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Evidence for an Impairment in the Conversion of Methionine to Cysteine in the Selenium-Deficient Chick (41130)

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Abstract. Experiments were conducted to determine the effects of dietary sulfur-containing amino acids on the etiology of nutritional pancreatic atrophy (NPA) in the Se-deficient chick. Supplementation of a crystalline amino acid diet containing 0.82% methionine with 0.4% L-cystine resulted in significant improvements in growth, efficiency of feed utilization and chick survival, whereas supplementation with 0.4% L-methionine had no effect. L-Cystine supplementation did not significantly affect plasma Se-dependent glutathione peroxidase activity, or prevent NPA. The addition of graded levels of L-cystine to a diet marginally adequate in methionine resulted in maximal gain in body weight and efficiency of feed utilization with 45 mmole half-cystine equivalents per kilogram. Supplementation of the same diet with equimolar amounts of L-methionine did not show significant improvements in these parameters unless the diet was also supplemented with Se. Plasma-free amino acid analyses showed significant reductions in the concentrations of cysteine, cystathionine, and homocysteine in Se-deficient chicks. These data indicate than an impairment in the metabolic synthesis of cysteine in the Se-deficient chick is responsible for a major portion of the growth depression observed with severe uncomplicated Se deficiency. This effect of selenium, however, is distinct from that in preventing NPA.

Atrophy and fibrosis of the exocrine pancreas, depressed appetite, poor growth and high rate of mortality are observed when Se-depleted chicks are fed a purified diet practically devoid (containing less than 0.015 ppm) of Se (1). Atrophy of the exocrine pancreas has been reported in the mouse fed a torula yeast diet low in cystine, and deficient in vitamin E (2). The degeneration of the mouse pancreas, similar in appearance to that observed in humans with kwashiorkor (3), was prevented by dietary supplements of selenium (0.15 ppm), vitamin E (500 IU/kg), or cystine (0.5%) (2). However, this condition has not been reproduced in the mouse or other mammalian species (4). Nutritional pancreatic atrophy (NPA) in the chick is prevented only by dietary Se and is thus associated strictly with uncomplicated Se deficiency.

The relationship of cystine to other Se–vitamin E-related deficiency diseases is well documented. Cystine plays a primary role in preventing nutritional muscular dystrophy (NMD) in vitamin E-deficient chicks. No other sulfur compound, including methionine, has been shown to possess equal antidystrophic activity (5). Factors (e.g., creatine and choline) which reduce the conversion of methionine to cysteine decrease the effectiveness of methionine in preventing NMD, whereas factors (e.g., guanidoacetic acid, nicotinamide) which enhance this conversion increase the effectiveness of methionine in preventing NMD (6). In addition, compounds such as mercaptoethylamine which metabolically spare cysteine reduce the severity of NMD, whereas compounds such as *S*-benzylcysteine which diminish the body store of cysteine increase the severity of this condition (6).

Schwarz (7) reported that cystine prevented dietary liver necrosis in the vitamin E-depleted rat; however, a portion of this effect was attributed by that author to Se contamination of the cystine used in that study. More recently, the addition of cystine to a diet adequate in total sulfur amino acids was shown to counteract a conditioned Se deficiency induced by elevated dietary copper (8).

The present experiments were conducted to determine: (a) whether the dietary requirement for sulfur-containing amino acids is altered in severe uncomplicated Se defi-

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ciency, and (b) whether there is a specific requirement for cysteine in the prevention of NPA in the Se-deficient chick.

Materials and Methods. Three experiments were conducted with day-old Se- and vitamin E-depleted male Single-Comb White Leghorn chicks produced from dams maintained on a low Se, low vitamin E practical diet (diet B(9)). Triplicate lots of 10 chicks each per treatment were housed in thermostatically controlled, wire-floored battery brooders with a 15 hr day. In the first experiment, the basal diet (Table I) was fed with or without additions of 0.4% L-methionine or 0.4% L-cystine in the presence or absence of supplemental Se (0.05 ppm, as Na_2SeO_3). Body weight, feed consumption, and mortality were recorded weekly for 28 days. At the end of that period, blood was obtained from each chick via anterior cardiac puncture, and the chicks were killed by cervical dislocation.

