given to individuals with low zinc levels, the number of T-cell lymphocytes circulating in the blood increases and the ability of lymphocytes to fight infection improves. However, high dosages of zinc evoke negative effects on immune cells and cause alterations similar to those observed with zinc deficiency. Furthermore, when peripheral blood mononuclear cells are incubated with zinc *in vitro*, the release of cytokines such

as IL-1 and -6, TNF- α , soluble IL-2-receptor (IL-2R) and IFN- γ is induced.⁷⁷ Zinc deficiency can change immune functions from predominantly Th1 responses to Th2 response and adversely influence the course of several diseases, including AIDS⁷⁸. Zinc is the structural component of a wide variety of proteins, neuropeptides, hormone receptors and polynucleotides. Among the best known zinc-dependent enzymes and hormones are copper-zinc-superoxide dismutase, an enzyme component of the antioxidant defence system and thymulin, which is essential for the formation of T-lymphocytes. Thymulin is a hormone which is produced by the epithelial cells of thymus. Zinc also inhibits the production of TNF, which is implicated in the pathophysiology of cachexia and wasting in AIDS⁷⁶.

Zinc in metalloproteins, particularly viral (v) and cellular (c) zinc finger proteins (ZFP) plays a crucial role in cell proliferation, neovascularization, apoptosis and viral infection. Zinc acts as a key agent in controlling viral and proliferative diseases. It is known that zinc deficiency, resulting from exposure of culture cells to membrane-permeable Zn^{2+} chelators can induce apoptosis in virally transformed cells, while normal cells remain unaffected under these conditions. Apoptosis is possibly due to simultaneous inactivation of vZFP and cZFP, which are essential for maintenance of cellular and viral structure and which are activated in virally transformed cells. Zinc metalloproteins may be useful to prevent transmission of viral diseases⁷⁹. Natural resistance-associated macrophage protein 1 (Nramp1) is a proton/divalent cation antiporter exclusively expressed in monocyte/macrophage cells with a unique role in innate resistance to intraphagosomal pathogens. In humans, it is linked to several infectious diseases, including HIV. Alter-Koltunoff *et al.*⁸⁰ have demonstrated that the restricted expression of Nramp1 is mediated by the macrophage specific transcription factor and have identified Myc interacting zinc finger protein 1 (Miz-1) as a new interacting partner.

Effect of zinc on rhinovirus infection: In absence of effective treatment for common cold, zinc has been used in its prevention or treatment. However, the effect of zinc treatment on the severity or duration of cold symptoms is debatable. A study of over 100 human subjects indicated that zinc lozenges decreased the duration of colds by one-half, although no differences were seen in the duration of fevers or the level of muscle aches⁸¹. In another study of 213 patients with recent onset of cold symptoms, 108 patients received zinc therapy and 105 received placebos. It was shown that zinc taken as nasal gel within 24 h of the onset of cold is effective in shortening the duration of symptoms of common cold⁸². The study by Novick *et al.*⁸³ showed that free ionic zinc (Zn^{+2}) in saliva shortens the duration and severity of common cold symptoms. It has been proposed that Zn^{+2} complexes with proteins of critical nerve endings and surface proteins of human rhinovirus (HRV) and interrupts nerve impulse and blocks docking of HRV

on intracellular adhesion molecule-1 (ICAM-1) on the somatic cells and thereby interrupts HRV infection. Since leukocyte function associated antigen-1 (LFA-1) binds leukocyte to cells through ICAM-1 initiating inflammation, Zn^{+2} is expected to block LFA-1/ICAM-1 binding and thereby suppress inflammation⁸³. Zinc ions may be an important anti-inflammatory factor because they can block the docking of both HRV and LFA-1 with ICAM-1.

On the other hand several studies have shown that zinc has no effect on common cold. The study by Takkauche *et al.*⁸⁴ suggested that the intake of zinc is not related to the occurrence of common cold in the population. Similarly, Marshall⁸⁵ also suggested that treatment with zinc lozenges did not reduce the duration of cold symptoms. Another study examined the effect of zinc supplements on cold duration and severity in over 400 randomized subjects. In their first group, a rhinovirus was used to induce cold symptoms. The duration of illness was significantly lower in the group receiving zinc gluconate lozenges, but not in the group receiving zinc acetate lozenges. None of the zinc preparations affected the severity of cold symptoms during the first three days of treatment. In the second study, which examined the effect of zinc supplements on duration and severity of natural colds, no differences were seen between individuals receiving zinc and those receiving a placebo (sugar pill). It suggests that the effect of zinc may be influenced by the ability of the specific supplement formula to deliver zinc ions to the oral mucosa⁸⁶. Turner⁸⁷ evaluated the effectiveness of intranasal zinc gluconate for prevention of experimental rhinovirus infection and illness, and observed that zinc has no effect on total symptoms score, rhinorrhoea, nasal obstruction, or the proportion of infected volunteers, who developed clinical colds. More detailed studies are needed to determine whether zinc compounds have any effect on common cold.

