with vit. B_{12} had the highest egg production. Hatchability of eggs from birds injected with B_{12} was within the normal range. No live chicks were obtained from pullets injected with penicillin from the 14th week. A 39% hatch was obtained from the eggs of pullets fed penicillin continuously. The B_{12} content of the liver and kidney tissues and of the egg yolks was directly related to the injection of the vitamin into the birds. 4. Feeding of penicillin caused a significant increase in the total number of intestinal micro-organisms as indicated by thioglycollate counts. