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# Effects of Dietary Zinc, Manganese, and Copper on Tissue Accumulation of Cadmium by Japanese Quail (41522)

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*Abstract.* The beneficial effects of a combined dietary supplement of Zn, Cu, and Mn in decreasing Cd absorption was previously reported. The purpose of this study was to investigate the individual and combined effects of these three elements. In the first two experiments, day-old Japanese quail were fed basal diets containing either requirement amounts of Zn (30 ppm) and Mn (12 ppm) and slightly above requirement levels of Cu (5 ppm). From Day 7 birds were fed either the basal diet or diets containing combinations at twice these concentrations; a 2 × 2 × 2 factorial design was used. $^{109}$Cd content and Cd concentration of these diets were 100 $\mu$Ci and 145 $\mu$g/kg, respectively. In the third experiment, day-old birds were fed either the basal diet or a basal diet containing $^{109}$Cd and single additional supplements of either Zn, Cu, or Mn. All birds were killed at 14 days of age. The Cd concentration was determined for the duodenum, jejunum–ileum, liver, and kidney. When the experimental diets were fed for 7 days, only Zn had a protective effect against Cd. Whereas none of the elements reduced the Cd concentration of the duodenum, Zn reduced the Cd in the jejunum–ileum, liver, and kidney by approximately 66, 21, and 11%, respectively. Cu and Mn caused occasional increases of Cd in some tissues. Feeding the experimental diets for 2 weeks resulted in similar responses. Zn nutrition appears to play an important role in protecting against dietary Cd absorption.

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Nutrients, especially the mineral elements, play an important role in the metabolism and toxicity of cadmium. The degree of toxicity of a given level of cadmium can be largely dependent on the level and sometimes the form of nutrients present in the diet. Nutrients that can reduce the toxicity of orally consumed cadmium are protein, zinc, copper, calcium, iron, and ascorbic acid (1, 2).

In humans, the potential hazards from cadmium in foods and water are related to long-term retention and renal tubular damage if cadmium reaches a critically high level. Many aspects are not well defined; however, concomitant nutrient intake is thought to be an important aspect in evaluating these hazards. For humans, the attenuating effect that these nutrients may exert in reducing the absorption, accretion, and the body burden of cadmium typically occurring in the diet and other low-level exposure sources is of great potential significance.

Iron (3, 4), ascorbic acid (4), and certain types of purified protein (5) reduce the absorption of low levels of dietary cadmium. We have reported that a combined supplement of zinc, manganese, and copper reduced the uptake (6) and long-term retention of $^{109}$Cd in Japanese quail fed very low levels of cadmium (7) similar to those in the diet of humans. However, the individual effects these three mineral elements played in cadmium absorption were not determined.

In these experiments we have investigated the individual as well as the combined effects of zinc, manganese, and copper on tissue accumulation of dietary cadmium. The supplemented amounts of these three elements were the same as those used before (6) and were equal to the amounts present in the basal diet. The levels of cadmium fed were approximately one-tenth the level that will elicit a mild cadmium toxicity in growing quail (8).

**Materials and Methods.** In the first two experiments, day-old Japanese quail (*Cotur-*

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*nix coturnix japonica*) of both sexes were fed a purified basal diet containing soy isolate and requirement levels of zinc (30 ppm), manganese (12 ppm), and slightly above-requirement levels of copper (5 ppm) (6). Conditions regarding animal care and exclusion of airborne and waterborne trace element contamination have been previously described (9).

On Day 7 the birds were wing banded and redistributed into groups of 10 each (6). During the experimental period, Days 7–14, birds were fed either the basal diet or the basal diet containing an additional (in mg/kg diet) 30 zinc, 12 manganese, and 5 copper alone or in all possible combinations; this was a 2 × 2 × 2 factorial design. Two experiments of this type were carried out. All diets contained 145 μg Cd/kg and 100 μCi of carrier-free (accelerator produced) $^{109}$Cd/kg (6). The level of cadmium present in the diet as a contaminant was 20 μg/kg (6). In the third experiment, four groups of birds, 9–10 per group, were fed from hatching to 14 days of age the identical basal diet or the basal diet containing a single element addition (in mg/kg diet) of 30 zinc, 12 manganese, or 5 copper. These diets also contained 145 μg Cd/kg as in the first two experiments, and 250 μCi $^{109}$Cd/kg.

