



20. Jahrestagung der GMS im Rahmen des Second International FESTEM Symposium on Trace Elements and Minerals in Medicine and Biology, 13. – 15. Mai 2004, in Neuherberg bei München

Die 20. Jahrestagung fand dieses Jahr als Teil des Spurenelement- und Mineralstoff Symposiums in Medizin und Biologie der Vereinigung der europäischen Spurenelementgesellschaften (FESTEM) statt. Während dieses 2 ½ tägigen Symposiums wurden Arbeiten von allen beteiligten Gesellschaften präsentiert, wobei vor allem der zweite Tag für Beiträge aus dem Bereich der GMS gedacht war. An diesem Tag fanden auch die

Präsidiumssitzung und danach die Mitgliederversammlung der GMS statt.

Für das wissenschaftliche Programm waren attraktive Themen vorgeschlagen worden, zu denen insgesamt zehn renommierte Wissenschaftler als Redner eingeladen waren.

Die Themen waren

- A. Analyse/Diagnose
- B. Molekularbiologie: Mechanismen und Auswirkungen
- C. Physiologische Prozesse, Gesundheit des Menschen
- D. Pathologische Prozesse, Krankheiten beim Menschen
- E. Epidemiologie/Prävention/Intervention
- F. Ernährung
- G. Umwelt, Toxikologie
- H. Landwirtschaft und Tiermedizin

Die eingeladenen Redner und ihre Vortragsthemen waren

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| <p style="text-align: center;">Bo Lönnerdal University of California, Davis, CA Trace Element Nutrition of Infants</p> |
| <p style="text-align: center;">Hannelore Daniel Molecular Nutrition Unit, Technical University Of Munich, Germany, Hochfeldweg 2, D-85350 Freising-Weihenstephan Assessing the Molecular Functions of Zinc in Mammalian Systems by Transcriptome, Proteome and Metabolite Analysis</p> |
| <p style="text-align: center;">N.I.Kaletina I.M.Sechenov Moscow Medical Academy, Department of Toxicological Chemistry, Moscow, Russia Biological Complexes of Trace Elements and their Implication in Personalized Medicine</p> |
| <p style="text-align: center;">Berislav Momčilović Institute For Medical Research And Occupational Health, POB 291, Zagreb, CROATIA The Metabolic Response to the Idiorrhhythmic Dose-Rate Variability in Trace Element Dietary Intake</p> |
| <p style="text-align: center;">Wolfgang Maret Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, TX 77555 Zinc Coordination Environments in Proteins Determine Cellular Zinc Functions</p> |
| <p style="text-align: center;">Milan Vašák Institute of Biochemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland Advances in Metallothionein Structure And Function</p> |
| <p style="text-align: center;">Rita Cornelis University of Ghent, Laboratory for Analytical Chemistry, Proeftuinstraat 86, B-9000 Ghent, Belgium Modern Approaches of Trace Element Analysis</p> |
| <p style="text-align: center;">Pier Luigi Nimis Department of Biology, University of Trieste (Italy) Recent Advances in the Monitoring of Heavy Metals Deposition in Plants</p> |
| <p style="text-align: center;">John D. Bogden Department of Preventive Medicine & Community Health UMDNJ-New Jersey Medical School, Newark, NJ, USA 07103 Trace Elements and The Immune System: Experimental and Clinical Studies</p> |
| <p style="text-align: center;">J.J. García (*), E. Martínez-Ballarín, S. Millán-Plano, J.L. Allué, C. Albendea, L. Fuentes and J.F. Escanero Hospital Clinico Universitario, Servicio de Bioquímica avd. Gomez Laguna s/n, Zaragoza, Spain EFFECTS OF TRACE ELEMENTS ON MEMBRANE FLUIDITY</p> |

Die **Zusammenfassungen** der eingeladenen Vorträge finden Sie etwas weiter unten nach dem „Rahmenprogramm“.

Vollständigen Zugriff auf die **Abstracts aller Beiträge** erhalten Sie unter der Internetadresse [http:// www.gsf.de/wwwspec/abstracts-festem2.html](http://www.gsf.de/wwwspec/abstracts-festem2.html) .

Gabriel Bertrand Medaille

Wie schon 2001 auf dem 1. FESTEM Symposium wurde auch dieses Mal die Gabriel Bertrand Medaille verliehen.

