Effect of zinc deficiency on appetite and plasma amino acid concentrations in the rat

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1. Levels of zinc in liver and plasma of the Zn-depleted rats fluctuated with the feeding cycle and were significantly higher at the bottom than at the top of the cycle. As Zn deficiency became more severe fluctuations in plasma Zn diminished. Concentrations of Zn in liver, in contrast to levels in plasma and femur, were not markedly lowered by day 15.

2. In contrast to *ad lib.*-fed (AL) and overnight-fasted (OF) controls, some pair-fed (PF) controls had elevated levels of Zn in liver and plasma.

3. Intakes of water and food were significantly correlated in Zn-deficient rats. Packed cell volumes were significantly higher for Zn-depleted than for AL and PF rats.

4. Food intakes and plasma glucose concentrations were related in AL, OF and PF control rats but not in Zn-deficient rats.

5. At day 15 of Zn deficiency the order of total plasma amino acid concentrations in the groups of rats was AL > Zn-deficient > OF > PF. Many of the differences between the AL and OF groups for individual plasma amino acids also appeared in the Zn-deficient group at the top and bottom of the feeding cycle. Differences in individual amino acid concentrations at the top and bottom of the feeding cycle tended to be opposite in the PF and the Zn-deficient group. Levels of tyrosine and tryptophan in plasma were correlated ($P < 0.05$) with the cyclic feeding pattern of the Zn-deficient group; however, the ratios tryptophan or tyrosine: sum of other large neutral amino acids did not correlate significantly with the eating habits of Zn-deficient rats.

The adverse effect of zinc deficiency on the appetite of animals has long been known (Todd *et al.* 1934). Anorexia is apparent within 2–3 d of the initiation of a severely Zn-deficient diet (Macapinlac *et al.* 1966; McConnell & Henkin, 1974). This reduction in food intake is accompanied by a pattern of cyclic feeding in rats that are given a Zn-deficient, high-protein diet (Mills *et al.* 1969; Chesters & Quarterman, 1970; Griffith & Alexander, 1972; Wilkins *et al.* 1972). The reason for the anorexia and cyclic feeding patterns in Zn deficiency is unclear.

Reports from a number of laboratories suggested that amino acid levels in plasma might be related to the control of appetite (Peng & Harper, 1970; Fernstrom *et al.* 1973; Tagliamonte *et al.* 1973; Curzon & Knott, 1974; Pardridge, 1977; Anderson, 1977; Anonymous, 1978; Tews *et al.* 1979). More specifically levels of tryptophan in plasma correlated with concentrations of tryptophan and serotonin in brain (Fernstrom *et al.* 1973; Tagliamonte *et al.* 1973; Curzon & Knott, 1974). Findings of Anderson (1977) suggested that in plasma ratio tryptophan: other large neutral amino acids influences intake of protein, and plasma tyrosine: other large neutral amino acids influences the intake of energy.

Zn deficiency impairs amino acid utilization and metabolism (Hove *et al.* 1937; Hsu *et al.* 1969*a*, *b*; Hsu & Anthony, 1970; Griffith & Alexander, 1972; Duerre *et al.* 1977; Fosmire & Sandstead, 1977). It seemed possible, therefore, that the cyclic feeding patterns of rats given high-protein, Zn-deficient diets might be related to the plasma ratio tryptophan or tyrosine or both: other large neutral amino acids. We therefore measured the amino acid levels in plasma and other factors that are associated with the cyclic feeding.

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MATERIALS AND METHODS

Diet

All rats were given a 200 g sprayed egg white/kg, biotin-enriched diet with a Zn content of less than 1 mg/kg (Luecke *et al.* 1968). The diet was modified by the omission of chlortetracycline hydrochloride and the addition of 1·0 g inositol/kg diet (Teklad Mills, Madison, Wisconsin).

