Evidence of a Possible Requirement for Nickel by the Chick[1] (34896)

FORREST H. NIELSEN[2] AND H. E. SAUBERLICH

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado 80240

Nickel has been shown to have a ubiquitous distribution in plant and animal tissues (1–3). Schroeder *et al.* (1) have discussed the possibility that nickel may be an essential element, suggesting that it may perform some physiological role in the animal body. Their reasoning is supported by several observations: (1) nickel shows biological activity *in vitro*, affecting certain enzymes; (2) it is present in plants and animals, including the newborn; (3) intestinal and hepatic barriers to nickel transport are implied; (4) there is little tendency for nickel to accumulate in tissues during a lifetime of exposure; and (5) evidence has been presented which indicates that nickel may have a role in pigmentation. Attempts have been made to show essentiality for nickel using methods of special diets (2, 4) and isotopic techniques (5). Definitive data for an *in vivo* role for nickel have not been obtained. The purpose of the studies to be reported here was to determine whether or not nickel performs a physiological role in the chick.

Materials and Methods. Day-old White Rock chicks[3] were used in each of two experiments. The birds were randomly distributed, without segregation according to sex, into groups of three each in experiment 1 and groups of four each in experiment 2. Each group was placed in a plastic cage fabricated in a manner similar to that described by Smith and Schwarz (6). Three of these cages containing the chicks were placed into each of two all-plastic isolators. The isolators were maintained at a temperature of 29°–32°.

The chicks in one isolator, 9 in experiment 1 and 12 in experiment 2, were fed a diet which contained <0.08 ppm nickel on an air-dried basis (determined by atomic absorption spectrophotometry[4]). This represented the low-nickel treatment group. The control group of chicks was housed in the other isolator in each experiment and was fed the same basal diet as the treatment group except that the control diet was supplemented with 5 ppm nickel as $NiCl_2 \cdot 6H_2O$. The compositions of the diets used in experiments 1 and 2 are presented in Table I. Compared to the diet for experiment 1, the following alterations were made in experiment 2: (1) corn oil was added to make the diet less hygroscopic; (2) additional arginine was provided since it has been shown that high levels of dietary arginine can augment a zinc deficiency (7); and (3) vitamin D_3 was added to the vitamin mix. The diets and glass-distilled water were provided *ad libitum* in polypropylene cups made from Erlenmeyer flasks.

At 3 weeks of age, the chicks were given a dose of radioactive nickel[5] as $^{63}NiCl_2$ by gavage. In experiment 1, five chicks from each isolator were given 20 μCi of ^{63}Ni; in experiment 2, nine chicks from each isolator were given 25 μCi of ^{63}Ni. The specific activity of the isotope used was approximately 5 Ci/g; thus, the doses contained approximately 4 and 5 μg of nickel in experiments 1 and 2, respectively.

In experiment 1, three chicks from each

[1] The principles of Laboratory Animal Care as promulgated by the National Society for Medical Research were observed.

[2] Present address: U. S. Department of Agriculture, Agriculture Research Service, Human Nutrition Research Division, Beltsville, Maryland.

[3] Pollard Hatchery and Feed, Denver, Colorado.

[4] Model 303, Perkin-Elmer Corporation, Norwalk, Connecticut.

[5] Cambridge Nuclear Corp., Cambridge, Massachusetts.

TABLE I. Composition of the Diets Employed.

Ingredients	Experiment 1	Experiment 2
	g	g
Skim milk powder[a]	500.0	500.0
Corn meal, degerminated, enriched, yellow[b]	475.1	419.6
L-Arginine	3.5	30.0
Glycine	10.0	10.0
Corn oil	—	20.0
Mineral mix[c]	10.4	10.4
Vitamin mix	1.0[d]	10.0[e]

[a] Nutritional Biochemicals Corp., Cleveland, Ohio.

[b] The Quaker Oats Co., Chicago, Illinois.

[c] Mineral mix contained: (in g) $Ca_3(PO_4)_2$, 8.75; NaCl, 1.5; $MnCO_3$, 0.125; ZnO, 0.025; KI, 0.0005.

[d] Vitamin mix contained: (in mg) vitamin A palmitate (250,000 IU/g), 4.0; DL-alpha tocopherol powder (250 IU/g), 4.0; menadione, 0.5; pyridoxine HCl, 1.0; folic acid, 0.9; and corn meal to 1.0 g.