Effects of zinc on hepatitis A virus infection: In an *in vitro* study it has been shown that zinc inhibits the replication of hepatitis A virus (HAV) in BSC-1 cells⁸⁸. However, studies by Bishop and Anderson⁸⁹ showed that zinc had no effect on the binding of HAV to the cultured cells.

Effects of zinc on hepatitis B virus infection: It is known that cytotoxic T-lymphocytes are responsible for viral clearance in chronic hepatitis B virus (HBV) infection. Zinc deficiency affects development of acquired immunity by preventing certain functions of T-lymphocytes (Table 2). The study by Gur *et al.*⁷³ suggested that zinc supplementation might improve hepatic encephalopathy by increasing the efficiency of the urea cycle. In this study they determined the hepatic zinc concentration in patients with chronic liver disease due to HBV and to ascertain the relationship between the severity of liver disease and hepatic zinc content. The results indicated that as the severity of liver damage increases, the hepatic zinc concentration decreases.

Transgenic mice that express the viral coat proteins of HBV in the liver display hepatocellular damage, inflammation, regeneration, hyperplasia and eventually neoplasia similar to those of people with chronic, active hepatitis caused by HBV infection. Hepatocellular regeneration, in the context of chronic injury and inflammation, is thought to expose dividing cells to excessive oxygen radicals, which is believed to lead to DNA damage and ultimately neoplasia. Metallothioneins are known to scavenge free radicals. Studies on mice that express excess metallothionein I (MT-I mice) and the HBV surface antigens (HBsAg) have shown that MT-I produces a beneficial effect on HBV-induced hepatitis⁹⁰.

Fota-Markowska *et al.*⁹¹ studied the serum zinc level dynamics in patients with acute hepatitis B and the early recovery periods. They observed significantly decreased serum zinc levels during hospitalization and that the differences in initial and early recovery periods compared to control groups were markedly at random. Ozbal *et al.*⁹² have reported that the mean baseline serum zinc, alanine aminotransferase and HBV-DNA values, histologic activity index, periportal necrosis and fibrosis scores are predictive of response to IFN- α 2b therapy. Their studies indicate that serum zinc levels may be used as a factor predicting response to IFN- α 2b therapy, and may help in identifying patients with a better chance of response. These findings are further supported by those of Selimoglu *et al.*⁹³, showing the relationship of serum zinc status to liver histopathology at the end of IFN therapy response. Kalkan *et al.*⁶⁴ have also shown the decrease in zinc levels in sera of patients with viral hepatitis (A, B, C, D, E).

Effect of zinc on hepatitis C virus infection: Nutritional status of zinc influences the effect of IFN on hepatitis C patients. This fact is supported by the study of Nagamine *et al.*⁹⁴, who observed that basal zinc levels in the serum are significantly lower in chronic hepatitis C patients. Administration of IFN- α to hepatitis C patients augments serum zinc reductions up to 40% in 8 h. Serum zinc level and zinc/copper ratio are higher in complete responders than in non-responders to IFN therapy at each time point.

T-lymphocytes and immunoregulatory cytokines play an important role in the host response to HCV infection. Takagi *et al.*⁹⁵ evaluated the synergistic effect of zinc supplementation on the response to IFN- α therapy in patients with intractable chronic hepatitis C. They showed that zinc supplementation enhances the response to IFN therapy, being most effective in infection with genotype 1b. In another study, Nagamine *et al.*⁹⁶ investigated the difference between two compounds of zinc, zinc sulphate and polaprezinc, on the effectiveness of IFN- α therapy. The data suggest that polaprezinc increases the therapeutic response of IFN- α . Grungreiff *et al.*⁹⁷ determined the clinical significance of the cytokines sIL-2R, IL-6, transforming growth factor (TGF)- β 1, neopterin, and of zinc in chronic HCV infection, in the serum of 16 patients before, during

and at the end of therapy with IFN- α , and after 6 months follow-up. The mean serum zinc concentrations were found to be slightly decreased in all three patient groups.

Effect of zinc on HIV infection: Zinc deficiency is the most prevalent micronutrient abnormality seen in HIV infection. Low levels of plasma zinc predict a three-fold increase in HIV-related mortality, whereas normalization has been associated with significantly slower disease progression and a decrease in the rate of opportunistic infections. Zinc deficiency characterized by low plasma zinc levels over time enhances HIV-associated disease progression, and low dietary zinc intake is an independent predictor of mortality in HIV-infected drug users. The amount of zinc supplementation in HIV infection appears to be critical, because deficiency, as well as excessive dietary intake of zinc, have been linked with declining CD4 cell counts and reduced survival⁹⁸. Further studies are needed to determine the optimal zinc supplementation level in HIV-infected patients.

