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Nickel-Copper Interrelationship in the Rat (39893)

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Nielsen (1) has discussed the possibility of nickel (Ni) interacting with other inorganic ions. Since Ni and copper (Cu) are closely related chemically, an interaction between these two metal ions in the biological system may exist. Copper is known to be involved in the pigmentation process (2) and evidence suggests that Ni may have a role with respect to pigmentation in certain species (3, 4). Both Ni and Cu can activate tyrosinase (5), an enzyme involved in melaninogenesis. However, Cu is much more effective than Ni in activating this enzyme (5). High levels of dietary Ni have been reported to decrease the activity of cytochrome oxidase, a Cu-containing enzyme (6), while high levels of dietary Cu have been reported to increase Ni excretion in cows fed diets high in Ni (7). Copper is essential for normal hemoglobin formation (2). Recently Ni-deficient rats were found to have reduced erythrocyte counts, hematocrit, and hemoglobin values (8). The present study was designed to study the interrelationship between Cu and Ni in rats fed diets low or adequate in Cu.

Materials and methods. Forty weanling male rats of the Sprague-Dawley strain initially weighing approximately 70 g were randomly divided into four groups. Prior to initiation of the experiment the rats were fed a stock diet *ad libitum*. Treatments were: (i) basal; (ii) basal + 20 ppm Ni; (iii) basal + 15 ppm Cu; and (iv) basal + 15 ppm Cu + 20 ppm Ni. The basal diet is shown in Table I and contained 0.1 ppm Ni and 0.95 ppm Cu. The diet was formulated to be deficient in Cu but based on previous work (3, 8) the basal diet would not be considered deficient in Ni. Nickel and copper were added to the basal diet as $NiCl_2 \cdot 6H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively. Rats were housed individually in stainless steel cages and offered distilled water and feed *ad libitum* in glass containers.

After 28 days five rats from each treatment were anesthetized and whole blood was collected from the abdominal aorta for hematological studies including microhematocrit, hemoglobin, and erythrocyte count. The five remaining rats from each treatment were anesthetized and serum samples were collected for the determination of ceruloplasmin oxidase activity. The entire liver, lung, heart, and kidney were removed from this group, blotted, weighed, and used for Cu analysis and liver iron (Fe) determination.

Hemoglobin was determined using Hycel[1] cyanmethemoglobin reagent with readings at 540 nm. Blood was stained with Wright's stain for erythrocyte counting. Ceruloplasmin was assayed as described by Rice (9) with results expressed as international units of enzyme activity. Entire tissues were prepared for mineral analysis by digestion in concentrated nitric acid followed by oxidation with hydrogen peroxide. Mineral concentrations were determined by atomic absorption spectrophotometry.[2]

All data were analyzed by analysis of variance with mean differences determined using the least significant difference (LSD) test (10). Significant differences cited in the text have a P value of less than 0.05.

Results. In comparison to animals fed the basal low Cu diet, those receiving Cu or Ni supplements, or both, gained more weight during the 28-day period (Table II) and had higher hematocrits (Table III). Responses in weight gains and hematocrits were not different between supplemented groups. Hemoglobin values were not increased by Ni but were by Cu, irrespective of Ni supplementation. Erythrocyte counts were increased significantly only by Cu alone and not by Cu in the presence of Ni.

Ceruloplasmin oxidase activity (Table IV)

[1] Hycel, Inc., Houston, Texas.

[2] Model 306, Perkin-Elmer, Corporation, Norwalk, Connecticut.

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was increased only by Cu supplementation, the effect of Ni being nonsignificant. As shown in Table V, Cu-supplemented groups had lower concentrations of liver Fe and higher liver Cu than those receiving no Cu supplementation, irrespective of dietary Ni level.

As shown in Table VI, heart and kidney Cu were increased in Cu-supplemented animals, with Ni having no effect. Lung Cu was not significantly affected by diet supplementation in comparison to the basal group. However, rats receiving the basal diet supplemented with Ni had lower lung Cu concentrations than animals receiving the Cu-supplemented diet without supplemental Ni.

Discussion. Rats receiving the Cu-deficient basal diet gained less than rats receiving the supplemented diets though all rats performed well in terms of growth. Evans and Abraham (11) found growth to be affected shortly after rats had been placed on a low Cu diet.

Consistent with previous studies (11, 12) were the decreased hematocrit and hemoglobin values found in the rats receiving the low Cu diet. Hematocrits have also been reported to be lowered during an Ni deficiency in the rat (8, 13) as well as in the chick (3). Ni supplementation of the low Cu diet resulted in a significant increase in hematocrit values. However, the addition of Ni to the 15 ppm Cu treatment resulted in no further increase in hematocrit or hemoglobin values over the adequate Cu diet. Red blood cell numbers were slightly lower in rats receiving neither Ni or Cu. Copper deficiency has been reported to result in a shorter erythrocyte survival time (14) as well as a decreased erythrocyte production (15).