The birds from all experiments were killed by decapitation at 14 days of age. The duodenum, the jejunal–ileal portion of the intestinal tissue, and the liver were removed, washed with 0.75% sodium chloride solution, and blotted (5). The kidneys were excised but were not rinsed. Tissues were solubilized in concentrated nitric acid, the radioactivity was measured by crystal scintillation spectrometry, and cadmium concentrations were determined (6). Zinc from these tissues was determined by conventional flame atomic absorption spectrophotometry after acid digestion (6).

Data from the first two experiments were combined and tested for significant effects using a 2 × 2 × 2 factorial analysis of variance (10). Data from the third experiment were tested for significance using Student's *t* test (10). All statements of effects are based on a probability of at least 95%.

**Results.** Birds from all experimental groups grew and developed normally. Neither the single supplements nor any combination of the three supplements, fed for either the 1- or the 2-week period, caused significant changes in growth.

The effects of the three elements fed during the second week on tissue cadmium concentrations are shown in Table I. Zinc fed either alone or in combination with copper or manganese, or both, caused less cadmium to be accumulated (an antagonistic effect) by the jejunum–ileum, the liver, and the kidneys than those tissues from their respective control groups. However, supplemental zinc had no measurable effect on the duodenal concentration of cadmium.

In contrast to zinc, neither copper nor manganese had an antagonistic effect on the cadmium concentration in any of the tissues studied. Moreover, birds fed supplemental copper and manganese often accumulated more cadmium in some tissues, a synergistic effect. The absolute amounts of cadmium found in tissues varied greatly depending on the supplement fed and the tissue studied. The order of increasing concentration and the relative concentration of cadmium found in the tissues of the nonsupplemented controls (Table I) with reference to the liver cadmium concentration as 1, was kidneys 2.4, jejunum–ileum 93, duodenum 180. The duodenum maintained a cadmium concentration that ranged from approximately 15 to 20 times the cadmium concentration of the diet, 145 μg Cd/kg. The jejunum–ileum maintained a cadmium concentration that ranged from approximately 3 to 14 times that of the diet depending on the supplement fed.

Small increases in tissue cadmium were associated with supplemental copper and occasionally with supplemental manganese in the first two experiments with cadmium-109 fed during 7–14 days. It was thought that more definitive effects of each supplement might occur with longer exposure to cadmium-109. Therefore, such an experiment was carried out.

It was suspected that the previously observed effects of copper and manganese would be more evident after a 2-week exposure to $^{109}$Cd.

The effects of the three individually supplemented elements fed for 2 weeks on the tissue concentration of cadmium are shown

TABLE I. EFFECTS OF ZINC, COPPER, AND MANGANESE ON CADMIUM CONCENTRATION OF TISSUES OF JAPANESE QUAIL FED CADMIUM FOR 1 WEEK[a]

| Supplement[b] | Number of birds | Duodenum (μg/g) | Jejunum–ileum (μg/g) | Liver (ng/g) | Kidney (ng/g) |
|---|---|---|---|---|---|
| — | 20 | 2.5 ± 0.17 | 1.3 ± 0.12 | 14 ± 0.8 | 33 ± 2.6 |
| Zn | 20 | 2.2 ± 0.15 | 0.42 ± 0.037 | 11 ± 0.7 | 27 ± 1.9 |
| Mn | 20 | 2.5 ± 0.14 | 2.1 ± 0.10 | 13 ± 0.8 | 30 ± 1.4 |
| Cu | 20 | 2.6 ± 0.16 | 2.0 ± 0.15 | 16 ± 1.1 | 36 ± 3.7 |
| Zn, Mn | 20 | 2.8 ± 0.15 | 0.72 ± 0.80 | 12 ± 0.6 | 28 ± 1.6 |
| Zn, Cu | 20 | 2.9 ± 0.17 | 0.63 ± 0.060 | 11 ± 0.6 | 30 ± 2.1 |
| Mn, Cu | 18 | 2.7 ± 0.17 | 1.8 ± 0.12 | 16 ± 0.8 | 39 ± 1.7 |
| Zn, Mn, Cu | 17 | 2.9 ± 0.14 | 0.71 ± 0.072 | 11 ± 0.7 | 32 ± 2.3 |
| Significant effects[c] | | Cu,** Zn × Mn × Cu* | Zn,†† Mn,** Cu,* Mn × Cu,** Zn × Mn × Cu†† | Zn,†† Cu,** Zn × Cu†† | Zn,†† Cu** |

