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Effects of Zinc, Iron and Copper Deficiencies on Cadmium in Tissues of Japanese Quail

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Experiments with young Japanese quail were conducted to determine whether combined moderate deficiencies of zinc, iron and copper would cause greater uptake and tissue retention of cadmium than the single deficiencies. Birds were fed the experimental diets containing 62 ppb cadmium from hatching to 16 days of age. On day 9 each bird received a dose of $^{109}CdCl_2$ in its diet. On day 10, the duodenal and jejunal-ileal tissues contained large amounts of cadmium, and there were many significant effects of treatment on cadmium-109 retention in the livers and kidneys. At day 16, zinc deficiency caused increased cadmium in the liver, whereas iron and copper deficiencies each caused increased cadmium in the kidneys. Combined deficiencies had little or no greater effect than single deficiencies and in some cases the combined effect was less than that of a single deficiency.

Introduction

For the average person who is not exposed to cadmium through environmental pollution or industrial exposure, the principal source of cadmium intake is food. The amount of this cadmium that is absorbed may be influenced by the form in the diet, the nutrient content of the diet, and the nutritional status of the individual. The major nutrients that interact with cadmium are zinc, iron, copper, selenium, calcium, vitamin D, ascorbic acid, and protein. In general, intakes of the nutrients below and above requirements have been associated with tissue levels or toxicity of cadmium that were increased and decreased, respectively. These areas have recently been reviewed (*1–3*).

The kidney is well established as the principal target organ for cadmium accumulation and damage (*4*). The liver and other tissues also accumulate cadmium and some of this slowly moves to the kidney. In the present study we addressed the question of whether combined deficiencies of zinc, iron and copper would have greater effects than single deficiencies on tissue accumulation of cadmium from diets containing 62 ppb cadmium, a level representative of diets in the United States.

Experimental Procedures

Day-old Japanese quail (*Coturnix coturnix japonica*) of both sexes from our stock colony were housed in continuously lighted, suspended stainless steel cages. The incandescent light bulbs provided heat. Environmental trace element contamination was minimized, and birds received diet and deionized drinking water *ad libitum*. The birds were wing-banded at 7 days of age. Control birds received a purified casein-gelatin diet with 20 ppm zinc, 100 ppm iron, 2.5 ppm manganese, 1.5 ppm copper and 300 ppm magnesium, which are required levels for this diet (*5*). Experimental groups received the same diet except that zinc and copper salts were omitted and iron was decreased to 25 ppm. The deficient diets contained 11 ppm zinc and 0.9 ppm copper, supplied primarily by the vitamin-free casein. Birds received the control diet and diets deficient in each element singly and in all possible combinations, a total of eight treatments. All diets contained a total of 62 ppb cadmium supplied as $CdCl_2$ (*5*). The background level of cadmium was negligible.

Fifteen birds were fed each diet from hatching. Birds were weighed at 7, 10, 14, and 16 days of

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age. On day 7, birds fed each diet were redistributed into two groups of six each so that body weight ranges and means of both groups were similar. Birds at the body weight extremes were discarded. On day 9 each bird received a compressed tablet of its diet (ca. 80–100 mg) with ca. 3.4 μCi of accelerator-produced, carrier-free $^{109}CdCl_2$ (New England Nuclear Corp., Boston, MA) added (*6*). The birds were not fasted before dosing, and the schedule permitted the six birds in one group fed each diet to be killed after 24 hr, on day 10. The remaining birds were killed at 16 day, 7 days after dosing.

On days 9 and 15, hemoglobin was determined and feather scores (quality and quantity) and feather pigment scores were recorded. Scores of 0, 0.5 (minimal adverse effect), 1, 2, 3 and 4 (maximal effect) were defined for each defect. Unfasted birds were killed by decapitation, and the duodenum, jejunum-ileum, liver, and kidneys were removed. All tissues except the kidneys were washed in 0.75% NaCl solution and blotted dry. Tissues were weighed, solubilized in nitric acid for determination of radioactivity, and digested with nitric and perchloric acids for mineral analysis (*6*). Radioactivity was measured in a NaI(Tl) crystal scintillation detector (Model 5285, Packard Instruments, Des Plaines, IL). Radioactivity was quantitated in each tablet prior to dosing and for 10 reference tablets in the original form and as solubilized in nitric acid to permit corrections between the two physical forms. Zinc, iron, manganese, copper, magnesium and calcium in dietary components and tissues and cadmium in the dietary $CdCl_2$ premix were determined by atomic absorption spectrophotometry (Model 5000, Perkin-Elmer Corp., Norwalk, CT). A replication of the first experiment was carried out with the same lots of all dietary components. The effects of treatment were evaluated by analysis of variance and Duncan's multiple range test. The scores were evaluated by the Wilcoxon signed rank test. The relationships between ratios of experimental/control cadmium values for the jejunum-ileum and kidney from this and other experiments were evaluated by correlation and multiple regression analysis. Calculations and statistical tests were performed by the computer programs of the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

Results

Effects of Deficient Diets on Physiological Responses

It is difficult to make comparisons for degree of deficiency for different nutrients; however, the severity of deficiencies in this study were mild to moderate (Table 1). Some mortality occurred during week 1 in the triply-deficient birds in the second experiment. The remaining birds, which were similar to birds in the first experiment, were assigned to the group killed at day 16. Otherwise, the responses of birds in both experiments were similar and the data were pooled for statistical analysis.

