# Toxicity of Ethionine in the Young Chick

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Day-old New Hampshire chicks maintained on a complete synthetic-type diet supplemented with 0.2% DL-ethionine grew very poorly, had a lowered survival rate, and developed fatty livers. Sex of the chick did not appear to influence the response to ethionine under these conditions. The toxic effects of ethionine could be totally overcome with additional methionine. Supplementary choline had a variable effect, whereas cystine and elemental sulfur increased the adverse effects of ethionine. A very high level of vitamin $B_{12}$ failed to decrease ethionine toxicity. Addition of methionine at levels of 2 to 4% of an adequate diet was increasingly depressive upon growth by 4 weeks of age. Ethionine did not alter the toxicity of these high levels of methionine.

Growth failure of rats and signs of marked toxicity when ethionine [2-amino-4-(ethylthio)-butyric acid, *S*-ethylhomocysteine] was added to a diet containing 5% casein and 19% lard (*4*) were reported by Dyer in 1938. The effects of ethionine could be reversed by additional dietary methionine, thus indicating antagonism of the ethyl analog against methionine. These initial observations have been succeeded by investigations of the metabolic and pathological effects of ethionine in several species by a number of workers. The response of the young chick to dietary ethionine has not been reported previously. The experiments reported herein were concerned with the toxicity of ethionine to the young chick, the effect of methionine and related compounds upon this toxicity, and the effect of ethionine upon toxic levels of methionine.

## Experimental Procedure

Day-old New Hampshire chicks from a commercial hatchery were distributed equally by weight into groups of six chicks each. The weights of the chicks used in each experiment were within a 5-gram range. Females were used throughout, except in two experiments where the response of males and females was compared. The chicks were kept in electrically heated batteries on wire mesh floors. Feed and water were supplied *ad libitum*. Chicks were weighed at weekly intervals and experiments were terminated at the end of 4 weeks.

The composition of the two synthetic-type diets used in these studies is given in Table I. Diet C2 contained adequate amounts of all nutrients known to be required by the young chick for optimal growth. Diet C15M was deficient in methionine. The cystine and methionine contents were calculated to be 0.09 and 1.1%, respectively, for diet C2 and 0.07 and 0.6% for diet C15M. All supplements were added to the diet at the expense of glucose. L-Cystine, DL-methionine, and DL-ethionine were used.

**Table I. Composition of Experimental Diets**

| Constituent | C2, G./Kg. | C15M, G./Kg. |
|---|---|---|
| Casein, vitamin-free | 200 | 150 |
| Gelatin | 80 | 130 |
| DL-Methionine | 3 | . . . |
| Corn oil | 40 | 40 |
| Chick salts A[a] | 60 | 60 |
| Vitamins[b] | 2 | 2 |
| Glucose (Cerelose) | 615 | 618 |

[a] Composition previously reported (*3*).
[b] Vitamins added as follows, mg./kg. diet: thiamine hydrochloride 8, riboflavin 8, calcium pantothenate 20, choline chloride 2000, nicotinic acid 100, pyridoxine hydrochloride 8, *d*-biotin 0.3, pteroylglutamic acid 3, vitamin $B_{12}$ 0.02, vitamin A acetate 3, vitamin $D_3$ 0.02, α-tocopherol acetate 10, 2-methyl-1,4-naphthoquinone 1.

At the end of the experiments chicks were killed by decapitation. The livers were removed, pooled by experimental group, and homogenized at a 1 to 3 dilution with water. The liver fat was extracted from an aliquot of the homogenate with a mixture of equal parts of absolute ethyl alcohol and acetone. The total lipide concentration of the extract was determined by the method of Bragdon (*2*), using palmitic acid as a standard. Another aliquot of the lipide extract was assayed for phosphorus by the method of Fiske and Subbarow as outlined by Umbreit, Burris, and Stauffer (*14*). Calculation of the phospholipide concentration was based on the assumption that its phosphorus content was 4%. Water content was determined by drying an aliquot of the homogenate in vacuo at 70° C. to constant weight.

## Results and Discussion

The effect of ethionine upon growth, mortality, and feed efficiency at 4 weeks of age can be seen in Tables II and III. Data from two experiments have been combined in Table III, as the results were similar. Ethionine fed in the complete diet C2 at 0.1% was nontoxic, whereas 0.2% depressed growth caused some mortality, and decreased feed efficiency. The addition of 0.1% ethionine to the methionine-low diet C15M resulted in toxicity comparable to that obtained with 0.2% ethionine in the complete diet.

The chicks receiving ethionine suffered loss of appetite, became less active, grew slowly, and developed an unkempt appearance. Very often the birds were semicomatose for 2 to 3 days before death. The chief abnormality seen in the chicks that were killed at 4 weeks of age was a pale yellow liver which usually had some small hemorrhagic areas. In a few of the chicks the kidneys were pale. Gizzard erosion and perosis, which have been associated with methionine and choline deficiencies, respectively, were not observed in chicks receiving ethionine. (A study of the histological changes associated with ethionine toxicity will be published later.)