TABLE I. COMPOSITION OF BASAL DIET FOR PRODUCTION OF PANCREATIC FIBROSIS

Ingredient	Percentage
Glucose monohydrate	65.41
Amino acid mix[1]	23.53
Corn oil	4.00
Monoolein	0.50
Linoleic acid	0.50
Vitamin mix[2]	0.20
Mineral mix[3]	5.56
Sodium taurocholate	0.10
Choline chloride, 70% solution	0.20
	100.00

[1] Amino acid mix supplied (per kg diet): (g) L-arginine·HCl, 11.6; L-glutamic acid, 122.7; glycine, 12; L-histidine, 4.7; L-isoleucine, 9.5; L-leucine, 16; L-lysine·HCl, 12; DL-methionine, 8.2; L-phenylalanine, 8.2; L-proline, 3; L-threonine, 8.2; L-tryptophan, 2.2; L-tyrosine, 7.0; L-valine, 10.0.

[2] Vitamin mix supplied (per kg diet): (IU) stabilized vitamin A palmitate, 16,250; vitamin D_3, 800; *dl*-α-tocopheryl acetate, 100 IU; (mg) menadione sodium bisulfite, 1.2; biotin, 0.2; vitamin B_{12}, 0.014; calcium pantothenate, 30; folic acid, 4; niacin, 50; pyridoxine, 10; riboflavin, 10; thiamin HCl, 20; ethoxyquin, 125; glucose to make 2.0 g.

[3] Mineral mix supplied (per kg diet): (g) $CaHPO_4$, 19.8; $CaCO_3$, 7.9; $NaHCO_3$, 11; $KHCO_3$, 11; $MnSO_4$, 0.2; $MgCO_3$, 5; $FeSO_4 \cdot 7H_2O$, 0.45; (mg) KIO_3, 10; $CuCl_2 \cdot 2H_2O$, 30, $ZnCl_2$, 150; $CoCl_2 \cdot 5H_2O$, 2; $NiCl_2$, 6; $Na_2MoO_4 \cdot 2H_2O$, 8; $Cr_2K_2(SO_4)_2 \cdot 24H_2O$, 1.

Plasma was prepared from whole blood by centrifugation at 2500*g* for 10 min. The pancreases of four chicks per treatment were immediately excised, fixed in 10% neutral-buffered formalin for histological examination, and scored for pancreatic atrophy and fibrosis as described previously (10). Selenium-dependent glutathione peroxidase activity was determined in plasma by the glutathione reductase-coupled assay of Paglia and Valentine (11) as modified by Lawrence and Burke (12). Selenium concentrations of the basal diet and in each lot of sulfur amino acid used to supplement that diet were determined by the fluorometric method of Olson *et al.* (13).

In the second experiment, chicks were fed the basal diet containing 0.47% L-methionine (this represented 30 mmole/kg, or 2% of total dietary protein) in the presence or absence of supplemental Se (0.10 ppm as Na_2SeO_3). Additions of L-cystine were made to the basal diet at 15, 30, 45, or 60 mmole half-cystine equivalents per kilogram; equimolar additions of L-methionine were also made in a separate treatment series. Additional treatments consisting of the highest level of sulfur-containing amino acids (60 mmole L-methionine or half-cystine equivalents/kg) supplemented with Se (0.1 ppm as Na_2SeO_3) were included as positive controls. Body weight, feed consumption, and mortality were recorded weekly for 21 days. At the end of that period, plasma SeGSHpx activity and pancreatic scores were determined as in Experiment 1.

In the third experiment, chicks were reared on the basal diet or that diet supplemented with 0.15 ppm Se (as Na_2SeO_3). At 14 days of age, five composite blood samples from two chicks per treatment were collected as described above. The concentration of free amino acids in plasma was determined by automated ion exchange chromatography according to Mondino *et al.* (14). Results were expressed as nanomoles amino acid per milliliter of plasma.

Data were evaluated statistically by Student's *t* test or by analysis of variance according to the method Snedecor and Cochran (15). When significant treatment effects were detected by the latter procedure,

treatment means were ranked by a 5% new multiple range test.[1]

