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Effects of Zinc Deficiency on Compositional Development and Protein Synthesis in Liver, Heart, and Kidney of the Suckling Rat (39669)

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Zinc deficiency causes growth depression in young animals, perhaps mediated through an impairment of normal nucleic acid and protein metabolism (1). Various investigators have examined whether the incorporation of several different amino acids into protein was depressed by zinc deficiency and some have obtained results suggesting that there is an effect on protein metabolism (2–4) while others have not observed differences in response to zinc deficiency (5–7). The conflict in these observations could be explained, in part, by differences in the tissues examined, the age of the animals studied, and the severity of the deficiency.

Depriving the nursing dam of adequate zinc results in growth retardation (8) and abnormalities in brain development of the offspring (9, 10). Since zinc deficiency appears to have its most severe consequences on rapidly growing tissues, we examined the incorporation of [^{14}C]leucine into the protein of liver, heart, and kidney, which are growing at different, although essentially linear rates during the 21-day suckling period (11). This provided information about the effects of zinc deficiency on growth and compositional development of these organs and on the rate of incorporation of the labeled amino acids into protein.

Materials and Methods. Dams of the Long–Evans strain were bred and maintained on a commercial laboratory diet until delivery. Shortly after parturition, the pups from several dams were withdrawn, mixed together, and randomly redistributed to the dams at eight pups each. The dams were divided into three groups and fed a zinc deficient, 20% sprayed egg white diet (12) which was modified to delete the antibiotic and include 1 mg inositol/kg diet.[1] The first group, zinc deficient, was fed the diet ad libitum and given distilled/demineralized water to drink. The second group was pair fed on an individual basis with the zinc deficient dams and given 25 mg Zn/l in their drinking water. The third group was given the diet *ad libitum* and the zinc supplemented drinking water.

On the days of analysis (6, 12, or 21 days of age), the pups were injected sc with [U-^{14}C]leucine (0.2 μCi/g body wt). After 20 min, the pups were decapitated, the liver, heart, and kidneys were removed and immediately placed in ice-cold saline. The tissues were weighed and homogenized, and aliquots were analyzed in duplicate for DNA (13), RNA (14), and protein (15), and the incorporation of [^{14}C]leucine was determined by liquid scintillation counting of an aliquot of the sample prepared for protein analysis. Acid-washed glassware was used throughout. Values cited in text represent the means ± standard errors and the significance of the differences was examined by use of Student's t test.

Results. The zinc deficient dams developed anorexia and cyclical feeding patterns were observed. By the fifth day, food consumption was significantly depressed (9.6 ± 5.1 g vs 26.4 ± 1.1 g, zinc-deficient vs ad libitum fed control, $P < 0.05$). The anorexia and cyclical feeding persisted during the balance of the experiment and this contributed to the impaired growth of the suckling pups (Table I). The extent of the growth depression increased postnatally since at 6 days of age the zinc deprived pups weighed 78.7% as much as the pups of *ad libitum* control dams, while their body weights were only 53.6% of the *ad libitum*

[1] Teklad Mills, Madison, Wisconsin, 53713. 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.

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TABLE I. WEIGHT OF SUCKLING RATS ON THE DAY OF ANALYSIS.[a]

	6 days of age		12 days of age		21 days of age	
	weight, g	% *ad lib.*	weight, g	% *ad lib.*	weight, g	% *ad lib.*
Zn def	7.8 ± 0.4[c] (12)	78.7	16.0 ± 0.7[c] (6)	74.2	19.6 ± 0.3[b,c] (6)	53.6
Pr fed	8.3 ± 0.3[c] (12)	82.8	18.8 ± 1.2[c] (6)	87.4	21.7 ± 0.8[c] (6)	59.3
Ad lib	9.9 ± 0.2 (12)		21.5 ± 1.0 (6)		36.6 ± 1.1 (6)	

[a] Values are the means ± the standard errors for the number of animals in parentheses.
[b] Significantly different from pair fed, $P < 0.05$.
[c] Significantly different from *ad lib.*, $P < 0.01$.

controls at 21 days of age. Pups from the pair fed controls tended to grow better than their zinc deficient counterparts, but, their body weights were significantly different only at 21 days of age. Pups of the pair fed controls grew less well than those of the *ad libitum* fed control dams at all ages examined.

The growth of liver, heart, and kidneys in the zinc deficient rat pups also was less than in pups from *ad libitum* fed controls (Table II). Although there was a trend for the organs to be smaller in zinc deficient animals at 6 days of age, only the kidney was significantly smaller when compared with the *ad libitum* fed controls. At 12 and 21 days of age, however, all three tissues were significantly smaller than those from the *ad libitum* fed controls. Tissues from pups suckled by pair fed dams were generally intermediate in weight between values from the zinc deficient and well fed controls, but were not usually significantly different from those of the zinc deficient animals. Compositional determination revealed some differences in response to zinc deficiency and undernutrition (Table II). DNA concentrations were higher in tissues from pups of zinc deficient and pair fed dams and tended to become more disparate with increasing age from the values obtained from pups from *ad libitum* fed controls. This effect was observed in all the tissues, but was most pronounced in the heart. Differences in RNA concentrations did not appear to follow a consistent pattern of responses to either zinc deficiency or undernutrition. Protein concentrations did appear to be elevated in response to zinc deficiency, but only in the liver.

The incorporation of the labeled amino acid into protein appeared to be influenced by the zinc status of the animal, and the effect was most pronounced in the youngest animals (Table III). In addition, the liver appeared to be considerably more severely affected than did kidney or heart. At 6 days of age, all three tissues from the pups suckled by zinc deficient dams displayed decreased incorporation of the leucine into protein. This effect did not appear to be due to undernutrition as the pups from pair fed controls did not show the reduced specific activity. At subsequent ages, the livers from the zinc deficient pups continued to display a reduced specific activity when compared with zinc adequate controls (although the difference was not statistically significant from the pair fed controls at 12 days of age). The heart at 12 and 21 days of age did not show differences in specific activity in response to dietary manipulations. The level of incorporation tended to be reduced in the kidneys from zinc deficient pups at 12 and 21 days of age, but the differences were not statistically significant.

Discussion. The dams fed the zinc deficient diet and not supplemented with zinc in the drinking water displayed signs of zinc deficiency including anorexia and cyclical feeding. It has been previously shown that the deficiency experienced by the nursing dams impairs milk production and decreases the zinc content of the milk (8). Pups from such dams display signs of zinc deficiency including reduced plasma and femur zinc and impaired growth (8–10).

The pups in this study also displayed reduced growth and the degree of growth reduction increased with age of the pups and thus with exposure to the deficiency. The growth depression affected the organs examined since they were smaller than those from pups of *ad libitum* fed controls, although the organ· weights were consonant with body size and as percentage of body weight did not vary significantly among the

TABLE II. WEIGHT AND COMPOSITION OF LIVER, HEART, AND KIDNEY OF SUCKLING RAT PUPS AT 6, 12, AND 21 DAYS OF AGE IN RESPONSE TO ZINC DEFICIENCY, PAIR FEEDING, OR FEEDING *ad lib.*[a]

	6 days						12 days						21 days					
	Weight (g)	DNA (mg/g)	RNA (mg/g)	RNA/DNA	Protein (mg/g)	Protein/DNA	Weight (g)	DNA (mg/g)	RNA (mg/g)	RNA/DNA	Protein (mg/g)	Protein/DNA	Weight (g)	DNA (mg/g)	RNA (mg/g)	RNA/DNA	Protein (mg/g)	Protein/DNA
Liver																		
Zn def	0.297	5.454	12.481[b,c]	2.326	157.3[b]	29.2	0.399[b,c]	4.961[b,c]	10.702[c]	2.163[b,c]	162.4[b,c]	32.8	0.668[c]	4.011[c]	9.514	2.397[c]	194.5[b,c]	47.6[b,c]
	±0.024	±0.281	±0.163	±0.150	±3.0	±1.6	±0.020	±0.116	±0.040	±0.048	±2.4	±0.3	±0.023	±0.206	±0.286	±0.098	±12.8	±1.7
Pr fed	0.265[c]	4.891	11.221	2.334	138.5	28.9	0.498[c]	4.577	10.753[c]	2.354[c]	154.6	33.9	0.700[c]	3.976[c]	9.518	2.393[c]	156.8[c]	39.4
	±0.006	±0.271	±0.239	±0.155	±3.2	±2.2	±0.036	±0.083	±0.067	±0.042	±1.6	±0.8	±0.030	±0.078	±0.261	±0.040	±4.7	±0.8
Ad lib.	0.323	5.007	11.475	2.266	148.7	29.3	0.605	4.519	11.461	2.530	151.0	33.4	1.331	3.465	9.317	2.692	132.6	40.6
	±0.005	±0.136	±0.191	±0.056	±4.0	±0.5	±0.030	±0.044	±0.091	±0.032	±3.4	±0.8	±0.038	±0.066	±0.166	±0.053	±1.4	±2.8
Heart																		
Zn def	0.051	5.172	5.296	1.035	115.1	22.4	0.077[c]	5.353	3.853[b,c]	0.722[b,c]	106.4[b,c]	20.2[c]	0.099[c]	3.827[c]	3.188	0.831[b,c]	131.6	34.4[c]
	±0.004	±0.173	±0.318	±0.087	±5.6	±1.4	±0.006	±0.237	±0.164	±0.024	±2.7	±1.4	±0.004	±0.100	±0.104	±0.021	±3.2	±0.6
Pr fed	0.046	5.038	4.713	0.953	104.2	19.2	0.078[c]	5.661[c]	4.543	0.805[c]	121.8	21.6[c]	0.104[c]	3.696[c]	3.327	0.903[c]	132.8	36.1[c]
	±0.003	±0.399	±0.282	±0.054	±5.1	±1.1	±0.005	±0.164	±0.098	±0.026	±2.2	±0.5	±0.004	±0.144	±0.082	±0.018	±3.0	±1.0
Ad lib.	0.060	4.993	5.299	1.065	111.9	22.5	0.103	5.009	4.443	0.890	122.2	24.5	0.187	2.862	3.203	1.21	132.0	46.2
	±0.004	±0.160	±0.141	±0.037	±3.4	±0.6	±0.005	±0.157	±0.080	±0.027	±2.0	±0.7	±0.012	±0.080	±0.078	±0.025	±2.4	±0.8
Kidney																		
Zn def	0.060[c]	9.169	7.413	0.812	101.6	11.1	0.104[c]	9.077	5.537[b]	0.611[c]	106.2	11.8	0.123[c]	9.666[b,c]	5.641[c]	0.585[b,c]	130.9	13.5[b,c]
	±0.004	±0.216	±0.114	±0.029	±2.3	±0.3	±0.004	±0.154	±0.054	±0.012	±2.0	±0.4	±0.003	±0.333	±0.114	±0.015	±5.2	±0.2
Pr fed	0.056[c]	8.833	6.893	0.780	100.0	10.8	0.112[c]	9.508[c]	5.991	0.630	107.7	11.3	0.131	8.648[c]	5.700[c]	0.660[c]	128.9	14.9[c]
	±0.002	±0.410	±0.356	±0.008	±0.5	±0.1	±0.006	±0.196	±0.147	±0.010	±3.5	±0.2	±0.005	±0.166	±0.071	±0.010	±1.3	±0.2
Ad lib.	0.068	9.067	7.120	0.788	100.2	11.1	0.132	8.601	5.536	0.643	102.3	11.9	0.191	8.146	6.054	0.744	136.4	16.7
	±0.002	±0.309	±0.143	±0.018	±1.7	±0.3	±0.003	±0.215	±0.187	±0.011	±3.6	±0.2	±0.007	±0.114	±0.083	±0.005	±2.0	±0.1

[a] Values represent means ± SEM for six animals in each group at each age.
[b] Significantly different from pair fed controls, $P < 0.05$.
[c] Significantly different from *ad libitum* fed controls, $P < 0.05$.

