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The aim of the present study was to evaluate the protective potential of zinc treatment on liver following nickel treatment to protein deficient rats. The various investigations carried out to achieve the objectives of the study included estimations of transaminases (AST and ALT), alkaline phosphatases, enzymes of mixed function oxidase system, enzymes involved in the antioxidant defense system, serum and liver trace-elemental analysis, radio tracer studies, SDS gel electrophoresis and histopathological studies both at the light and electron microscopic levels.

Body weights

We observed that the body weights of normal control and zinc treated rats increased progressively throughout the study. Protein deficiency resulted in a significant (p<0.001) decline in the body weight after 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 animals (Tandon et al, 1998). Many other workers have also reported the decrease in body weight due to protein deficiency (Eisenstein and Harper, 1991; Wang et al, 1995 and Bauman et al, 1988). 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 earlier been reported (Ambrose et al, 1976; Sunderman et al, 1978, Cempel and Janicka, 2002). Das KK and Das Gupta S, 1997, observed significant decrease in the body and organ weight of both normal protein-fed and protein-restricted groups of animals following nickel sulfate administration. The decrease in body weight may not solely be attributed to protein deficiency alone as the Ni treatment alone...
has also 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 toxicity (Clary, 1975; Dieter et al, 1988; Cempel and Janicka, 2002). Nickel diminishes the DNA and RNA polymerase activity and decreases DNA replication fidelity (Sirover and Leob, 1976) which in turn can reduce the protein synthesis. To substantiate this contention Das KK and Das Gupta S; 2000, has observed the decrease in nucleic acids and protein concentration following nickel toxicity. Zinc treatment to the protein deficient and Ni treated protein deficient rats tended to improve the body weight growth though it was not significant. Similar protective effects of zinc in improving the body weight gain of the animals have also been emphasized in other studies, where radiations or carbontetrachloride was used to cause liver injury (Anttinen et al, 1984; Dhawan and Goel, 1994; Yao et al, 2001; Chen, 2001). The protective effects zinc could be attributed to its ability to reduce collagen accumulation in liver and also it exerts critical physiological role in regulating the structure and function of cell.

Serum total protein and albumin/globulin (A/G) ratios

Serum total proteins and albumin were estimated in all the groups in order to assess the severity of protein deficiency. Protein deficient and nickel treated protein deficient rats showed significant reduction (p<0.01) in serum total protein and albumin which is in agreement with the earlier reports (Jennings et al, 1992; Davenport et al, 1994: Das and Das, 2000). Davenport et al, 1994 demonstrated that in protein deficient states, the reduction in serum albumin contents were due to depletion in amino acid precursors of albumin synthesis. In their study, total protein values paralleled albumin values in the protein deficient group, whereas
globulins remained normal. They indicted that the decrease in total protein contents was due to reduced albumin in the LP fed group. Tower et al, 1983 have earlier reported that the toxic effects of heavy metals like lead on the cellular as well as that on the protein metabolism could cause an overall reduced protein synthesis as evident in present study.

In our study, nickel treatment has not caused any significant change in the levels of albumin and protein, however a small decrease was definitely there. Nickel in earlier reports, has also been able to cause significant depression in protein levels (Sreedevi et al, 1992; Kusal and Shkuntla, 2000).

Administration of Zn to the animals of protein deficient and combined PD+Nickel groups tended to restore the serum albumin levels, though it was non-significant. However, Zn treatment to animals of nickel treated brought the observed small depressed levels of serum albumin, serum total proteins and A/G ratio to normal range. This property of Zn could be attributed to its role in the induction of metallothionein (Zn binding protein) thereby regulating the amino acid precursors for albumin synthesis (Dhawan et al, 1992; Zhou et al, 1993; Yang et al, 1995; Tekeli, 2002).

**Hepatic protein Contents**

Protein deficient (PD), Ni treated and combined PD+Ni treatment groups, showed a highly significant (P<0.001) reduction in the hepatic protein contents as compared to normal control group. However, zinc administration to the G-6 (PD+Zn) and G-7 (Ni+Zn) rats helped in raising the hepatic protein contents (p<0.001) and were brought to normal limits as compared to their respective controls. But zinc could not restore the protein contents in G-8 (PD+Ni+Zn) to within normal levels however it did help in raising the protein contents appreciably. These observations regarding the hepatic protein contents following various treatments in the present study could be explained on the
similar guidelines as mentioned earlier for serum protein contents. Since, the dietary intake of protein is low in protein deficient and nickel treated protein deficient group, therefore, Zn might have induced the metallothionein thereby leading to increased hepatic protein contents (Shrivastava et al, 1993; Dhawan and Goel, 1994).

**SDS-PAGE**

A specific polypeptide band of approximately 97 KDa was observed to be of very weak intensity in the samples from nickel treated (G-3), nickel treated protein deficient (G-5), nickel and zinc treated (G-7) and nickel and zinc treated protein deficient (G-8) animals in proportion to the electrophoretic intensity of the normal control sample in lane A. It may be because nickel is impairing the production of this particular protein by acting at the levels of both transcription as well as translation processes resulting in decrease of the RNA and protein content. This contention is supported by the findings of Kusal and Shkuntla, 2000.

**Alkaline Phosphatase (ALP)**

Alkaline phosphatase (ALP), a phosphodiesterase is composed of a group of enzymes with a wide substrate specificity to catalyze the hydrolysis of monophosphate esters at alkaline pH with broad specificity towards different structurally related substrates (Duncan and Prasse, 1986).

The actual substrates in vivo are not known but are postulated to be either ethanolamine phosphate or phosphatidylethanolamine (Kachman and Moss, 1976). ALP is found in abundance in osteoclasts in bone, liver, parenchymal cells and bile duct epithelium, where enzyme activity is associated with the transport of phosphate across the cell membrane.

The serum alkaline phosphatase activity got significantly depressed following protein deficiency as compared to normal control rats. Our results are in confirmity with Kumari et al, 1993 whereby they have reported a decrease in the level of serum alkaline phosphatase
activity, in protein energy malnourished children. They ascribed the
decline to the slow rate of protein synthesis and amino acid
mobilization. Nickel treatment also depressed the alkaline phosphatase
activity significantly. Combined treatment of Ni and PD also caused
significant depression in the serum alkaline phosphatase activity as
compared to normal controls. Further, Whanger, 1973; Donskay et al.
1986; Athar et al., 1987; Novelli et al., 1995 reported the inhibition of ALP
due to cholestasis and necrotic liver disorder following intoxication with
nickel.