Changes in size and composition of the liver are presented in Table IV. Ethionine caused an increased ratio of liver size to body weight and fatty infiltration of the liver, whereas the water content was essentially unaffected. The fat-free, moisture-free liver residue, which represents chiefly protein, was higher per unit body weight when ethionine was fed. In experiment I the liver fat of chicks fed diet C2 was higher than has been found in other experiments. Fatty infiltration of the liver has been observed as early as the second week of the experiment. Acute ethionine toxicity in rats has been shown by others to produce marked fatty infiltration of the liver, particularly in females (*5*, *6*). On the other hand, chronic ethionine toxicity in the rat and mouse has been characterized chiefly by growth depression, and in most instances the fat content of the liver was normal (*7*, *9*, *12*, *13*). Wachstein and Meisel (*15*) found some fatty infiltration of the livers of mice that had been fed 0.5% ethionine for 28 days or longer.

The phospholipide content of the chick liver (Table IV) was slightly lower when ethionine was fed. The same difference has been observed in other groups, but it is not clear without further study whether this represents a real depression. Under conditions of acute ethionine toxicity in young female rats, Jensen, Chaikoff, and Tarver (*8*) found no change in phospholipide concentration of the liver, even though the total lipide was markedly increased.

The effects of methionine and related compounds on the toxicity of ethionine are presented in Tables II, III, and IV. Addition of 1% methionine to the complete diet C2 was effective in reversing all the adverse effects of 0.2% ethionine—e.g., growth depression, increased mortality, poor feed efficiency, hypertrophy, and fatty infiltration of the liver. The growth of chicks fed diet C2 (Table III) was below that usually observed with this diet (see Table II), so the improvement in growth upon addition of 1% methionine cannot be interpreted to mean that diet C2 is deficient in this amino acid. With diet C15M the addition of 0.4% methionine overcame the growth depression of 0.1% ethionine in the diet; however, this quantity of methionine was not sufficient to overcome the combination of ethionine toxicity and methionine deficiency of diet C15M. With this diet, levels of cystine and elemental sulfur equivalent to 0.4% methionine appeared to increase the adverse effects of ethionine. Choline and the very high level of vitamin $B_{12}$ were without effect. In the experiment using adequate diet C2 (Tables II and IV) cystine again appeared to depress further the growth of the ethionine-fed chicks. The 1% level of choline decreased the effect of ethionine alone as well as the more severely toxic combination of cystine and ethionine.

Cystine has not been shown to afford protection against ethionine. Stekol and Weiss (*12*) reversed the growth depression of dietary ethionine in rats equally well by feeding choline alone or combined with cystine. These workers (*13*) reported similar findings with weanling C3H mice fed a complete diet containing 30% casein. They found that choline alone overcame ethionine toxicity; however, a lower level of choline fed with

**Table II. Dietary Supplements Altering Ethionine Toxicity**

(6 chicks per group)

| Supplement: Ethionine, % | Supplement: Other | 4-Wk. Wt., G. ± S. E.[a] | Mortality | Feed Efficiency[b] |
|---|---|---|---|---|
| | | Diet C2 | | |
| 0.0 | None | 400 ± 12 | 0 | 0.597 |
| 0.2 | None | 170 ± 24 | 1 | 0.468 |
| 0.2 | 0.3% cystine | 122 ± 27 | 2 | 0.410 |
| 0.2 | 1% choline | 252 ± 17 | 0 | 0.538 |
| 0.2 | 1.5% choline | 210 ± 19 | 0 | 0.500 |
| 0.2 | 0.3% cystine + 1% choline | 206 ± 23 | 0 | 0.526 |
| | | Diet C15M | | |
| 0.0 | None | 198 ± 33 | 0 | 0.439 |
| 0.1 | None | 129 ± 12 | 0 | 0.345 |
| 0.0 | 0.4% methionine | 334 ± 24 | 0 | 0.579 |
| 0.1 | 0.4% methionine | 224 ± 15 | 0 | 0.608 |
| 0.1 | 0.4% cystine | 72 ± 12 | 3 | 0.305 |
| 0.1 | 0.084% sulfur[c] | 77 ± 13 | 2 | 0.306 |
| 0.1 | 0.3% choline | 134 ± 19 | 0 | 0.381 |
| 0.1 | 1% choline | 119 ± 11 | 0 | 0.354 |
| 0.1 | 0.18 mg. vit. $B_{12}$/kg. | 100 ± 11 | 0 | 0.311 |

[a] S. E. calculated by method of Mantel (*10*).

[b] Feed efficiency $= \frac{\text{g. wt. gain}}{\text{g. food consumed}}$.

[c] Sulfur equivalent to 0.4% methionine.

