NIQUEL DEFICIENCY IN CHICKS AND RATS: EFFECTS ON LIVER MORPHOLOGY, FUNCTION AND POLYSOMAL INTEGRITY

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Nickel is an essential nutrient for animals. It has the following characteristics and biological behaviors which are consistent with essentiality: (1) It is a low molecular weight transition element which forms chelates. Thus it is chemically suitable for biological functions. (2) It is ubiquitous in the earths' crust (0.008%) and in sea water (0.1-0.5 ppb), therefore, it has been generally available to life forms throughout evolution. (3) It is present in relatively constant amounts in plants and animals. (4) It is non-toxic to animals orally except in astringent doses. (5) Homeostatic mechanisms are implied by serum levels, excretion rates, and its tendency not to accumulate. (6) Inadequate amounts of nickel in the diets of experimental animals repeatedly result in an impairment of metabolic processes. These effects correlate with morphologic abnormalities. The first five of these features have been reviewed (Schroeder, et al., 1962; Underwood, 1971; Nielsen, 1971 and 1973; Nielsen and Ollerich, 1973). The last aspect has been the subject of our research for the past several years. This presentation will describe some of these studies.

The first evidence that nickel is essential was obtained from experiments with chicks (Nielsen and Sauberlich, 1970; Nielsen and Higgs, 1971; Nielsen, 1971). Feeding a diet containing less than 40 ppb nickel resulted in an apparently characteristic deficiency syndrome. When compared with controls given a supplement of 3-5 ppm nickel, the deficient chicks showed: (1) pigmentation changes in the

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shank skin; (2) thicker legs with slightly swollen hocks; (3) dermatitis of the shank skin; (4) a less friable liver which appears to have been related to the fat content; and (5) an enhanced accumulation of a tracer dose of $^{63}$Ni in liver, bone and aorta. These findings were observed under conditions which produced suboptimal growth. The abnormalities in leg structure and the dermatitis of the shank skin were inconsistent.

Sunderman et al. (1972) attempted to confirm these findings by feeding a diet containing 44 ppb to chicks raised in a slightly different environment. While they found no gross effects, they did observe ultrastructural changes in the liver. These included dilation of the perimitochondrial rough endoplasmic reticulum in 15 to 20% of the hepatocytes.

In a single experiment with chicks, Leach (1973) observed a growth response with nickel. Later attempts to obtain the growth response were unsuccessful.

Wellenreiter et al. (1970) fed a diet containing 80 ppb nickel to reproducing quail and saw no gross symptoms except an inconsistent positive effect of nickel on breast feathering in birds which were fed a diet relatively deficient in arginine but otherwise adequate in protein.

To clarify and extend the above observations, improvements have been made in the experimental environment and a diet has been formulated with a nickel content of 3-4 ppb. With this diet and environment, we have consistently been able to produce a nickel deficiency in chicks and rats (Nielsen and Ollerich, 1973; Nielsen, 1973).

It has been found that after 3½ weeks, the gross appearances of the deficient and control chicks are similar except for the difference in pigmentation of shank skin which had been observed previously (Nielsen and Sauberlich, 1970; Nielsen and Higgs, 1971; Nielsen, 1971). All chicks at 3½ weeks weigh 350 to 400 g. Leg structure and skin abnormalities which occur are inconsistent. The other gross sign, decreased friability of the liver, which had been observed in our earlier studies is consistently found in the deficient chicks which are raised under the improved experimental conditions.

