Influence of Vitamin E and Bile Compounds on Cysteine Metabolism in Nutritional Muscular Dystrophy in Chicks* (32838)

J. N. HATHCOCK,[1] M. L. SCOTT, AND J. N. THOMPSON
Department of Poultry Science and Graduate School of Nutrition, Cornell University, Ithaca, New York 14850

An adequate dietary supply of either vitamin E or sulfur amino acids prevents nutritional muscular dystrophy in chicks. Previous work has shown that certain non-sulfur compounds can affect the development of this disease by altering the rate of methionine to cysteine conversion (1). Those results indicated that cysteine or some compound derived from it, and not methionine, is responsible for the sulfur amino acid involvement in the prevention of nutritional muscular dystrophy in chicks.

This report concerns experiments designed to further investigate the specificity of cysteine in this function.

Experimental. Duplicate lots of day-old, white Plymouth Rock X Vantress chicks were used for each dietary treatment, with 5 male and 5 female chicks per lot. The chicks were reared in electrically-heated battery brooders with wire-mesh floors. Feed and water were supplied *ad libitum*. The composition of the basal diet used in the experiments is presented in Table I; it is a modification of a diet by Hathcock and Scott (1). Ethoxyquin and sodium selenite were included in this diet at levels high enough to prevent encephalomalacia and exudative diathesis, respectively, while allowing the production of muscular dystrophy (2,3).

The severity of the dystrophic lesions was determined at 4 weeks of age by killing the chicks, removing the skin from the breast and visually scoring the lesions on a 0–4 scale.

Cysteine to taurine conversion rates *in vivo* were estimated by orally administering 11 μC of cystine-^{35}S in chicks and determining the rate of incorporation of counts into the bile salts. Samples of bile were obtained after 24 and 48 hours by killing the chicks and removing the gallbladders. The various components of the bile samples were separated on thin layer plates, according to the method of Kottke *et al.* (4). After the appropriate spots

TABLE I. Composition of Basal Diet.

Ingredient	%
Casein	6.00
Isolated soybean protein	7.00
Torula yeast	10.00
Glucose monohydrate[a]	57.25
Cellulose[b]	3.00
Stripped lard[c]	5.00
Vitamin mixture[d]	0.74
Mineral mixture[e]	6.04
Amino acid mixture[f]	4.97
	100.00

[a] Cerelose, Corn Products Co., Argo, Ill.

[b] Solka-Floc, Brown Company, Berlin, N. H.

[c] From Distillation Products Industries, Rochester, N. Y.

[d] The vitamin mixture supplied the following per kg of diet: (in mg) thiamin, 10; riboflavin, 10; pyridoxine • HCl, 4.5; niacin, 50; folic acid, 4; Ca pantothenate, 20; menadione, 1; choline Cl, 1490; ethoxyquin, 125; and (in μg) biotin, 200; vitamin B_{12}, 20; and (in IU) vitamin A, 5600; vitamin D_3, 4110.

[e] The mineral mixture supplied the following per kg of diet: (in gm) $CaHPO_4 \cdot 2H_2O$, 27.22; $CaCO_3$, 13.55; KH_2PO_4, 8.68; NaCl, 7.13; $KHCO_3$, 2.10; and (in mg) Cu (acetate)$_2 \cdot H_2O$, 34; $MnCl_2 \cdot 4H_2O$, 466; $FePO_4 \cdot 4H_2O$, 267; MgO, 850; KI, 2.6; ZnO, 69; $CoCl_2 \cdot 6H_2O$, 1.7; $Na_2MoO_4 \cdot 2H_2O$, 8.3; $Cr_2K_2(SO_4)_4 \cdot 24\ H_2O$, 48; Na_2SeO_3, 0.22.

[f] The amino acid mixture supplied the following per kg of diet: (in gm) L-arginine • HCl, 10; glycine, 14; L-glutamic acid, 16; L-lysine • HCl, 2.5; L-tyrosine, 1.5; L-tryptophan, 1.2; L-phenylalanine, 0.5; L-leucine, 1.5; L-isoleucine, 0.5; DL-methionine, 2.0.

* These investigations were supported in part by Public Health Service Research Grant NB 05632, and by grants from the Muscular Dystrophy Associations of America, Inc., the Selenium-Tellurium Development Association, Inc., and by Hoffmann-La-Roche, Inc.