Design of Experiment

Male Long-Evans rats (55–65 g) which were purchased commercially (Charles River Breeding Laboratory, Wilmington, Massachusetts) were housed individually in Plexiglas cages in a laminar flow rack located in a humidity- and temperature-controlled room (25°, 40–50% humidity) with 12-h periods of light and dark. Food intake, water consumption and weight changes were measured between 08.00 and 09.00 hours. Wasted food was collected on papers and weighed at the same time. Wastage was taken into account for pair-feeding (PF), because Zn-deficient rats were significantly more wasteful than either PF ($P < 0{\cdot}01$) or *ad lib.* fed AL; ($P < 0{\cdot}02$) groups.

For the 7 d stabilization all rats were fed the Zn-deficient diet *ad lib.* and 25 mg Zn/l as zinc acetate in the drinking water. To minimize trace metal contamination silicon stoppers (Rodhelm-Reiss Inc., Ronsell Rubber Products Division, Belle Mead, New Jersey) were used in the water bottles. The rats were assigned to four groups after the stabilization period (day 0). One group was given the Zn-deficient diet *ad lib.* and distilled deionized drinking-water (zinc-deficient rats). Three zinc-adequate control groups were fed the zinc-deficient diet plus zinc acetate in their drinking water. Each rat in the first control group was individually pair-fed (PF) an amount of diet that equalled the amount of diet eaten by the Zn-deficient rat on the previous day. The second control group (AL) was fed *ad lib.* The third control group (OF) was fed *ad lib.* and fasted overnight before slaughter. The rats were kept on this regimen for either 15 or 20 d. They were then anaesthetized with diethyl ether and bled by cardiac puncture with heparinized (Eli Lilly and Co., Indianapolis, Indiana) syringes. Packed cell volume was measured on a small portion of whole blood. The plasma was quickly separated from the blood cells by centrifugation and refrigerated.

Within 1 h after cardiac puncture, 0·5 ml plasma was mixed with an equal volume of 100 g sulphosalicylic acid/l containing lithium acetate buffer pH 2·2 (Beckman Instruments Inc., Palo Alto, California) and S-β(4-pyridylethyl)-L-cysteine (Sigma Chemical Co., St Louis, Missouri) as the internal standard. The mixture was allowed to stand for 60 min at 4°, then centrifuged at 10000 *g* for 20 min at room temperature. The supernatant fractions were clarified through 0·45 μm filters (Millipore Corp., Bedford, Massachusetts) and held at −70° before the amino acids were determined with an amino acid analyzer (Beckman Model 119CL; Beckman Instruments Inc., Palo Alto, California). Within 1 h after collection, plasma tryptophan was assayed spectrofluorometrically by the method of Denckla & Dewey (1967) as modified by Bloxam & Warren (1974). Within 24 h, refrigerated plasma was assayed for plasma glucose with glucose oxidase (*EC* 1.1.1.47) and a glucose analyzer (Beckman Model 2; Beckman Instruments Inc., Fullerton, California) as described by Kadish *et al.* (1968). Bromcresol green was used to determine plasma albumin concentration (Doumas & Biggs, 1972).

Tissue Zn was measured by flame atomic absorption spectroscopy (Varian Model AA-6; Varian-Techtron Pty Ltd, Melbourne, Australia). Absorbance was measured at 213·9 nm and the values were compared with those of Zn solutions prepared from a certified reference solution containing 1000 μg Zn/l (Apgar, 1972). Plasma Zn was determined after dilution of the plasma with 3 vol. of distilled water. Two methods of ashing tissue and diet samples

for Zn analysis were used. Femurs were cleaned of soft tissue with stainless steel scapels, dried overnight at room temperature, stored at −20° for 30–60 d, boiled with 30 ml chloroform–methanol (2:1 v/v), dried overnight, weighed and then dry-ashed at 450° in quartz crucibles (Vycor crucibles, Corning Glass, Corning, New York) in a muffle furnace. After complete ashing, 0·35 ml concentrated, high-purity nitric acid (Ultrex, J. T. Baker Chemical Co., Phillipsberg, New Jersey) was added to the bones and the crucibles were returned to the furnace until the ash was oxidized. The resulting white ash was dissolved in 10 ml of 1 M-hydrochloric acid and analysed for Zn. Liver was wet ashed as described by Allen *et al.* (1977). Freeze-dried liver samples (0·4–0·5 g) were digested in approximately 30 ml of concentrated HNO_3 and 0·5 ml concentrated sulphuric acid. The mixture was heated to a gentle boil, until it emitted white fumes. Digestion was completed by addition of 10 ml hydrogen peroxide (300 ml/l). Both the dry and wet ashing procedures were utilized in the determination of Zn in the diet and the values obtained were similar ($P < 0·0001$).