TABLE III. Incorporation of ^{14}C-leucine into Tissues of Rat Pups Suckled by Zinc Deficient, Pair Fed or *ad lib* Fed Dams at 6, 12, or 21 Days of Age.[a]

	Liver			Heart			Kidney		
	6 days	12 days	21 days	6 days	12 days	21 days	6 days	12 days	21 days
Zn def	3.706[b,c]	3.981[c]	4.006[b,c]	1.748[b,c]	2.363	1.711	3.094[b,c]	3.173	3.165
	±0.183	±0.111	±0.143	±0.112	±0.194	±0.097	±0.161	±0.098	±0.119
Pr fed	5.504[c]	4.002[c]	4.754[c]	2.692	2.210	1.952[c]	5.839	3.096	3.511
	±0.138	±0.204	±0.209	±0.182	±0.188	±0.118	±0.746	±0.200	±0.132
Ad lib.	4.735	4.643	5.339	2.438	2.178	1.672	3.969	3.292	3.466
	±0.204	±0.083	±0.101	±0.068	±0.066	±0.039	±0.253	±0.040	±0.089

[a] Values are means ± SEM for the specific activities in (dpm/mg protein) 10^{-3} for six samples in each group at each age.
[b] Significantly different from pair fed controls, $P < 0.05$.
[c] Significantly different from *ad libitum* fed controls, $P < 0.05$.

three groups. No organ system, of those examined, appeared to be selectively damaged in terms of growth.

Effects of zinc deficiency or undernutrition on the composition of the three organs appeared to increase with age of the pups. At 6 days of age, differences between the groups appeared very minimal, but by 21 days of age, the differences were greater and involved all three organs. DNA concentrations were elevated suggesting a reduced cell size and RNA/DNA ratios were reduced perhaps implying a reduction in RNA and protein synthesis. The protein/DNA ratio, frequently used as a measure of cell size, was depressed in both heart and kidney, but was increased in liver. This apparent ambiguity is not readily explained. Ratios of RNA and protein to DNA as indicators of cell size must be interpreted with caution, however, as zinc deficiency has been demonstrated to interfere with the biosynthesis of all of these constituents under various circumstances (1). Possibly, one of the constituents could be affected disproportionately and thus alter the ratio in a way not affecting cell size.

The incorporation of radioactive leucine appeared to be decreased in response to zinc deficiency. In contrast to compositional alterations, the effects on this incorporation were most pronounced at the earlier ages. We observed the greatest depression in leucine incorporation at a time when synthesis of protein by the liver is at a comparatively low level. Protein synthesis appears to be maximal around day 20 and is depressed before day 10 (16). The diminished incorporation, which we observed, could be due to decreased synthesis or to increased degradation of the amino acid. Increased oxidation of leucine in zinc deficient animals has been described (17, 3). Variations in pool size may also be an important consideration. The free amino acid pools in the liver vary during postnatal development and increase markedly before 20 days of age (16). It is not possible from the present work to establish the exact cause for the decreased incorporation we observed.

Summary. Some effects of zinc deficiency on the growth and development and protein synthesis in liver, heart and kidney of suckling rat pups have been assessed. The deficiency impaired the growth of the pups and organs, although this may have been largely due to undernutrition because values were similar to those obtained with pups from dams pair fed, and given adequate zinc. Zinc deficient pups displayed a reduced incorporation of leucine into protein in the three organs, particularly at 6 days of age; this effect was most pronounced in the liver.

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