In contrast to the gross signs, a number of abnormalities in biochemical indices of metabolism are consistently found in the nickel deficient chicks. They include a decreased oxygen uptake by liver homogenates in the presence of α-glycerophosphate, an increase in liver total lipids, and a decrease in liver lipid phosphorus. In addition, a preliminary study has shown an increase in total lipids and lipid phosphorus in the heart.
Figure 1. Hepatic cell from a nickel supplemented chick (3 ppm nickel). Compare mitochondria (M) with those in figure 2. Endoplasmic reticulum (ER). Nucleus (N). Uranyl acetate and lead citrate. X 21,600.
Figure 2. Hepatic cell from a nickel deficient chick (4 ppb nickel). Swelling of mitochondria (M) was evident in numerous hepatic cells. The swelling was in the compartment of the matrix and appeared to cause fragmentation of cristae. Note the dilated cisterns of the rough endoplasmic reticulum (ER) and dilated perinuclear space (PS). Nucleus (N). Uranyl acetate and lead citrate. X 21,600.
Ultrastructural abnormalities in the hepatocytes are also consistently found. Some of these abnormalities are illustrated in figures 1 and 2. These abnormalities are similar to, though more extensive than, those described by Sunderman et al. (1972). They include dilation of the cisterns of the rough endoplasmic reticulum and swelling of the mitochondria. The swelling is in the compartment of the matrix and is associated with fragmentation of the cristae. Other changes include a dilation of the perinuclear space and pyknotic nuclei.

In rats, nickel apparently affects reproduction as seven first generation nickel deficient females which were mated had a significant number of dead pups (15%), while no mortality occurred in the pups of six nickel adequate controls. Nine second generation nickel deficient female rats which were mated had a 19% loss of pups. This finding was somewhat confounded by the fact that the eight controls had a 10% loss. This was, however, roughly only one-half of the loss in the nickel deficient group. During the suckling stage, the nickel deficient pups have a less thrifty appearance and are less active than control pups. To assess this

Figure 3. The activity of six each of nickel deficient and supplemented rats from matched litters of the third generation.
last observation, a Stoelting activity monitor \(^2\)\(^3\) has been used to measure the activity of matched litters in the second and third generation. Figure 3 indicates that the nickel deficient pups are indeed less active.

Liver oxidative ability has been measured in 12 deficient and 12 control second generation male rats. Grossly, the livers of the deficient rats were a muddy brown color compared with a red brown color of controls. They also had a less distinct substructure and were less friable. As was the case with chicks, the nickel deficient rat liver homogenates oxidized α-glycerophosphate less well than did liver homogenates from control rats.

It has recently been reported that nickel deficiency can be produced in swine (Anke et al., 1973), as well as in chicks and rats. Some of the signs include impaired reproduction and offspring with a less thrifty appearance characterized by a sparse rough hair coat and parakeratosis-like changes in skin. First generation nickel-deficient piglets grow poorly.

Thus, our studies and those of others have established that nickel deficiency can be experimentally produced in three species of animals. The findings do not, however, provide more than a meager insight as to the metabolic function of nickel.

It is known that nickel may complex with macromolecules. For example, it is present in the metalloprotein nickeloplasmin which has been isolated from the serum of rabbits and man (Nomoto et al. 1971 and Sunderman et al. 1971).

Nickeloplasmin is a macroglobulin which has an estimated molecular weight of \(7.0 \times 10^2\) and contains approximately 0.8 g atoms of Ni/mole. Disc gel and immunoelectrophoresis have shown that purified nickeloplasmin is an α-1 macroglobulin in rabbit serum and an α-2 macroglobulin in man. The functions of this protein are unknown. It is known that nickel can activate numerous enzymes in vitro. Examples include arginase (Hellerman and Perkins, 1935), tyrosinase (Lerner et al. 1950), desoxyribonuclease (Miyaji and Greenstein, 1951), acetyl coenzyme A synthetase (Webster, 1965), and phosphogluconatase (Ray, 1969). These studies, however, do not necessarily indicate that nickel is a specific activator of a specific enzyme in vivo.

\(^2\)Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

\(^3\)Stoelting Company, Chicago, Illinois.
Nickel also complexes with DNA and RNA. Significant concentrations of nickel have been found in DNA (Wacker and Vallee, 1959; Eichhorn, 1962) and RNA (Wacker and Vallee, 1959; Wacker et al. 1963; Sunderman, 1965) from phylogenetically diverse sources. It has been suggested that nickel and other metals which are present may contribute to the stabilization of the macro-molecular structure of the nucleic acids. Nickel has been shown to stabilize RNA (Fuwa et al. 1960) and DNA (Eichhorn, 1962) against thermal denaturation. In addition, it is extraordinarily effective in the preservation of tobacco mosaic virus RNA infectivity (Wacker, et al. 1963; Cheo et al., 1959). Other studies have suggested that nickel may have a role in the preservation of the compact structure of ribosomes (Tal, 1968, 1969a, 1969b). Nickel will protect ribosomes from in vitro thermal denaturation at low temperatures and will restore the sedimentation characteristics of E. coli ribosomes which have been denatured with EDTA.

Because these in vitro studies are consistent with a role for nickel in the metabolism of RNA, we conducted a study of the effects of nickel deficiency on rat liver polyribosomes and the activities of liver RNA polymerase and RNase.

MATERIALS AND METHODS

To bring out the effects of nickel deprivation, successive generations of Sprague-Dawley rats were raised. Thus, the animals were exposed to deficiency throughout fetal, neonatal and adult life. The rats were housed in plastic cages placed inside a controlled environment such as an all plastic rigid isolator\(^4\), or a laminar flow animal rack\(^5\). The feed and water cups were also plastic. Thus, the only materials the animals came into contact with were plastic, and the air entering the system was filtered to remove contaminating dust.

All equipment used in the experiments was cleaned as follows: (1) washed with detergent and tap water, (2) soaked in a 1:10 solution of radiacwash\(^6\) for 1 hour or more, (3) rinsed with distilled water and (4) rinsed with high purity water\(^7\) (18 megohms-cm resistance).

\(^4\)Germfree Laboratories, Inc., Miami, Florida.
\(^5\)Carworth Division of Becton, Dickinson, and Co., New City, New York.
\(^6\)Atomic Products Corp., Center Moriches, L.I., New York, New York.
\(^7\)Produced by a "Super Q High Purity Water System", Millipore Corp., Bedford, Massachusetts.
The diet used was the same as described previously (Nielsen, 1973). It was based on dried skim milk, ground corn and corn oil. The diet contained approximately 4 ppb nickel on an air dried basis. Control rats were fed the basal diet supplemented with 3 ppm nickel as NiCl$_2$·6H$_2$O. The diets and high purity water were given ad libitum.

The age, sex and generation of rats used in the experimental procedures are given in the results and discussion.

The sucrose density gradients of the liver post-mitochondrial supernatants were obtained by homogenizing the liver after diluting 1:3 (w/v) with a medium containing 0.25 M sucrose, 5 mM Tris·HCl, 25 mM KCl and 5 mM MgCl$_2$, at pH 7.4. The supernatants obtained

![Graph showing sucrose density gradients](image)

**Figure 4.** Representative sucrose density gradients of liver post-mitochondrial supernatants obtained from six nickel deficient (4 ppb nickel) and six supplemented (+3 ppm nickel) rats.
after centrifugation at 4400 x g, at 4°C, were made 0.5% with respect to deoxycholate and centrifuged at 4687 x g, at 4°C. The supernatants were then layered over a linear 15.5-35.5% (w/v) sucrose density gradient and centrifuged for 160 minutes at 284,000 x g, at 4°C. The gradients were monitored at 260 nm.

The RNA polymerase was analyzed in intact nuclei by using the high Mg, low salt technique as described by Fosmire et al. (1973).

RNase was determined by the method of Gagnon and de Lamirande (1973) as modified by Fosmire (1973). Protein was determined by the biuret method after samples (0.1 ml) were dispersed with 0.4 ml of 1% NaCl and 0.5 ml of 10% sodium deoxycholate. Statistical analyses was performed by the "t" test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Sucrose density gradients of liver post-mitochondrial supernatants were prepared from six each of control and deficient rats. Three second generation males, approximately 6 weeks of age from different litters and three third generation females, approximately 6-10 weeks of age from different litters were used. The findings were all similar to the profiles shown in figure 4. It is evident that there is a decrease in polyribosomes with a relative increase in the monosomes in the nickel deficient rat liver.

For the RNA polymerase analyses, two first generation male rats approximately 5 weeks of age, and three third generation male rats approximately 6-10 weeks of age, from each of the nickel deficient and supplemented groups were used. For the RNase analyses, five first generation male rats approximately 4 weeks of age from each the nickel deficient and supplemented groups were used.