Acid-washed glassware was used throughout the study. Mean values and standard deviations are given and the significance of the differences were examined by use of Student's *t* test or linear regression.

RESULTS

Weight gain, intake of food and water and food efficiency ratios for the 15 d experiment are shown in Table 1. Food intakes of Zn-deficient rats was 63·6% of the intake of AL control animals and was significantly depressed ($P < 0·01$) at day 4 and later, although values are not shown. The efficiency of food utilization was significantly less for Zn-deficient than for either AL ($P < 0·0001$) or Zn-adequate, PF control rats ($P < 0·0002$). Although the values are not presented comparable results were obtained for the 20 d experiment. Zn-deficient rats displayed typical symptoms of Zn-deficiency such as alopecia, depigmentation of hair, dermatitis of paws, anorexia, growth retardation and cyclic feeding. Fig. 1 illustrates food consumption, and weight changes of a representative Zn-deficient and AL control rat and the cyclic feeding pattern of the Zn-deficient rat.

Although values are not presented daily intakes of water and food were closely correlated ($P < 0·001$) for the Zn-deficient rats but not for AL and PF rats. However, total water consumption over the 15 d period was similar for the three groups (Table 1). After 15 d the Zn-deprived animals had a packed cell volume of $0·439 \pm 0·023$ which was significantly higher than PF ($0·406 \pm 0·038$, $P < 0·02$) and the AL control ($0·395 \pm 0·016$, $P < 0·001$) values. OF rats displayed higher packed cell volumes than the AL group ($P < 0·01$). The differences in packed cell volumes were also evident at 20 d.

The mean (±SD) daily Zn intake was 490 ± 133 μg for PF and 480 ± 45 μg for AL rats. Plasma Zn concentrations at 15 and 20 d are shown in Table 2. After 15 d, plasma Zn values for Zn-deficient rats, which were eating the least food, were 46·5% of those for AL rats and 23·8% of those for AL-fed rats at the top of the cycle. After 20 d plasma Zn values of Zn-deficient rats at the bottom of the feeding cycle were 28·6% of the values for AL rats and 18·0% of values for AL rats at the top of the cycle. The difference between plasma Zn values at the bottom and top of the feeding cycle was significant at both 15 and 20 d ($P < 0·02$). Plasma Zn level of Zn-deficient rats was significantly lower at both the top and bottom of the feeding cycle on day 20 than on day 15 ($P < 0·05$).

In liver, in contrast to plasma, Zn levels were not markedly lowered by 15 d of Zn deficiency (Table 3). However, liver Zn levels in Zn-deficient rats apparently reflected the plasma Zn changes at the top and bottom of the feeding cycle, i.e. the liver Zn was significantly higher ($P < 0·02$) at the bottom ($97·3 \pm 12·1$ μg/g) than at the top of the cycle ($75·0 \pm 11·3$ μg/g). Liver Zn was significantly higher ($P < 0·01$) in OF ($117·0 \pm 10·9$ μg/g) than in AL ($91·9 \pm 3·8$ μg/g) rats. Liver Zn was approximately 50% higher in PF than in

