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Blood and Liver Concentrations of Glutathione, and Plasma Concentrations of Sulfur-Containing Amino Acids in Chicks Fed Deficient, Adequate, or Excess Levels of Dietary Cysteine (41594)

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Abstract. Experiments were conducted with young male chicks to examine the influence of cysteine status on blood and liver glutathione (GSH) concentrations and plasma sulfur amino acids. The equivalence of cystine, cysteine, and GSH as sources of dietary cysteine for the chick was reconfirmed. Whole-blood GSH was unresponsive to dietary cysteine level, but liver GSH increased as dietary cysteine increased from a deficient level to the required level. Excess levels of cysteine (two or four times the chick's requirement) elicited no further increase in hepatic GSH. Plasma cystine concentration increased markedly (i.e., ninefold) and in a linear fashion while plasma cysteine increased only modestly (i.e., twofold) as dietary cysteine level increased.

Glutathione (L-γ-glutamyl-L-cysteinylglycine, i.e., GSH) was first detected in yeast in 1888 and its structure confirmed in 1935. It is ubiquitous in body tissues and is located almost exclusively inside cells (1). It is the most abundant thiol in the body. Tateishi and coworkers (2, 3) have proposed two hepatic pools of GSH, one with a high turnover rate which serves as a readily accessible reservoir of cysteine, and the other with a slower turnover which is much more difficult to deplete. Thus, fasting results in a rapid fall in liver but not erythrocyte GSH in rats (4), and refeeding methionine or cystine alone, quickly repletes hepatic GSH (5).

In addition to serving as an endogenous source of cysteine, GSH is fully active as an exogenous source of cysteine (6, 7). Some workers have suggested that cysteine (or cystine) *per se,* irrespective of methionine, may be a dietary essential for premature infants. This conclusion has been arrived at more through indirect (8–10) evidence with premature infants (e.g., low hepatic cystathionase activity and low plasma cystine and taurine levels) than through direct (11, 12) evidence (i.e., reduced growth when diets adequate in methionine but devoid of cystine are fed parenterally). Nonetheless, clinicians are keenly interested in finding a readily available source of cysteine for addition to parenteral solutions, since cysteine itself, tends to be unstable, and cystine cannot be used due to its low solubility. Hence, GSH which has a very acceptable lemon-like flavor and is also freely soluble in water could be a very desirable source of exogenous cysteine for parenteral solutions.

The work reported herein was designed to compare the efficacy of L-cysteine, L-cystine, and GSH as dietary sources of L-cysteine activity for both protein acretion (i.e., growth) and tissue GSH formation. Excess dietary cysteine was also studied to ascertain its effect on hepatic GSH and on plasma sulfur-containing amino acids.

Experimental Procedure. Male chicks resulting from the cross of New Hampshire males and Columbian females were used in both experiments. Care of chicks prior to initiation of experiments as well as allotment procedures have been described previously (13). Quadruplicate (experiment 1) or triplicate (experiment 2) groups of five male chicks were placed on each treatment diet during assay periods of 8 (experiment 1) or 12 days (experiment 2). Chicks were housed in heated, thermostatically controlled, starter batteries in an environmentally controlled laboratory room with 24-hr lighting. Feed and water were provided *ad libitum.*

The chemically defined basal diet (14) fed in both experiments was adequate in methionine, 0.30% L-methionine (7), but devoid of cyst(e)ine. In experiment 1, L-cystine, L-cysteine·HCl·H_2O and reduced glutathione

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TABLE I. GROWTH AND TISSUE GLUTATHIONE LEVELS IN CHICKS FED CYSTEINE-DEFICIENT DIETS SUPPLEMENTED WITH CYSTINE, CYSTEINE, OR GLUTATHIONE (EXPERIMENT 1)[a]