Table 1. Physiological responses indicative of deficiencies.[a]

	Body weight, g		Feather score, 0–4		White feather score, 0–4		Hemoglobin, g/dL	
Deficiency	10 days	16 days	9 days	15 days	9 days	15 days	9 days	15 days
Zn	30 ± 0.8	47 ± 1.0	1.5 ± 0.3*	1.6 ± 0.3*	0 ± 0	0 ± 0	11 ± 0.3	13 ± 0.2
Fe	30 ± 0.6	48 ± 1.0	0.6 ± 0.1	0.6 ± 0.2	0 ± 0	0 ± 0	6 ± 0.2*	7 ± 0.2*
Cu	31 ± 0.8	52 ± 1.0	0.9 ± 0.2	0.8 ± 0.1*	0.3 ± 0.2	1.4 ± 0.3*	10 ± 0.3	13 ± 0.3
Zn Fe	29 ± 0.7*	47 ± 0.9	1.9 ± 0.4*	2.3 ± 0.4*	0 ± 0	0 ± 0	7 ± 0.2*	8 ± 0.2*
Zn Cu	30 ± 1.0	48 ± 1.4	2.6 ± 0.3*	3.0 ± 0.3*	0.1 ± 0.1	1.8 ± 0.4*	11 ± 0.5	12 ± 0.2
Fe Cu	29 ± 1.0*	44 ± 1.5*	1.1 ± 0.2*	1.0 ± 0.3*	0.5 ± 0.2	1.7 ± 0.3*	6 ± 0.3*	6 ± 0.3*
Zn Fe Cu	25 ± 0.9*	41 ± 1.0*	3.7 ± 0.2*	3.2 ± 0.3*	0 ± 0	0.9 ± 0.2*	6 ± 0.6*	7 ± 0.2*
None	32 ± 0.8	49 ± 1.6	0.4 ± 0.1	0.4 ± 0.2	0 ± 0	0 ± 0	11 ± 0.3	12 ± 0.3
Multiple analysis of variance, p[b]								
Zn	<0.01	<0.01	—	—	—	—	NS	<0.001
Fe	0.0001	0.0001	—	—	—	—	0.0001	0.0001
Cu	<0.01	<0.05	—	—	—	—	NS	<0.01
Zn Fe	NS	NS	—	—	—	—	NS	<0.02
Zn Cu	NS	NS	—	—	—	—	NS	NS
Fe Cu	NS	<0.001	—	—	—	—	NS	NS
Zn Fe Cu	NS	NS	—	—	—	—	NS	NS

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10.
[b]NS = nonsignificant; — = no comparisons.
*Significantly different from control (no deficiency), $p < 0.05$.

Table 2. Weight and cadmium-109 retention of the duodenum.[a]

Deficiency	Weight, mg		^{109}Cd retention, %	
	10 days	16 days	10 days	16 days
Zn	397 ± 16	461 ± 41*	29 ± 2.1	6.0 ± 1.2*
Fe	364 ± 12	487 ± 22*	32 ± 2.1	7.6 ± 0.9*
Cu	415 ± 16	622 ± 31	21 ± 3.0	1.4 ± 0.2
Zn Fe	340 ± 17	469 ± 24*	32 ± 3.0	9.4 ± 1.2*
Zn Cu	397 ± 18	567 ± 21	31 ± 2.2	3.2 ± 0.5
Fe Cu	341 ± 18	513 ± 30	35 ± 3.0	6.8 ± 1.1*
Zn Fe Cu	351 ± 24	453 ± 21*	43 ± 5.1*	6.3 ± 0.4*
None	383 ± 14	590 ± 29	29 ± 3.5	1.0 ± 0.2
Multiple analysis of variance, p[b]				
Zn	NS	<0.001	<0.05	<0.0001
Fe	0.0001	0.0001	<0.001	<0.0001
Cu	NS	NS	NS	<0.01
Zn Fe	NS	NS	NS	<0.02
Zn Cu	NS	NS	<0.05	<0.02
Fe Cu	NS	NS	<0.02	NS
Zn Fe Cu	NS	NS	NS	NS

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10.
[b]NS = nonsignificant.
*Significantly different from control, $p < 0.05$.