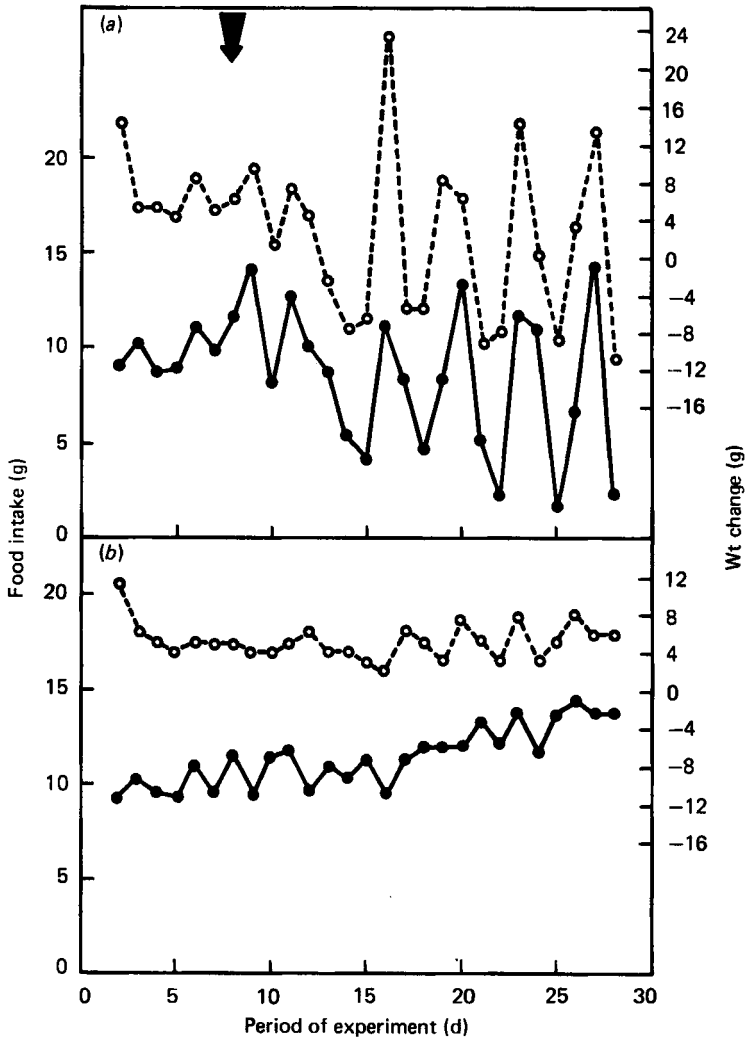

Fig. 1. Weight change (g; ○---○) and food intake (g; ●——●) for a rat given a zinc-deficient diet and distilled water (*a*) and a rat given a Zn-deficient diet and 25 mg zinc/l in the drinking water (*b*). From days 0 to 7, both rats were stabilized by feeding a Zn-deficient diet and 25 mg zinc/l in the drinking water. ⬇ On day 7 the previously-mentioned regimen was imposed.

AL or Zn-deficient rats. These values may reflect trends observed in the plasma of PF rats at 15 d, i.e. a number of PF rats displayed extremely high plasma Zn concentrations (Table 2).

Correlation was not significant between Zn level in femur and plasma in any group for either 15 or 20 d. At day 15 femur Zn in Zn-deprived rats was $117{\cdot}9 \pm 10{\cdot}4$ μg/g, less than half of that for AL control rats ($266{\cdot}9 \pm 18{\cdot}9$ μg/g), and at day 20 was significantly less ($94{\cdot}6 \pm 8{\cdot}1$ μg/g, $P < 0{\cdot}001$) than the 15 d Zn-deficient rats.

At day 20 plasma glucose concentrations differed significantly ($P < 0{\cdot}01$) between the top (148 ± 16 mol/l) and bottom (119 ± 11 mol/l) of the feeding cycle in the PF but not in the Zn-deficient rats (148 ± 19 *v.* 141 ± 13 mol/l). However, plasma glucose was significantly lower ($P < 0{\cdot}02$) for Zn-deficient rats at the bottom (141 ± 13 mol/l) of the feeding cycle

Table 1. *Weight gain, intakes of food and water, and food efficiency ratios of Zn-deficient, pair-fed and* ad lib.*-fed rats for a* 15 *d regimen*

(Mean values and standard deviations)

Group						
Feeding regimen	No.	No. of rats	Wt gain (g)	Total food intake (g)	Total water intake (ml)	Food efficiency ratio
Zn-deficient	1	15	17·1 ± 8·1	132·1 ± 12·0	280·4 ± 51·9	0·128 ± 0·060
Pair-fed	2	15	26·9 ± 6·0	125·7 ± 10·5	269·2 ± 45·0	0·214 ± 0·044
Ad lib.-fed	3	10	64·3 ± 11·9	183·3 ± 20·3	296·8 ± 54·9	0·349 ± 0·036