Diet	Gain (g)[b]	Gain/feed (g/kg)[b]	Tissue GSH	
			Liver (μmole/g)[c]	Blood (μmole/ml)
1. Basal (B)[d]	36	380	0.82	0.87
2. B + 0.10% L-cystine	89	584	1.39	0.96
3. B + 0.20% L-cystine	106	689	1.98	0.92
4. B + 0.10% L-cysteine[e]	89	584	1.38	0.86
5. B + 0.20% L-cysteine	107	668	3.57	0.94
6. B + 0.256% GSH[f]	90	582	1.87	0.86
7. B + 0.512% GSH	103	700	1.96	0.76
Pooled SEM	3.1	9.3	0.369	0.089

[a] Mean of quadruplicate groups of five male chicks during the period 8 to 16 days posthatching; average initial weight was 68 g.
[b] Quadratic ($P < 0.01$) response to all sources of cysteine.
[c] Linear ($P < 0.05$) response to all sources of cysteine.
[d] Contained 0.30% L-methionine and no cysteine or cystine.
[e] Provided as L-cysteine · HCl · H_2O.
[f] 0.256% and 0.512% GSH are isosulfurous to 0.10% and 0.20% L-cystine, respectively.

were compared as sources of dietary cysteine.[2] Two levels of each compound, isosulfurous to 0.10 and 0.20% L-cysteine, were fed in addition to the unsupplemented basal diet. The levels of cyst(e)ine supplementation were chosen to provide levels of cysteine activity below the chick's dietary requirement. Graded levels of L-cysteine · HCl · H_2O providing from 0 to 0.96% L-cysteine, a level four times the chick's requirement (15) for maximum growth, were fed in experiment 2.

Chick weight gain and efficiency of feed conversion were determined in both experiments. At termination of each experiment, chicks were bled by heart puncture and liver samples rapidly excised, weighed, and placed on ice until homogenized. Chicks had access to feed immediately prior to being bled. In experiment 1, liver and whole-blood GSH were measured by the method of Tietze (16). Liver GSH was also measured in experiment 2. Plasma cysteine was determined by the method of Gaitonde (17) and concentrations of plasma free methionine and cystine were determined using an automated amino acid analyzer (Model 119CL, Beckman Instruments, Palo Alto, Calif.).

Data were subjected to appropriate analysis of variance procedures (18); in experiment 2, treatment means were compared by the least significant difference method when the F value for treatment from the analysis of variance was significant.

Results. Gain and efficiency of gain (G/F) responded quadratically ($P < 0.01$), and liver GSH responded linearly ($P < 0.05$) to graded increments of each cysteine source (Table I). All sources of dietary cysteine were utilized equally. Blood GSH was unresponsive to the cysteine deficiency and it did not increase when increasing levels of the cysteine sources were fed.

Two deficient levels (0 and 0.12%), an adequate level (0.24%), and two excess levels (0.48 and 0.96%) of cysteine were fed to chicks in experiment 2 (Table II). Gain and G/F responded linearly ($P < 0.01$) to cysteine addition up to 0.24%. The 0.24% level of cysteine produced maximal growth, but a slightly higher level was necessary to maximize feed efficiency. At four times the L-cysteine requirement for maximal growth (i.e., 0.96%), weight gain but not G/F decreased. Liver GSH increased in going from cysteine deficiency to cysteine adequacy, but levels of cysteine above the chick's dietary requirement for maximum

[2] L-cystine, L-cysteine · HCl · H_2O, and GSH were purchased from Ajinomoto, U.S.A., New York, N.Y., Nutritional Biochemicals Corp., Cleveland, Ohio, and United States Biochemical Corp., Cleveland, Ohio, respectively.

TABLE II. EFFECT OF GRADED LEVELS OF CYSTEINE ON PERFORMANCE AND TISSUE LEVELS OF SULFUR COMPOUNDS (EXPERIMENT 2)[a,b]

Diet	Gain (g)	Gain/feed (g/kg)	Liver GSH (μmole/g)	Plasma AA (nmole/ml)		
				Cysteine	Cystine	Methionine
1. Basal (B)[c]	57[w]	347[w]	1.34[w]	11[w]	9[v]	42
2. B + 0.12% L-cysteine	148[x]	527[x]	1.49[wx]	13[wx]	23[w]	37
3. B + 0.24% L-cysteine	196[z]	632[y]	3.40[y]	15[x]	35[x]	35
4. B + 0.48% L-cysteine	198[z]	668[z]	2.48[wxy]	20[y]	61[y]	25
5. B + 0.96% L-cysteine	168[y]	647[yz]	3.09[xy]	24[x]	81[z]	36
Pooled SEM	5.3	7.3	0.525	0.8	3.5	4.2

[a] Mean of triplicate group of five male chicks during the period 9 to 21 days posthatching; average initial weight wsa 81 g.

[b] Means within a column not having a common superscript differ significantly ($P < 0.05$).

[c] Contained 0.30% L-methionine and no cysteine or cystine; cysteine added as L-cysteine · HCl · H_2O.

performance (i.e., 0.24%) brought about no further increase in hepatic GSH.

Plasma cystine concentration increased markedly (i.e., ninefold) and in a linear ($P < 0.01$) fashion while plasma cysteine increased only modestly (i.e., twofold) as dietary cysteine level increased. Plasma methionine was unresponsive to cysteine intake. Other essential amino acids, as expected, decreased in plasma as dietary cysteine was increased from 0 to 0.24%, after which they tended to remain constant.

Discussion. The finding of equivalence (for chick growth and feed utilization) of cystine, cysteine, and GSH as dietary cysteine sources confirms an earlier report from our laboratory (7).

Lack of response of whole-blood GSH to cysteine status in the chick confirms the observed failure of fasting and refeeding regimens to alter rat erythrocyte GSH levels (4). Charkey *et al.* (19) reported that blood GSH was not affected, but liver GSH levels increased in chicks when the methionine-deficient diet they were fed was supplemented with DL-methionine. To our knowledge, however, this is the first report of the response of chick liver GSH and plasma methionine, cystine and cysteine to variable levels of dietary cysteine. Cho *et al.*[3] have reported a study of similar design in which rat erythrocyte GSH was unaffected, but spleen, thymus, muscle, and liver GSH increased in response to dietary cysteine additions. However, their basal diet contained what we have estimated[4] to be an inadequate level of methionine for the growing rat. Our results with chicks fed a methionine-adequate diet show maximal hepatic GSH occurring at the same level of dietary cysteine as is needed for maximal growth. Thus, dietary cysteine in excess of the level required for maximum chick growth is without value for increasing liver GSH stores and therefore gives no metabolic advantage with respect to GSH's protective, transport, coenzymatic, and protein synthetic functions.

The linear responses of plasma cystine and cysteine to levels of dietary cysteine between zero and four times the chick's requirement are in contrast to the classical idea that plasma levels of the deficient amino acid remain low due to rapid removal of the deficient amino acid by tissues until the requirement for growth is met (20). We had hypothesized that the cysteine requirement for maximal growth might be lower than that required for maximal hepatic GSH storage, as was observed by Robbins *et al.* (21) in their investigation of histidine status as measured by growth rate, plasma-free histidine, and breast muscle carnosine. In their studies, plasma histidine lev-

[3] Cho ES, DeKrey NG, Stegink LD. Tissue glutathione as a cyst(e)ine reservoir during cystine depletion. Fed Proc 40:847, 1981 (Abstr.).

[4] Unpublished data from our laboratory indicate a methionine requirement of 0.24% (in the presence of excess cystine) for weanling male rats fed a chemically defined diet.

els remained low until muscle carnosine content was maximized; but, the chick's histidine requirement for maximal growth was lower than that for maximal muscle carnosine. Clearly the same type of relationship does not exist for cysteine nutriture as determined by growth rate, liver GSH, and plasma cysteine and cystine.

Even though chicks were fed graded levels of cysteine in experiment 2, cystine rather than cysteine was the predominant form accumulating in the plasma. Crawhall and Segal (22) have reported that cystine predominates over cysteine in plasma of rats fed a commercial diet, but they did not examine the influence of various levels of dietary cyst(e)ine on plasma levels of cysteine and cystine.

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