Der diesjährige Preisträger war Bo Lønnerdal.

Er war von den wissenschaftlichen Beiräten/Komitees der Mitgliedsgesellschaften von FESTEM unter mehreren möglichen Preisträgern ausgewählt worden.

Ferner wurden drei Posterpreise verliehen.

Rahmenprogramm

Im Rahmen der Tagung fanden auch gesellige Abende statt, welche vom zwanglosen Treffen zum Kennenlernen (Icebreaker) im *Hofbräuhaus* München, einem Abendessen im historischen Restaurant *Spöckmeier* oder einer gemeinsamen Stadtführung im historischen Altstadt kern reichten. Ganz zum Abschluss traf sich ein Teil der Teilnehmer nochmals zum gemeinsamen Abendessen beim *Hofer - dem Stadtwirt*. Es war auch dafür gesorgt, dass während der gesamten Veranstaltungen das gesamte Programm des Münchner Tourismusbüros am Registrationsbüro individuell gebucht werden.

Zusammenfassungen der eingeladenen Beiträge

TRACE ELEMENT NUTRITION OF INFANTS

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Newborn infants are exposed to widely varying intakes of trace elements, but little is known about their ability to homeostatically adjust to these intakes. Recent discoveries of several metal ion transporters in the small intestine are likely to enhance our understanding of molecular mechanisms regulating trace element absorption. Iron absorption is regulated by divalent metal ion transporter 1 (DMT1) and ferroportin 1 (FPN1). Studies on human infants have shown that young infants cannot regulate Fe absorption, whereas older infants can. Our studies on infant rat pups show that there is no regulation of DMT1 and FPN1 at young age, but that this develops at older age. These findings may explain adverse effects of iron supplementation on growth in young human infants. Zinc absorption in the small intestine is regulated by ZnT1, ZnT2, ZnT4 and ZIP4 and Zn status affects the expression of these transporters in an attempt to achieve Zn homeostasis. Copper absorption is regulated by the Cu transporters Ctr1, Atp7A and Atp7B, and exposure to Cu at early age affects the expression and

cellular localization of these proteins, affecting Cu uptake and transport. Manganese concentrations of infant formulas are considerably higher than in breast milk. As Mn is absorbed in the small intestine by Fe transporters and Fe status usually is low, Mn absorption and retention is high. High Mn retention during infancy has in animal studies been shown to result in high brain Mn and neurodevelopmental impairment, similar to what is observed in ADHD children. Further studies are needed to explore whether there is such a correlation in human infants. The consequences of trace element interactions during infancy also need to be investigated in more detail.

**ASSESSING THE MOLECULAR FUNCTIONS OF ZINC IN MAMMALIAN SYSTEMS BY
TRANSCRIPTOME, PROTEOME AND METABOLITE ANALYSIS**

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Zinc is essential for the structural and functional integrity of cells and plays a pivotal role in the control of gene expression. To understand the role of zinc in cell systems, we have studied zinc deficiency effects in human HT-29 cells in culture and an animal model (rats) under force feeding conditions. Microarray analysis showed that a low intracellular zinc concentration caused major alterations in the steady-state mRNA levels of several hundred genes. Northern blot analysis and/or real-time RT-PCR confirmed the array results for most of the selected target genes. Regulated genes encoded mainly proteins involved in central pathways of intermediary metabolism and growth control but also of signalling, cell cycle control, vesicular trafficking, cell-cell interaction, cytoskeleton and transcription control. In zinc-deficient rats differential analysis of tissues including liver, brain, muscle, intestine and kidney demonstrated very specific changes in gene expression with only a very few genes showing identical alterations along the tissues. Proteome analysis performed in rat livers by 2D-PAGE followed by peptide mass fingerprinting via MALDI-TOF MS identified a large set of proteins with altered steady state expression level that not necessarily showed the same kind or regulation at the transcript level. However, studies on changes in metabolite levels as predicted based on transcriptome profiling confirmed that clusters of proteins involved in the same pathways indeed caused substantial changes in the corresponding hepatic metabolic pathways such as lipacidogenesis, lipid sequestration and patterns of fatty acids. In HT-29 cells zinc-deficiency caused alterations in the steady-state mRNA levels of 309 genes out of 10.000 targets encoding proteins of similar gene classes as found in the rat studies including 19 genes involved in transcription control with several zinc finger-containing transcription factors. The Kruppel-like factor 4 here showed the most pronounced changes at the mRNA and protein level. Our findings on zinc deficiency demonstrate that the molecular mechanisms by which cellular functions are altered at a low zinc status, occur via pleiotropic effects

on gene expression but the pattern of zinc-affected genes may represent a reference for defining the zinc regulon in mammalian cells.

