Ineffectiveness of Nickel in augmenting the hepatotoxicity in protein deficient rats
Nutrición Hospitalaria, vol. 20, núm. 6, noviembre-diciembre, 2005, pp. 378-385
Grupo Aula Médica
Madrid, España

Available in: http://www.redalyc.org/articulo.oa?id=309225559018
Abstract

This study was designed to determine the toxic effects of nickel sulfate on the biochemical and elemental profile of liver in protein deficient rats. Nickel sulfate in the dose of 800mg/l in drinking water was administrated to Sprague Dawley (S.D) normal control as well as protein deficient rats for a total duration of eight weeks. The effects of nickel treatment and protein deficiency when given separately and in combination were studied on rat liver marker enzymes like Alkaline phosphatase (ALP), Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT) and also on the status of essential elements in rat liver. Protein deficient, Ni treated as well as combined protein deficient and nickel treated rats showed significant reductions in the body weight and hepatic protein contents as compared to normal control rats. Hepatic alkaline phosphatase activity and alanine aminotransferase showed a significant elevation in rats subjected to protein deficiency, nickel treatment and combined protein deficiency and nickel treatment. As regards to hepatic levels of aspartate aminotransferase a significant elevation was observed in protein deficient and nickel treated protein deficient animals. Nickel administration to normal and protein deficient rats has resulted in a significant increase in concentrations of nickel, phosphorus and sulfur in liver tissue. The concentration of zinc and copper in liver tissue decreased significantly in protein deficient, nickel treated and nickel treated protein deficient animals. Tissue iron concentrations were found to be decreased in protein deficient animals, but the concentrations of iron got elevated significantly in nickel treated and nickel treated protein deficient animals. It has been observed that nickel is ineffective in augmenting the hepatotoxicity in protein deficient rats.

Original

EFICACIA DEL NÍQUEL EN AUMENTAR LA HEPATOTOXICIDAD EN RATAS CON DEFICIENCIA EN PROTEÍNAS


Institute of Physiology and Experimental Pathophysiology, Friedrich-Alexander University, Erlangen-91054, Germany. Department of Biophysics, Panjab University, Chandigarh-160014, India. Umweltforschungszentrum Leipzig-Halle, Leipzig, Germany für Physik und Geowissenschaften, Universität Leipzig, Leipzig, Germany. *Department of Biophysics Panjab University Chandigarh, India.

NUTR. HOSP. (2005) XX (6) 378-385
ISSN 0212-1611 • CODEN NUHOEQ

Correspondencia: P. Sidhu
E-mail: pardeepsidhu@yahoo.co.uk
Introduction

Nickel is a common respirable-sized particulate pollutant and a widespread environmental contaminant. Nickel is widely used in various industrial processes and is present in many consumer products. Nickel has been shown to interact with multiple cellular pathways, including immune function, metallo-enzyme expression, and DNA repair mechanisms. Nickel exposure has been linked to various health effects, including respiratory and gastrointestinal diseases, cardiovascular disease, and cancer. In view of the increasing incidences of cancer, cardiovascular, respiratory and gastrointestinal diseases, poisoning due to heavy metal toxicity, the analysis of dietary intake of heavy metals, the study of their role in the manifestation of functional disorders of different organs, and elucidation of their mechanism is of special importance.

Malnutrition and this may be associated with adverse functional disorders of body metabolism, which is liable to be exaggerated in conditions of heavy metal poisoning. Persons afflicted with protein malnutrition are at a higher risk and are more likely to develop nickel toxicity. The protein calorie malnutrition (PEM), is a prominent cause of malnutrition in the developing countries like India. In the developing countries like India, the population with protein calorie malnutrition is very high. Children suffering from severe protein energy malnutrition have very low levels of the thymulin hormone, which is a sensitive indicator of zinc status in man. The decreases in thymulin levels are associated with severe PEM, which affects the synthesis of the mRNA suggesting that a defect occurs at a pre-transcriptional level that results in a diminution of the concentration of mRNA1. PEM is characterized by hypoalbuminemia, and anemia in malnourished children6. Bhaskaram and Hemalatha, 19957 observed that children suffering from severe protein energy malnutrition were deficient in a variety of micronutrients. Protein deficiency was induced in the animals of this group by maintaining them on the laboratory prepared diet to which the protein content was reduced to 3% and thereafter the animals were randomly and equally divided into four groups each having ten animals. Thereafter, the animals were randomly and equally divided into four groups each having ten animals. The animals were housed in polypropylene cages in the Animal House, Panjab University, Chandigarh. The animals were fed with diet containing normal protein contents (20%) as G-1, Normal Control. G-2, Protein deficient, (PD) rats were fed with diet containing normal protein contents (20%) and given in table below. Thereafter, the animals were randomly and equally divided into four groups each having ten animals. Thereafter, the animals were randomly and equally divided into four groups each having ten animals. The animals were housed in polypropylene cages in the Animal House, Panjab University, Chandigarh. The animals were fed with diet containing normal protein contents (20%). Composition of the diet used was as described by Kaur et al., 1992 and given in table below.

Materials and methods

Animals

Rats in the weight range of 110-120 g of Sprague Dawley (S.D.) strain were procured from the Central Animal House of the Department of Biophysics, under hygienic conditions and were acclimatized for at least one week before putting them on different treatments. Animals in this group served as normal controls and were fed with diet containing normal protein contents (20%). Grouping of animals was done as follows: G-1, Normal Control. G-2, Protein deficient, (PD) rats subjected to protein deficiency. G-3, Nickel treated. G-4, Nickel treated and nickel treated protein deficient animals. Nickel has been shown to interact with number of cellular dysfunctions2,3. Nickel breaks down the immunity by affecting the T-cell system and suppresses the activity of natural killer cells in rats and mice10,11. Nickel mobilizes and trace elements including iron, zinc, copper, manganese in experimental animals4,5. Alterations in the levels of zinc, manganese, copper, calcium and magnesium in tissue needs further research. Since, nutrients take for the toxic and protective elements and their levels in body are maintained by the organism by maintaining them on the laboratory prepared diet to which the protein content was reduced to 3%.
pared protein deficient diet with 8% protein contents. Composition of the diet used was as described by Kaur et al., 1992 (16) and given in table below.

G-3, Nickel treated, (Ni)
Animals in this group were given nickel in the form of NiSO₄. 6H₂O at a dose level of 800 mg/L in drinking water and the animals had free access to the drinking water containing nickel and the normal diet.

G-4, Ni+PD treated
Animals in this group were given protein deficient diet as given to G-2 animals and in addition were subjected to Ni treatment as mentioned for G-3 animals.

<table>
<thead>
<tr>
<th>Composition of diets (weight %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Casein (g)</td>
</tr>
<tr>
<td>Starch (g)</td>
</tr>
<tr>
<td>Sucrose (g)</td>
</tr>
<tr>
<td>Cellulose (g)</td>
</tr>
<tr>
<td>Corn oil (ml)</td>
</tr>
<tr>
<td>Vitamin mixture (g)</td>
</tr>
<tr>
<td>Salt mixture (g)</td>
</tr>
</tbody>
</table>

The treatments of rats continued for a period of eight weeks. At the end of the treatment, the animals were weighed and were sacrificed by exsanguination under light anesthesia. Livers were removed immediately and were perfused and rinsed in normal saline (NaCl, 9 g/l/w/v). One lobe was preserved by freezing for the determination of various trace elements and the other was processed immediately for various biochemical studies.

Biochemical Estimations

Protein
Protein assay was done by the method of Lowry et al., 1951 (17).

Estimation of liver marker enzymes in liver
The enzyme activity of Alkaline Phosphatase (ALP) was measured by the method of Wooton (18) and the enzyme activities of aspartate aminotransferase (AST) and Alanine Aminotransferase (ALT) were estimated according to the procedure of Reitman and Frankel (19).

Elemental analysis of liver samples
Estimations of various elements in the liver samples of different groups was done using Energy Depressive X-ray Fluorescence (EDXRF) technique. The elements estimated were calcium, phosphorus, iron, copper, manganese, zinc, cadmium, lead, nickel, and sulfur. The EDXRF technique is one of the most suitable analytical methods to analyze trace elements because of its properties such as non-destructive, sensitivity up to ppm and multi-elemental analysis.

– Sample Preparation for EDXRF
The liver tissues of all the animals were oven dried at 70°C to a constant weight and then ground with the help of Agate Pestle and Mortar. 300 mg dried powder of the tissue so obtained was weighed and mixed with equivalent amount of Hoechst Wachs (wax) to make self supporting pellets. The pellets were made by using a specially designed pure steel dye and a hydraulic press from Paul Weber, Germany. A force of approximately 45 KN (Kilo newtons) was applied at the dye top in order to make pellets of uniform thickness.

– EDXRF Setup
In the present work, the pellets of tissues were analyzed using an EDXRF X-Lab, 2000 to determine the levels of various elements. The X-lab, 2000 spectrometer involved a 0.4 kw Pd anode X-ray tube as source of excitation. The power of the X-ray tube was adjusted on line for each individual measurement by the spectrophotometer software, to secure optimum acquisition parameters for the current analysis. presently, different X-ray energies and excitation modes are being used but the most important mode used was of 40 kV and excitation used was polarized X-Ray. A Si (Li) detector coupled with computer (Pentium, 600 MHz, software package SPECTRO X-LABPRO 2.2) was used to collect the fluorescent X-ray spectra from the samples. The X-ray tube, secondary exciter, target and the Si (Li) detector were placed in a triaxial geometry mode. This geometry was used to minimize the background due to scattered photons.

Results
The results of all the experiments conducted during the current study are depicted in various tables. All the results of various treatment groups have been compared with their normal controls. Results of nickel + protein deficient (G-4) treated group have been compared with the results of the protein deficient group (G-2) also.

Statistical Analysis
The statistical significance of the values has been determined by using one way analysis of variance.
Body weights

The variations in the body weights of the animals subjected to different treatments are shown in Table I. It was observed that the protein deficiency resulted in a significant (p < 0.001) decrease in the body weights after eight weeks, when compared to normal control rats. Nickel treatment to normal control rats resulted in some decrease (p < 0.05) in the body weights but nickel treatment to protein deficient rats resulted in appreciable reduction (p < 0.001) in the body weights as compared to normal control rats.

Hepatic protein Contents

The hepatic protein contents in various treatment groups expressed as mg g⁻¹ tissue are shown in Table I. Protein deficient, Ni treated as well as combined protein deficient and nickel treated rats showed significant (P < 0.001) reductions in the hepatic protein contents as compared to normal control rats.

Alkaline phosphatase

Hepatic alkaline phosphatase activity showed a significant elevation (p < 0.01) in rats subjected to protein deficiency, nickel treatment and combined protein deficiency and nickel treatment as shown in Table II.

Aspartate Aminotransferase

As regards to hepatic levels of AST, a significant (p < 0.001) elevation was observed in protein deficient and nickel treated protein deficient animals (Table II).

Alanine Aminotransferase

Table II depicts the hepatic observations of ALT where significant (p < 0.001) elevation in ALT levels has been observed in protein deficient, nickel and nickel treated protein deficient animals.

Hepatic concentration of various elements

The concentrations of various elements have been depicted in Table III. The nickel administration to normal and protein deficient rats has resulted in a significant (p < 0.001) increase in concentrations of nickel in liver tissue. The concentration of zinc and copper in liver tissue got decreased significantly (p < 0.001) in protein deficient, nickel treated and nickel treated protein deficient animals. Tissue iron concentrations were found to be decreased in protein deficient animals, but the concentrations of iron got elevated significantly (p < 0.001) in nickel treated and nickel treated protein deficient animals. It has been observed that selenium got decreased significantly (p < 0.001) in protein deficient, nickel treated and nickel treated protein deficient animals when compared to normal animals. The elevation of selenium in nickel treated protein deficient animals was also significantly (p < 0.05) higher when compared to protein deficient animals.

### Table I

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (Grams)</th>
<th>Hepatic Protein (mg g⁻¹ tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1 Normal Control</td>
<td>199 ± 7</td>
<td>156.31 ± 5.38</td>
</tr>
<tr>
<td>G-2 Protein Deficient</td>
<td>146 ± 29 a3</td>
<td>112.37 ± 5.02a3</td>
</tr>
<tr>
<td>G-3 Nickel Treated</td>
<td>167 ± 20 a1</td>
<td>140.96 ± 2.03a3</td>
</tr>
<tr>
<td>G-4 Protein Deficient + Nickel</td>
<td>141 ± 30 a3, b1</td>
<td>120.00 ± 9.48a3, b1</td>
</tr>
</tbody>
</table>

Values are Mean ± SD.

By Newman-Keuls Test.

a1p < 0.05, a2p < 0.01 and a3p < 0.001 in comparison to G-1.

b1p < 0.05, b2p < 0.01 and b3p < 0.001 comparison of G-4 with G-2.

### Table II

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatic Alkaline Phosphatase (nmoles phenol produced min⁻¹ mg⁻¹ protein)</th>
<th>Hepatic Aspartate Aminotransferase (µ moles of pyruvate formed min⁻¹ g⁻¹ tissue)</th>
<th>Hepatic Alanine Aminotransferase (µ moles of pyruvate formed min⁻¹ g⁻¹ tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1 Normal Control</td>
<td>1.03 ± 0.06</td>
<td>2.59 ± 0.17</td>
<td>3.02 ± 0.05</td>
</tr>
<tr>
<td>G-2 Protein Deficient</td>
<td>1.67 ± 0.09 a3</td>
<td>3.50 ± 0.09a3</td>
<td>4.75 ± 1.07a3</td>
</tr>
<tr>
<td>G-3 Nickel Treated</td>
<td>1.36 ± 0.07 a3</td>
<td>2.50 ± 0.04</td>
<td>5.79 ± 0.77 a3</td>
</tr>
<tr>
<td>G-4 Protein Deficient + Nickel</td>
<td>1.55 ± 0.11 a3, b2</td>
<td>3.20 ± 0.15a3, b3</td>
<td>5.77 ± 0.48a3, b3</td>
</tr>
</tbody>
</table>

Values are Mean ± SD.

By Newman-Keuls Test.

a1p < 0.05, a2p < 0.01 and a3p < 0.001 in comparison to G-1.

b1p < 0.05, b2p < 0.01 and b3p < 0.001 comparison of G-4 with G-2.
Significant (p < 0.001) decrease has been observed in potassium concentration in nickel treated and nickel treated protein deficient animals. On the other hand phosphorus and sulfur concentrations were found to be increased significantly in nickel treated and nickel treated protein deficient animals.

Discussion

We observed a significant (p < 0.001) decline in the body weights of rats subjected to protein deficiency for a period of eight weeks, when compared to normal control rats. Loss in body weight is characteristic of protein malnutrition. In an earlier report from our laboratory it has been observed that protein deficiency leads to significant growth retardation in animals2. Many other workers have also reported the decrease in body weight due to protein deficiency20,21. It has been seen in these reports that retardation in body weight growth over a period is not due to low intake of diet but deficiency in protein intake.

Nickel treatment to normal control and protein deficient rats resulted in marked reduction in the body weights as compared to normal control rats. The reduction in body weights following nickel treatment has also been reported earlier22,23. The decrease in body weight may not solely be attributed to protein deficiency alone as the Ni treatment alone also has caused significant decline in body weight. The decrease in body weight due to nickel treatment has been connected by researchers to be not due to low intake of diet consumption of the rats following toxic treatment with nickel, vis a vis normal rats, and thus it is anticipated that this effect could possibly be due to the overall increased degeneration of lipids and proteins as a result of nickel toxicity23,24.

Rats in protein deficient, Ni treated and combined PD+Ni treated groups, showed a highly significant (P < 0.001) reduction in the hepatic protein contents as compared to rats of normal control group. Davenport y cols., 199425 demonstrated that in protein deficient states, the reduction in protein contents are due to depletion in amino acid precursors. Nickel in earlier reports, has also been able to cause significant depression in protein levels26,27. Nickel diminishes the DNA and RNA polymerase activity and decreases DNA replication fidelity28 which in turn can reduce the protein synthesis.

Hepatic alkaline phosphatase activity followed a significant elevation due to protein deficiency and nickel treatment. This elevation could be anticipated to the reason that ALP is bound to the intracellular membranes, and does not leak out with the increased permeability of the cell membranes. Moreover, Hultberg and Disaksson, 198329 proposed that activated macrophages including the Kupffer cells are the cellular source for the increased levels of ALP in conditions of liver damage. Davenport y cols., 199425 also postulated that many hepatic and extrahepatic conditions could also result due to protein-restricted diets that in a way caused increased production of alkaline phosphatase isoenzymes from bone and hepatobiliary source.

The aminotransferases are intracellular enzymes, which are active in operating the reversible exchange of aminoacids between alpha–amino and alpha-keto acids. As all the naturally occurring amino acids can undergo amino transfer reactions thus this class of intracellular enzymes is important for maintaining the balance of amino acids in the body. In protein deficient states the aminotransferase activity is expected to increase due to the increased need for amino acids for protein synthesis. Nickel treatment has also been shown to cause a decrease in the activity of these enzymes23,24.

Table III

<table>
<thead>
<tr>
<th>Group/Element</th>
<th>Nickel</th>
<th>Zinc</th>
<th>Copper</th>
<th>Iron</th>
<th>Selenium</th>
<th>Potassium</th>
<th>Phosphorus</th>
<th>Sulfur</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1 Normal Control</td>
<td>4.10 ± 1.36</td>
<td>57.93 ± 5.9</td>
<td>12.61 ± 2.14</td>
<td>194 ± 29</td>
<td>2.81 ± 0.34</td>
<td>3.8 ± 0.5</td>
<td>1.7 ± 0.4</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>G-2 Protein Deficient</td>
<td>3.60 ± 1.63</td>
<td>40.28 ± 5.13</td>
<td>7.07 ± 1.31</td>
<td>149 ± 39</td>
<td>1.05 ± 0.11</td>
<td>3.0 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>G-3 Nickel Treated</td>
<td>12.08 ± 2.66</td>
<td>35.23 ± 4.07</td>
<td>4.88 ± 1.87</td>
<td>367 ± 37</td>
<td>1.46 ± 0.29</td>
<td>1.6 ± 0.6</td>
<td>2.9 ± 0.2</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>G-4 Protein Deficient + Nickel</td>
<td>13.45 ± 4.21</td>
<td>39.80 ± 5.15</td>
<td>5.86 ± 1.84</td>
<td>305 ± 73</td>
<td>1.58 ± 0.32</td>
<td>1.3 ± 0.0</td>
<td>2.8 ± 0.4</td>
<td>2.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are Mean ± SD
By Newman-Keuls Test
a1p < 0.05, a2p < 0.01 and a3p < 0.001 in comparison to G-1
b1p < 0.05, b2p < 0.01 and b3p < 0.001 comparison of G-4 with G-2
tions were noticed by Kumari et al., 1993 who estimated that metals that have similar chemical and physical properties would often interact biologically and antagonize or embellish each other's function. They proposed that metals that have similar chemical and physical properties would often interact biologically and antagonize or embellish each other's function. During the course of this study, we have also observed a decrease in the concentrations of these metals due to lead intoxication.

In the present study, nickel concentration has been found to be increased in liver tissue following the administration of nickel to normal and protein deficient rats. Our results are in agreement with earlier reports. During the course of this study, we have also observed a decrease in the concentrations of these metals due to lead intoxication.

The most significant pathological and biochemical state involving abnormalities in the regulation of trace element metabolism. This can be due to inadequate dietary intake, increased requirements or excretion, or changes in the distribution of these essential trace elements in the body can have both nutritional and toxicological consequences with regard to the metabolism of other metals. They have both nutritional and toxicological consequences with regard to the metabolism of other metals. Those metals which are essential for many enzymes in numerous metabolic pathways; the growth of microorganisms for over hundred years, contain protein deficient animals. The observed increased activities of hepatic AST and ALT in the animals given protein deficient diet are in conformity with the conclusions drawn in the present study. Significant inhibition is observed in copper concentrations due to lead intoxication.

Copper depletion is associated with depressed hepatic Cu-Zn superoxide dismutase (SOD) activity and it is well known that copper contents in the present study which is in agreement with earlier reports. We have also observed decrease in the activities of hepatic AST and ALT in the animals given protein deficient diet are in conformity with the conclusions drawn in the present study. Significant inhibition is observed in copper concentrations due to lead intoxication.

Furthermore, a large number of zinc-containing enzymes and proteins have been recognized as having important function in the regulation of trace element metabolism. This can be due to inadequate dietary intake, increased requirements or excretion, or changes in the distribution of these essential trace elements in the body can have both nutritional and toxicological consequences with regard to the metabolism of other metals. Those metals which are essential for many enzymes in numerous metabolic pathways; the growth of microorganisms for over hundred years, contain protein deficient animals. The observed increased activities of hepatic AST and ALT in the animals given protein deficient diet are in conformity with the conclusions drawn in the present study. Significant inhibition is observed in copper concentrations due to lead intoxication.

In the present study, nickel concentration has been found to be increased in liver tissue following the administration of nickel to normal and protein deficient rats. Our results are in agreement with earlier reports. During the course of this study, we have also observed a decrease in the concentrations of these metals due to lead intoxication.
in the form of enhanced ALP, AST and ALT levels. It has been reported that nickel inhibits the Na-K-ATPase60.

This inhibition of ATPase leading to decreased levels of potassium levels in nickel treated and nickel treated protein deficient animals. It may possibly be due to increased requirement of phosphorus either due to the increased mobilization from bones.