[a] Mean values ± SEM from experiments 1 and 2. The cadmium concentration of the various tissues was determined radiochemically using the known specific activity of $^{109}$Cd in the diet. Total cadmium present in the diets fed to quail the second week after hatching was 145 μg/kg. The concentration of $^{109}$Cd fed during the second week was 0.1 μCi/g diet. Control values at the end of the experiment (14 days of age) for body weight, duodenum, jejunum–ileum, liver, and kidney weight (g) were 40.0 ± 1.23, 0.64 ± 0.020, 0.73 ± 0.031, 1.5 ± 0.06, and 0.42 ± 0.016, respectively. Values for these tissues from other groups did not vary significantly ($P < 0.05$) from those of the controls. All values given for tissue cadmium concentration are on a fresh weight basis.

[b] When added, the various supplements supplied either an additional (mg/kg diet) 30 zinc, 12 manganese, or 5 copper.

[c] Significant effects by analysis of variance: synergistic effect, *($P < 0.05$), **($P < 0.01$); antagonistic effect, †($P < 0.05$), ††($P < 0.01$).

in Table II. As before, zinc had a protective effect against cadmium accumulation. Lower concentrations of cadmium were found in the jejunum–ileum, liver, and kidneys in birds fed zinc. As was observed in experiment 1, the duodenal cadmium concentration was not affected by supplemental zinc. Supplemental manganese had no effect. Supplemental copper again resulted in increased cadmium in the jejunum–ileum; however, in the liver and kidney it had no effect.

The effects of supplemental zinc, copper, and manganese on the tissue concentration of zinc are shown in Table III. Supplemental zinc had little effect on the zinc concentration of the tissues studied. Only the jejunal–

TABLE II. EFFECTS OF ZINC, COPPER, AND MANGANESE ON CADMIUM CONCENTRATION OF TISSUES OF JAPANESE QUAIL FED CADMIUM FOR 2 WEEKS[a]

| Supplement[b] | Number of birds | Duodenum (μg/g) | Jejunum–ileum (μg/g) | Liver (ng/g) | Kidney (ng/g) |
|---|---|---|---|---|---|
| — | 10 | 2.2 ± 0.20 | 1.4 ± 0.21 | 22 ± 1.9 | 45 ± 3.4 |
| Zn | 9 | 2.3 ± 0.09 | 0.60 ± 0.03†† | 16 ± 0.9† | 35 ± 2.8† |
| Mn | 9 | 1.9 ± 0.24 | 1.5 ± 0.16 | 20 ± 1.4 | 38 ± 2.8 |
| Cu | 10 | 2.5 ± 0.23 | 2.1 ± 0.15* | 24 ± 1.7 | 45 ± 3.3 |

[a] Mean values ± SEM from experiment 3. The cadmium concentration of the various tissues was determined radiochemically using the known specific activity of $^{109}$Cd in the diet. Total cadmium present in the diets fed to quail for 2 weeks was 145 μg/kg. The concentration of $^{109}$Cd fed during this 2-week period was 0.25 μCi/g diet. Control values ± SEM at the end of the experiment (14 days of age) for the weight (g) of the whole bird, duodenum, jejunum–ileum, liver, and kidney were 42.3 ± 1.96, 0.63 ± 0.023, 0.73 ± 0.045, 1.5 ± 0.07, and 0.45 ± 0.035, respectively. Values for these tissues from other groups did not vary significantly ($P < 0.05$) from those of the unsupplemented group. Mean values were significantly different from the unsupplemented group: synergistic effect *($P < 0.05$), **($P < 0.01$); antagonistic effect †($P < 0.05$), ††($P < 0.01$). All values given for tissue cadmium concentration are on a fresh weight basis.

[b] When added, the various supplements supplied either an additional (mg/kg diet) 30 zinc, 12 manganese, or 5 copper.

TABLE III. EFFECTS OF ZINC, MANGANESE, AND COPPER ON ZINC CONCENTRATION ($\mu$g/g) OF TISSUES OF JAPANESE QUAIL FED CADMIUM FOR 2 WEEKS[a]

| Tissue[b] | Supplement[c] | | | |
|---|---|---|---|---|
| | — | Zn | Mn | Cu |
| Duodenum | 33 ± 1.5 | 35 ± 1.6 | 29 ± 1.0* | 29 ± 0.3* |
| Jejunum–ileum | 26 ± 1.2 | 33 ± 1.2† | 25 ± 0.4 | 27 ± 0.5 |
| Liver | 19 ± 0.8 | 19 ± 0.3 | 19 ± 0.7 | 22 ± 1.1 |
| Kidney | 19 ± 0.2 | 19 ± 0.2 | 18 ± 0.2 | 19 ± 0.3 |

[a] Mean values ± SEM from one experiment. Values for zinc reported here were from the same groups of birds as those reported in Table II. The total cadmium and $^{109}$Cd present in the diets fed to the quail for 2 weeks were 145 $\mu$g/kg diet and 0.25 $\mu$Ci/g diet, respectively. Mean values were significantly different from the unsupplemented group: decrease *($P < 0.05$), increase †($P < 0.01$). Only zinc was determined in these tissues.

[b] All values are on a fresh weight basis.

[c] When added, the various supplements supplied either an additional (mg/kg diet) 30 zinc, 12 manganese, or 5 copper.

ileal zinc concentration was increased by supplemental zinc. Supplemental manganese and copper caused a decrease in the zinc concentration of the duodenum, whereas the zinc concentrations of other tissues were unaffected by additional manganese or copper.

**Discussion.** In previous studies (6, 7) we had observed a protective effect of a combined supplement of zinc, copper, and manganese on the uptake and retention of very low levels of dietary cadmium, similar to the level used here. These protective effects included the reduced accretion of cadmium by the liver and kidneys, two of the most important target organs in the body (11). In the study reported here, in which the effects of the individual elements were gauged by changes in tissue concentration of cadmium, only zinc provided protection against cadmium. Supplements of copper and manganese were without any beneficial effect and occasionally caused increases in the cadmium concentration in some indicator tissues. Copper has been reported to be a protective agent against cadmium absorption (12) and toxicity (13). However, those studies were carried out using either higher levels of cadmium or copper-deficient diets. Copper in the basal diet was present entirely as a contaminant, primarily in the soy protein. This level of copper (5 ppm) is thought to be in excess of the copper requirement. The copper requirement of young coturnix quail, using a mixture of casein–gelatin as a protein source, is approximately 1.5 ppm (14). There appears to be no direct evidence in the literature that manganese is beneficial against the absorption of very low levels of cadmium in the diet, i.e., less than 1 $\mu$g Cd/g. However, when cadmium was fed at high or toxic levels, manganese concentrations of several tissues were reduced (15), suggesting that some type of manganese–cadmium interaction might exist. Similar protective effects for zinc against cadmium absorption were reported by Fox *et al.* (5). Feeding graded dietary zinc in a casein–gelatin protein-based diet was associated with marked decreases in duodenal and jejunal–ileal cadmium concentration. Zinc also caused a decrease in liver cadmium; however, there was no effect on the cadmium concentration in the kidneys. In one of two experiments with required levels of zinc intake, the concentration of cadmium in the liver was higher with the soy isolate diet than with the casein gelatin diet (5).

The effects reported here resulted from feeding two times the requirement for zinc and manganese and a somewhat greater excess of copper. The data demonstrate the importance of zinc on cadmium absorption and its probable beneficial action on the health impact of dietary cadmium.

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