BIOLOGICAL COMPLEXES OF TRACE ELEMENTS AND THEIR IMPLICATION IN PERSONALIZED MEDICINE

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Metabolism, transport, deposition and elimination of ions of metals are attributed to their ability to form complexes with endogenous and exogenous ligands. Intensity and specific features of the response of the organism to the imbalance of trace elements /TE/ or ligands /L/ are individual and depend on the genetic mechanisms of *metal-ligand* homeostasis /MLH/. The survey of developing technologies in tissue typing shows that in 2-3 years it will be technologically possible to determine the significant variations of TE levels' ratios and, in perspective, - to determine all such variations that correlate with genetic information obtained from DNA. *Individual elementogram is a dynamic indicator of MLH* that is used for further planning of the patients' personalized treatment.

We are developing an alternative way of creating new biologically active substance, which is based on the integral approach to the results of the experiments dealing with the dynamics of TE composition of the organism at specific pathology; with the changes in metal and ligand /drug/ properties during the formation of the complex; with the ability of cell membranes to recognize structures similar in composition to natural biomolecules; with the induction of correspondence in enzyme systems. We have synthesized and patented in the Russian Federation more than a 100 new bioactive substances that are made up on the principle of N-glycosilated and coordinated structures of Zn, Co, Cu, Ni, Fe, Mn with drug ligands. Summary "stress" and specific elementograms were obtained using ICP-MS method in the experimental modeling of various diseases in different biological objects. The specific elementogram correlates with blood biochemistry values and can be used as an additional diagnostic test. X-ray analysis of the synthesized complexes of TE with drugs revealed that their structures resemble the active centers of a number of enzymes. For example, the site of specificity of Zn biocomplex with metronidazol /Zn-MN/ is identical to the active center of carbonic anhydrase C in: coordination bond of Zn with three MN molecules via N³ of the imidazole ring, tetrahedral structure of the complex, the water molecule in the inner sphere of the complex. Binding sites of metal ions with different bioligands can "trap" superoxide anion radicals; suppress their formation by direct inhibition of the enzymes involved in the process of enzymatic production of reactive oxygen species; incorporate into hydrophobic core of membranes and reduce the fluidity of membrane lipids; comprise steric barriers for the transport of free radicals, i.e. reduce the rate of membrane peroxidation process. Tetrahedral structure of the complexes is complementary to the conformation of carbon backbone

chain of fatty acids of phospholipids. The library of pharmacological properties of TE biocomplexes is significantly enlarged compared to the ligands and their mechanical mixtures /antibacterial, fungicide, antitrichomonal, antioxidant, immunomodulating, wound healing, radioprotective properties/.

TE biocomplexes acquire the ability to restore the impairment of MLH caused by factors of various nature; they act at low and ultra-low concentrations /the phenomenon of the “restored activity” observed in substances upon the decrease of the dose/; they promote the development of compensatory adaptive reactions in liver, spleen and kidneys.