Table 3. Weight and cadmium-109 retention of the jejunum-ileum.[a]

Deficiency	Weight, mg		^{109}Cd retention, %	
	10 days	16 days	10 days	16 days
Zn	462 ± 20	598 ± 35	16 ± 1.6	5.5 ± 0.86*
Fe	444 ± 25	631 ± 23	8 ± 1.3*	2.3 ± 0.40
Cu	475 ± 18	664 ± 30	8 ± 1.0*	0.9 ± 0.15
Zn Fe	522 ± 18	701 ± 26	17 ± 2.2	7.2 ± 0.83*
Zn Cu	515 ± 26	732 ± 23	18 ± 1.2*	5.0 ± 0.89*
Fe Cu	467 ± 26	626 ± 32	12 ± 1.7	1.8 ± 0.36
Zn Fe Cu	512 ± 19	693 ± 34	21 ± 1.3*	6.4 ± 0.60*
None	496 ± 30	653 ± 33	13 ± 1.0	0.7 ± 0.11
Multiple analysis of variance, p[b]				
Zn	NS	NS	0.0001	0.0001
Fe	NS	NS	NS	<0.002
Cu	NS	NS	NS	NS
Zn Fe	NS	NS	NS	NS
Zn Cu	NS	NS	NS	NS
Fe Cu	NS	NS	<0.01	NS
Zn Fe Cu	NS	NS	NS	NS

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10.
[b]NS = nonsignificant.
*Significantly different from control, $p < 0.05$.

The defective feathering that is characteristic of zinc deficiency occurred in all zinc-deficient groups, with similar severity at 9 and 16 days. Significant absence of feather pigment, a copper deficiency sign previously shown in quail (7), occurred only in birds that were copper deficient. This effect was definite only at 16 days. Hemoglobin was significantly decreased only in birds fed the iron-deficient diets. The interactions among treatments that are shown by the multiple analysis of variance in this and subsequent tables will be considered later.

The tissue weights are presented in Tables 2, 3, 4 and 5. More significant decreases occurred in tissue weights than in body weight; however, there were fewer differences from the control groups when tissue weight was related to body weight (data not shown). The exception was the jejunum-ileum, for which treatments caused no changes in absolute weight.

Table 4. Weight and cadmium-109 retention of the liver.[a]

Deficiency	Weight, g		^{109}Cd retention, %	
	10 days	16 days	10 days	16 days
Zn	1.2 ± 0.05	1.6 ± 0.09*	0.23 ± 0.02*	0.57 ± 0.04*
Fe	1.1 ± 0.04*	1.5 ± 0.04*	0.15 ± 0.01	0.35 ± 0.04
Cu	1.2 ± 0.05	1.8 ± 0.07	0.23 ± 0.03*	0.30 ± 0.02
Zn Fe	1.1 ± 0.03*	1.5 ± 0.05*	0.17 ± 0.01	0.57 ± 0.04*
Zn Cu	1.3 ± 0.05	1.7 ± 0.05	0.20 ± 0.01*	0.46 ± 0.04
Fe Cu	1.1 ± 0.04*	1.4 ± 0.08*	0.12 ± 0.01	0.26 ± 0.02*
Zn Fe Cu	1.0 ± 0.04*	1.4 ± 0.04*	0.16 ± 0.04	0.43 ± 0.03
None	1.3 ± 0.05	1.9 ± 0.12	0.11 ± 0.00	0.37 ± 0.03
Multiple analysis of variance, p[b]				
Zn	NS	NS	<0.01	0.0001
Fe	0.0001	0.0001	<0.002	NS
Cu	NS	NS	NS	0.0001
Zn Fe	NS	NS	NS	NS
Zn Cu	NS	NS	<0.05	NS
Fe Cu	NS	NS	<0.02	NS
Zn Fe Cu	NS	NS	<0.001	NS

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10.
[b]NS = nonsignificant.
*Significantly different from control, $p < 0.05$.

Table 5. Weight and cadmium-109 retention of the kidneys.[a]

Deficiency	Weight, mg		^{109}Cd retention, %	
	10 days	16 days	10 days	16 days
Zn	336 ± 12	476 ± 33	0.11 ± 0.01	0.40 ± 0.02
Fe	333 ± 12*	543 ± 20	0.18 ± 0.02*	0.67 ± 0.05*
Cu	337 ± 10	583 ± 20	0.17 ± 0.01*	0.60 ± 0.04*
Zn Fe	328 ± 22*	534 ± 17	0.11 ± 0.01	0.56 ± 0.05*
Zn Cu	357 ± 14	536 ± 24	0.13 ± 0.01*	0.50 ± 0.04
Fe Cu	327 ± 13*	497 ± 25	0.16 ± 0.01*	0.71 ± 0.08*
Zn Fe Cu	323 ± 22*	464 ± 18*	0.17 ± 0.02*	0.62 ± 0.04*
None	383 ± 18	549 ± 26	0.09 ± 0.01	0.41 ± 0.04
Multiple analysis of variance, p[b]				
Zn	NS	<0.01	<0.05	<0.02
Fe	<0.01	NS	<0.002	0.001
Cu	NS	NS	0.0001	<0.01
Zn Fe	NS	NS	NS	NS
Zn Cu	NS	NS	NS	NS
Fe Cu	NS	<0.002	NS	NS
Zn Fe Cu	NS	NS	<0.001	NS

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10.
[b]NS = nonsignificant.
*Significantly different from control, $p < 0.05$.

Effects of Deficient Diets on Cadmium Retention and Essential Mineral Levels

The retention of cadmium-109 in the duodenum at 10 days was increased only in the triply-deficient birds, whereas at 16 days most groups deficient in zinc or iron had significantly more cadmium-109 than the control group (Table 2). This contrasts markedly with the amounts of essential elements in the duodenum (Table 6). Many of the deficiencies caused decreases of zinc, iron, copper and calcium at 10 days. Most of the effects remained at 16 days except for calcium. At 16 days, simple zinc deficiency was associated with normal duodenal zinc but with increased iron, copper and calcium, as compared with the controls.

Cadmium-109 retention in the jejunum-ileum was reduced by single deficiencies of iron and copper at 10 days but increased by zinc-copper and zinc-iron-copper deficiencies (Table 3). The latter two effects persisted at 16 days, at which time there was also increased cadmium-109 retention with deficiencies of zinc and zinc-iron. The

Table 6. Zinc, iron, copper and calcium in the duodenum.[a]

	Zn, ppm		Fe, ppm		Cu, ppm		Ca, ppm	
Deficiency	10 days	16 days	10 days	16 days	10 days	16 days	10 days	16 days
Zn	25 ± 0.5*	32 ± 3.2	64 ± 8.4	120 ± 20.0*	2.4 ± 0.13	2.9 ± 0.29*	150 ± 7	159 ± 10
Fe	35 ± 1.3	32 ± 1.1	15 ± 0.3*	16 ± 0.3*	1.7 ± 0.10*	1.9 ± 0.05*	116 ± 12*	125 ± 13
Cu	34 ± 1.6	34 ± 1.2	39 ± 3.4*	76 ± 9.1	1.4 ± 0.06*	1.4 ± 0.05*	135 ± 12*	144 ± 9
Zn Fe	24 ± 0.7*	25 ± 0.4*	15 ± 0.5*	15 ± 0.3*	2.0 ± 0.05*	2.1 ± 0.08	107 ± 8*	121 ± 9
Zn Cu	23 ± 0.5*	23 ± 0.4*	40 ± 4.0*	75 ± 9.1	1.4 ± 0.05*	1.3 ± 0.08*	122 ± 10*	137 ± 14
Fe Cu	35 ± 1.4	34 ± 1.6	19 ± 0.6*	18 ± 0.8*	1.3 ± 0.06*	1.1 ± 0.04*	130 ± 14*	114 ± 9
Zn Fe Cu	23 ± 1.0*	26 ± 0.7*	20 ± 2.8*	20 ± 1.4*	1.4 ± 0.05*	1.3 ± 0.07*	107 ± 9*	107 ± 6
None	36 ± 2.3	35 ± 1.3	64 ± 11.4	87 ± 11.8	2.3 ± 0.10	2.2 ± 0.11	173 ± 15	144 ± 11
Multiple analysis of variance, p[b]								
Zn	<0.001	0.0001	NS	NS	<0.02	<0.005	NS	NS
Fe	NS	NS	0.0001	0.0001	<0.01	0.0001	<0.001	<0.002
Cu	NS	NS	<0.02	NS	0.0001	0.0001	NS	NS
Zn Fe	NS	NS	NS	NS	NS	NS	NS	NS
Zn Cu	NS	<0.02	NS	NS	NS	<0.02	NS	NS
Fe Cu	NS	<0.01	<0.001	<0.02	0.0001	<0.01	<0.02	NS
Zn Fe Cu	NS	<0.05	NS	NS	NS	NS	NS	NS

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10.
[b]NS = nonsignificant.
*Significantly different from control, $p < 0.05$.

Table 7. Zinc, iron, copper and calcium in the jejunum-ileum.[a]