Differences between weight gain values for groups nos. 1 and 2, $P < 0·02$; 1 and 3, $P < 0·0001$; 2 and 3, $P < 0·0001$.
Differences between total food intake values for groups nos. 1 and 3, $P < 0·0001$; 2 and 3, $P < 0·0001$.
Differences between food efficiency ratio values for groups nos. 1 and 2, $P < 0·0002$; 1 and 3, $P < 0·0001$; 2 and 3, $P < 0·0001$.

Table 2. *Plasma zinc concentrations in rats given a Zn-deficient diet for* 15 *or* 20 *d with or without Zn supplementation in the drinking water*

(Mean values with their standard deviations)

Period on diet (d)...	Plasma Zn (μg/l)							
	15				20			
Group	No. of rats	Range	Mean	SD	No. of rats	Range	Mean	SD
Zn-deficient								
B	7	46·0–113·7	82·7	25·0*‡	8	24·0–60·3	47·0	12·0†‡
T	8	28·3–57·6	42·2	10·5*§	7	22·2–41·2	29·6	6·3†§
Pair-fed								
B	7	166·1–315·0	227·8	47·1	8	149·4–606·4	292·1	142·4
T	7	139·9–234·5	187·9	29·8	7	157·2–483·2	221·4	119·1
Ad lib.-fed	10	149·7–201·0	177·7	18·1	10	135·8–191·1	164·3	16·1
Overnight-fasted					5	154·2–178	163·0	9·9

B, bottom of cycle; T, top of cycle.
* Comparison of the top and bottom of the cycle was significant at 15 d ($P < 0·01$).
† Comparison of the top and bottom of the cycle was significant at 20 d ($P < 0·02$).
‡ Comparison of the bottom of the cycle on days 15 and 20 was significant ($P < 0·02$).
§ Comparison of the top of the cycle on days 15 and 20 was significant ($P < 0·03$).

than for AL rats (168 ± 24 mol/l) and was lowest of all (103 ± 18 mol/l) for OF rats. These trends were also present after 15 d.

Total amino acid concentrations in plasma are compared in Table 4. In Zn-deficient rats plasma amino acids tended to be lower at the bottom than at the top of the feeding cycle. In PF rats, however, plasma amino acids tended to be higher at the bottom than at the top of the cycle. Relative total plasma amino acid concentrations in the AL and OF groups were similar to values from Zn-deficient rats at the top and bottom of the feeding cycle, i.e. the values tended to be higher at the top than at the bottom of the feeding cycle.

In AL and OF groups levels of many individual amino acids were similar to those in the Zn-deficient group at the top and bottom of the feeding cycle respectively. Urea, threonine, serine, asparagine, alanine, citrulline, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, ornithine and arginine were lower in OF than in AL rats and were lower in Zn-deficient rats at the bottom than at the top of the feeding cycle. This

Table 3. *Liver zinc concentrations in rats given a Zn-deficient diet for* 15 *d with or without Zn supplementation in the drinking water*

(Mean values and standard deviations)

Group	Liver zinc (μg/g dry wt)			
	No. of rats	Range	Mean	SD
Zn-deficient				
B	7	79·1–113·9	97·3	12·1*
T	8	62·4–95·6	75·0	11·3*
Pair-fed				
B	7	119·2–182·8	145·1	20·3†
T	8	104·7–148·7	123·9	14·3†
Ad lib.-fed	10	86·3–96·4	91·9	3·8†
Overnight-fasted	6	96·7–125·9	117·0	10·9

B, bottom of cycle; T, top of cycle.
* Comparison of the top and bottom of the cycle was significant ($P < 0·02$).
† Comparison of pair fed with *ad lib.* values was significant ($P < 0·0001$).

similarity in plasma amino acid concentrations for the Zn-adequate control and the Zn-deficient rats was not found for glutamic acid and histidine. Glutamic acid levels were depressed in Zn-deficient rats both at the top and bottom of the feeding cycle, but were elevated in OF rats. Glutamic acid was similar in the Zn-deficient rats and in PF controls. Histidine was depressed in OF rats but was elevated both at the top and bottom of the feeding cycle in Zn-deficient rats. Apart from glutamic acid, alanine, tyrosine and histidine, individual amino acids in PF rats were higher at the bottom than the top of the feeding cycle; this was in contrast to the Zn-deficient rats.