THE METABOLIC RESPONSE TO THE IDIORRHYTHMIC DOSE-RATE VARIABILITY IN TRACE ELEMENT DIETARY INTAKE

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Nutrition may be described as a series of time discrete dose-rate events in which a specific nutritional information evolves in time and space from one food intake till the next one. Idiorrhhythmic dose-rate (ID-R) feeding is a scrupulously defined model for the study of variability in a meal and nutrient partitioning, and in clear distinction to other generally intuitive descriptive concepts. The ID-R approach requires that average dietary Zn concentration (Modulo, M) over the entire experiment (Epoch, E) is kept constant across different groups, whereas idiorrhhythms ($I = nM/n^{th}$) involve offering the diet with n times the Modulo only every n^{th} day with Zn-deficient diet offered on other days. An animal receiving a standard Zn adequate diet of $12 \mu\text{g Zn g}^{-1}\text{d}^{-1}(M_{12})$ every third day separated by two days with no Zn in the diet would have $I = 3M_{12}/3 = 36 \mu\text{g Zn every } 3^{rd} \text{ day} = 36/3$. The ID-R yields a different metabolic response than that elicited by the classical dose-response feeding model, which is based on a continuous intake of a diet with a constant nutrient composition. The ID-R revealed that the metabolic efficiency of dietary Zn is not constant, but depends upon the ID-R of Zn intake, and could vary by as much as 50%. If the non-caloric nutrient Zn is in a short supply in the diet over a given period of time, it is better to indulge in Zn ample diet from time to time, than to moderate on a Zn deficient diet providing the same total amount of Zn all the time. The metabolic efficiency of bone zinc deposition was dependent on Zn modulo and Zn idiorrhhythm in a non-linear fashion, indicating that the observed difference is due to the partitioning of metabolizable Zn. The ID-R coupling of Zn dose to frequency shows a limited capacity of excessive dietary zinc to compensate for a previous deficient intake. The limit was three times standard Zn diet ($I = 36/3$) and five times high Zn diet ($I = 220/5$) for M_{12} and M_{48} over a 48 day long Epoch, respectively. Both M and I limits the capacity of extra Zn to maintain body growth and femur and incisors Zn concentration. Since the adequate amount of Zn can be recovered over a period of several days, Zn does not need to be present in our diet every day; with such "multi-day" Recommended Dietary Allowances (RDA's) it is possible to

develop a whole set of adequate diets and block *menues* beyond the current daily concept of RDA's. Appropriate ID-R coupling of Zn dosing to the frequency may either induce or bypass intestinal metallothionein induction, depending upon the particular M and I, and hence improve or worsen Zn absorption from the gastrointestinal tract. For the first time it was possible to study the interaction among the trace elements at the physiological level of their dietary intake, and to provide evidence how, due to the homeostatic regulation, RDA's and especially Safety Limits should be set in a range format and not as a single cut-off numbers. The current international programmes of the intermittent trace element supplementation are seriously flawed since they ignore the fact that any supplementation is *ipso facto* an ID-R situation and hence not arbitrary in the dosing schedule (I), in the total dose consumed (M), and in the length of supplementation (E). The ID-R with non-energetic nutrient Zn generates an M and I dependent cycling of body weight loss, when Zn deficient period is long enough, followed by a catch-up growth after Zn dosing. Apparently, ID-R of Zn is a potent external synchronizer to the growth which may have a clinical potential. In the real life situation, cyclical changes in the body weight gain and loss may represent an early sign of nutritional deficiency due to the subtle metabolic disturbances.

ZINC COORDINATION ENVIRONMENTS IN PROTEINS DETERMINE CELLULAR ZINC FUNCTIONS

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Estimates of the number of zinc proteins in humans are now possible and a functional annotation of the zinc proteome can begin (1). The *catalytic* and *structural* roles of zinc in hundreds of enzymes and thousands of so-called “zinc finger” protein domains have provided a molecular basis for the numerous biological functions of this essential element. Additional, *regulatory* functions of zinc/protein interactions are being recognized. They include roles of the zinc ion in signal transduction (2), in controlling the architecture of protein complexes (3), and in redox-active zinc sites, where the binding and release of zinc is under redox control (4). Moreover, a considerable number of proteins participate in cellular zinc homeostasis, e.g. membrane transporters and cellular storage, sensor, and trafficking proteins. These proteins have evolved with mechanisms to handle zinc ions rather specifically and selectively. They perform their functions with a remarkably modest set: One redox state of the zinc ion and nitrogen, oxygen, and sulfur ligands from the side chains of histidine, glutamate/aspartate, and cysteine. By permutation of the ligands in this set, biology has fully explored the functional potential of the zinc ion and established different coordination environments that modulate the chemical characteristics of the zinc ion, control the kinetics of its binding, and allow it to

be either metabolically active or inert. Insights into all these functions are building an understanding of why zinc is so critical for such a multitude of life processes.