[1] Present address: Department of Biochemistry, School of Medicine, St. Louis University, St. Louis, Missouri.

TABLE II. Effects of Cholic Acid, Taurocholic Acid, and Taurine on Muscular Dystrophy.

Treatments[a]	Muscular dystrophy score[b] (4 week)
Expt. 1	
Basal diet	1.0[e] ± 0.07[d]
+ cholic acid	1.9[f] ± 0.09
+ taurocholic acid	0.6[g] ± 0.03
+ taurine	0.7[g] ± 0.07
Expt. 2	
Basal diet	1.3[e] ± 0.05
+ cholic acid	2.2[f] ± 0.11
+ taurocholic acid	0.6[g] ± 0.05
+ taurine	0.6[g] ± 0.13
+ DL-methionine	0 [h] ± 0
+ methionine + cholic acid	0.2[h] ± 0.05

[a] Each additive to the basal diet was at a concentration of 9.3 mmoles/kg, except DL-methionine at 0.2%.

[b] Scores on a 0–4 scale; values shown are means for 20 chicks.

[c] Values with same superscripts are not significantly different ($p \leq .05$) by Duncan's test. (7)

[d] Standard error of means.

were removed from the plates, the bile salts were leached out by a Hyamine–methanol (1:1) mix. The ^{35}S contents were then determined by liquid scintillation counting.

The taurine excretion rates were determined by analyzing the urine of 3 chicks per treatment. These chicks were surgically altered to allow collection of the urine separate from the feces, according to the procedure described by Newberne *et al.* (5). Taurine was assayed by the method of Pentz *et al.* (6).

Results and Discussion. Since one of the normal metabolic fates of cysteine is oxidation and decarboxylation to taurine, and since taurocholate is the only bile salt produced by the chicken, it appeared reasonable that the metabolic supply of cysteine might be affected by dietary supplements of cholic acid, taurocholic acid, and taurine. Specifically, addition of cholic acid would be expected to increase the rate of conversion of cysteine to taurine, and dietary taurocholic acid and taurine would be expected to slow that rate. Furthermore, if cysteine is the sulfur compound necessary for the prevention of muscular dystrophy, then dietary cholic acid should increase the severity of the dystrophic lesions and taurocholic acid and taurine should have beneficial effects. Experiments designed to investigate this hypothesis were performed.

In the first experiment cholic acid, taurocholic acid, and taurine were fed at dietary concentrations of 9.3 mmoles/kg. The results (Table II, Exp. 1) show that cholic acid approximately doubled the severity score, while taurocholic acid and taurine lowered the scores. A subsequent experiment confirmed these results (Table II, Exp. 2).

Another experiment was performed to determine whether the dietary supplements of cholic acid, taurocholic acid, and taurine were actually altering the rate of cysteine to taurine conversion, as hypothesized. Rates of incorporation of orally administered L-cystine-^{35}S counts into bile salts were used as a measure of the relative rates of this conversion. The results are presented in Table III. Dietary cholic acid greatly increased the incorporation of counts into the bile salts at both 24 and 48 hours after dosing. Dietary supplements of taurocholic acid and taurine caused greatly reduced rates of incorporation of counts into the bile. The results are presented as counts per mg of bile because the bile pool sizes and turnover rates in the chicken were not known. Also, the muscular dystrophy scores reconfirmed the earlier results. These results show that treatments which increase the incorporation of cystine sulfur into bile acids accentuate the dystrophy, and that treatments which decrease the rate of that incorporation decrease the severity of the dystrophic lesions.

Since vitamin E is an antioxidant and since sulfhydryl groups are easily oxidized, vitamin E-deficient chicks, compared to normal chicks, might be expected to oxidize cysteine and excrete taurine at increased rates. To test this hypothesis, three experiments were performed in which chicks were surgically altered at 4 weeks of age to allow collections of urine separate from feces. Urine samples from these chicks were analyzed for taurine. The results of the first two experiments (Table IV) show that the vitamin E-supplemented groups excreted only 1.5–2.3 μmoles of taurine/100 gm of body weight per day, while the basal

TABLE III. Muscular Dystrophy Scores, Taurine Excretion Rates, and Cystine to Taurine Conversion Rates.