The ratio, plasma tryptophan: other large neutral amino acids did not differ significantly among groups (Table 5). The ratio, plasma tyrosine:phenylalanine, however, was significantly lower in PF and OF rats than in the Zn-deficient and AL animals. Plasma tyrosine:phenylalanine also differed significantly between the top and bottom of the cycle for PF but not for Zn-deficient rats. Trends were similar in plasma tyrosine:other large neutral amino acids.

Plasma albumin concentrations for all groups were within normal range at day 15 but were significantly depressed for the Zn-deficient group ($30·8 \pm 1·1$ g/l, $P < 0·001$) at day 20.

DISCUSSION

Young male rats given a high-protein, Zn-deficient diet displayed typical signs of Zn deficiency including anorexia and cyclic feeding within 3–4 d. Intakes of water and food were correlated closely. Packed cell volumes were significantly higher in Zn-deficient than in AL and PF control rats. This is in agreement with previous studies, which found increased packed cell volumes in Zn-deficient rats (Macapinlac *et al.* 1966; Apgar, 1975). This haemoconcentration effect may be due to dehydration caused by zinc deficiency (McKenzie *et al.* 1975).

In 15 d of deficiency, femur Zn fell to less than half the AL control values. Plasma Zn fell similarly and fluctuated with the feeding cycle. The correlation between the amount of food consumed and plasma Zn levels of rats given a high protein Zn-deficient diet agrees with a report by Chesters & Quarterman (1970). We observed a similar relationship; liver Zn reflected both plasma Zn and food intake in Zn deficient rats, i.e. liver Zn levels were significantly lower at the top than at the bottom of the feeding cycle.

Table 4. *Plasma amino acid concentrations (nmol/ml) in Zn-deficient and control rats after 15 d*
(Mean values and standard deviations)

Group...	Zn-deficient				Pair-fed (PF)				*Ad-lib.*-fed (AL) (10)		Overnight-fasted (OF) (5)	
	T (6)		B (9)		T (6)		B (9)					
Plasma amino acids	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Taurine	467	75	486	179	357	52	405	86	517	87	497	86
Urea	10001	1406	8618	2805	4089	2414	6181	1883	12249	2144	7311	635
Threonine[a, c]	895	79	710	257	297	76	594	230	905	209	473	110
Serine[a]	681	88	578	106	488	141	624	159	575	147	453	35
Asparagine[a, b, c]	932	231	598	138	429	100	605	109	873	348	439	40
Glutamic acid	239	63	238	36	293	26	238	55	348	43	424	45
Glycine[b, c]	597	82	730	67	706	144	1005	207	662	122	625	63
Alanine[a, b]	1360	100	900	273	866	129	822	150	1382	210	775	94
Citrulline[a, b, c]	143	9	120	12	127	13	177	29	200	26	138	21
Valine[a, b, c]	515	103	301	79	182	33	274	75	577	112	330	29
Methionine[a, b]	141	26	85	20	57	8	66	11	164	35	88	7
Isoleucine[a, b, c]	197	43	141	33	86	16	137	47	240	49	176	15
Leucine[a, b]	298	56	224	40	176	19	230	64	324	69	250	10
Tyrosine[a, b]	201	37	136	30	93	17	93	13	226	52	131	17
Phenylalanine[a, b, c]	135	17	109	18	85	11	115	20	145	21	116	8
Tryptophan[a, b, c]	128	27	86	11	56	6	74	13	126	30	86	11
Ornithine[c]	122	31	99	18	82	16	123	19	107	14	85	7
Lysine	861	192	837	122	655	108	886	213	792	104	918	77
Histidine[a]	174	32	159	58	105	12	108	17	128	13	104	7
Arginine[a, b, c]	368	70	266	65	211	27	255	33	340	14	275	23
Total[a]	18440	1796	15412	3274	9437	2984	12965	2808	20880	2842	13715	499

T, top of cycle; B, bottom of cycle; *a*, AL *v.* OF; *b*, Zn-deficient T *v.* Zn-deficient B; *c*, pair-fed T *v.* pair-fed B; *a, b, c*.
Differences between groups were significant $P < 0{\cdot}05$.