The results of the RNA polymerase and RNase analyses are shown in Table I.

These preliminary results indicate that nickel deficiency apparently causes a significant increase in liver nuclear RNA polymerase activity and in the active, and possibly the total, alkaline RNase/protein. These findings substantiate the hypothesis that nickel has a role that is interrelated with certain aspects of nucleic acid, especially RNA metabolism.
<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>RNA Polymerase</th>
<th>RNase, pH 7.8 Total</th>
<th>RNase, pH 7.8 Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni Def</td>
<td>5</td>
<td>7.7 ± 1.2</td>
<td>0.99 ± 0.04</td>
<td>0.44 ± 0.001</td>
</tr>
<tr>
<td>(4 ppb)</td>
<td></td>
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</tr>
<tr>
<td>Ni Supl</td>
<td>5</td>
<td>4.2 ± 1.5</td>
<td>0.82 ± 0.06</td>
<td>0.035 ± 0.002</td>
</tr>
<tr>
<td>(+3 ppm)</td>
<td></td>
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</tbody>
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1 Significantly different (P < .05) from +3 ppm Ni group.
2 ± SEM
3 Significantly different (P < 0.10) from +3 ppm Ni group.
4 Significantly different (P < 0.01) from +3 ppm Ni group.
An increase in these 2 enzymes, and a decrease in polysomes, in nickel deficiency suggests the following hypothesis. The structural stability of the polysome is abnormal, or there is an impairment in the formation of polyribosomes through the combination of ribosomes with messenger RNA. The resultant subnormal quantity of polyribosomes may induce the production of additional messenger RNA via increase RNA polymerase activity. This additional RNA which is not readily incorporated into the polyribosomes stimulates the activity of RNase by its presence in the cytoplasm.

Since a major portion of the polysomes is attached to membranes, it is possible the defect is in this relationship. This is suggested by the ultrastructural abnormalities which occur in the livers of the nickel deficient chicks. The swollen mitochondria may indicate that there is an abnormality in the mitochondrial membrane. The dilation of the perinuclear space and the presence of pyknotic nuclei may be indicative of abnormalities in the nuclear membrane. Finally, the cisterns of the endoplasmic reticulum - a network of membrane bound cavities - to which the polysomes attach, are dilated. This finding is consistent with an abnormality in these membranes. Other supportive evidence includes the finding that the level of phospholipids in liver is decreased by nickel deficiency (Nielsen, 1973; Nielsen and Ollerich, 1973). Phospholipids are an integral part of the various membranes of cells.

Other support for the hypothesis that nickel has a role in the metabolism or function of certain cell membranes is provided by in vitro studies with isolated tissues. Nickel can substitute for calcium in certain steps of the excitation-contraction coupling of isolated skeletal muscle (Frank, 1962; Fischman and Swan, 1967). Other investigators have found that nickel can also substitute for calcium in the excitation process of the isolated nerve cell (Blaustein and Goldman, 1968; Hafeman, 1969). It has been suggested that nickel can substitute for calcium in the complex with a membrane ligand, such as the phosphate of a phospholipid and thus may influence the processes of nerve transmission and muscle excitation and contraction. Though these speculations are attractive, our current knowledge allows us to say little more with confidence than that nickel complexes with macromolecules, and that it thus appears to influence their structure and function.

SUMMARY

Substantial evidence has accumulated which shows that nickel
is an essential nutrient for animals. Nickel deficiency repeatedly results in an impairment of a function, or functions, as evidenced by ultrastructural degeneration, by reduced oxidative ability, and by the increased total lipids and decreased lipid phosphorus in the liver of the chicks. Swine deprived of nickel show impaired reproductive performance and the young produced are less thrifty and do not grow as well as nickel supplemented controls. Rats deprived of nickel show a reduced oxidative ability in the liver and abnormalities in the liver polysomal profile. Preliminary results also indicate increased liver nuclear RNA polymerase and increased total and active alkaline RNase/protein.

It is suggested on the basis of available data that nickel, because it can complex with macromolecules, has a role in the metabolism of membranes, and in the metabolism of RNA.

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