	Zn, ppm		Fe, ppm		Cu, ppm		Ca, ppm	
Deficiency	10 days	16 days	10 days	16 days	10 days	16 days	10 days	16 days
Zn	22 ± 0.4*	25 ± 1.9*	66 ± 4.8	92 ± 8.7*	1.8 ± 0.04	2.1 ± 0.19*	260 ± 26	189 ± 14*
Fe	26 ± 0.7	28 ± 1.2	24 ± 2.2*	16 ± 0.8*	1.8 ± 0.07	1.6 ± 0.08	205 ± 12	151 ± 12
Cu	26 ± 0.8	30 ± 1.2	40 ± 3.7*	34 ± 2.8	1.3 ± 0.04*	1.3 ± 0.05*	190 ± 13	120 ± 6
Zn Fe	21 ± 0.5*	19 ± 0.8*	23 ± 1.7*	22 ± 1.3*	1.7 ± 0.06	1.6 ± 0.08	228 ± 15	134 ± 6
Zn Cu	20 ± 0.5*	20 ± 0.6*	51 ± 2.6	70 ± 6.5*	1.3 ± 0.08*	1.1 ± 0.06*	207 ± 17	131 ± 11
Fe Cu	26 ± 1.1	29 ± 1.1	24 ± 1.6*	17 ± 0.8*	1.2 ± 0.06*	1.0 ± 0.03*	198 ± 14	154 ± 9
Zn Fe Cu	18 ± 0.9*	21 ± 0.3*	18 ± 1.9*	22 ± 0.8*	1.2 ± 0.08*	1.2 ± 0.02*	193 ± 2	156 ± 6
None	27 ± 1.2	31 ± 1.1	59 ± 6.3	42 ± 2.1	1.8 ± 0.06	1.8 ± 0.07	208 ± 16	144 ± 17
Multiple analysis of variance, p[b]								
Zn	0.0001	0.0001	NS	0.0001	NS	NS	NS	NS
Fe	NS	<0.01	0.0001	0.0001	NS	<0.001	NS	NS
Cu	<0.05	NS	<0.001	<0.02	0.0001	0.0001	<0.05	NS
Zn Fe	NS	NS	<0.05	0.0001	NS	NS	NS	<0.02
Zn Cu	NS	NS	NS	NS	NS	NS	NS	NS
Fe Cu	NS	<0.01	<0.01	<0.01	NS	NS	NS	<0.001
Zn Fe Cu	NS	NS	NS	NS	NS	<0.01	NS	NS

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10.
[b]NS = nonsignificant.
*Significantly different from control, $p < 0.05$.

highest cadmium retention occurred when zinc was decreased in the diet. The concentration of zinc in the jejunum-ileum was decreased at 10 and 16 days with all diets deficient in zinc (Table 7). Jejunal-ileal iron also was decreased by diets deficient in iron; however, zinc deficiency caused an increase in tissue iron at 16 days. Copper in the jejunum-ileum was decreased at 10 and 16 days with all diets that were deficient in copper. Zinc deficiency caused a significant increase in copper at 16 days. The calcium content of the jejunum-ileum was not affected by dietary treatment except for an increase with zinc deficiency at 16 days.

Compared with the controls, cadmium-109 retention in the liver at 10 days was increased by deficiencies of zinc, copper and zinc-copper, whereas at 16 days, cadmium was increased by deficiencies of zinc and zinc-iron but decreased by deficiency of iron-copper (Table 4). In the liver, significant decreases in zinc concentrations occurred at 16 days with deficiencies of iron, zinc-

Table 8. Zinc, iron, manganese, copper and calcium in the liver.[a]

Deficiency	Zn, ppm		Fe, ppm		Mn, ppm		Cu, ppm		Ca, ppm	
	10 days	16 days	10 days	16 days	10 days	16 days	10 days	16 days	10 days	16 days
Zn	19 ± 0.5	21 ± 1.3	120 ± 13*	103 ± 13*	2.1 ± 0.14	2.1 ± 0.21*	4.0 ± 0.27*	3.9 ± 0.27	44 ± 5	33 ± 2
Fe	19 ± 1.0	19 ± 0.6*	35 ± 4*	32 ± 2*	1.5 ± 0.10	1.4 ± 0.09	3.2 ± 0.16	3.5 ± 0.17	70 ± 13*	63 ± 11*
Cu	19 ± 0.4	20 ± 0.8	153 ± 19	183 ± 13*	1.7 ± 0.19	1.9 ± 0.14	2.5 ± 0.13*	2.5 ± 0.14*	35 ± 2	36 ± 4
Zn Fe	19 ± 0.5	18 ± 0.7*	34 ± 1*	31 ± 1*	1.4 ± 0.10*	1.7 ± 0.15	3.4 ± 0.11	3.9 ± 0.16	99 ± 8*	63 ± 8*
Zn Cu	21 ± 1.4	19 ± 0.6*	118 ± 9*	162 ± 20	1.9 ± 0.13	2.0 ± 0.11	2.6 ± 0.14*	2.6 ± 0.16*	46 ± 7	30 ± 0
Fe Cu	23 ± 2.9	20 ± 0.6	44 ± 3*	48 ± 2*	1.4 ± 0.09*	1.2 ± 0.08*	2.1 ± 0.10*	2.2 ± 0.21*	102 ± 15*	67 ± 8*
Zn Fe Cu	19 ± 1.0	20 ± 1.1	63 ± 12*	58 ± 6*	1.2 ± 0.22*	1.5 ± 0.14	2.6 ± 0.08*	2.3 ± 0.10*	49 ± 7	79 ± 10*
None	21 ± 1.2	22 ± 1.3	180 ± 33	143 ± 24	1.9 ± 0.16	1.6 ± 0.10	3.4 ± 0.19	3.6 ± 0.06	34 ± 1	33 ± 2
Multiple analysis of variance, p[b]										
Zn	NS	NS	NS	NS	NS	<0.002	<0.01	<0.05	NS	NS
Fe	NS	<0.05	0.0001	0.0001	0.0001	0.0001	<0.01	NS	0.0001	0.0001
Cu	NS	NS	NS	0.0001	NS	NS	0.0001	0.0001	NS	NS
Zn Fe	NS	NS	<0.02	NS	NS	NS	NS	NS	NS	NS
Zn Cu	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS
Fe Cu	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS
Zn Fe Cu	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10. Control values for Mg were 243 ± 5 ppm at 10 days and 243 ± 5 ppm at 16 days; no values were significantly different from the control at 10 days; liver from birds with deficiencies of Zn, Fe Cu, and Zn Fe Cu were significantly higher than the control at 16 days.