Table 5. *Plasma tyrosine:phenylalanine, plasma tyrosine:sum of the other large neutral amino acids (NAA) and plasma tryptophan:other large neutral amino acids after 15 d of the feeding regimen*

Group	No. of rats	Tyrosine:phenylalanine[a, c, d, g]		Tyrosine:NAA[c]		Tryptophan:NAA[d, g]	
		Mean	SD	Mean	SD	Mean	SD
Zn-deficient	15	1·355	0·264	0·161	0·027	0·061	0·014
B	9	1·265	0·265	0·161	0·034	0·055	0·013
T	6	1·489	0·195	0·160	0·017	0·070	0·011
Pair-fed	15	0·937	0·212	0·134	0·035	0·048	0·008
B	9	0·824	0·228	0·116	0·018	0·046	0·007
T	6	1·107	0·228	0·162	0·038	0·050	0·009
Ad-lib.-fed	10	1·573	0·336	0·164	0·040	0·068	0·012
Overnight-fasted	5	1·130	0·155	0·130	0·013	0·062	0·007

B, bottom of cycle; T, top of cycle; *a*, AL *v.* OF; *b*, Zn-deficient T *v.* Zn-deficient B; *c*, pair-fed T *v.* pair-fed B; *d*, Zn-deficient *v.* AL; *e*, Zn-deficient *v.* PF; *f*, Zn-deficient *v.* OF; *g*, AL *v.* PF; *h*, PF *v.* OF.
a, *b*, *c*, *d*, *e*, *f*, *g*, *h*; differences between groups are significant $P < 0·05$.

The extremely high levels of plasma Zn observed in some PF, Zn-adequate controls might be related to tissue catabolism caused by starvation (Henry & Elmes, 1975). Spencer & Samachson (1970) suggested that the Zn in the zincuria of prolonged starvation is from the liver. In our rats, however, Zn in the liver of PF controls was elevated approximately 50%, rather than depressed; apparently excess Zn was from another source. Possibly muscle breakdown is involved in the metabolic response to starvation, as in response to injury (Cuthbertson *et al.* 1972).

Values for plasma glucose for PF, AL and OF Zn-adequate controls, confirms that under normal conditions plasma glucose correlates closely with food intake. In Zn-deficient rats, however, plasma glucose and food intake were not correlated. This difference might be related to changes in the release of or response of Zn-deficient rats to insulin (Quarterman *et al.* 1966; Boquist & Lernmark, 1969; Huber & Gershoff, 1973). Chesters & Quarterman (1970) reported that an eating pattern differed between Zn-deficient (nibblers) and the PF control rats (meal eaters), which might explain some of the differences in plasma glucose between those groups.

Total plasma amino acids were significantly higher in Zn-deficient rats than in PF, Zn-adequate controls, which agrees with the findings of Griffith & Alexander (1972). Many of the differences for individual amino acids between the top and bottom of the feeding cycle in Zn-deficient rats were mirrored by a comparison of AL and OF control rats; when rats ate food the level of most amino acids in plasma increased, but when rats fasted the level decreased, exceptions being glutamic acid and histidine. For the PF. Zn-adequate controls the trend was reversed; levels of most plasma amino acids were depressed at the top and elevated at the bottom of the feeding cycle. In PF rats the plasma amino acids probably increased at the bottom of the feeding cycle because body protein was degraded and amino acids were released into the plasma as reported during starvation (Francesconi *et al.* 1972). Apparently amino acid homoeostasis differed between the Zn-deficient and the control rats.