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ADVANCES IN METALLOTHIONEIN STRUCTURE AND FUNCTION

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Metallothioneins (MTs) is a class of ubiquitously occurring low molecular weight cysteine- and metal-rich proteins possessing sulfur-based metal clusters. In these compounds, clusters are usually formed through the preferential coordination of d^{10} metal ions such as zinc, copper and cadmium by an array of closely spaced cysteine thiolate ligands. The preservation of these clusters in an increasing number of three-dimensional structures of these proteins signifies the importance of this structural motif. Much of our understanding of the biological actions of MTs has arisen from the studies of their chemical and structural properties. There is a large body of evidence for a number of vital roles for this pleiotropic protein. MTs are involved in sequestration of environmental toxic metals cadmium and mercury. They also play a significant role in the chemotherapy of certain cancers, both in the development of tolerance to chemotherapeutics and as an adjunct to reduce toxic side effects. Recent studies make it clear that MTs, the major intracellular zinc-binding proteins, play a critical regulatory role in zinc uptake, distribution, storage, and release. Apart from their role in zinc metabolism, a number of studies suggest their involvement in a number of biological processes, among others, protection against reactive oxygen species, adaptation to stress, protection against brain injury, antiapoptotic effects or regulation of neuronal outgrowth. Thus, in the postgenomic era it is becoming increasingly clear that MTs fulfils protean functions, the relative importance of which depends very much on specific requirements of the particular organism. This should not be unexpected, as their unique structural characteristics, potent metal binding, and striking chemical reactivity have bearing on numerous biochemical processes. The advances in the structure and function of MTs will be discussed.

MODERN APPROACHES OF TRACE ELEMENT ANALYSIS

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The latest trends in trace element analysis appear to be sophisticated refinements of plasma detection techniques, of calibration methods and of the validation of results. Parallel to this there is a continuous increase in elemental speciation analysis, with the development of advanced separation techniques, but as this is not the topic of this symposium, I will not dwell on it.

Whereas the old fashioned graphite furnace and even flame atomic absorption spectrometry still fulfill expectations in routine trace element analysis of clinical samples, plasma spectrometry (inductively coupled plasma atomic emission and –ICP mass spectrometry) has become the leading detection technique in research.

Nowadays trace element determinations in liquid samples such as serum and urine may often be performed after simple dilution by plasma spectrometry. Usually total trace element analyses in tissues request a digestion step. Plasma techniques do not require a complete mineralisation of the specimens. An acid or enzymatic treatment will suffice to solubilize the matrix as the final mineralisation occurs in the plasma at elevated temperatures (about 7 000 K).

A very economic and fast way of trace element analysis of solid samples exploits electro thermal vaporization (ETV) in a graphite oven, followed by the introduction of the vapors into the plasma of an ICP-MS. The main hurdle here is the calibration, because the matrix that is carried along strongly influences the signal detection. Internal calibration with a spike of a specific isotope of another element with similar mass is not effective. A convenient way to circumvent the problem is the continuous introduction in the gas- flow of a spike of the element to be determined enriched in one of the isotopes. This is evidently only applicable for multi-isotopic elements. It also may require high resolution sector-field CP-MS.

Measuring the very low concentrations in which trace elements occur in human serum using a quadrupole ICP-MS (resolution 1 mass) is often hindered by poly-atomic interferences creating isobars. This is , e.g., the case for Cr, V, ... In these cases high resolution sector-field MS is needed to ensure reliable data, albeit with reduced sensitivity when very high resolution is needed. A new way to substantially eliminate such poly-atomic interferences in quadrupole measurements consists of using a dynamic reaction cell. Prior to the introduction of the nebulized sample into the mass spectrometer, the

vapour is bombarded in this reaction cell with a suitable gas to annihilate poly-atomic masses. One example is the measurement of vanadium that occurs in serum at a concentration of only 10 ng/L. The naturally occurring ^{51}V (isotopic abundance 99,750) is heavily interfered by $^{35}\text{Cl}^{16}\text{O}$. This interference can be eliminated through bombardment with ammonia.

The use of laser ablation –ICP-MS for measuring trace elements in tissue or in electrophoresis gels opens interesting perspectives. Because calibration is not evident, many research centers are developing ingenious ways to solve this problem.

It may be interesting to quote that nowadays there is a trend to no longer determine actinides and fission products by measuring the radioactivity they emit, but through mass spectrometry of the isotopes. This is, of course, only possible with dedicated equipment.