Copper is currently believed to exert its effect on hemoglobin metabolism through ceruloplasmin. Osaki *et al.* (16) first suggested that ceruloplasmin catalyzed the oxidation of ferrous Fe into ferric Fe for incorporation into apotransferrin, thus enhancing the transport of iron to the site of hemoglo-

TABLE I. COMPOSITION OF BASAL DIET[a].

	Percentage
Spray dried egg white	20.0
Sucrose	57.3
Solka floc	3.0
Corn oil	10.0
Vitamin mix[b]	5.0
Mineral mix[a]	4.7

[a] Basal diet contained 0.1 ppm Ni and 0.95 ppm Cu.

[b] Vitamin-glucose mix, g/100g of diet: thiamine HCl, 0.001; riboflavin, 0.0006; Ca pantothenate, 0.0016; pyridoxine HCl, 0.0004; niacin, 0.0025; biotin, 0.00024; folic acid, 0.00005; B_{12}, 0.000002 (in mannitol); choline chloride, 0.15; menadione, 0.00004; α-tocopherol-succinate, 0.00327; retinyl palmitate, 0.004; calciferol, 0.00025; glucose, 4.85.

[c] Mineral mix, g/100g of diet: $CaHPO_4$, 2.7160; NaCl, 0.3900; KCO_3, 1.2370; $MgSO_4$, 0.2972; $MnSO_4 \cdot H_2O$, 0.0123; $FeSO_4 \cdot 7H_2O$, 0.0249; $CoCl_2 \cdot 6H_2O$, 0.0040; NaF, 0.0007; KI, 0.0005; $Na_2MoO_4 \cdot 2H_2O$, 0.005; $SnCl \cdot 2H_2O$, 0.0004; Na_2SeO_3, 0.000022; $CrCl_3 \cdot 6H_2O$, 0.0002; ZnO, 0.0062; $NaSiO_3 \cdot 9H_2O$, 0.0500; NH_4VO_3, 0.00005.

TABLE II. EFFECT OF VARYING LEVELS OF DIETARY COPPER AND NICKEL ON 28-DAY GAINS.

Added copper (ppm)	Added nickel (ppm)	Gain (g)
0	9	142.1 ± 5.6[a]
0	20	160.9 ± 5.0[b]
15	0	162.0 ± 5.6[b]
15	20	173.5 ± 5.6[c]

[a] Mean ± SEM.

[b] Significantly different from basal diet ($P < 0.05$).

[c] Significantly different from basal diet ($P < 0.01$).

TABLE III. HEMATOLOGICAL PARAMETERS IN RATS RECEIVING VARYING LEVELS OF COPPER AND NICKEL.

Added copper (ppm)	Added nickel (ppm)	Hematocrit (%)	Hemoglobin (g/100 ml)	Erythrocyte count (cells × 10^3/mm^3)
0	0	38.1 ± 1.0[a]	10.7 ± 0.3	6070 ± 114
0	20	41.5 ± 0.9[b]	11.7 ± 0.6	6670 ± 170
15	0	43.8 ± 1.6[c]	13.0 ± 0.2[c]	6970 ± 197[b]
15	20	43.5 ± 1.1[c]	12.8 ± 0.4[c]	6440 ± 123

[a] Mean ± SEM.

[b] Significantly different from basal diet ($P < 0.05$).

[c] Significantly different from basal diet ($P < 0.01$).

bin synthesis. Ceruloplasmin activity was decreased substantially in the present study as a result of the low dietary Cu intake. The decreased ceruloplasmin activity in the rats fed the low Cu diets was associated with an increased hepatic Fe and a decreased hepatic Cu concentration. Other workers have found Fe to accumulate in the liver (11, 12) and spleen (12) during a Cu deficiency. Ceruloplasmin appears to be involved in the mobilization of stored iron (11).

It is unclear why Ni supplementation to the low Cu diet resulted in an increased weight gain and hematocrit. Nickel addition to the low Cu diet also appeared to have only slight influence on hemoglobin concentration, erythrocyte numbers, and ceruloplasmin activity. One possible explanation is that Ni by substituting for Cu at certain biological sites preferentially spared Cu for vital functions. Nickel tended to decrease tissue Cu levels especially in the low Cu group. Since Cu is transported by ceruloplasmin the slight increase in ceruloplasmin activity due to Ni may reflect an increased mobilization of Cu from certain organs. These data are suggestive only and can be verified only by further experimentation. A more likely possibility is that Ni may play a role independent of Cu in Fe transfer, storage, hemoglobin, or red blood cell formation. Kirchgessner and Schnegg (8) recently found Fe absorption to be greatly impaired in Ni-deficient rats. Hematocrits, hemoglobin and erythrocyte numbers, and liver Fe were also reduced as a result of Ni deficiency (8).