HIV replicates preferentially in Th-0 and Th-2 cells but not in Th-1 cells, which contain more zinc⁷⁸. This may be because the zinc ion is known to inhibit intracellular HIV replication⁷⁸. Zinc is involved in the replication of the HIV virus at a number of sites⁹⁹. In HIV infection, serum level of zinc is frequently diminished. Wellinghausen *et al.*¹⁰⁰ studied the zinc level in 79 HIV-1 seropositive patients and found zinc deficiency in 23% of the patients associated with a low CD4 cell count, high viral load and increased neopterin and IgA levels. Earlier, Reich and Church¹⁰¹ had reported zinc deficiency in HIV-infected adults and suggested that zinc may act as an antiviral agent. Following oral zinc treatment, six out of 13 patients showed normal serum zinc level and only two had increased CD4⁺ cell numbers. Studies by Koch *et al.*¹⁰² have shown severe zinc deficiency in 29% and borderline levels in an additional 21% of AIDS patients. Zinc levels were not associated with the length of HIV seropositivity, CD4 count and degree of malnutrition. The mitogenic effect of zinc on lymphocyte proliferation response has been observed in HIV-1-positive individuals. Zinc treatment of PHA stimulated peripheral blood mononuclear cell cultures has been shown to enhance ³H-thymidine incorporation significantly in both asymptomatic and symptomatic groups. A decreased percentage of apoptotic cells could be identified in cell cultures from HIV-1 positive individuals submitted to zinc treatment compared to cells treated only with PHA¹⁰³. Extracellular matrix zinc plays an important role against the HIV infection. The zinc-bound form of thymulin (active thymulin, Zn FTS) is strongly reduced in stage IV of the disease with decrease in CD4⁺ cell count and zincemia values and increase in unbound form of thymulin (inactive, FTS). *In vitro* addition of zinc to plasma sample induces a recovery of the thymulin active form, suggesting low zinc bioavailability as the cause of impaired thymic functions with consequent CD4⁺ depletion¹⁰⁴.

The HIV-1 transcriptional regulatory protein, Tat is a pleiotropic factor that represses expression of the human manganese-superoxide dismutase. Tat increases oxidative stress, as shown by decreased glutathione and NADPH levels. These changes enhance proliferation and apoptosis and alter the activity of zinc thiolate containing proteins such as Sp-1. The zinc chelator TPEN sensitizes HeLa-tat cells to apoptosis. In these cells binding of the zinc-containing factor Sp1 to its DNA sequence is higher than in parental cells. It is observed that Sp-1 DNA interaction decreases more rapidly in the HeLa-tat cells after TPEN treatment. Tat protein, via direct or indirect mechanism, increases proliferation, sensitizes cell to apoptosis, and changes the conformation of Sp-1, affecting its ability to bind to its cognate DNA sequence and to retain its zinc¹⁰⁵.

Effect of zinc on CBV infection: Infection with human CBV in the murine model results in viral replication and inflammation in the pancreas and myocardium. Infected mice develop a pronounced decrease in zinc concentration in the serum on day 1. On day 3, concentrations of several trace elements, including copper, zinc, manganese, etc. show pronounced changes in both the serum and the pancreas⁴⁹. Earlier, Funseth *et al.*¹⁰⁶ observed increased levels of manganese, cobalt and copper and decreased levels of zinc in the myocardium. Determination of some of these trace elements in the plasma may be useful to indicate target tissue involvement in the early pre-inflammatory stage of an infectious disease. Some of these elements are important nutrients for the immune system, while others may be associated with the development of disease complications, such as cardiac arrhythmias. But the pathogenic significance of these changes in trace metals, preceding the development of pancreatitis or myocarditis is not known and warrants further studies.

Effect of zinc on infectious pancreatic necrosis virus infection: Zinc acts as an environment stressor and increases susceptibility of grouper (a fish) to infectious pancreatic necrosis virus (IPNV) infection. ZnCl₂ has been used to treat groupers before and after virus infection. Cumulative mortalities in the experimental group were 96–100% within 42 days. Only 5–15% mortality was observed in most of the groups that were exposed to zinc or virus infection alone. The study indicates that an IPNV with only low pathogenicity could cause high mortality in groupers when combined with zinc¹⁰⁷.

Effect of zinc on polio virus infection in vitro: Marchetti *et al.*¹⁰⁸ have investigated the inhibitory activity of different milk proteins on poliovirus infection in Vero cells. Viral cytopathic effect was prevented by the lactoferrins in a dose-dependent manner. Further experiments were carried out in which lactoferrins fully saturated with ferric, manganese or zinc ions were added to the cells during different phases of viral infection. All lactoferrins were able to pre-

vent viral replication, but the strongest inhibition was with zinc-lactoferrin which is the sole compound capable of inhibiting a phase of infection subsequent to virus internalization into the host cells.

Effect of zinc on other viral infections: Infection of many cultured cell types with Sindbis virus triggers apoptosis through a commonly utilized caspase activation pathway. The results of Lin *et al.*¹⁰⁹ suggest that Sindbis virus may activate apoptosis by reducing intracellular zinc superoxide dismutase levels, thus defining a novel redox signalling pathway by which viruses can trigger cell death. In an earlier study, zinc ions were reported to inhibit the replication of Sindbis virus in chicken embryo fibroblast cultures¹¹⁰. Bergstrom *et al.*¹¹¹ have shown that sodium 2-mercaptoethanesulfonate-Zn(II) complex has modest antiviral activity against vesicular stomatitis virus respiratory syncytial virus, Junin virus and Tacaribe virus, but is cytotoxic.