[b]NS = nonsignificant.

*Significantly different from control, $p < 0.05$.

Table 9. Zinc, iron, copper and calcium in the kidneys.[a]

Deficiency	Zn, ppm		Fe, ppm		Cu, ppm		Ca, ppm	
	10 days	16 days	10 days	16 days	10 days	16 days	10 days	16 days
Zn	17 ± 1.0	21 ± 2.1	92 ± 6.4*	98 ± 7.2*	2.3 ± 0.26*	3.1 ± 0.31	109 ± 19	56 ± 6*
Fe	18 ± 0.9	18 ± 0.2	32 ± 3.1*	40 ± 3.6*	2.7 ± 0.10	2.5 ± 0.09	70 ± 8	52 ± 3*
Cu	17 ± 0.9	18 ± 0.4	61 ± 2.2	67 ± 3.5	2.0 ± 0.21*	2.0 ± 0.06*	72 ± 6	47 ± 2*
Zn Fe	18 ± 0.8	18 ± 0.3	32 ± 2.1*	41 ± 2.3*	2.9 ± 0.25	2.9 ± 0.09	88 ± 12	49 ± 2*
Zn Cu	18 ± 0.5	17 ± 0.4	92 ± 12.6*	81 ± 4.9	2.0 ± 0.09*	2.2 ± 0.14	70 ± 6	104 ± 31
Fe Cu	22 ± 1.3	20 ± 1.2	41 ± 5.0*	37 ± 2.2*	2.0 ± 0.14*	2.3 ± 0.15	144 ± 14*	52 ± 3*
Zn Fe Cu	16 ± 0.3	19 ± 1.0	33 ± 1.8*	38 ± 2.9*	2.1 ± 0.09*	2.4 ± 0.19	82 ± 14	60 ± 7*
None	19 ± 0.7	19 ± 0.6	61 ± 2.4	70 ± 4.0	2.9 ± 0.12	2.7 ± 0.12	75 ± 10	119 ± 26
Multiple analysis of variance, p[b]								
Zn	<0.01	NS	<0.001	<0.001	NS	<0.05	NS	NS
Fe	NS	NS	0.0001	0.0001	NS	NS	NS	<0.01
Cu	NS	NS	NS	<0.02	0.0001	0.0001	NS	NS
Zn Fe	NS	NS	0.0001	<0.001	NS	NS	<0.05	NS
Zn Cu	NS	NS	NS	NS	NS	NS	<0.001	<0.002
Fe Cu	NS	<0.01	NS	NS	NS	NS	<0.001	NS
Zn Fe Cu	<0.001	NS	NS	NS	NS	NS	NS	<0.02

[a]Mean values ± SE; N = 11 or 12 except 6 for triple deficiency, day 10. Control values for Mn were 1.15 ± 0.06 ppm at 10 days and 1.24 ± 0.05 ppm at 16 days. No other values were significantly different from the respective controls. Control values for Mg were 197 ± 6.7 ppm at 10 days and 211 ± 6.4 ppm at 16 days. No other values were significantly different from their respective controls.

[b]NS = nonsignificant.

*Significantly different from control, $p < 0.05$.

iron and zinc-copper (Table 8). All treatments except copper deficiency caused decreases in liver iron as compared with controls. The same effects were observed at 16 days except for an increase in iron with copper deficiency and no effect of zinc-copper deficiency. At 10 days, liver manganese was decreased by all combined deficiencies involving iron, whereas at 16 days only iron-copper deficiencies decreased tissue manganese, and zinc deficiency caused an increase in manganese. Compared with controls liver copper was increased at 10 days by zinc deficiency but decreased by all diets deficient in copper. Only the latter effects occurred at 16 days. Calcium in the liver was increased by all diets deficient in iron.