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, 1983 proposed
that activated macrophages including the Kupffer cells are the cellular
source for the increased levels of ALP in conditions of liver damage.
Thus it could be speculated that increased ALP activity in liver might
also be due to contribution from the phagocytic cells. Davenport et al,
1994 also postulated that many hepatic and extrahepatic conditions
could result due to protein-restricted diets that in a way caused
increased production of alkaline phosphatase isoenzymes from bone
and hepatobiliary source.

Alkaline phosphatase is the prototype of those enzymes that
reflect the pathological reductions in bile flow. Following bile duct
ligation, the activity of this enzyme has been reported to increase in liver
due to the de novo synthesis of the membrane bound form of the
enzyme (Kaplan, 1986). Also, biliary excretion corresponds to
approximately 3% per day of the total alkaline phosphatase activity in
the rat liver (Laruso and Fowler, 1979). Thus in light of all these details,
it sounds increasingly plausible to speculate that increased alkaline
phosphatase activity in liver might be due to contribution from the
phagocytic cells and decreased bile secretion in nickel induced liver toxicity. However, it seems difficult to conjecture why the serum ALP has been observed to be marginally depressed whereas hepatic ALP has been increased. This could be due to the increased hydrolysis of serum ALP that might be needed to combat the protein deficiency state.

Role of Zn in regulating the altered alkaline phosphatase level could be evaluated to its property as an important co-factor for the expression of enzyme activity and thereby helping in smooth transportation of phosphates across cell membranes (Conard and Burton, 1978). This restoration in the levels of alkaline phosphatase activities to near normal limits subsequent to zinc treatment could be ascribed to the antioxidative potential of the zinc as has been delineated by us previously (Dhawan et al, 1992; Dhawan and Goel, 1994). Earlier in our study, a great deal of improvement has been observed in the histoarchitecture of damaged hepatocytes by zinc. This improvement with structural integrity as a result of zinc supplementation might have lead to the regulation of levels of alkaline phosphatase activity.

Aminotransferases

The aminotransferases are intracellular enzymes, which are active in operating the reversible exchange of amino acids 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 (aminotransferases) form an important link between protein and carbohydrate metabolism (Plaa and Hewitt, 1989).

Serum levels of activities of serum aspartate aminotransferases showed a statistically significant increase in the enzyme activity in protein deficient rats. The AST activity also got increased significantly ($p<0.01$) following nickel treatment to normal rats and protein deficient rats for a period of 2, 4 and 8 weeks. However, simultaneous zinc treatment to nickel treated animals appreciably reduced the observed increased AST levels in protein deficient and PD + nickel treated rats.
but the levels of AST could not be reverted to within normal levels in 2 and 4 weeks treatments. However, it could effectively brought back the raised levels of serum AST to within normal range in nickel treated rats after 8 weeks of treatment.

As regard to hepatic levels of AST, significant (p<0.001) elevation was observed in protein deficient and nickel treated protein deficient animals. However, zinc was able to restore the elevated levels to normal range.

Activity of serum ALT in the protein deficient rats, was found to be raised significantly (p<0.001) after 4 and 8 weeks of treatment as compared to normal control rats. In our results, Ni treatment to G-3 animals resulted in a significant elevation of serum ALT at 4 and 8 weeks. Combined treatment of protein deficiency and Ni to the G-5 rats also resulted in increase in serum ALT (p<0.01) at 4 and 8 weeks when compared to the values of normal control rats. Interestingly, zinc administration to the G-6 (PD+Zn), G-7 (Ni+Zn) and G-8 (PD+Ni+Zn) animals resulted in alleviating the raised levels of serum ALT to some extent but they were still different from normal rats. Hepatic levels of ALT got significantly (p<0.001) elevated in protein deficient, nickel and nickel treated protein deficient animals. However, zinc restored the altered levels to normal range in all the groups.

The observed increased activities of serum and hepatic AST and ALT in the animals given LP diet are in conformity with the study of Pond et al, 1992. They reported that the increase in serum ALT activity and total bilirubin concentration in pigs fed a protein deficient diet suggested altered liver function, although microscopic anatomy revealed no evidence of excessive fat accumulation or of pathologic changes. Similar observations were noticed by Kumari et al, 1993 who estimated ALT and AST activities in the serum samples of 30 cases of pediatric PEM. Their observations also noticed a significant increase in marker enzymes of liver in these cases. These authors explained that
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during hepatobiliary disorder, amino acids are released from exaggerated tissue breakdown. In order to metabolize these amino acids, the process of transmutation gets enhanced leading to increased activity of the related enzymes AST and ALT. On the contrary, Davenport et al., 1994 did not observe any alteration in the activities of serum AST and ALT in protein deficient rats. The rise in the activity of both the transaminases with a concomitant increase in serum bilirubin is suggestive of the acute liver injury (Tokha-El-Sherif, 1970).

Zimmerman, 1984 identified that the extent of elevation of both AST and ALT depends upon their specificity for and sensitivity to different types of liver dysfunction. AST is also present in high concentrations in the heart, skeletal muscles and kidney apart from the liver, therefore elevated levels of AST might reflect some kind of non-specific or extrahepatic dysfunction also. However, ALT is a liver specific enzyme and is present in the liver in abundant quantities, therefore it is more specific marker of hepatocellular function. ALT is reported to be more exclusive in liver and is specific marker as compared to AST, which might reflect some kind of non-specific indication of liver functions.

A substantial increase in the serum both these transaminases (AST and ALT) have been observed following Ni treatment. This could be attributed to the hepatic damage due to long term Ni administration resulting in increased the synthesis of functional enzymes from the biomembranes (Misra et al., 1990). According to other mechanisms, an increase in serum AST and ALT activities is normally indicative of leakage of these enzymes which may be due to tissue damage following nickel toxicity resulting in altered membrane permeability, the latter being necessary condition for the release of these enzymes from the tissue into the plasma (Srivastava et al., 1993). Drugs that can damage the liver have been shown to increase these activities of AST and ALT in the serum (Vatal and Aiyar, 1988). Leonard et al., 1986
further stressed that there was an excellent correlation between the severity of quantified histological damage produced by a toxin and the elevation in serum ALT activity in rats. Therefore, it appears that not only is it possible to detect raised ALT levels in the presence of liver injury, but also under some circumstances, the severity of the lesion can be approximated by the elevation in the enzyme activity. In the present study, this fact was further corroborated as we have observed marked disturbances in the hepatic histoarchitecture of nickel intoxicated animals.