Increased liver iron concentration of iron in liver tissue. It can also be speculated that the nickel treatment leads to the formation of a Ni-selenide excretable complex59. Selenium, which is an essential trace metal and is a major cation of intracellular fluid, and functions in glutathione metabolism pathways, which could have been adversely affected in stress conditions. When serum iron levels exceed the iron binding capacity of transferrin a disturbance in the marker enzymes of rat liver following administration of nickel in the present study.

The formation of a Ni-selenide excretable complex59. Selenium act antagonistically and the detoxifying effect of selenium on nickel toxicity seems to be due to the fact that the detoxifying effect of selenium on nickel toxicity seems to be due to the fact that selenium is an important constituent of many enzymes and the detoxification of selenium by the liver is the major site of detoxification of selenium. It is possible that nickel could have lead to alterations in the membrane permeability of hepatocytes especially with regard to potassium channels or has caused inhibition of ATPase leading to decreased levels of potassium levels in nickel treated and nickel treated protein deficient animals. It has been reported that nickel and protein deficiency (PD) and nickel treatment which could be thought of due to impaired hepatic elimination of a severe oxidative stress status in the tissue, thus as observed in our study may lead to the development of liver iron concentrations.

Conclusion

The findings indicate that prenatal nickel dosing does not enhance the signs of nickel toxicity in rats.

Acknowledgments

This work was supported by grant from IUC-DAE, Kolkata and ICMR, New Delhi.

References


17. Lowry OH, Rosebrough NJ, Farr AL and Randall J: Protein the hepatotoxicity in protein deficient rats. Ineffectiveness of nickel in augmenting
18. Wooton IDP: In: Microanalysis in Medical Biochemistry (4th
15. Anke M, Kronemann H, Grappel B, Hening A, Meissner D
20. Wang G, Yu S and Bao C: Effect of different levels of protein feeding and microvillus membrane glycosylation in normal and
24. Dieter MP, Jameson CW, Tucker AN, Luster MI, French JE,
30. Plaa GL and Hewitt WR: Detection and evaluation of chemi-
26. Sreedevi P, Sivaramakrishan B, Suresh A, Radhakrishanaiah
37. Dhawan DK and Goel A: Protective role of zinc on rat liver
58. Rukgauer M, Neugebauer RJ, Plecko T: The relation between
57. Boisier X, Schon M, Sepulveda A, Basualdo A, Cornejo P,
40. Severa J, Yskocil, Fiala Z and Cizara M: Distribution of nickel in
19. Riechmann H, Tschierske A and Speck C: Nickel and heavy metals (Cd, Pb and Hg) on haematology and serum bio-
15. Forrest HN, Thomas JZ, Michael EC, Duane RM: Nickel De-
55. Forrest HN, Thomas JZ, Michael EC, Duane RM: Nickel De-
44. Vallee BL, Galden A: The metallo-biochemistry of Zn enzy-
42. Vallee BL, Falchuk KH: The biochemical basis of zinc phy-
19. Riechmann H, Tschierske A and Speck C: Nickel and heavy metals (Cd, Pb and Hg) on haematology and serum bio-
22. Fiala Z, Yakubikova M, Vlcek V, Bartek J: Distribution of nickel in
43. Forbes RM: Use of laboratory animals to define physiological
48. Huang CJ, Fwu ML: Degree of protein deficiency affects the
49. Burnett FM: A possible role of zinc in the pathology of de-
50. Dhawan D, Singh B, Chand B, Singh N, Mangal PC, Trehan
51. Rhim SL, Zhang XF, Goodrowe C, Tran D, Fisher SK: The influence of dietary zinc on the activities of metallothionein-
52. Galan P, Hereberg S, Tovitouy Y: The activity of tissue enzy-
53. Aggette PJ: Physiology and metabolism of essential trace ele-
55. Forrest HN, Thomas JZ, Michael EC, Duane RM: Nickel De-
57. Boisier X, Schon M, Sepulveda A, Basualdo A, Cornejo P,
58. Rukgauer M, Neugebauer RJ, Plecko T: The relation between
57. Boisier X, Schon M, Sepulveda A, Basualdo A, Cornejo P,
40. Severa J, Yskocil, Fiala Z and Cizara M: Distribution of nickel in
19. Riechmann H, Tschierske A and Speck C: Nickel and heavy metals (Cd, Pb and Hg) on haematology and serum bio-
49. Burnett FM: A possible role of zinc in the pathology of de-