Results. *Experiment 1.* The addition of 0.4% L-cystine to the Se-deficient basal diet resulted in a significant ($P < 0.05$) increase in body weight gain and efficiency of feed utilization, and a significant reduction ($P < 0.05$) in mortality (Table II). The addition of 0.4% L-methionine had no significant effect ($P > 0.05$) on these same parameters. In this experiment, the basal diet was apparently adequate (in normal circumstances) with respect to total sulfur-containing amino acids because the addition of 0.4% L-methionine or 0.4% L-cystine to the Se-supplemented diet did not significantly improve chick growth or feed efficiency. The methionine and cystine used in these experiments were found to contain 0.294 and 0.588 ppm Se, respectively; therefore, these materials contributed less than 0.0012 and 0.0024 ppm Se, respectively, and resulted in a maximal Se concentration of the basal diet of 0.017 ppm. These levels of supplemental Se are considerably below that required to produce a growth response, regardless of form of Se (9). Histological examination of the pancreases at 28 days of age showed that L-cystine supplementation did not prevent NPA. The duodenal loops of these chicks were larger than those of the Se-deficient or the methionine-supplemented animals. Nevertheless, both the gross and microscopic appearances of the pancreases from all Se-deficient chicks did not appear to be affected by dietary supplementation with sulfur-containing amino acids. Addition of L-methionine or L-cystine to the basal diet did not significantly affect ($P > 0.05$) plasma SeGSHpx activity. Therefore, L-cystine promoted a growth response in these chicks without affecting Se status.

Experiment 2. Supplementation of the basal diet with 45 mmole half-cystine equivalents per kilogram resulted in a maximal gain in body weight (Table III). Pancreases of chicks from this treatment, however,

TABLE II. RESPONSE OF Se-DEPLETED CHICKS TO DIETARY SULFUR-CONTAINING AMINO ACIDS (EXPERIMENT 1)

Dietary treatments			28-day performance			Plasma	Pancreas
Se (ppm)	L-Met (%)	L-Cys[1] (%)	Gain (g)	Feed/gain	Mortality (%)	SeGSHpx[2]	score[3]
0.0	0.82	0	$27.1 \pm 2.3^{a[3,5]}$	$6.54 \pm 0.40^{a[4,5]}$	$66.7 \pm 6.7^{c[4]}$	$0.09 \pm 0.05^{a[4,5]}$	$4.19 \pm 0.24^{b[4-6]}$
0.05	0.82	0	193.0 ± 4.5^{c}	2.19 ± 0.10^{c}	0.0 ± 0.0^{c}	3.39 ± 0.32^{c}	1.18 ± 0.02^{a}
0.0	1.22	0	27.7 ± 1.9^{a}	6.37 ± 0.30^{a}	63.6 ± 3.3^{a}	0.0 ± 0.0^{a}	4.27 ± 0.15^{b}
0.05	1.22	0	188.1 ± 3.4^{c}	2.09 ± 0.20^{c}	6.7 ± 6.7^{c}	2.35 ± 0.31^{b}	1.08 ± 0.02^{a}
0.0	0.82	0.4	101.7 ± 7.2^{b}	3.12 ± 0.40^{b}	40.0 ± 5.7^{b}	0.0 ± 0.0^{a}	4.00 ± 0.31^{b}
0.05	0.82	0.4	187.7 ± 3.7^{c}	2.27 ± 0.05^{c}	3.3 ± 3.3^{c}	2.17 ± 0.39^{b}	1.31 ± 0.19^{a}

[1] L-Cystine.
[2] nmole NADPH·min^{-1}.
[3] Score: 1 = normal, 5 = completely fibrotic.
[4] Mean ± SEM, for three replicates of 10 chicks each per treatment.
[5] Means with like superscripts are not significantly different ($P > .05$).
[6] Mean ± SEM for 12 chicks per treatment.

[1] SAS-76 Statistical Analytical System, SAS Institute, Inc., Raleigh, N.C., employed with the Cornell University Computer System.

TABLE III. RESPONSE OF Se-DEPLETED CHICKS TO DIETARY L-CYSTINE SUPPLEMENTATION (EXPERIMENT 2)