Last but not least there is the rising use of electrospray mass spectrometry to measure the larger molecules trace elements sometimes form part of. Very well documented are the organo-arsenicals and organo-selenium compounds

RECENT ADVANCES IN THE MONITORING OF HEAVY METALS DEPOSITION IN PLANTS

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Human activities release large amounts of exchangeable elements into the environment and have become a major factor in altering biogeochemical cycles. Increasing body burdens of potentially toxic elements in organisms, even from remote regions of the Northern Hemisphere far from significant sources of local pollution, have underlined the importance of establishing reliable monitoring systems at different scales. A reliable appraisal of pollutant concentrations in such an extremely variable compartment as the atmosphere needs a statistical approach based on a large number of samples in both time and space. The high costs of establishing and managing automatic monitoring networks often limit the number of sampling stations and/or the number of pollutants considered. Thus, although very reliable, data from instrumental recording may be statistically weak and their integration with diffusion models cannot give reliable information about the deposition and impact of atmospheric pollutants on terrestrial ecosystems.

Biological accumulators are organisms that reflect the chemical content of their environment. Biological monitoring with accumulator organisms provides an essential adjunct to instrumental recording. Hundreds of studies carried out over the last 30 years confirm that several organisms can be used as reliable accumulators of airborne inorganic contaminants. The use of bioaccumulators is

comparatively cheap, enabling coverage of large and remote areas, and provides current and retrospective information on the integrated effects of atmospheric pollutants and other environmental factors. In order for biological monitoring to be widely accepted and profitably used, several techniques still need some efforts towards standardisation.

This paper concentrates on some basic methodological issues related to the use of bioaccumulators:

- 1) Data variability, both among and within species. Particular stress will be given to sampling and preparation of the material as a source of bias, and to the different responses which can be obtained by using different organisms in the same survey area.
- 2) Establishment of scales for the interpretation of metal concentrations in organisms in terms of environmental alteration (deviation from background conditions).
- 3) Problems related to sampling strategies and data quality assurance, with the main stress on the main source of bias in bioaccumulation studies, i.e. field work.

Each of these points will be illustrated with practical examples deriving from several case-studies.

TRACE ELEMENTS AND THE IMMUNE SYSTEM: EXPERIMENTAL AND CLINICAL STUDIES

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There is a substantial body of evidence that documents the vital role of the immune system in protecting against infectious diseases and cancer, as well as evidence that it also influences the occurrence and course of other chronic illnesses. Because of its large mass and high rate of cellular turnover, the immune system is a major user of essential micronutrients, including trace elements. Of the nine essential trace elements, there is considerable evidence that supports the importance of copper, iron, selenium, and zinc for maintaining immune functions. Some experimental and clinical evidence for the impact of these four trace elements, especially zinc, on immunity and infection will be presented. This evidence supports several general conclusions:

1. Patients with a severe trace element deficiency, for example of zinc in the disease acrodermatitis enteropathica, have substantially compromised cellular immunity and are at high risk for serious and life-threatening opportunistic infections.
2. Mild to moderate deficiencies can impair immunity, but the effects are often subtle.
3. Supplementation with trace elements to improve immune functions is most likely to be beneficial in those who have some degree of deficiency.
4. Supplementation with trace elements as single nutrients may lead to micronutrient imbalances, especially in populations with multiple underlying deficiencies. As in studies done in

Bangladesh, this may occur even with low dose supplementation, but is more likely to occur with higher doses.

5. Cross-sectional studies demonstrate a number of significant associations between trace element nutrition and various measures of immunity, but some of these may not be causal relationships.
6. Ingestion of high doses of zinc supplements for prolonged periods of time is likely to impair immunity by interfering with copper nutrition and possibly by other mechanisms.
7. Routine use of single nutrient trace element supplements by healthy people is unlikely to improve immunity, and may interfere with the beneficial effects of other micronutrients on immunity. However, there is evidence that routine ingestion of a multivitamin/mineral supplement that includes moderate quantities of essential trace elements can result in improvements in immune functions in older people.