Increased retention of cadmium-109 in the kidneys was observed with diets deficient in iron and copper singly and in most other combinations (Table 5). Zinc concentration in the kidney was not affected except for an increase with deficiencies of iron-copper and a decrease with deficiencies of zinc-iron-copper at day 10 (Table 9). Zinc

deficiency caused increased iron in the kidneys at 10 and 16 days, and zinc-copper deficiencies caused increased iron at 10 days. All other deficient diets except copper alone caused decreases in tissue iron as compared with controls. Copper concentrations in the kidneys were decreased by copper deficiency alone or in combination with zinc or iron at 10 days, whereas at 16 days only the copper deficient diet caused decreased tissue copper. Calcium in the kidneys was increased by iron-copper deficiency at 10 days but was decreased by all deficient diets except zinc-copper at 16 days.

Concentrations of essential elements in the tissues from control birds were similar at 10 and 16 days. The following changes in concentrations occurred between 10 and 16 days: decreases in jejunal-ileal iron ($p < 0.05$) and calcium ($p < 0.02$).

Effects on Cadmium Retention of Combined Deficiencies Compared with Single Deficiencies

These interactive effects of deficient elements on cadmium were assessed by multiple analysis of variance (ANOVA) and by comparisons between means for combined deficiencies with those for each single deficiency by Duncan's multiple range test. Statistically significant differences by the latter ($p < 0.05$) are not shown in the tables. The major effects only will be considered below. For the duodenum, more effects of treatment at day 16 were shown by ANOVA than by Duncan's test (Table 2). Zinc and iron deficiencies were responsible for increased cadmium retention; however, retention with zinc-iron deficiency was not greater than with iron alone. Retention of cadmium with zinc-copper deficiency was less than with zinc deficiency alone. At day 10, the effects of iron and copper deficiencies in decreasing jejunal-ileal cadmium were negated when combined with zinc deficiency. At day 16, zinc was the dominant factor increasing cadmium retention.

Several interactions between the essential element deficiencies affected cadmium retention in the liver at 10 days of age (Table 4). Most of these had disappeared by 16 days, when zinc was the predominant deficiency that increased cadmium. The combined deficiencies of zinc-copper and zinc-iron-copper resulted in cadmium retention that was less than with zinc deficiency alone.

By ANOVA the only significant interaction affecting cadmium in the kidney was between zinc, iron, and copper at 10 days (Table 5). The combined deficiencies of zinc-iron and zinc-copper resulted in cadmium retention that was significantly less than with iron and copper deficiencies, respectively. The combined deficiencies of iron and copper had no greater effect on cadmium than either deficiency alone. These interactions were not present at 16 days. Although cadmium retention was not different from the control with zinc deficiency alone, there was a significant effect of zinc by ANOVA at both 10 and 16 days (Table 5). This apparent protection of the kidney against cadmium accumulation by zinc deficiency is probably not important when the effects of zinc deficiency in increasing cadmium in the liver are considered. The liver cadmium would be expected to move to the kidney.

Discussion

The major conclusions to be drawn from these data are that zinc deficiency caused an increase of cadmium in the liver and that deficiencies of either copper or iron caused increased cadmium retention in the kidneys. The combined deficiencies did not produce additive effects on cadmium retention. In some cases the effect of combined deficiencies was less than that of a single deficiency, e.g., zinc-iron and zinc-copper deficiencies resulted in lower kidney cadmium than single deficiencies of iron and copper, respectively.

There were several significant effects of experimental treatments on various measurements that do not appear to be related either directly or indirectly to cadmium retention. Examples include the increased iron concentrations in some zinc-deficient tissues and increased calcium in iron-deficient livers. Such effects might be relevant to cadmium retention under different experimental conditions; however, they will be excluded from detailed discussion here.

In a further attempt to interpret the data, correlation coefficients were calculated for tissue mineral levels for all groups at 10 days versus tissue cadmium retention at 10 and at 16 days and tissue mineral at 16 days versus tissue cadmium at 16 days. The latter provided more significant correlations; some of these data are shown in Table 10. These data in many cases reaffirm effects already presented, such as the relationship between jejunal-ileal cadmium and liver cadmium, an effect ascribable primarily to zinc deficiency (Tables 3 and 4). Other relationships, such as those between liver manganese and liver and kidney cadmium, are not otherwise apparent.

The small intestine is probably the principal site at which the elemental deficiencies affect cadmium uptake and retention. The extent to

Table 10. Correlation between tissue element concentrations at 16 days and tissue cadmium retention at 16 days.

Tissue	Element	r^a Duodenum	Jejunum-ileum	Liver	Kidneys
Duodenum	Zn	−0.210‡	−0.386*	−0.312†	NS
	Fe	−0.376	NS	NS	−0.460*
	Cu	NS	NS	0.387*	−0.348*
	Cd	1.000*	0.590*	0.398*	0.341*
Jejunum-ileum	Zn	—	−0.551*	−0.416*	NS
	Fe	—	NS	0.265†	−0.467*
	Cu	—	NS	0.312‡	−0.325‡
	Ca	—	NS	0.233†	NS
	Cd	—	1.000*	0.707*	NS
Liver	Fe	—	—	NS	−0.266†
	Mn	—	—	0.257‡	−0.267†
	Cu	—	—	0.402*	NS
	Cd	—	—	1.000*	NS
Kidneys	Fe	—	—	0.228‡	−0.384*
	Cu	—	—	0.336‡	NS

[a]There were no significant correlation coefficients for tissue elements and tissue Cd as follows: duodenal Ca; liver Zn, Mg and Ca; and kidney Zn, Mn, Mg and Ca. NS = nonsignificant: — = no comparisons.
*$p < 0.001$.
†$p < 0.01$.
‡$p < 0.05$.