Dietary treatments			21-day performance		Mortality	Plasma	Pancreas
Se (ppm)	L-Met (mmole/kg)	½ Cys-cys[1] (mmole/kg)	Gain (g/chick)	Feed/gain	(%)	SeGSHpx[2]	score[3]
0	30	0	19.3 ± 3.4^{c}[4,5]	5.50 ± 1.0^{a}	40.0 ± 10.0^{a}	0.25 ± 0.07^{d}	3.85 ± 0.11^{a}[5,6]
0	45	0	21.7 ± 4.2^{c}	5.09 ± 0.8^{a}	60.0 ± 11.5^{a}	0.25 ± 0.04^{d}	—
0	60	0	30.1 ± 0.5^{c}	$4.02 \pm 0.5^{a-d}$	50.0 ± 20^{a}	0.07 ± 0.07	—
0	75	0	20.0 ± 2.8^{c}	5.54 ± 0.75^{a}	40.0 ± 5.8^{a}	0.13 ± 0.03^{d}	3.77 ± 0.19^{a}
0	90	0	28.9 ± 6.1^{c}	$3.64 \pm 0.18^{b-e}$	56.6 ± 12.01^{a}	0.22 ± 0.03^{d}	—
0	30	15	28.7 ± 4.1^{c}	$4.33 \pm 0.77^{a-e}$	50.0 ± 10.0^{a}	0.30 ± 0.03^{d}	—
0	30	30	55.0 ± 7.4^{d}	$2.96 \pm 0.40^{e-f}$	36.6 ± 8.8^{a}	0.15 ± 0.04^{d}	—
0	30	45	111.6 ± 2.1^{b}	$2.03 \pm 0.08^{d-f}$	40.0 ± 20.0^{a}	0.25 ± 0.18^{d}	3.29 ± 0.34^{b}
0	30	60	84.3 ± 1.6^{c}	$2.28 \pm 0.11^{d-f}$	40.0 ± 10.0^{a}	0.13 ± 0.11^{d}	—
0.1	30	0	77.1 ± 5.0^{c}	$2.75 \pm 0.18^{c-f}$	3.3 ± 3.33^{b}	3.07 ± 0.82^{c}	1.24 ± 0.04^{c}
0.1	90	0	123.5 ± 2.41^{b}	$2.00 \pm 0.01^{e-f}$	0.0 ± 0.0^{b}	5.22 ± 1.5^{b}	1.22 ± 0.01^{c}
0.1	30	60	135.6 ± 1.3^{a}	1.79 ± 0.10^{f}	0.0 ± 0.0^{b}	7.49 ± 0.19^{a}	1.56 ± 0.01^{c}

[1] L-Cystine as half-cystine equivalents.
[2] nmole NADPH·min^{-1}.
[3] Score 1 = normal, 5 = completely fibrotic.
[4] Mean ± SEM for three replicates of 10 chicks each per treatment.
[5] Means with like superscripts are *not* significantly different ($P > 0.05$).
[6] Mean ± SEM for 12 chicks per treatment.

showed NPA lesions at Day 21. L-Cystine supplementation at 60 mmoles half-cystine equivalents per kilogram significantly depressed gain in body weight compared to the 45 mmole/kg level. The addition of L-methionine to the basal diet did not significantly ($P > 0.05$) affect body weight gain or pancreatic histology at any level of supplementation. Selenium supplementation resulted in significant ($P < 0.05$) improvements in body weight gain and SeGSHpx activity and reductions in mortality and pancreatic fibrosis scores at 21 days of age. Supplemental Se also prevented the growth depression observed with the highest level of supplemental L-cystine. This treatment, in fact, resulted in growth significantly greater ($P < 0.05$) than that observed with supplementation of Se plus an equimolar amount of methionine.

Experiment 3. Se-deficient chicks were found to have significantly depressed ($P < 0.05$) levels of cysteine and cystathionine, but significantly elevated ($P < 0.05$) levels of histidine and phenylalanine in plasma as compared to Se-supplemented controls (Table IV). Homocysteine was not detected in Se-deficient chick plasma samples; however, it was qualitatively observed in all Se-supplemented control samples. The findings have been confirmed in recent studies[2] showing that Se-deficient chicks have less than 29% of the free homocysteine concentrations observed in plasma of Se-adequate chicks. Plasma concentrations of methionine and all other free amino acids analyzed were not affected ($P > 0.05$) by Se supplementation.

TABLE IV. EFFECT OF DIETARY Se ON PLASMA FREE AMINO ACID CONCENTRATIONS[1]

Amino acid	Selenium supplementation (nmole·ml^{-1})	
	0 ppm	0.15 ppm
Aspartic acid	123.3 ± 20[2]	118.5 ± 4[2]
Threonine	1703.6 ± 168	2043.0 ± 11
Serine	735.3 ± 54	606.0 ± 20
Glutamic	680.2 ± 208	535.5 ± 40
Proline	178.6 ± 6	163.6 ± 11
Glycine	1069.3 ± 128	877.2 ± 61
Alanine	1101.8 ± 184	1604.9 ± 70
Valine	431.5 ± 32	437.4 ± 37
Lysine	527.0 ± 121	381.8 ± 30
Histidine	160.0 ± 17*	111.4 ± 7
Methionine	485.2 ± 39	488.5 ± 14
Cystathionine	11.7 ± 1*	23.8 ± 3
One-half cystine	36.7 ± 3*	55.1 ± 4
Isoleucine	156.3 ± 42	158.8 ± 17
Leucine	342.7 ± 28	314.2 ± 32
Phenylalanine	140.5 ± 10*	90.1 ± 11

[a] Samples drawn on Day 14 of experiment.
[b] Mean ± SEM for five composite samples from each of two chicks per treatment.
* Significant treatment effect ($P < 0.05$).