Effect of copper

Copper is essential for a variety of biochemical processes and is needed for certain critical enzymes to function in the body. Copper is also involved in the functioning of the nervous system, in maintaining the balance of other useful metals in the body such as zinc and molybdenum, and other body functions. The main source of copper is through diet and is present in mineral-rich foods like vegetables, legumes, nuts, grains, fruits and chocolate. Copper is a natural element found in the earth's crust and the surface water. Groundwater that is used for drinking purposes also contains copper. Certain foods and water kept for an extended period of time in copper ware may contain copper transferred from their surface. Copper is an integral part of many important enzymes involved in a number of vital biological processes (Table 1). Although normally bound to proteins, copper may be released and become free to catalyse the formation of highly reactive hydroxyl radicals that have capacity to initiate oxidative damage and interfere with important cellular events. Zinc removes copper from its binding site, where it may cause free radical formation¹¹².

Effect of copper on avian myeloblastosis virus infection: Cupric complexes inhibit DNA synthesis catalysed by avian myeloblastosis virus (AMV) reverse transcriptase. The inhibitory effect is seen even after initiation of polynucleotide synthesis. Infection of the one-day-old chicks with AMV pretreated with cupric complexes prevents symptoms of leukaemia due to virus inactivation¹¹³.

Effect of copper on HSV infection: Arthington *et al.*¹¹⁴ have demonstrated the effect of copper deficiency on acute-phase protein concentrations, superoxide dismutase activity, leukocyte numbers, and lymphocyte proliferation in heifers (young cow) inoculated with live bovine HSV-1. Their data

indicate that copper deficiency alters the acute-phase protein response to viral infection and may affect lymphocyte responsiveness to mitogen stimulation. Studies indicate that copper has no significant role during some of the infections. It has been suggested that hypocuparaemia and hypocuprosis are not consistent features of Border disease and thus have no aetiological significance¹¹⁵.

Effect of copper on infectious bovine rhinotrachitis virus infection: Data reported by Gengelbach *et al.*¹¹⁶ indicate that dietary levels of copper can affect body temperature and feed intake responses of calves to intranasal inoculation with live infectious bovine rhinotrachitis virus disease by affecting plasma TNF and other cytokines.

Effect of copper on hepatitis virus infection: Serum concentration of copper plays an important role in the interrelation among immunoglobulins IgA, IgM and IgG in subjects with various forms of liver diseases¹¹⁷. Copper accumulation in fibrotic livers caused by chronic HCV infection may contribute to hepatic injury. Hatano *et al.*¹¹⁸ have shown that the hepatic copper contents increased with the progression of hepatic fibrosis and concluded that the presence of copper may enhance HCV infection. The study by Pramoolsinsap *et al.*¹¹⁹ on serum levels of copper in young adult patients in the early icteric phase of acute HBV infection has shown significantly elevated serum copper levels indicating alteration of copper metabolism during the acute icteric phase of uncomplicated hepatitis. Kalkan *et al.*⁶⁴ have studied serum trace elements, including copper in sera of patients with viral hepatitis (A, B, C, D, E) cases and the controls. They have shown elevation in copper levels and have suggested that this probably resulted from defence strategies of organism and induced by hormone-like substances. The role of copper during hepatitis virus infection needs further investigations.

Effect of copper on retrovirus infection: Role of murine acquired immunodeficiency syndrome (MAIDS) on the mineral status of liver, heart and muscle has been investigated in C57BL/6 mice. Retrovirus infection which has not proceeded to murine AIDS results in a significant increase in heart Cu and Zn concentration compared with uninfected mice. Early retrovirus infection alters tissue micronutrient levels, and may thus contribute to immunological changes¹²⁰.

Effect of copper on Semliki Forest and other viral infection: Treatment of chicken embryo fibroblast tissue cultures with copper, nickel and cobalt salts enhances the plating efficiency of Semliki Forest virus. This augmented plaque formation may be due to a higher adsorption rate of virions to the cell surface under the influence of the transition metal ions. The plating efficiency of West Nile virus in chicken-embryo fibroblasts and, to a lesser degree, of poliovirus type 1 and 2 in KB-cells is also enhanced by copper sulphate¹²¹.

Effect of cobalt

Cobalt is an important constituent of vitamin B₁₂, which is needed to maintain normal bone marrow function for producing erythrocytes. The food sources of cobalt are meat, dairy products and green leafy vegetables. Cobalt chelates act as antiviral agents.

Effect of cobalt on CBV infection: CBV infection may result in viral replication, subsequent inflammation and changed trace element levels in the myocardium. Funseth *et al.*¹⁰⁶ studied trace element levels in the plasma and heart of adult male A/J mice during the pre-inflammatory stage of CBV myocarditis for cobalt, copper, manganese, selenium, zinc, etc. In the heart, the levels decrease for cobalt and selenium and are increased for manganese and copper. Decreased levels of zinc were noted in the plasma, whereas increased levels of manganese, cobalt and copper were seen. Determination of some of these trace elements in the plasma may be a useful indicator of target tissue involvement in the early pre-inflammatory stage of an infectious disease. Some of these elements may be associated with the development of disease complications, such as cardiac arrhythmias¹⁰⁶.