Possibly the levels of amino acids in plasma of the Zn-deficient rats were affected by abnormalities in amino acid utilization or excretion. Hove *et al.* (1937) suggested that in Zn-deficient rats the utilization of absorbed nitrogenous products is less efficient. This phenomenon apparently is generally true. Zn deficiency in tomatoes inhibits protein synthesis (Wood & Sibly, 1952) and free amino acids accumulate (Possingham, 1956). Zn

is required for protein synthesis in *Euglena gracilis* (Wacker, 1962) and *Rhizopus nigricans* (Wegner & Romano, 1963). Zn deficiency inhibits incorporation of amino acids into the proteins of a number of rat tissues that differ in susceptibility (Macapinlac *et al.* 1966; Hsu *et al.* 1969*b*; O'Neal *et al.* 1970; Hsu & Woosely, 1972; McClain *et al.* 1973; Hsu *et al.* 1974; Duerre *et al.* 1977; Fosmire & Sandstead, 1977). Possibly, the apparent homoeostatic changes and elevated levels of plasma histidine in our Zn-deficient rats reflect impaired utilization of amino acids. Abnormalities that might contribute to depression of protein synthesis are depressed activity of RNA polymerase (*EC* 2.7.7.6) (Terhune & Sandstead, 1972), abnormal formation or stability of polysomes (Fosmire *et al.* 1976) and possibly the production of an unusual RNA polymerase that can change the base content of the RNA produced, as was observed in *E. gracilis* (Falchuk *et al.* 1977). Apparently the rate of oxidation of some amino acids also was enhanced in zinc deficiency (Theuer & Hoekstra, 1966; Hsu *et al.* 1969*a*). Urinary excretion of ^{35}S increased in zinc-deficient rats that were injected with [^{35}S]methionine (Hsu & Anthony, 1970), but the conversion of alanine to urea decreased (Griffith & Alexander, 1972). Depressed urea synthesis is related to depressed activity of ornithine transcarbamylase (*EC* 2.1.3.3) (Burch *et al.* 1975) and, as a consequence, plasma ammonia (Prasad *et al.* 1978) may be elevated in Zn-deficient animals. In our rats, however, the urea cycle apparently functioned normally as evidenced by the depression of plasma citrulline levels during Zn deficiency. Greeley (1979), however, found that plasma citrulline levels were significantly elevated in marginal Zn deficiency in pregnant rats, which might be explained by the extra stress of pregnancy.

Levels of tyrosine and tryptophan in plasma were correlated with their levels and the levels of catecholamines and serotonin respectively in the brain (Fernstrom & Wurtman, 1971; Wurtman *et al.* 1974; Gibson & Wurtman, 1978). The uptake of tyrosine or tryptophan by the brain is influenced by the relative values for tyrosine or tryptophan: other large neutral amino acids (Fernstrom *et al.* 1975; Fernstrom & Faller, 1978). These relationships apparently have implications for the control of appetite. Anderson & Ashley (1976) found that tyrosine:phenylalanine, but not tyrosine:neutral amino acids is correlated with energy intake and that tryptophan:neutral amino acids ratio is correlated with protein intake (Ashley & Anderson, 1977). We found no significant differences among the experimental groups in tryptophan:other large neutral amino acids in plasma. Also, we found no relationship between food intake and either the tyrosine:phenylalanine or tyrosine:neutral amino acids in the plasma of Zn-deficient rats.

Our findings suggest that in Zn-deficient rats levels of plasma tyrosine, tryptophan and other large neutral amino acids are correlated with the intake of food, but tyrosine or tryptophan:other large neutral amino acids is not. In fact, many of the differences in the plasma amino acids in the Zn-deficient rats between the top and bottom of the feeding cycle resembled differences in plasma amino acids between the AL and OF control rats. These variations in plasma amino acid concentrations appear to be a consequence, rather than a controlling mechanism, of food intake. In our Zn-deficient rats only glutamic acid and histidine deviated from this pattern. Plasma glutamic acid was depressed and histidine was elevated during Zn deficiency. Our findings do not support the hypothesis that appetite control is mediated by changes in plasma amino acid concentrations but do suggest that Zn deficiency *per se* directly affects appetite.

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