Summary. Weanling rats were fed a basal low Cu diet (0.95 ppm Cu, 0.1 ppm Ni) or the basal diet supplemented with 20 ppm Ni or 15 ppm Cu or with both. The responses to supplementing the basal diet with Cu were increases in weight gain, hematocrit, hemoglobin, erythrocyte count, ceruloplasmin, and Cu content of liver, heart, and kidney. Supplementation of the basal diet with Ni increased weight gains and hematocrits to the same extent as did Cu. Responses to Cu were not modified by simultaneous Ni supplementation but responses to Ni were seen only in low Cu diets.

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TABLE IV. SERUM CERULOPLASMIN ACTIVITY IN RATS RECEIVING VARYING LEVELS OF COPPER AND NICKEL.

Added copper (ppm)	Added nickel (ppm)	Ceruloplasmin (IU)
0	0	6.1 ± 0.6[a]
0	20	9.6 ± 1.4
15	0	33.2 ± 3.0[b]
15	20	35.7 ± 4.1[b]

[a] ± Mean ± SEM.
[b] Significantly different from non-Cu-supplemented groups ($P < 0.01$).

TABLE V. LIVER IRON AND COPPER IN RATS RECEIVING VARYING LEVELS OF COPPER AND NICKEL.

Added copper (ppm)	Added nickel (ppm)	Fe (μg/g)[a]	Cu (μg/g)[a]
0	0	148.9 ± 20.8[b]	1.61 ± 0.26
0	20	119.2 ± 9.8	1.47 ± 0.21
15	0	75.2 ± 5.7[c]	3.66 ± 0.12[c]
15	20	71.1 ± 5.4[c]	3.47 ± 0.14[c]

[a] Micrograms per gram of fresh tissue.
[b] Mean ± SEM.
[c] Significantly different from non-Cu-supplemented groups ($P < 0.01$).

TABLE VI. HEART, LUNG, AND KIDNEY COPPER IN RATS RECEIVING VARYING LEVELS OF COPPER AND NICKEL.

Added copper (ppm)	Added nickel (ppm)	Cu (μg/g)[a]		
		Heart	Lung	Kidney
0	0	3.17 ± 0.32[b]	2.03 ± 0.21	3.97 ± 0.30
0	20	3.09 ± 0.24	1.48 ± 0.18	3.75 ± 0.18
15	0	6.58 ± 0.86[d]	2.27 ± 0.26[c]	5.22 ± 0.25[d]
15	20	5.60 ± 0.50[d]	1.99 ± 0.15	5.73 ± 0.23[d]

[a] Micrograms per gram of fresh tissue.
[b] Mean ± SEM.
[c] Significantly different from 0 Cu, 20 ppm Ni group ($P < 0.05$).
[d] Significantly different from non-Cu-supplemented groups ($P < 0.01$).

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1. Nielsen, F. H., *in* "Newer Trace Elements in Nutrition" (W. Mertz and W. E. Cornatzer, eds.), p. 215. Marcel Dekker, New York (1971).
2. Underwood, E. J., "Trace Elements in Human and Animal Nutrition," p. 57. Academic Press, New York (1971).
3. Nielsen, F. H., Myron, D. R., Givand, S. H., and Ollerich, D. A., *J. Nutr.* **105,** 1607 (1975).
4. Kikkawa, H., Ogita, Z., and Fujito, S., *Science* **121,** 43 (1955).
5. Lerner, A. B., Fitzpatrick, T. B., Calkins, E., and Summerson, W. H., *J. Biol. Chem.* **187,** 793 (1950).
6. Weber, C. W., and Reid, B. L. *J. Anim. Sci.* **28,** 620 (1969).
7. Valuiskii, P. P., *Mikroelem. Zhivotnovod. Rastenievod.* **1966,** 38 (1966).
8. Kirchgessner, M., and Schnegg, A., *Bioinorganic Chem.* **6,** 155 (1976).
9. Rice, E. W., *Anal. Biochem.* **3,** 452 (1962).
10. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).
11. Evans, J. L., and Abraham, P. A., *J. Nutr.* **103,** 196 (1973).
12. Elvehjem, C. A., and Sherman, W. C., *J. Biol. Chem.* **98,** 309 (1932).
13. Nielsen, F. H., Myron, D. R., Givand, S. H., Zimmerman, T. J., and Ollerich, D. A., *J. Nutr.* **105,** 1620 (1975).
14. Bush, J. A., Jensen, W. N., Athens, J. W., Ashenbrucker, H., Cartwright, G. E., and Wintrobe, M. M., *J. Exp. Med.* **103,** 701 (1956).
15. Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E., and Wintrobe M. M., *Blood* **1,** 1053 (1952).
16. Osaki, S., Johnson, D. A., and Frieden, E., *J. Biol. Chem.* **241,** 2746 (1966).

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