Effect of cobalt on HSV infection: Vellema *et al.*¹²² investigated the effect of cobalt supplementation on the immune reactivity in vitamin B₁₂-deficient lambs by comparing the humoral and cell-mediated immune responses against bovine HSV-1 and *Mycobacterium paratuberculosis*. The results demonstrated a significantly lower lymphoblastic response in non-supplemented lambs compared with supplemented ones. No differences were found in total and differential white blood cell counts, in total protein, albumin, alpha-, beta- and gamma-globulin and in antibody production against bovine HSV-1.

Cobalt(III) Schiff base complexes have been shown to inhibit the replication of ocular herpes virus¹²³. The study by Asbell *et al.*¹²⁴ shows that all CTC series of cobalt chelate complexes inhibit HSV-1 replication *in vitro*, CTC-96 being best. Topical CTC-96 application is effective in diminishing the symptoms of disease and corneal surface virus. Schwartz *et al.*¹²⁵ reported that the CTC series of cobalt chelates display *in vitro* and *in vivo* activity against HSV-1 and HSV-2. Furthermore, CTC-96 inhibits plaque formation by varicella-zoster virus and vesicular stomatitis virus as efficiently as by HSV-1. Collectively, these experiments suggest that CTC-96 is a broad-spectrum inhibitor of infection by enveloped viruses and that it inhibits HSV-1 infection at the point of membrane fusion independent of the type of virus and cellular receptors present.

Effect of cobalt on encephalomyocarditis and other virus infection: Cobalt sulphate given orally does not inhibit the protective activity of New Castle disease virus against encephalomyocarditis virus induced mortality, but excess

cobalt inhibits the protective activity of poly I/poly C against encephalomyocarditis virus-induced mortality¹²⁶. Another study has reported cobalt deficiency in young calves suffering from tick-borne fever virus infection¹²⁷.

Effect of cobalt on hepatitis virus infection: Activity of cobalt-activated acylase in the serum was investigated in 120 children aged between 3 months and 15 years suffering from viral hepatitis. No differences were found in the mean values of acylase activity¹²⁸.

Effect of manganese

Manganese is an antioxidant nutrient and is important in the breakdown of amino acids and the production of energy. It is essentially required for the metabolism of vitamin B-1, C and E and for activation of various enzymes which are important for proper digestion and utilization of foods. Manganese acts as a catalyst in the breakdown of fats and cholesterol and also helps in the nourishment of the nerves and the brain. It is necessary for normal skeletal development and maintains sex hormone production. The best natural sources of manganese are whole grains, cereal products, nuts and green leafy vegetables. Dairy products, meat, fish and poultry are poor sources. A deficiency can cause poor reproductive performance, growth retardation, abnormal formation of bone and cartilage and an impaired glucose tolerance.

Effect of manganese on measles virus infection: Measles virus infection of B-cells results in marked alterations in proliferation and immunoglobulin production. The mitochondrial protein, manganese superoxide dismutase (MnSOD) is upregulated in B-cells during measles virus infection. Intracellular MnSOD inhibits proliferation of B-cells and decreases the titre of virus produced from infected cells. MnSOD may play an important role in the alteration of immune function during the infection of B-cells with measles virus¹²⁹.

Effect of manganese on polio virus infection: Marchetti *et al.*¹⁰⁸ have shown that lactoferrin saturated with manganese inhibits the replication of poliovirus in Vero cells. Viral RNA-dependent RNA polymerases exhibit great sequence diversity. Only six core amino acids are conserved across all polymerases of positive-strand RNA viruses of eukaryotes. Arnold *et al.*¹³⁰ have analysed the divalent cation specificity of poliovirus RNA-dependent RNA polymerase, 3D(pol). In the presence of Mn(2+), 3D(pol) activity is increased by greater than ten-fold relative to that in the presence of Mg(2+). The ability of 3D(pol) to catalyse RNA synthesis *de novo* is also stimulated approximately ten-fold using Mn(2+), and the enzyme is capable of utilizing a DNA template for primer-independent RNA synthesis. While exploring the function of conserved residues, aspara-

gine 297 in the prototypic poliovirus polymerase 3D(pol), Crotty *et al.*¹³¹ have identified three viable mutants with noncanonical amino acids at this conserved position. The viruses exhibited Mn(2+)-dependent RNA replication and viral growth. The finding that strictly conserved residues in the nucleotide-binding pocket of the polymerase can be altered in a manner that supports virus production, suggests that drugs targeting this region of the enzyme will still be susceptible to the problem of drug-resistant escape mutants.

Effect of manganese on Japanese encephalitis virus infection: Japanese encephalitis virus (JEV) infection is commonly associated with inflammatory reaction and neurological disease that occurs in the infected animals and humans. Reactive oxygen species have been implicated as a critical mediator for inflammation and disease. Liao *et al.*¹³² have investigated the change in redox potential in glial cells following JEV infection. JEV infection induces the generation of superoxide anion and nitric oxide in rat cortical glial cells. Manganese superoxide dismutase, but not copper/zinc superoxide dismutase is activated by JEV infection. In addition, the increased superoxide dismutase activity is also apparent in acutely or persistently JEV infected continuous cell lines. These results suggest that cellular factors regulating oxidative pathway may play roles in responding to JEV infection. Uchil and Satchidanandam¹³³ have reported that *in vitro* the JEV replication complex synthesizes viral RNA utilizing a semiconservative and asymmetric mechanism. Among divalent cations, Mg(2+) is essential and exhibits cooperative binding for its two replicase-binding sites. Mn(2+), despite having sixfold higher affinity for the replicase, elicited only 70% of the maximum Mg(2+)-dependent activity, and deficit of either cation lead to the synthesis of incomplete RNA products.

Effect of manganese on adenovirus infection: Adenovirus gene therapy is a promising tool in the clinical treatment of many genetic and acquired diseases. However, it also causes pathogenic effects in organs such as the liver. The redox-sensitive transcription factors AP-1 and NF-kappa B have been implicated in these effects. Zhang *et al.*¹³⁴ have studied the mechanisms of adenovirus-mediated AP-1 and NF-kappa B activation and the possible involvement of oxidative stress in adenovirus transduction. For this, rats were injected with either replication-defective recombinant adenovirus with DNA containing the cytomegalovirus promoter region only (AdCMV), adenovirus containing human MnSOD cDNA (AdMnSOD), or the vehicle. Compared to the vehicle and AdCMV transduction, MnSOD gene transfer yielded a fivefold increase in liver MnSOD activity 7 days postinjection. MnSOD overexpression abolishes this activation. Glutathione/glutathione disulphide ratios are decreased by adenovirus transduction and restored by MnSOD overexpression. These data indicate that cellular transduction by recombinant adenovirus stimulates AP-1

DNA binding activity. The results suggest that MnSOD overexpression decreases AP-1 DNA binding activity by regulating intracellular redox status, with the possible involvement of Ref-1 in this redox-sensitive pathway. Overexpression of MnSOD has been postulated as one possible mechanism of protection from oxidative damage and free radicals. Cullen *et al.*¹³⁵ have reported that enforced expression of MnSOD by adenovirus transfection in the rapid growing cell line MIA PaCa-2 increases MnSOD immunoreactivity and MnSOD activity and decreases growth rate.

Effect of manganese on pox virus infection: Martins and Shuman¹³⁶ have suggested that the baculovirus and pox virus triphosphatases are a distinct lineage within the metal-dependent RNA triphosphatase family. Synergistic activation of the lymphoid enhancer factor (LEF)-4 triphosphatase by manganese and magnesium suggests a two-metal mechanism of gamma phosphate hydrolysis.

Effect of manganese on encephalomyocarditis, semliki forest and Venezuelan equine encephalitis virus infection: Seth *et al.*¹³⁷ have investigated the impact of exposure of toxic doses of manganese on encephalomyocarditis and Semliki Forest virus, and Venezuelan equine encephalitis virus infection. Pretreatment with a single oral dose of manganese increased the susceptibility of mice to a sub-lethal infection of these viruses as observed by increased severity of symptoms and mortality. An early onset of virus infection was found in brains of manganese-treated animals.

Effect of manganese on HBV infection: Urban *et al.*¹³⁸ investigated the metal ion preferences of HBV polymerase for both the protein-priming and reverse transcription activities of this enzyme and found that reverse transcription of HBV is dependent on magnesium, however, protein-priming is strongly favoured by manganese ions.

Effect of manganese on Epstein-Barr virus infection: During the course of acute Epstein-Barr virus (EBV) infection, there is a rise in oxygen radical production. As a consequence, the production of oxygen radical scavenger MnSOD is increased. Patients with acute EBV infections regularly develop autoantibodies against MnSOD that are able to inhibit the enzyme activity *in vitro*. Two main epitopes p(no15) and p(no30) of MnSOD show sequence homologies with EBV-encoded proteins. Thus, a molecular mimicry causes the occurrence of cross-reactive anti-MnSOD antibodies that are able to block the protective effects of MnSOD in a model for oxidative damage produced by xanthine/xanthine oxidase in EAhy926 endothelial cells¹³⁹. Thus, these autoantibodies may contribute *in vivo* to clinical symptoms by accumulation of toxic oxygen radicals.

Effect of manganese on HSV infection: *In vitro* bypass of damaged DNA by replicative DNA polymerases is usually

blocked by helix-distorting or bulky DNA lesions. Villani *et al.*¹⁴⁰ have reported that substitution of the divalent metal ion Mg²⁺ with Mn²⁺ promotes quantitative replication of model DNA substrates containing the major cis-platin or N-2-acetylaminofluorene adducts by the catalytic subunit (UL30) of the replicative DNA polymerase of HSV. The ability of Mn²⁺ ions to confer bypass of bulky lesions was not observed with other replicative DNA polymerases of the B family, such as bacteriophage T4 or delta polymerases. Manganese induced a conformational change in the structure of UL30 bound to the platinated substrate. Taken together, the latter findings suggest a mechanism by which manganese might allow UL30 to efficiently promote translesion DNA synthesis *in vitro*.

Effect of molybdenum

Molybdenum is an essential nutrient needed in trace amounts by animals and humans. Tissue content of molybdenum is low, with the highest concentrations in the liver, kidney, adrenal gland and bone. Molybdenum forms oxides and is a component of a pterin coenzyme essential for the activity of xanthine oxidase, sulfite oxidase, and aldehyde oxidase. These enzymes share a common 'molybdenum cofactor'. Food sources of molybdenum are legumes, cereals, organ meat and leafy vegetables. The concentration of molybdenum in plants is directly related to its concentration in the soil. Hard water is an important source for some people¹⁴¹⁻¹⁴³.

Effect of molybdenum on IBRV infection: The effects of supplementing a diet deficient in molybdenum on phagocytic cell function and disease resistance of calves inoculated intranasally with live IBRV have shown that dietary levels of molybdenum and copper can affect body temperature and feed intake responses to disease by affecting TNF and other cytokines¹¹⁶.

Effect of molybdenum on HSV infection: Molybdenum induces copper deficiency. Ceruloplasmin, a copper-dependent acute phase protein, increases after challenge in control but not in copper-deficient heifers (young cow). Erythrocyte SOD activity is reduced in copper-deficient heifers. The challenge with bovine HSV-1 has no effect on SOD activity. The lymphocyte proliferative response to PHA stimulation is decreased in copper-deficient heifers following bovine HSV-1 challenge. No difference is detected when lymphocytes are stimulated with Concanavalin A or pokeweed mitogens¹¹⁴. Therefore, it is possible that molybdenum-induced copper deficiency can alter the acute phase protein response to viral infection, which may affect lymphocyte responsiveness to mitogen stimulation.

Effect of molybdenum on retrovirus infection: Inouye *et al.*¹⁴⁴ have reported that a novel heteropolyoxomolybdate

(PM-104) is associated with potent anti-HIV activity by interfering with virus adsorption and/or penetration into the cells. It also blocks the replication of HSV-1 and HSV-2. They have suggested that the antiviral properties of PM-104 could be attributed to the combined effect of europium atoms and its peculiar three-dimensional anion structure. In an interesting study, a screening for inhibitors of HIV-1 among various types of isopolyoxomolybdates and heteropolyoxomolybdates was carried out using an *in vitro* assay system measuring the cytopathogenicity of HIV-1 in CD4⁺ human MT-4 cells. It was found that a novel heteropolyoxomolybdate (PM-104) is associated with potent anti-HIV-1 activity. PM-104 interferes with virus infection during early stages of adsorption and/or penetration into the cells. In addition to the cytopathic effect of HIV-1 on MT-4 cells, syncytium formation between mock-infected MOLT-4 cells and MOLT-4 cells chronically infected with either HIV-1 or HIV-2 is also suppressed by PM-104. The antiviral properties of PM-104 could be attributed to the combined effect of europium atoms and its peculiar three-dimensional anion structure.

Effect of molybdenum on other viral infections: Tonew *et al.*¹⁴⁵ have reported significant antiviral activity of bis cyclopentadienyl titanium dichloride *in vitro* against a number of enveloped DNA and RNA viruses, for example, inhibition of vaccinia, herpes virus, orthomyxoviruses, paramyxovirus and rhabdovirus. The compound bis cyclopentadienyl molybdenum dichloride has no antiviral action on vaccinia and influenza viruses. Furthermore, PM-104 also blocks the replication¹⁴⁴ of HSV-1 and HSV-2.

Effect of chromium

Chromium is a naturally occurring metal found commonly in the environment in trivalent (Cr III) and hexavalent (Cr VI) forms. Cr(VI) compounds have been declared as a potent occupational carcinogens among workers in chrome plating, stainless steel, and pigment industries¹⁴⁶. On the contrary, Cr(III) salts such as chromium polynicotinate, chromium chloride and chromium picolinate are used as micronutrients and nutritional supplements and have been demonstrated to exhibit a significant number of health benefits in animals and humans. Various cells of animals have the capacity to reduce toxic Cr(VI) to non-toxic Cr(III)¹⁴⁷. Food sources of chromium are fruits, vegetables, vegetable oils, whole grains, seeds, brewer's yeast, etc. Chromium plays an important role in glucose and cholesterol metabolism and is thus essential to man and animals. Chromium is an essential nutrient required by the human body to promote the action of insulin in body tissues, so that the body can use sugars, proteins and fats. Chromium picolinate has been used to control blood sugar in diabetes and may reduce cholesterol and blood pressure levels. Chromium increases insulin binding to cells, insulin receptor number

and activates insulin receptor kinase leading to increased insulin sensitivity. It also has beneficial effect on both muscle strength and body composition^{13,148,149}.

Effect of chromium on sandfly fever virus infection: Acute infection with sandfly fever virus reduces availability of circulating chromium, which may contribute to the altered glucose metabolism characteristic of acute infection even in the presence of elevated insulin levels and other hormonal changes¹⁵⁰.