[e] Vitamin mix contained: (in mg) vitamin A palmitate (250,000 IU/g), 4.0; DL-alpha tocopherol powder (250 IU/g), 4.0; menadione, 0.5; pyridoxine HCl, 1.0; folic acid, 0.9; vitamin D_3 (40,000,000 IU/g), 0.01; and corn meal to 10 g.

treatment group (low-nickel and control) were sacrificed 6 hr after isotope administration; and two chicks, 24 hr after isotope administration. In experiment 2, three chicks from each treatment group were sacrificed at each of three time intervals after isotope administration: 6, 24, and 48 hr. Immediately prior to sacrifice by decapitation, the birds were weighed and observed for gross abnormalities. The blood was collected in a centrifuge tube containing 0.1 ml of heparin (100 units). A portion of the blood was centrifuged to facilitate separation of red blood cells from the plasma. Other tissues removed at sacrifice were: tibia, kidney, spleen, liver, duodenum, gizzard, lung, muscle, skin, heart, aorta, and feather. All tissues were frozen until ^{63}Ni analysis could be made. For the analysis, a bone sample was a 100-mg portion including the marrow from the center of the tibia shaft. The tibias were measured for length and widths with calipers, using the largest length and the smallest diameter. To facilitate the measurements, the flesh was removed from the bone by rubbing the shaft with cheesecloth.

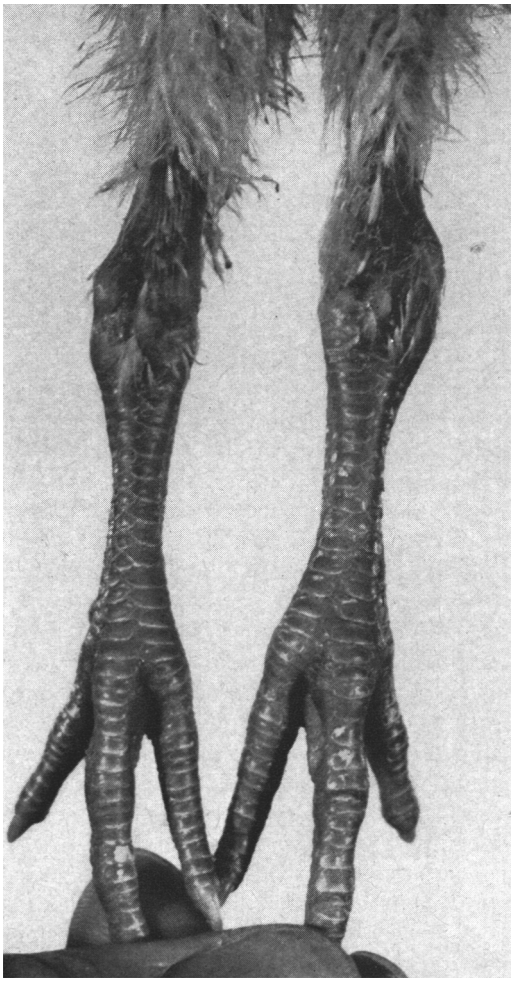

FIG. 1. Effect of dietary nickel upon the leg development of chicks. The leg on the left is from a control chick fed a diet supplemented with 5 ppm nickel. The leg on the right shows the thickened leg bone and swollen hock typical of those chicks fed a diet containing <0.08 ppm nickel. Both chicks were approximately the same weight.

Sample preparation and counting procedure were similar to the method of Smith and Hackley (5) with the counting being done in a liquid scintillation counter[6]. Statistical analysis was by the *t* test (8).

Results. The gross appearance of the chicks was affected by the amount of nickel

[6] Model Mark 1, Nuclear Chicago Corp., Des Plaines, Illinois.

in the diet in both experiments. One of the influences of nickel was upon the pigmentation of the legs. Chicks fed the low-nickel diet (<0.08 ppm) showed a bright orange-yellow color in their legs. By comparison, the chicks fed the nickel-supplemented diet had a pale brown-yellow color in their legs. Another difference noted between the low-nickel and control chicks concerned leg development. Figure 1 shows that the chicks fed the low-nickel diet had slightly thickened legs compared to the control birds. Also, the low-nickel chicks appeared to have somewhat swollen hock joints. Compared to the control chicks, the low-nickel chicks appeared to walk with a slightly abnormal clumsy gait.

Table II shows the results of the measurements of tibia bone size, expressed as the ratio of the length to width. This length: width ratio was significantly reduced in the chicks fed the low-nickel diet compared to the chicks fed supplemental nickel. Also presented in Table II are the average final body weights of the chicks. There was no significant effect of dietary nickel upon body weight.

In experiment 1, the administered ^{63}Ni appeared to be retained to a greater extent in the tissues of the chicks fed the low-nickel diet than in those of the control chicks. This appeared to be evident for bone, kidney, liver, heart, and aorta at both the 6-hr and 24-hr interval after dosage. As a result of these observations, a more detailed study was made on the distribution of ^{63}Ni in tissues in experiment 2 (Table III). In this experiment, the low-nickel chicks retained a significantly greater amount of ^{63}Ni in the liver, spleen, aorta, and, possibly, bone than did the control birds at the 6-hr interval after isotope administration. At the 48-hr time period, the low-nickel chicks showed a significantly higher isotope concentration in the bone.

TABLE II. Body Weights and Tibia Length: Width Ratios of Chicks Fed Different Levels of Nickel.

Experiment[a]	Dietary nickel (ppm)	Body[b] weights (g)	Length:width ratio of tibia
1	<0.08[c]	130	—
1	5.00	128	—
2	<0.08[c]	121	17.62[d]
2	5.00	109	18.38[d]

[a] Mean of 5 chicks in experiment 1; 10 chicks in experiment 2.

[b] Body weights at 3 weeks of age.

[c] Nickel content by analysis of diet with no supplemental nickel.

[d] Significant difference at the 0.05 level.

Several tissues took up relatively large amounts of ^{63}Ni during the 6 hr after dosage, including bone, kidney, liver, and aorta. A large amount of isotope was still retained by the bone, kidney, and liver 48 hr after administration. Conversely, very little ^{63}Ni was found in muscle and blood (red blood cells and plasma) after the 6-hr time period.

Discussion. The data presented herein appear to support the contention that nickel may have a physiological role in chicks with respect to pigmentation and leg structure. Kikkawa *et al.* (9) proposed that color was dependent upon the presence of specific metals. They showed that the yellow and white pigments from rabbit hair contained nickel. Their *in vitro* evidence led to the conclusion that nickel is especially important in white pigmentation. The data reported in this paper provide *in vivo* evidence that dietary nickel affects the yellow color in the legs of the chick.

Other investigators have attempted to find abnormalities in rats and mice fed low-nickel diets (2, 4). Growth rate was monitored in those studies. With chicks, in the present study, dietary nickel had no significant effect upon growth rate. However, nickel did appear to influence bone formation in chicks. Not only did the low-nickel chicks appear to have thickened legs and swollen hocks, the length:width ratios of the tibias from the low-nickel chicks were significantly lower than those from chicks fed the nickel-supplemented diet. The fact that leg abnormalities have not been noted by previous workers in either rats or mice fed low-nickel diets is not surprising. Leg abnormalities normally occur in chicks fed either zinc-deficient or manganese-deficient diets, whereas rats

TABLE III. Distribution of ^{63}Ni in Selected Tissues at Time Intervals After a Single Dose (Experiment 2).[a]

Tissue	Hours after dose					
	6		24		48	
	Ni Low[b]	Ni High[c]	Ni Low	Ni High	Ni Low	Ni High
	(% dose/g fresh tissue)					
Bone	0.296[d]	0.128[e]	0.101	0.076	0.098[j]	0.041[k]
Epiphyseal plate	0.102	0.070	0.023	0.024	0.015	0.017
Primary spongiosa	0.134	0.072	0.034	0.023	0.029	0.024
Hyaline cartilage	0.096	0.043	0.017	0.014	0.016	0.008
Blood	0.041	0.035	0.004	0.005	0.002	0.002
Red blood cells	0.021	0.015	0.002	0.002	0.002	0.001
Plasma	0.054	0.044	0.044	0.006	0.003	0.002
Duodenum	0.035	0.044	0.008	0.008	0.006	0.005
Kidney	0.069	0.292	0.291	0.073	0.193	0.141
Spleen	0.044[l]	0.023[m]	0.021	0.020	0.017	0.010
Liver	0.103[o]	0.042[p]	0.062	0.039	0.069	0.035
Lung	0.052	0.030	0.012	0.011	0.019	0.010
Muscle	0.018	0.017	0.003	0.004	0.003	0.003
Skin	0.051	0.048	0.019	0.022	0.023	0.023
Aorta	0.157[q]	0.053[r]	0.026	0.016	0.021	0.025
Heart muscle	0.020	0.029	0.016	0.023	0.009[s]	0.052[t]
Feather	0.032	0.035	0.040	0.049	0.064	0.046
Gizzard lining	0.102[f]	0.196[g]	0.008[h]	0.016[i]	0.006	0.005

[a] Each value represents the mean of three chicks.

[b] Ni Low indicates those chicks fed the basal diet which contained <0.08 ppm nickel on an air-dried basis.

[c] Ni High indicates those chicks fed the basal diet supplemented with 5 ppm nickel.

[d,e,f,g,h,i] Significant at the 0.1 level.

[j,k,l,m,o,p,q,r,s,t] Significant at the 0.5 level.

fed either of these types of diets usually show no gross leg defects. Perhaps a similar phenomenon occurs in the case of nickel.

Smith and Hackley (5) found an apparent selective uptake of ^{63}Ni in their studies with rats fed a diet containing 3.3 ppm nickel. They suggested, however, that the selective uptake may have been due to a difference in blood volume of the various tissues. This could have contributed to the differences as their isotope was administered iv and, thus, the blood was relatively high in ^{63}Ni even 16 hr after isotope administration. In the present experiments, after an oral dose, a relatively small amount of ^{63}Ni was found in the blood, especially for periods of time greater than 6 hr after dosage. Still, relatively large amounts of ^{63}Ni were found in kidney, bone, and liver at all time intervals studied; whereas, muscle retained very little of the isotope. Therefore, it apears that other factors than just blood volume or blood level of nickel are affecting the apparent selective uptake of nickel by the tissues. Furthermore, the low-nickel chicks retained significantly greater amounts of ^{63}Ni in the liver, aorta, and, possibly, bone at the 6-hr time period than did the control chicks. Several other tissues from the low nickel chicks appeared to retain greater amounts of ^{63}Ni than similar tissues from the control birds, but these differences were not significant. These data could be interpreted as indications that homeostatic mechanisms are trying to maintain nickel in sufficient quantities in various tissues in which it may have an important physiological role.

Due to the limitations of the analytical

method, the level of nickel in the low-nickel diet is reported as <0.08 ppm. The data would seem to suggest that this dietary concentration of nickel is low enough to demonstrate that nickel does perform a physiological function in the growing chick. Efforts are being made to determine the exact concentration of nickel in the diet and to formulate a diet of lower nickel concentration than the one presently used. Only then can it be fully determined whether nickel is an essental element; and, if so, the exact level of nickel required to prevent changes from normal physiological function.

Summary. After 3 weeks on a dried skim milk–corn meal-based low-nickel diet (<0.08 ppm Ni), chicks which were maintained in an all-plastic controlled environment system had the following symptoms: (1) a change in leg pigmentation; (2) thickening of the long bones; (3) swelling of the hock joint; and (4) a significantly reduced length:width ratio of the tibia. All symptoms were prevented by the supplementation of 5 ppm nickel to the diet. Also, chicks fed the low-nickel diet accumulated a significantly greater amount of ^{63}Ni in the liver, spleen, aorta, and possibly bone during the 6 hr after oral dosage than did control birds. This evidence supports the hypothesis that nickel has a physiological role in the chick.

The authors express their appreciation to Gerhard J. Isaac for performing the statistical analyses and to Dr. R. P. Dowdy for his constructive criticisms in the preparation of the manuscript.

1. Schroeder, H. A., Balassa, J. J., and Tipton, I. H., J. Chronic Dis. **15**, 51 (1961).

2. Schroeder, H. A., Balassa, J. J., and Vinton, W. H., Jr., J. Nutr. **83**, 239 (1964).

3. O'Dell, G. D., Miller, W. J., and Moore, S. L., Fed. Proc. **29**, 696 (Abstracts) (1970).

4. Smith, J. C., Jr., *in* "Trace Substances in Environmental Health - II" (D. D. Hemphill, ed.), p. 223. Univ. Missouri, Columbia, Mo. (1969).

5. Smith, J. C., and Hackley, B., J. Nutr. **95**, 541 (1968).

6. Smith, J. C., and Schwarz, K., J. Natr. **93**, 182 (1967).

7. Coleman, B. W., Reimann, E. M., Grummer, R. M., and Hoekstra, W. G., Fed. Proc. **28**, 761 1234567 (Abstracts) (1969).

8. Snedecor, G. W., and Cochran, W. G., tistical Methods," p. 91. Iowa State Univ. Press, Ames, Iowa (1967).

9. Kikkawa, H., Ogita, Z., and Fugito, S., Science **121**, 43 (1955).

Received Feb. 27, 1970. P.S.E.B.M., 1970, Vol. 134.