Table 11. Cadmium-109 retention in the duodenum plus jejunum-ileum and liver plus kidneys with projected increases in the liver plus kidneys between days 16 and 22.[a]

	^{109}Cd retention, %				Projected: liver + kidneys	
	Duodenum + jejunum-ileum		Liver + kidneys		Increase	Total
Deficiency	10 days	16 days	10 days	16 days	16–22 days	22 days
Zn	45 ± 3	11.5 ± 2.0*	0.34 ± 0.03*	0.97 ± 0.04	0.22	1.19
Fe	40 ± 3	9.9 ± 1.1*	0.33 ± 0.03*	1.02 ± 0.08*	0.23	1.25
Cu	29 ± 3*	2.3 ± 0.4	0.40 ± 0.05*	0.90 ± 0.05	0.04	0.94
Zn Fe	49 ± 4	16.6 ± 1.8*	0.28 ± 0.01	1.13 ± 0.07*	0.44	1.57
Zn Cu	49 ± 3	8.2 ± 1.3*	0.33 ± 0.03*	0.96 ± 0.07	0.13	1.09
Fe Cu	47 ± 4	8.6 ± 1.4*	0.28 ± 0.02	0.97 ± 0.09*	0.15	1.12
Zn Fe Cu	64 ± 5*	12.7 ± 0.8*	0.33 ± 0.06*	1.05 ± 0.06*	0.18	1.23
None	42 ± 4	1.7 ± 0.2	0.20 ± 0.01	0.78 ± 0.06	0.02	0.80
Multiple analysis of variance, p^b						
Zn	0.0001	0.0001	NS	<0.05	—	—
Fe	<0.001	0.0001	NS	<0.01	—	—
Cu	NS	<0.05	NS	NS	—	—
Zn Fe	NS	NS	NS	NS	—	—
Zn Cu	<0.02	NS	NS	NS	—	—
Fe Cu	<0.01	NS	<0.01	NS	—	—
Zn Fe Cu	NS	NS	<0.001	NS	—	—

[a]Mean values ± SE for measured values. Projected values were calculated from means.
[b]NS = nonsignificant; — = no comparisons.
*Significantly different from control, $p < 0.05$.

which intestinal tissue concentrations represent the dynamic transport processes or even the overall absorption is not known. In the cases of the essential elements, there were decreases of each element in both the duodenum and jejunum-ileum with each deficiency at 10 and 16 days, except for zinc at 16 days. If cadmium binds to the same ligands as the essential elements, each deficiency may provide a greater opportunity for cadmium binding. Such ligands would include metallothionein in the case of zinc and copper and ferritin in the case of iron. The mechanism(s) controlling cadmium absorption under these conditions may not be element-specific. The absence of additive effects with combined deficiencies supports this possibility.

The mechanisms of zinc absorption (*8,9*) and the interactions of cadmium and copper at the site of intestinal absorption have been reviewed (*10*). In almost all studies of cadmium metabolism and interactions with other elements, the amounts of cadmium have been two or more orders of magnitude in excess of typical human intake (ca. 50 μg/day); however, such a low level was used in this study. Recent studies on the role of transferrin in iron absorption (*11*) and a high molecular weight plasma protein that binds copper in the early phases of copper absorption (*12*) provide additional pathways for investigation of cadmium interaction with each of these elements.

In an earlier experiment with Japanese quail, whole body retention of cadmium-115m was followed for 50 days (*13*). The rapid phase of cadmium loss took approximately 2 weeks. A supplement of zinc, copper and manganese, at levels in excess of requirement, which decreased cadmium-115m retention, produced an effect at 1 week that was similar to that at 2 weeks. From these data we have assumed that a maximal effect of a dietary treatment would be present 7 days after administration of a labeled cadmium dose. In the present study, most of the cadmium-109 had cleared the small intestine at 16 days (7 days after dosing); however, very large proportions of cadmium still remained in birds fed some of the deficient diets (Table 11). Based on the cadmium loss for the duodenum plus jejunum-ileum and the uptake by the liver and kidney between 10 and 16 days, projected liver and kidney increases during the next 6 days make it appear likely that by 22 days the liver and/or kidney cadmium of experimental groups would differ still more from the control group. It is not known where this cadmium was localized within the intestinal tissue and whether it would be ultimately transported to the liver and kidneys or lost into the intestine during cellular desquamation.

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