EFFECTS OF TRACE ELEMENTS ON MEMBRANE FLUIDITY

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According to the Fluid Mosaic Model, a biological membrane is a two-dimensional fluid of oriented proteins and lipids. The lipid bilayer is the basic structure of all cell and organelle membranes, and proteins are inserted in different parts of the bilayer (1). Phospholipids are the major form of lipids in the eukaryotic cells. They are amphipathic molecules with hydrocarbon regions that tend to cluster together away from water and polar parts that like to associate with water. Phospholipids are asymmetrically distributed on the two sides of the membrane: phosphatidylethanolamine and phosphatidylserine are located preferentially on the inner leaflet and lecithin and sphingomyelin on the outer leaflet. The fatty-acid chains of phospholipids contain numbers of carbon atoms, mostly in the range 14-24. Some membranes, particularly plasmatic membranes, have a significant proportion of the hydrophobic molecule cholesterol. By contrast, the membranes of subcellular organelles, such as mitochondria or nuclei, rarely contain much cholesterol (2).

Fluidity is the quality of ease of movement and represents the reciprocal value of membrane viscosity (3, 4). In general, the term means a combination of different types of mobility of membrane components. These include: lateral diffusion of molecules in the plane of the membrane, transversal diffusion of molecules from one monolayer to the other, and flexibility of acyl chains (5). The modulators of lipid fluidity can be divided into chemical and physical effectors: a) the length and degree of unsaturation of the fatty acid chains; b) the nature of polar head groups, which influences the mobility of the hydrocarbon chains; c) the content of cholesterol; d) the level of membrane protein; e) the temperature; f) the presence of natural or synthetic amphipathic substances in the bilayer (6, 7). Fluid properties of biological membranes are essential for

numerous cell function including cell growth, solute transport, signal transduction and membrane-associated enzymatic activities (8-12). Even slight changes in membrane fluidity may cause aberrant function and pathological processes (13, 14).

Fluorescence polarization anisotropy of diphenylhexatriene derivatives, as well as electron paramagnetic resonance using fatty acids spin-label agents have been extensively used in the assessment of membrane fluidity in a variety of biological membranes (3). There are several evidences suggesting that trace elements may influence membrane fluidity. Incubation of human erythrocyte ghosts with Cu^{2+} ions decreased membrane fluidity, possibly because these cations may interact with phosphatidylcholine groups located in the outer leaflet (15). However, controversial results were obtained in copper-deficient rats (16, 17).

Although iron, the most abundant transition metal in human body, is needed for numerous physiological processes, e.g., oxygen transport, a mixture of hydrogen peroxide and ferrous salt may oxidize many different organic molecules because it generates hydroxyl radicals by the Fenton reaction. Polyunsaturated fatty acids, present in the membranes are particularly sensitive to the aggressive behaviour of unstable free radicals. Thus, iron is frequently used to induce lipid peroxidation and consequently, membrane rigidity, in a variety of biological membranes (18-21). Figure illustrates a time-course study carried out in our lab that demonstrates an increase in malondialdehyde and 4-hydroxy-alkenals concentrations, which indicates lipid peroxidation, and membrane rigidity in hepatic microsomes exposed to iron. These observations are in agreement with a significant reduction of polyunsaturated as well as a parallel increase of saturated fatty acids in hepatic mitochondrial and plasma membrane phospholipids of rats with chronic dietary iron overload (22).

Elevated concentrations of zinc ions modified lipid composition and fluidity in erythrocyte plasma membrane of carps (23). In addition, oral zinc supplementation increased platelet membrane microviscosity in Alzheimer's disease patients (24). By contrast, selenium supplementation could stabilize membranes against the rigidity due to aging (25), a protective action that is interpreted in terms of the activity of selenium containing glutathione peroxidase (26). Finally, Belagyi y cols. shown that the addition of chromium (VI) on eukaryotic plasma membranes increased rotational mobility of the 5- and 14-doxyl stearic spin probes, which indicates an increase in the membrane fluidity (27).

In addition to the effects of trace elements, *in vitro* exposure of membrane to toxic heavy metals may also disturb its fluidity. Thereby, using fluorescence polarization measurements, an increase in polarization and anisotropy, i.e., decreased fluidity, were obtained in erythrocyte ghosts and placental membranes treated with chloride salts of cadmium, mercury and lead (28, 29). These data suggest that the interaction of heavy metals with cellular membranes may contribute to explain, at least partially, the toxicity associated with these metals.

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Legend to the figure

Time-course studies of MDA+4-HDA concentrations (▼) and membrane fluidity (●) in rat hepatic microsomal membranes exposed to 0,2 mM FeCl₃, 1,7 mM ADP and 0,2 mM NADPH incubated at 37°C. Data were obtained from four independent experiments and expressed as the mean.