Treatments[a]	Muscular dystrophy scores[b] (4 week)	Net cpm/mg of bile	
		24 hours	48 hours
Basal diet	1.0[e,c] ± 0.10[d]	14.3[e] ± 0.8[d]	22.4[e] ± 3.1
+ cholic acid	2.1[f] ± 0.12	79.3[f] ± 6.1	65.1[f] ± 7.5
+ taurocholic acid	0.5[g] ± 0.02	4.4[g] ± 0.2	5.7[g] ± 1.0
+ taurine	0.7[g] ± 0.08	2.1[g] ± 0.2	3.1[g] ± 0.5

[a] See footnote *a*, Table II.
[b] See footnote *b*, Table II.
[c] See footnote *c*, Table II.
[d] See footnote *d*, Table II.

and cystine-supplemented groups showed a relative taurinuria. The taurinuria of the dystrophic basal groups could have been due to either muscle degeneration or vitamin E deficiency. The taurinuria of the nondystrophic, cystine-supplemented groups could have been due either to the presence of extra cystine or the absence of vitamin E in the diet. A third experiment designed to clarify these relationships was performed. The results (Table IV, Exp. 3) indicate two reasons for taurinuria in chicks: muscular dystrophy and high cystine intake. Vitamin E was not capable of eliminating taurinuria in cystine-supplemented chicks. The possibility remains, however, that vitamin E could act to decrease taurine excretion in chicks which are deficient in cystine, and that the taurinuria in the group fed the basal diet could be due to either muscle degeneration or vitamin E deficiency.

TABLE IV. Taurine Excretion Rates in Muscular Dystrophy.

Treatments[a]	Mean muscular dystrophy score	Urinary taurine (μmoles/100 gm body wt. per 24 hours)
Expt. 1		
Basal diet 2	0.7[d,b] ± 0.08[c]	6.0[d] ± 0.20
+ L-cystine	0 [e] ± 0	7.2[d] ± 0.55
+ vitamin E	0 [e] ± 0	1.5[e] ± 0.29
Expt. 2		
Basal diet 2	1.0[d] ± 0.07	6.4[d] ± 0.68
+ L-cystine	0 [e] ± 0	8.8[d] ± 0.37
+ vitamin E	0 [e] ± 0	2.3[e] ± 0.09
Expt. 3		
Basal diet 2	0.8[d] ± 0.10	8.2[d] ± 0.95
+ L-cystine	0 [e] ± 0	8.7[d] ± 0.61
+ vitamin E	0 [e] ± 0	3.2[e] ± 0.13
+ L-cystine + vitamin E	0 [e] ± 0	9.4[d] ± 1.10

[a] L-Cystine at 0.16%, vitamin E as *d*-α-tocopheryl acetate at 40 mg/kg of feed.
[b] See footnote *c*, Table II.
[c] See footnote *d*, Table II.

Summary. The results of these experiments support the hypothesis that cysteine is the sulfur compound, or the precursor of a compound, involved in the prevention of nutritional muscular dystrophy in the chicken. Dietary cholic acid increases the rate of cysteine to taurine conversion and at the same time accentuates the dystrophy. Taurocholic acid and taurine decrease the rate of cysteine to taurine conversion and also partially alleviate the dystrophy. Vitamin E supplementation greatly reduces the taurine excretion rate, compared to that of the dystrophic chicks fed the basal diet.

1. Hathcock, J. N. and Scott, M. L., Proc. Soc. Exptl. Biol. Med. **121**, 908 (1966).
2. Scott, M. L., Vitamins Hormones **20**, 105 (1962).
3. Scott, M. L. Nutr. Abstr. Rev. **32**, 1 (1962).
4. Kottke, B. A., Wollenweber, J., and Owen, C. A., J. Chromatog. **21**, 439 (1966).
5. Newberne, P. M., Laerdal, O. A., and O'Dell, B. L., Poultry Sci. **36**, 821 (1957).
6. Pentz, E. I., Davenport, C. H., Glover, W., and Smith, D. D., J. Biol. Chem. **228**, 433 (1957).
7. Duncan, D. B., Biometrics **11**, 1 (1955).

Received Sept. 21, 1967. P.S.E.B.M., 1968, Vol. 127.