Effect of chromium HSV infection: Arthington *et al.*¹⁵¹ have observed that chromium-supplementation does not alter stress responses of calves experimentally inoculated with bovine HSV-1. Rectal temperatures are elevated but are not affected by chromium treatment. Secretion of ACTH, cortisol or plasma TNF- α is not affected by chromium treatment, although clear circadian variation in ACTH and cortisol occurs. No difference is detected in the concentrations of trace minerals excreted daily in the urine, lymphocyte proliferative response to mitogen stimulation and neutrophil bactericidal function. The acute phase proteins, ceruloplasmin and fibrinogen, also are not affected by treatment or viral challenge. However, it will be interesting to study the effects of chromium on human herpes virus infection.

Effect of chromium on IBRV infection: Kegley *et al.*¹⁵² have reported that when steers are inoculated with IBRV intranasally, average daily gain from day 0 to day 80 is increased by supplemental chromium. Transportation of steers increased the ratio of neutrophils to lymphocytes. Supplemental chromium did not affect rectal temperature after the IBRV challenge or the antibody response to IBRV or porcine red blood cells. Supplemental chromium did not affect any immune response that was measured. According to another report, supplemental chromium has no effect on antibody response to IBRV, parainfluenza 3 and bovine respiratory syncytial virus. However, it enhanced the antibody titres of calves in response to the bovine viral diarrhoea vaccine. On the other hand, chromium did not show any significant effect on antibody responses as well as on antibody titres of calves following vaccination with *Pasteurella haemolytica*¹⁵³. These findings suggest that supplemental chromium can enhance humoral response of market-transmit-stressed calves, but its enhancement on vaccine efficacy is antigen-dependent and variable.

Effect of nickel

Nickel is an essential micronutrient. The best sources of nickel include oatmeal, legumes, nuts, cocoa, whole wheat bread, and some leafy vegetables such as kale and lettuce. Nickel is found in blood and tissues at consistent levels, and is also associated with DNA and RNA in amounts that suggest

physiological significance. Nickel is required for normal growth and reproduction in animals, and presumably in human beings as well. It appears to have a role in the modulation of the immune system and in development of brain.

Effect of nickel on CBV infection: Ilback *et al.*¹⁵⁴ investigated immunotoxic effect of a ten-week low dose administration of nickel chloride (NiCl₂) prior to CBV infection. This dose did not influence CBV-induced mortality. Seven days after inoculation, impulse-counting showed that the infection induced increase of ⁶³Ni in pancreas and heart. Nickel tends to increase spleen B- and T-cell activities, but thymocyte activity remained unaffected. The activity of spleen NK cells decreased, whereas there was an increased blood cell activity. The number of cytotoxic T-cells, helper T-cells and Mac 2+ cells in the lesions of heart decreases with the nickel treatment¹⁵⁴. These results suggest that nickel may contribute to the progression of target organ pathology in CBV infection-induced diseases of an auto immune and/or inflammatory character, such as diabetes and myocarditis.

A potentially toxic metal such as nickel can affect the magnitude of inflammatory lesions in the heart of CBV-infected mice. New target organs for nickel during this infection were the heart, pancreas and lungs in which inflammatory lesions were present. This increased uptake was correlated with the disturbed function of immune cells and an increased inflammatory reaction. Nickel has a direct effect on immune cells that resulted in changed natural killer cell activity and decreased mobilization of macrophages, CD4+ and CD8+ cells into the inflammatory lesions¹⁵⁵.

Effect of nickel on murine cytomegalovirus infection: When female C3H/HeJ or CD+ mice are infected with a sublethal dose of murine cytomegalovirus and then exposed to nickel chloride, enhanced mortality and a reduction in virus augmented NK cell activity are observed at doses as low as 10 mg NiCl₂/kg, i.m.¹⁵⁶.

Conclusion

Although it is widely recognized that essential trace elements are required for the differentiation, activation and performance of numerous functions of immune cells, the specific roles of these micronutrients remain largely undefined. New insights about the participation of zinc, selenium, iron and copper in the selection, maturation and early activation events of the immune cells have been obtained by judicious use of available tools in cell biology, molecular genetics and array technology. Randomly controlled clinical and community trials demonstrate that zinc supplementation can enhance immunocompetence and decrease the incidence and severity of some infections in individuals with diagnosed or suspected mild zinc defi-

ciency. These existing results inspire to evaluate the potential benefits of supplementation programmes with trace elements status as cost-effective means of reducing the risk of infectious diseases. Several trace elements have immunomodulatory functions and thus influence the susceptibility of a host to various viral infections. Some trace metals have antiviral activity, while others may alter the genome of the viruses enhancing their virulence. Further research, both basic and applied, is needed to assess properly the possible role of malnutrition in contributing to the emergence of novel viral diseases.

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ACKNOWLEDGEMENTS. U.C.C. was an Emeritus Scientist of the Council of Scientific and Industrial Research, New Delhi. R.S. is a Senior Research Fellow of the Indian Council of Medical Research, New Delhi.

Received 9 February 2004; revised accepted 31 July 2004