**Table III. Toxicities of Ethionine and Methionine with Diet C2**

| Supplement, %: Ethionine | Supplement, %: Methionine | 4-Wk. Wt., G. ± S. E. | No. Chicks/ Mortality | Feed Efficiency |
|---|---|---|---|---|
| 0.0 | 0 | 331 ± 14 | 12/0 | 0.532 |
| 0.1 | 0 | 300 ± 6 | 6/0 | 0.567 |
| 0.2 | 0 | 119 ± 18 | 12/4 | 0.369 |
| 0.0 | 1 | 391 ± 12 | 6/0 | 0.642 |
| 0.0 | 2 | 270 ± 13 | 12/0 | 0.582 |
| 0.0 | 3 | 176 ± 19 | 6/0 | 0.469 |
| 0.0 | 4 | 100 ± 7 | 12/3 | 0.346 |
| 0.2 | 1 | 361 ± 11 | 6/0 | 0.633 |
| 0.2 | 2 | 236 ± 11 | 12/0 | 0.533 |
| 0.2 | 3 | 156 ± 16 | 6/1 | 0.465 |
| 0.2 | 4 | 113 ± 8 | 12/1 | 0.498 |

## Table IV. Liver Changes Associated with Dietary Ethionine

(6 chicks per group)

| Supplements to Diet C2: Ethionine, % | Supplements to Diet C2: Other | Liver Weight: Per chick, g. | Liver Weight: % B. W.[a] | Liver Analysis, % Fresh Wt.: Water | Liver Analysis, % Fresh Wt.: Total fat | Liver Analysis, % Fresh Wt.: Phospholipide |
|---|---|---|---|---|---|---|
| | | Experiment 1 | | | | |
| 0.0 | None | 11.4 | 3.5 | 70.1 | 10.1 | ... |
| 0.2 | None | 6.0 | 5.9 | 72.3 | 13.3 | ... |
| 0.0 | 1% methionine | 11.0 | 2.8 | 71.4 | 6.7 | ... |
| 0.0 | 2% methionine | 8.7 | 3.6 | 70.9 | 7.2 | ... |
| 0.0 | 3% methionine | 5.3 | 3.0 | 71.2 | 7.8 | ... |
| 0.0 | 4% methionine | 3.2 | 3.3 | 72.2 | 7.0 | ... |
| 0.2 | 1% methionine | 12.1 | 3.4 | 69.6 | 9.6 | ... |
| 0.2 | 2% methionine | 7.6 | 3.4 | 71.2 | 9.0 | ... |
| 0.2 | 3% methionine | 4.4 | 2.8 | 70.2 | 6.9 | ... |
| 0.2 | 4% methionine | 4.1 | 3.2 | 70.6 | 8.1 | ... |
| | | Experiment 2 | | | | |
| 0.0 | None | 8.6 | 2.2 | 73.8 | 5.3 | 3.0 |
| 0.2 | None | 11.8 | 6.9 | 68.8 | 14.6 | 2.1 |
| 0.2 | 1% choline | 10.1 | 4.0 | 71.5 | 10.7 | 2.6 |
| 0.2 | 1.5% choline | 10.1 | 4.8 | 73.2 | 10.4 | 2.4 |
| 0.2 | 0.3% cystine | 8.2 | 6.7 | 69.2 | 16.6 | 2.2 |
| 0.2 | 0.3% cystine + 1% choline | 10.6 | 5.1 | 71.0 | 12.8 | 2.3 |

[a] Body weight.

## Table V. Response of Male and Female Chicks to Ethionine on Diet C2

(6 chicks per group)

| Expt. No. | Ethionine, % | 4-Wk. Wt., G. ± S. E.[a]: Females | 4-Wk. Wt., G. ± S. E.[a]: Males | Mortality: Females | Mortality: Males |
|---|---|---|---|---|---|
| 1 | 0.0 | 400 ± 12 | 390 ± 17 | 0 | 0 |
| | 0.2 | 170 ± 24 | 149 ± 44 | 1 | 2 |
| 2 | 0.0 | 386 ± 15 | 341 ± 21 | 0 | 0 |
| | 0.2 | 120 ± 26 | 109 ± 14 | 0 | 1 |

[a] S. E. calculated by method of Mantel (*10*).

cystine was even more effective. On the other hand, Rice and others (*11*) found that cystine increased ethionine toxicity in the guinea pig, as is reported here for the chick.

The influence of sex upon the chick's response to ethionine was investigated (Table V). In both experiments males and females responded to ethionine in a similar manner. Greater susceptibility to the influences of ethionine in females than in males has been reported for adult rats, whereas sex differences were slight or nonexistent in young rats (*6*). These results in the young chick do not preclude different susceptibilities of adult male and female birds to ethionine.

Methionine has been shown to be the most toxic amino acid to the chick when added at a level of 4% to a complete diet (*7*). The chick fed ethionine may be considered comparable to a methionine-deficient chick in many respects. It appeared possible, therefore, that ethionine might be effective in reducing the toxicity of high levels of methionine. Results of this experiment are presented in Table III. Levels of supplemental methionine from 2 to 4% of diet C2 progressively depressed growth of the chick, whereas 1% methionine did not affect growth. When these same levels of methionine were fed in the presence of 0.2% ethionine, growth was essentially the same as when the methionine supplement alone was fed. It was concluded that with complete diet C2 0.2% ethionine had no effect on toxic levels of methionine.

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