Discussion. The results of the present experiments show that a component of the syndrome originally designated by Thompson and Scott (1) as uncomplicated Se deficiency in the chick is actually a secondary complication of a metabolic cysteine deficiency. Therefore, the dietary conditions under which uncomplicated Se deficiency is presently defined need to be modified. This observation has been overlooked because previous studies of uncomplicated Se deficiency in chicks have employed 0.82% methionine as the sole dietary source of sulfur-containing amino acids (1, 10, 16, 17). This level of methionine is considered to exceed the requirement of the chick to provide both methionine and cysteine, as the normal chick can utilize methionine for cysteine synthesis at a rate rapid enough to meet its physiological needs for growth (18). In past studies, beneficial effects of dietary cystine in Se deficiency have been attributed to possible Se contamination of sources of that amino acid (2). Because experimental diets used to study uncomplicated Se deficiency have been formulated to minimize all possible sources of Se contamination, cystine (a frequent source of Se contamination) has been omitted from such formulas. Cysteine has been previously shown to play a primary role in other vitamin E- and Se-related deficiency conditions (5, 6, 8). The present observation that cysteine synthesis may be impaired in severe uncomplicated Se deficiency suggests an additional function for Se, and a possible relationship of uncomplicated Se deficiency with other conditions in the chick, such as nutritional muscular dystrophy.

[2] M. W. LaVorgna and G. F. Combs, Jr., unpublished research, 1980.

The growth depression associated with severe uncomplicated Se deficiency in the chick is recognized to have both an appetitive component and a metabolic component (19). The effect of Se upon appetite was found to be responsible for two-thirds of this growth depression and the residual growth effect was thought to result from the metabolic effects of Se upon: (a) fat digestion, (b) enteric absorption, and/or (c) postabsorptive utilization of nutrients. In the present experiments, 82% of the Se-supplemented control chick growth rate could be restored by an *ad libitum* intake of the cystine-supplemented diet *in the absence of Se*. These results show that the postabsorptive utilization of methionine accounts for a considerable portion of the growth depression resultant of Se deficiency, and suggest that cysteine deficiency per se might also play a role in appetite regulation previously attributed to Se alone. The fact that cystine supplementation did not prevent NPA nor affect the SeGSHpx status of the Se-deficient chick, despite improving both growth and efficiency of feed utilization, supports previous findings which indicated that the effect of Se upon appetite was independent from its effect in preventing NPA (19). This observation, however, does not alter the fact that NPA is the only pathological condition resultant of uncomplicated Se deficiency.

The finding that Se-deficient chicks had decreased concentrations of free cysteine, cystathionine, and homocysteine, but normal concentrations of free methionine, in plasma further supports the hypothesis that a severe Se deficiency results in impairment of methionine transsulfuration. In that experiment, chicks were fed 0.82% methionine as the sole source of metabolic cysteine. An impairment in the transsulfuration pathway would be expected to result in decreased concentrations of the end product of the pathway along with intermediates beyond the site of impairment. Elevated serine (and glycine) concentrations could be indicative of a decreased production of cystathionine as the former amino acid condenses with homocysteine to form cystathione, the immediate precursor to cysteine. In recent findings,[3] the oxidation of [$^{14}CH_3$]methionine to $^{14}CO_2$ has been found to be significantly increased in Se-deficient chicks. Supplementing the Se-deficient diet with L-cystine reduced the rate of methionine methyl oxidation to levels comparable to those of Se-adequate chicks. Supplementing the Se-deficient diet with an equimolar amount of methionine did not reduce the oxidation rate in Se-deficient chicks. These results are consistent with the hypothesis that a Se-dependent reaction exists in the metabolic conversion of methionine to cysteine.

Whether the impairment in methionine transsulfuration results from the lack of a distinct seleno-component of this pathway or from indirect effects of a severe SeGSHpx deficiency is presently unknown and remains to be elucidated. The phenotypic effect of the impairment is primarily reduced growth which can be attributed to a metabolic cysteine deficiency as evidenced by the growth-promoting effect of L-cystine and decreased plasma one-half cystine concentration, as well as cystathione and homocysteine. The present results also indicate that the site of this impairment may be one or more of the initial steps of methionine transsulfuration resulting in "uncoupled" single carbon metabolism or increased methionine transamination.

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