The normalization of AST and ALT activities following Zn supplementation to protein deficient and Ni intoxicated rats are in agreement with the available reports (Goering and Klaassen, 1984; Dhawan et al., 1992; Dhawan and Goel, 1994). These reports emphasized the hepatoprotective efficacy of zinc under CCI₄ induced liver injury, as zinc treatment helped in the maintaining the homeostasis through regulation of protein synthesis. Srivastava et al., 1993 had indicated that Zn also helps in the stabilization of biomembranes in hepatic disorders following nickel toxicity.

**Lipid Peroxidation**

Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) and because of its biomedical implications, the process has been the subject of strenuous research. Its occurrence in biological membranes causes impaired membrane function, impaired structural integrity (Gutteridge, 1988; Halliwell and Gutteridge, 1989), decrease in fluidity, and inactivation of a number of membrane bound enzymes and protein receptors. Lipid peroxidation is an autocatalytic free-radical process and could be responsible for DNA damage (Shirali et al., 1994).

A significant increase in malondialdehyde products was observed in the protein deficient groups in the present study which suggested that low protein diet intake might result in enhanced LPO in liver. These
results seems to be in agreement with the previous findings (Pelissier et al, 1990; Huang and Fwu, 1993; Tandon et al, 1998) whereby these workers suggest that the rats fed on a low protein diet might be more susceptible to peroxidative tissue damage under the influence of oxidative stress. The increased LPO could be attributed to the reduction in detoxifying hyperperoxides in protein deficient conditions. Moreover, the degree of depressions of detoxifying hyperperoxides in protein deficient conditions might also be correlated with the degree of protein deficiency (Huang and Fwu, 1993).

Nickel treatment to normal control and protein deficient rats also indicated increase in the hepatic LPO. Increase in lipid peroxidation following nickel treatment has also been reported earlier (Misra et al, 1990; Shirali et al, 1994; Chen et al, 1998). Current studies on cellular injury by environmental toxicants implicate peroxidation of polyunsaturated fatty acids (PUFA), leading to the degradation of phospholipids in a variety of pathological states, which has been anticipated as an index of cellular deterioration (Tappel, 1973; Aboudonia, 1981).

The normalization of LPO due to Zn administration could be attributed to its antiperoxidative property. Srivastva et al, 1993 suggested that Zn-MT serve as an efficient antagonist in inhibiting nickel-mediated lipid peroxidation compared to Cd-MT or Ag-MT. Studies have shown that Zn causes inhibition of both endogenous as well as induced lipid peroxidation to stabilize biomembranes (Chvapil et al, 1972; Dreosti and Patrick, 1987; Dhawan et al, 1992; Srivastava et al, 1993).

**Antioxidative Enzymes**

To protect themselves against free radicals, cells have evolved antioxidant defenses and repair systems to prevent the accumulation of oxidatively damaged molecules. The antioxidant defense system include enzymes like glutathione peroxidase (GPx), catalase,
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glutathione reductase (GR), glutathione-s-transferase (GST), superoxide dismutase (SOD), as well as small molecules such as ascorbic acid, reduced glutathione (GSH) and uric acid (Capel, 1988; Chow, 1989; Cadenas, 1989). Catalase is a ubiquitous enzyme and is a major component in primary antioxidant enzyme system, which catalyzes the decomposition of $H_2O_2$ to $H_2O$ and sharing this function with glutathione peroxidase (GPx). Glutathione peroxidase on the other hand is located in the cytosol and mitochondrial matrix and catalyzes the reduction of $H_2O_2$ and lipid and nonlipid hydroperoxides to oxidized glutathione (GSSG) using two molecules of GSH. Further oxidized, GSSG is reduced back to GSH by glutathione reductase (GR), which utilizes NADPH regenerated by glucose-6-phosphate dehydrogenase.

In the present study, after subjecting the rats to protein deficiency, nickel treatment and protein deficiency along with nickel treatment, the hepatic activity of catalase, glutathione peroxidase and glutathione reductase got raised but superoxide dismutase activity was found to be inhibited. Darmon et al, 1993 also observed the increase in activity of catalase in low protein diet fed rats. Zhu et al, 1993 accounted the high levels of GPx following protein deficiency due to its low utilization and increase in synthesis. The enhanced levels of glutathione reductase has been reported earlier also in rats fed protein restricted diet (Ayala et al, 1991; Pelissier et al, 1990). The phagocytosis of particulate nickel compounds and the dissolution of the particles inside the cell and the resulting oxidative stress produced in the nucleus is a key component of the nickel carcinogenic mechanism (Costa et al, 2002). Chen et al, 2002 observed that antioxidant catalase provides protection against the oxidative stress induced by nickel by reducing the elevation of nickel induced oxidants. The observed elevation in the activities of both GPx and GR in present study under low protein diet and nickel toxicity, may be due to the mechanism of enhanced synthesis of these enzymes under these conditions which are actively involved in reducing the $H_2O_2$. 

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generation. The increase in activity of glutathione reductase, glutathione peroxidase and catalase following nickel treatment has been reported earlier also (Arthar et al., 1987;Ahmed et al, 1999;Randhawa et al, 2001). Rodriguez et al, 1991 also observed enhancement in hepatic GPx activity following nickel treatment. The increase in levels of these enzymes may be as a consequence thereof to combat the formation of reactive oxygen species in protein deficiency or nickel induced oxidative stress. Each of these antioxidative enzymes might trigger a response and an alteration in any of these, altogether disturbs, the oxidant defense system against free radicals (Michiels et al, 1994). Moreover, hepatic cells are subjected to high oxidative stress and peroxidative tissue damage because of combined protein deficiency and nickel toxicity.

The observed increase of LPO in protein deficient and nickel treated group seems to be associated with decrease in SOD activity as SOD inhibits hydrogen peroxide by scavenging free oxide molecule (Fridovich, 1995). Our results regarding the significant depression of SOD following restriction of protein diet are in agreement with earlier reports (Pelissier et al, 1990;Rana et al, 1996). Deneke et al, 1983 reported a significant reduction in liver superoxide dismutase (SOD) activity in rats fed 35% casein. The decrease in activity of SOD following nickel treatment has also been reported earlier (Rodriguez et al, 1996; Das et al 2001). Following zinc treatment the altered levels of enzymes tended to be normalized because of antioxidant property of zinc.

**Reduced Glutathione (GSH) and Glutathione-S-Transferase (GST)**

Glutathione-s-transferases (GSTs) form a group of enzymes that are present in high concentrations in the cytosol and catalyze a wide variety of substitution reactions in which glutathione (GSH) replaces an easily displaced group on the xenobiotic and thus prevents the subsequent toxic reactions (Siddiqui et al, 1990). This reaction involves
a compound with an electrophilic atom and GST facilitates the nucleophilic attack of glutathione thiolate on this electron deficient atom of the hydrophobic compound. GSH plays an important role in intracellular protection against toxic compounds, reactive oxygen species, and free radicals (Meister, 1984). Reduced glutathione (GSH) protects the liver microsomes against the effects of reactive (peroxides and oxygen) intermediates which are formed by Cytochrome $P_{450}$ system as well as lipid peroxidation (Burk et al, 1983). GSH further serves as both nucleophilic and an efficient reducing agent by interacting with numerous electrophilic and oxidizing compounds such as $H_2O_2$, $O_2$ and OH and nullifies the peroxide damage. Further, GSH is known to be metabolized by the enzyme viz. Glutathione peroxidase (GPx), glutathione reductase (GR) and gamma glutamyl transpeptidase ($\gamma$-GT). GSH is a substrate for GPx that catalyzes the reduction of $H_2O_2$ and other lipid peroxides. The oxidized glutathione (GSSG) produced in this reaction is subsequently reduced to GSH by NADPH via enzyme GR (Israel et al, 1992).

LP diet caused marked decrease in the levels of GSH which is in agreement with earlier studies (Claude et al, 1987; Ayala et al, 1991; Hum et al, 1992). Ayala et al, 1991 observed that in rats fed on a low protein diet, supplemented with all essential amino acids except methionine, there was a decrease of GSH levels. They proposed that low intracellular concentration of cysteine available for GSH synthesis and feed back inhibition of gamma glutamyl cysteine synthetase may be responsible for inhibition in the activities under protein deficient conditions. The low protein diet results in increase of hepatic levels of glutathione degrading enzyme, gamma glutamyl transferase resulting in decrease of concentration of glutathione (Taniguchi and Cherian, 1990). Because GSH is an important component of the detoxification mechanism its depressed activity in protein-deficient and Ni toxic conditions would therefore lead to decreased detoxification capacity of
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Further reduction in GSH levels in protein deficient and nickel toxicity is understandable in the light of elevation of GPx under these conditions.

The present observations of decline in GSH levels in nickel treated and protein deficient groups are in coherence with the earlier reports. Tandon et al., 1998 has observed decrease in the levels of GSH in following low protein diet while Teissier et al, 1994;Das et al, 2001 and Iscan et al, 2002 reported the similar trends following nickel toxicity. Rodriguez et al, 1996 observed upto 68% decrease in GSH following nickel treatment. It can be attributed to the activation of enzyme glutamyl transpeptidase ($\gamma$-GT) to replenish intracellular glutathione on the sinusoidal surface of the liver cells (Chow, 1979).

We have observed increase in GST following nickel and protein deficiency treatment. The increase in glutathione-s-transferase following nickel toxicity suggests the ability of the tissue to cope against the toxic effects of nickel by increasing its detoxifying capacity (Mohammed et al, 1987;Athar et al, 1987;Iscan et al, 1994;Ahmed et al, 1999). Ramdath and Golden, 1993 observed increase in activity of GST in malnourished children. Cho et al, 2000 stated that antioxidant response element (ARE)-binding activity of protein calorie malnutrition rats gets increased, which in turn results in activation of certain GST mRNAs resulting in increase of GST.

The observed normalization of GSH levels and GST activity following Zn treatment could be because of its property to induce metallothionein (S-rich protein) as a free radical scavenger, or its indirect action in reducing the levels of oxygen reactive species, however its mechanism for these actions remains to be elucidated, thereby protecting the important thiols in toxic conditions (Seagrave et al, 1983).
Biokinetics of $^{65}\text{Zn}$

Biological half-lives of $^{65}\text{Zn}$ in whole body and liver

To study the overall status of the $^{65}\text{Zn}$ as a function of time in the whole body under different dietary protein regimens and nickel treatment, we have estimated the biological half-life of $^{65}\text{Zn}$ which is an indication of turnover of zinc. The present study showed that $^{65}\text{Zn}$ followed two-compartment kinetics. The two components of the biological half-lives of the radiotracers have been resolved viz. the faster component $T_{b1}$ and the slower component $T_{b2}$. $T_{b1}$ represented the rapid elimination of presumably radionuclide mainly through urine and feces. $T_{b2}$, which is the slower component, plays an important role in the radiobiological consideration (Halford et al., 1983) and represents turnover of radionuclide already incorporated into tissue, and specifically bound to metallothionein and certain enzymes (Odum, 1961; Van Liew, 1962; Vallee and Falchuk, 1993).

Lowe et al., 1991 identified two major zinc pools in the body namely, liver and plasma. In biological systems, very little zinc exists in the free form. It carries out its function as a divalent cation primarily when bound to enzymes and other proteins. Zinc in the plasma is bound mainly to albumin, but other proteins such as macroglobulin, transferrin, ceruloplasmin, haptoglobin and gamma globulins also bind significant amounts of zinc (Prasad and Oberleas, 1970), and lysine are among the aminoacids with significant zinc binding capacity. The biological essentiality of zinc implies the existence of homeostatic mechanisms that regulate its absorption, cellular uptake, distribution among intracellular compartments and macromolecules, as well as excretion. Such mechanisms are needed to maintain the broad range of biochemical functions that are dependent on zinc proteins and enzymes (Vallee and Falchuk, 1993).

The most versatile means of management of heavy metal poisoning has been the chelation therapy where body reduces burden...
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of toxic metals by promoting their excretion. The treatment of nickel (Ni) toxicity using trace metals such as zinc has earlier been studied by many workers (Waalkes et al., 1985; Srivastava, 1993).

The observed increase in slow and fast component of biological half lives of $^{65}\text{Zn}$ in whole body and increase in fast component in liver indicates slow turnover of Zn in protein deficient rats. This could be attributed to increased metallothionein induction to compensate for low protein synthesis and enhanced LPO as well as oxidative stress in these rats. Here it is worth mentioning that important functions of metallothionein include activation of apoenzymes, detoxification/protective mechanism against excessive exposures to toxic metals, homeostatic control of zinc metabolism, and intracellular antioxidant (Thornalleyh and Vasak, 1985).

The present study showed reduced $T_{\text{b2}}$ in liver of rats treated with nickel, which is the reflection of faster turnover of $^{65}\text{Zn}$ from liver. The decrease in biological half-lives of $^{65}\text{Zn}$ under Ni treatment could be explained due to competitive replacement of radioactive Zn by continuous treatment of Ni in this group. Nickel as described previously is another prevalent element with which Zn has very significant interaction in biological and toxic reactions. The nickel-zinc interactions have been studied at absorptive as well as the enzymatic sites. Zinc occurs in many hydrolytic enzymes, including DNA and RNA polymerases and displacement of zinc by nickel appears to be associated with deactivation of critical enzymes (Gitlitz et al., 1975). The uptake of nickel in the intestinal wall was suppressed by concomitant perfusion with high levels of zinc thus indicating the antagonistic action against each other (Foulkes and McMullen, 1986).

Therefore, it could be explained based on above reports that Zn administration to protein deficient and nickel treated rats might have enhanced the metallothionein levels as well as improved the histoarchitecture of hepatocytes in these rats thereby improving the
retention of $^{65}$Zn following zinc supplementation to protein deficient, nickel treated and nickel treated + protein deficient rats. Zinc associated with metallothionein during this stage reflects physiological changes in demand for zinc required for nucleic acids and protein metabolism (Panemangalore et al, 1983).

**Biodistribution of $^{65}$Zn**

In the present study, liver has been shown to have the maximum percentage of $^{65}$Zn uptake amongst the organs under study, followed by kidney, small intestine, large intestine, blood and brain. Earlier, Spencer et al, 1965 reported that $^{65}$Zn concentration was highest in liver and decreased with time in all organs. The $^{65}$Zn concentration in kidney was also high and it might be due to rich vascularization of the kidney, the presence of carbonic anhydrase and the possible deposition of protein bound zinc (Spencer et al, 1965).

Peak levels of $^{65}$Zn in liver had been reported within 3-4 hr of isotope administration (Methfessel and Spencer, 1973; Lowe et al, 1991). The turnover rate of $^{65}$Zn in liver was found to be very rapid and some of $^{65}$Zn released from the liver may be taken up by bone and muscle (Spencer, 1965).

The percentage uptake of $^{65}$Zn decreased in intestine, spleen, liver, kidney and bone in protein deficient and nickel treated group as compared to normal control group. Methfessel and Spencer, 1968 reported that LP diet depressed tissue uptake of $^{65}$Zn from ligated intestinal segments. According to Van Campen and House, 1974 one important consequence of protein depletion may be a secondary zinc deficiency. Nickel may be affecting the intestinal absorption of zinc at intestinal level. Nickel apparently affects zinc metabolism as evidenced by altered urinary excretion patterns (Clary, 1975) and organ distribution (Whanger, 1973) of zinc following nickel exposure. The other line of argument would be that during protein deficiency, the levels of metallothionein and other enzymes having zinc as a co factor would go
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down thus indicating low affinity for $^{65}$Zn and further our study has indicated increased half life of slow and fast component in whole body indicating its slower turnover which substantiates low uptake of $^{65}$Zn. We observed increased uptake of $^{65}$Zn in brain of protein deficient as well as nickel treated protein deficient rats when compared to normal rats. Zinc has been known to be associated with neurotransmission, which is based on the belief that cytoplasmic zinc is complexed to biogenic amines that are involved in neurotransmitter function (Vallee and Falchuk, 1993). It has been reported that LP diet leads to low levels of gamma-amino butyrate and glutamic acid in the brain (Raja Lakshmi et al., 1965). Therefore, the high uptake of $^{65}$Zn in protein deficient conditions could be to overcome the adverse effects of protein deficiency in neurotransmission. Nickel treatment to rats also indicated depression in uptake of $^{65}$Zn in liver, brain and intestine which would be due to some mechanism operating resulting in high mobilization of metallothionein as a result of nickel treatment. Administration of zinc to the protein deficient and nickel treated rats has greatly improved the uptake of $^{65}$Zn in such conditions. This also suggests that zinc supplementation might have helped in stimulating the levels of metallothionein and related enzymes having zinc as a co-factor.

Status of essential elements in serum and liver

It is now well authorized that the liver has an important function in the regulation of trace element metabolism (Burnett 1981; Failla and Kiser, 1983; Dhawan and Goel, 1996). Further trace elements serve as cofactors for many enzymes in numerous metabolic pathways; therefore, changes in the distribution of these essential elements in the body can have both nutritional and toxicological consequences with regard to the metabolism of other metals (Dhawan and Goel, 1994). Those metals which are essential for maintenance of the structural and functional integrity of the living organisms are found in all living systems and are conserved within strict concentration limits in the systems
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(McCall et al, 1971). However, imbalances in the supply of any of the essential elements in the body can have both nutritional and toxicological consequences with regard to the metabolism of other metals. They can further be responsible for the development of clinical signs of trace element deficiencies or can modify the susceptibility to metal toxicity (Hill and Matrone, 1970). It is well insinuated that metals that have similar chemical and physical properties would often interact biologically and antagonize or embellish each other's function (Singh et al, 1994). In view of all these facts, we have made an effort in the present study to investigate the effects of various treatments on the trace elemental composition of different essential elements and we further aimed at to delineate any possible correlation amongst them to reach to some definite conclusion.

The gastrointestinal absorption is the primary route of absorption for most of the essential and pollutant metals and it mainly takes place in the cells of intestinal mucosa. Nutritional stress in the form of protein deficiency is quite prevalent in the developing countries and it has been shown that nutritional status of an individual may have a profound influence on the absorption of metals. Persons afflicted with protein malnutrition are deficient in a variety of micronutrients (Wallwork et al, 1983).

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 (Jiachen et al., 1986, Nieborer et al, 1992; Severa et al, 1995; Obone E et al, 1999; Cempel and Janicka, 2002). However, administration of zinc along with these treatments altered the nickel levels and the nickel levels were brought to near normal levels, thereby showing the antagonistic effects of zinc towards nickel toxicity. Indications of biological antagonism between nickel and zinc has also been reported earlier (Whanger 1973; Clary 1975; Babich and Stotzky 1982; Waalkes et al 1985; Fisher
at al, 1986). Concurrent zinc exposure can reduce the toxic effects of nickel (Michael et al, 1985; Kazimierz et al, 1988). The decrease in the concentration of nickel following zinc administration may be because the uptake of nickel in the intestinal wall is suppressed by concomitant perfusion with high levels of zinc (Foulkes and McMullen, 1986).

Although zinc had been known to be essential for the growth of microorganisms for over hundred years, the advances in the knowledge of zinc chemistry and biochemistry have been explored only during the last two decades (Vallee and Falchuk, 1993). Furthermore, a large number of zinc-containing enzymes and proteins have been recognized to participate in the metabolism of proteins, nucleic acids, carbohydrates and lipids. Consequently, zinc deficiency is the most significant pathological and biochemical state involving abnormalities in the metal’s metabolism. This can be due to inadequate dietary intake, increased requirements or excretion, conditioned deficiency or genetic disorders (Forbes, 1984; Vallee and Galden, 1984; Aggette, 1985).

In present study, observation of depressed Zn levels in serum and in the liver of protein deficient rats are in conformity with previous studies (Wallwork et al, 1982; Martel et al, 1992; Tandon et al, 1999). We have also observed decrease in serum as well as hepatic zinc concentration following nickel toxicity in the present study, which may be because nickel mobilizes and promotes the excretion of copper, zinc and manganese from organs and promotes storage of chromium in organs (Henry et al, 1974; Cempel and Janicka, 2002). Abnormalities in zinc metabolism leading to its deficiency are generally attributed to various factors like, malabsorption, malnutrition, decreased intestinal zinc binding factors or the increased excretion of the zinc via the gastrointestinal tract or via urine are of common occurrence in chronic liver disorders (McClain and SuLe, 1983). The depression of zinc in condition of nickel toxicity also corroborates the hypothesis that the action of zinc on nickel is antagonistic in nature which further gets
validated by the fact that in the present study where we have seen that by supplementing nickel treated rats with zinc, the levels of nickel have gone down considerably. The infrequent replacement of zinc by nickel could probably be related to geometric considerations. Most zinc-containing metalloenzymes require a tetrahedral arrangement, whereas the 4-coordinate complexes of nickel are generally octahedral (Waalkes et al, 1985). Zinc is an integral part of many metalloenzymes such as alcohol dehydrogenase, including DNA and RNA polymerases, collagenase, carboxypeptidase, superoxide dismutase and the displacement of zinc by nickel appears to be associated with deactivation of critical enzymes (Gitlitz et al, 1975; Nieboer et al, 1984). Furthermore, it has been implicated in a few reports that plasma zinc levels are essentially more sensitive indicators for the deficient conditions, rather than hepatic suppression of the metal ion (Huber and Gershoff, 1970). The results of the current study for lowered serum zinc concentrations could be explained on the basis that either it is excessively being utilized in providing antioxidant defense mechanism or there is some defect in the absorption/metabolism of zinc in toxic conditions created by nickel or protein deficiency. Moreover, inhibited serum alkaline phosphatase activity observed in the present study may also be indicative of low Zn levels as it acts as cofactor for the alkaline phosphatase. Studies carried out in animals and humans had shown that zinc is essential for utilization of aminoacids (Sandstead, 1981). Conversely, protein malnutrition plays a major role on liver zinc depletion (Martel et al, 1992). Thus protein and zinc nutrition are interdependent.

Zinc has been found to be associated with metal binding proteins that are known to regulate the functions of zinc as well as copper. Metallothionein also plays a role in the detoxification of heavy metals and stabilize membranes (Vallee and Falchuk, 1993). Thus it may be possible that in case of nickel treatment the levels of metallothionein
(Zn-MT) could have been altered resulting in the decreased levels of zinc (Srivastava, 1993).

Protein restricted diet led to marked reduction in copper contents in the present study which is in agreement with earlier reports (Wallwork et al., 1983; Martel et al., 1992; Tandon et al., 1999). Copper depletion is associated with depressed hepatic Cu-Zn superoxide dismutase (SOD) activity and it is well known that protein deficiency results in reduction in SOD activity (Huang and Fwu, 1993). Above all, we have also seen depressed activities of SOD in the study. Since Ni and Cu are closely related chemically, an interaction between these two metals may exist. Significant inhibition is observed in copper concentrations, following nickel and protein deficient treatment. High levels of dietary Ni have been reported to decrease the levels of tissue copper and activities of a Cu-containing enzymes like cytochrome oxidase (Spears et al., 1977; Burnett, 1981; Cempel and Janicka, 2002). In an earlier study from our lab, hepatic Cu levels have also been found to be decreased following lead treatment (Dhawan et al., 1995). Since the larger fraction of serum copper is transported by the serum copper enzyme ceruloplasmin, the slight increase in ceruloplasmin activity due to Ni may reflect the increased mobilization of Cu from certain organs, which may be the reason for increase in Cu concentrations in serum following protein deficiency and nickel treatment in present study (Schreiber V et al., 1984).

The estimation of metal dependent enzymes helps in understanding the underlying mechanism of metal toxicity and the associated organ disorders. A recent study has suggested that patients undergoing kidney hemodialysis could be at higher risk for either moderate to severe Cu toxicity. Blood indexes in such subjects revealed high plasma Cu levels (Hosokawa S et al., 1985). Copper containing erythrocyte superoxide dismutase (SOD) activities were also reported to be higher which indicates enhanced episodes of free radical mediated
oxidation (Emenaker et al, 1996). Zinc supplementation to protein deficient and nickel treated animals resulted in normalized zinc and copper concentrations in liver and serum which may be due to increase in production of Zn-MT, major metallothionein for the detoxification of nickel toxicity. Further, the levels of nickel have also been observed to be depressed when zinc was supplemented to nickel treated as well as combined nickel+protein deficient treated rats. The reduction of nickel levels following zinc treatment indicates the alleviation of toxic effects of nickel, which got manifested in cooperation with the restoration of activities of many enzymes.

Manganese (Mn) though incorporated in a very few metalloenzymes but it activates a variety of other enzymes including hydroxylases, kinases, decarboxylases, transferases and phosphatases as well as some dehydrogenases and peptidases. Two Mn dependent metalloenzymes present in humans are pyruvate decarboxylase and mitochondrial superoxide dismutase (SOD). As per the literature, it is crucial to assess the biological role of Mn, since loss of SOD activity has been reported in Mn deficient rats with abnormal elongation and cristal disorientation of mitochondria from heart, liver and kidney (Hurley, 1981). In our study, we have observed significantly low levels of Mn in protein deficient, Ni treated and nickel treated protein deficient rats and an inhibition in SOD activity in this group signifies its biological role and an association of the metal and the enzyme. Since manganese is co-factor for SOD, the depressed levels of Mn may have been resulted because of low concentration of SOD in the present investigations or the biological turnover of Mn could have been increased under these conditions. Low protein diet feeding caused reduction in manganese levels and is in agreement with the earlier studies (Martel et al, 1992, Takeda et al, 1996). Decrease in the manganese concentration following nickel treatment, has also been reported earlier (Jerry et al, 1986). Schroeder et al stated that nickel
mobilizes and promotes the excretion of copper, zinc and manganese from different organs (Henry et al, 1974). Following zinc treatment, we observed increase in manganese concentration.

Iron plays an important role, not only in oxygen delivery to the tissues, but also as a cofactor with several enzymes involved in energy metabolism and thermoregulation (Rosenweig and Volpe, 1999). Further, the enzymes cytochromes P450 and b5 involve the metal in the metabolism of compounds such as steroids and in the degradation of xenobiotics (Galan et al, 1984; Aggette, 1985).

The hepatic Fe contents, in the present study, were found significantly depressed in protein deficient rats as compared to normal control group. Reduction in Fe contents following protein deficiency has previously been reported by Klavins et al, 1962 and Tandon et al, 1999. They concluded that protein plays an important role in the absorption of iron from the gastrointestinal tract and protein deficiency results in decreased absorption of iron.

During the course of this study, we have also observed increased hepatic iron contents in the animals, which were given toxic treatment with nickel, which has also been reported earlier (Forrest et al, 1979). Pronounced increase in iron concentration at a higher dose of nickel could be because of nickel functioning as a cofactor that facilitates the intestinal absorption of the iron by enhancing its compellation to a lipophilic molecule (Nielsen et al, 1984). Increased hepatic iron levels in response to the toxic conditions established by nickel also suggests that the body requirement of iron has been so severe that more and more of this metal ion is transported to the liver, as it serves as a cofactor for many key enzymes involved in various energy generation pathways, which could have been adversely affected in stress conditions. When serum iron levels exceed the iron binding capacity of transferrin a β1-glycoprotein synthesized in the liver, the circulating free iron initially accumulates in Kupffer cells and later in the hepatocytes resulting in the
increased concentration of iron in liver tissue. It can also be speculated that the nickel treatment leads to the activation of host defense system which results in the enhancement of hepatic iron content so as to combat the toxic conditions created by nickel administration. Another possible reason for increased liver iron concentrations could be thought of due to impaired hepatic elimination of this metal from the liver. As the animals are exposed to profound stress, increased body requirements of iron seen to be reasonably convincing. Hepatic iron overload as observed in our study may lead to the development of a severe oxidative stress status in the tissue, thus contributing to the concomitant liver injury as reported by Boisier et al, 1999. This fact gets strengthened by the observation of the histoarchitecture at EM level where the hepatocytes have undergone some changes in the cellular organelles. However, zinc treatment to protein deficient, nickel treated and nickel treated protein deficient animals, has lead to considerable regulation of Fe levels but they still were different from normal animals. It has been reported that zinc prevents the iron induced lipid oxidation (Zago and Oteiza, 2001).

Selenium, which is an essential trace metal and inactivates sulfhydryl groups in certain enzymes and is also a component of glutathione peroxidase (Rukgauer, 2001). This ubiquitous enzyme located in both cytosol and mitochondrial matrix uses glutathione to reduce organic hydroperoxides, thereby, prevents oxidative damage to various cell organelles. In the present study, it was found to be decreased significantly in protein deficient (G-2), nickel treated (G-3) and nickel treated protein deficient (G-5) animals. It has been reported that nickel and selenium act antagonistically and the detoxifying effect of selenium on nickel toxicity seems to be due to the formation of a Ni-selenide excretable complex (Khandelwal S et al, 1990) which seems to have been marginalized due to high dose of nickel in the present study. Observations regarding depressed hepatic Se levels following
PD and Ni treatments in the present study could also be seen in relation to rise in the hepatic glutathione peroxidase (GPx) activity and reduced glutathione levels. This indicates that mechanism of GPx synthesis gets stimulated to prevent oxidative tissue damage following protein deficiency and nickel intoxication. However, zinc administration to these groups raised the selenium levels significantly as compared to respective controls but these are still lower than the concentration in normal control animals. This observation corroborates with earlier studies where it was seen that essential micronutrients selenium and zinc interact with one another to regulate the cellular homeostatic zinc system (Jacob et al, 1998; Maret W, 2003).

Potassium is an essential intracellular ion involved in cellular homeostasis and electrical conduction. We have observed a statistically significant decrease in potassium levels in nickel treated (G-3) and nickel treated protein deficient (G-5) animals. Rai et al, 1990 has also observed the loss of K⁺ and Na⁺ by Nickel. As potassium is a major cation of intracellular fluid, and functions in balance with the extracellular ionized sodium to maintain normal osmotic pressure and water balance hence 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. Its deficiency affects the activity of muscles and transmission of electrochemical impulses. It has been reported that nickel inhibits the Na-K-ATPase (Rubanyi et al, 1982). Administration of zinc to nickel treated (G-7) and nickel treated protein deficient (G-8) animals restored the lowered values to normal levels, emphasizing that Zn might have a regulatory effect on the Na⁺/K⁺ transport across the biomembranes through cellular ATPase and also Zn²⁺ is an integral part of the voltage-gated potassium channels (Jahng et al, 2002) which supports our hypothesis. The increase in intracellular
Discussion

Potassium concentrations following zinc administration has also been reported earlier (Chan and Cherian, 1992).

Phosphorus, which is an essential part of key cellular nucleoproteins such as DNA, has also been found to increase significantly in nickel treated (G-3) and nickel treated protein deficient (G-5) animals. It may possibly be due to increased requirement of phosphorus either due to the inhibitory effects of nickel on DNA synthesis leading to hyperplasia or increased mobilization from bones. Zinc treatment in these conditions restored the altered levels of phosphorus near to normal concentrations and decrease in concentrations is significant when compared to respective controls.

Sulfur is an important constituent of many amino acids like methionine, cysteine, cystine, homocysteine, homocystine, and taurine and also of enzymes like S-adenosylmethionine (SAM) and glutathione (GSH), (Parcell, 2002). Sulfur concentrations got elevated in nickel and PD+Ni treated animals, which might be to counter the toxic effects of nickel. Administration of zinc to these animals tried to restore the imbalance sulfur levels.

Morphological studies of blood cells

Erythrocytes are fundamentally capable of few stereotypic responses to a variety of environmental perturbations, which are sometimes considered to be of vital physiological significance. Furthermore, it is implicated that modifications of the shape and size of the erythrocytes represent the most common morphologic abnormalities that occur in pathologic conditions (Barnhart et al, 1983). Since Scanning Electron Microscope (SEM) provides details of the cell shape it is possible to use it to ascertain the morphological defects of red blood cells in different etiological conditions. Keeping this in view, the scanning study was undertaken to ascertain the importance of altered RBC morphology to the toxic effects of nickel and protein deficiency and possible protective effects of zinc in this regard.
Discussion

During the course of present investigations, drastic alterations in the red cell morphology were noticed in the animals, which received nickel and protein deficient treatment for 8 weeks. The prominent features were the transformation of the normal discocytic appearance of RBCs to many different forms viz., echinocytes, spherocytes, stomatocytes, acanthocytes etc. These altered forms of blood cells were evident at the same time and are indicative of the severity of the toxic treatments. The possible explanation of these altered forms of blood cells could be attributed to the general causes of the abnormalities in the red blood cell shapes which stems from vital reasons such as abnormal erythropoiesis in stem cells and bone marrow which seems to be primary target of nickel toxicity (Morse et al, 1977; Dieter et al, 1988; Morais et al, 1999), inadequate haemoglobin formation and damage to the red cells after they leave bone marrow because of nickel affecting the erythrocyte membrane lipid bilayer, accelerates erythrocyte aging, decrease water permeability across erythrocyte membranes, decrease erythrocyte thermostability, deformability and the rate of O₂ release by erythrocytes (Tkeshelashvili et al, 1989) or increased erythropoiesis by bone marrow to compensate for anemic conditions (Bessis, 1972).

Protein energy malnutrition lowers the rate of amino acid transport to RBC's due to their lower concentration in plasma or shift of transport mechanism from active transport to exchange diffusion which results in decrease of total essential and non-essential RBC's amino acids (el-Shobaki FA et al, 1980).

It has also been reported that the changes in the membrane lipid composition is the key reason for such deformations in blood cell shape in response to various adverse treatments (Sherman, 1979). The results of these modifications in membrane of the infected cell are manifested by the rheologic properties of the cells, whereby they cannot traverse the microvasculature, which leads to accelerated pitting and clearance within the spleen. In our present study, marked lipid peroxidation was...
noticed, which is in corroboration with this assumption. Analogous results were reported by Arutjunov et al., 1981 while studying toxic anaemias caused by exposures to occupational toxic agents and they concluded that most of the RBCs transformed to either spherocytes or schizocytes.

As regards the animals given zinc treatment, significant improvement in the morphology of the erythrocytes was noticed thereby indicating the protective effects of zinc could be attributed to its antiperoxidative potential, whereby, it maintains the membrane lipid composition and ultimately the normal shape of these cells.

**Histoarchitectural studies of liver**

Hepatic failure may occur when there is massive necrosis of hepatocytes, which may be caused by many agents that are direct hepatotoxins or it can be achieved even by the indirect action of certain agents, which cause anoxia and later perpetuate their effects by liver damage. During the course of present investigations, it was observed that the hepatic histoarchitecture of the nickel treated and protein deficient animals resulted in vacuolization of the hepatocytes and sinusoids got dilated appreciably, in comparison to the normal rats. The number of binucleated cells were also more in these animals, in proportion to the normal control animals. In the earlier studies also similar observations were recorded following nickel toxicity (Mathur et al., 1977) and following protein deficiency (Krustev et al., 1982; Lieber 1983). The present observations indicated marked changes in the overall histoarchitecture of liver in response to nickel treatment, which could be explained on the basis that nickel manifested its toxic effects primarily by the generation of more oxidative stress on the body. The resulting effect was production of increased number of free radicals, which cause significant amount of lipid peroxidation and thus cause damage to the various membranous components of the cell. The necrotic conditions observed in these animals are in good agreement.
Discussion

with our observed biochemical studies, where we have noticed increased levels of serum AST and ALT as high levels of these enzyme are indicative of hepatic injury.

Electron microscopic observations of the present study with regard to protein deficiency revealed that nuclear membranes of the hepatocytes were broken, and nuclei were altered, nucleoli got retracted. There was decrease in the number of mitochondrial population, few mitochondria were enlarged. There was reduction in the number of SER and RER and lots of fat globules were present. This is inconsonant with the study of Svoboda and Hicocinson, 1964; Ghaidally, 1982 and Sakuma et al, 1987. According to Ghaidally, 1982 protein starvation induces accumulation of lipid globules in the SER apparently by withholding the protein necessary for turning lipid into lipoprotein and thus for releasing it from the hepatocyte. It further causes swelling and rupture of mitochondria, presumably by depleting the protein-enzymes of their cation pumps and finally it produces first a hypertrophy of the ribosomal pars granularis of the nucleolus and later a shrunken, segregated pars granularis presumably because it eventually leads to deficiency of the enzyme-protein needed for RNA synthesis. In the present study also, we have noticed a significant decrease in phase I and phase II drug metabolizing enzymes, which possibly be due to the deficiency of RER leading to the reduced synthesis of enzyme-protein, needed for RNA synthesis.

Furthermore, electron microscopic observations of the present study with regard to nickel toxicity revealed predominant effects on the mitochondria, endoplasmic reticulum, and glycogen content. Ours is probably the only study so far to examine the effects of nickel on the ultrastructure of liver and we have observed reasonably convincing correlation between the extent of hepatic damage and various biochemical indices envisaged in this study. In our study, nickel treatment to rats resulted in significant effect on nuclear structures of
hepatocytes whereby their membranes were broken and shapes got deformed. These effects are suggestive of the decreased rate of DNA synthesis as there are reports that nickel affects the DNA synthesis by diminishing the DNA and RNA polymerase activity and decreases DNA replication fidelity (Sirover and Leob, 1976). Moreover, marked swelling and increase in the number of mitochondria was another prominent feature of the present study in nickel intoxicated animals and which is indicative of the increased energy requirements of the cells in an effort to overcome the noxious effects of the nickel.

In view of all these facts, the observed effects of nickel toxicity and protein deficiency on hepatic histoarchitecture seem mainly due to the increased membrane disintegration as a result of enhanced lipid peroxidation. This further initiates a chain of events, due to which body requirements of energy are increased and have direct bearing on the mitochondrial and nuclear structures.

Administration of zinc to nickel treated and protein deficient animals resulted in normalizing the hepatic histoarchitecture quite appreciably. Such hepatoprotective effects of zinc have also been observed by us earlier while evaluating its potential in CCl4 toxicity (Dhawan and Goel, 1994). Similar results are evident in this study, and could well be attributed to the antioxidant effects of zinc, which maintains the membrane integrity either by some direct mechanism or indirectly by scavenging the free radicals responsible for increased lipid peroxidation. The normalization of histoarchitecture of liver following zinc administration may also be attributed to the recovery accorded by zinc in regulating the levels of different enzymes as indicated in our study as well as the increased synthesis of metallothionein. The increased levels of metallothionein following zinc administration have also been reported earlier (Santon A et al, 2003). Zn-MTs protect membrane configuration and prevent the damage on the membrane fluidity and permeability (Xu L et al, 2000;Ali MM et al, 2002).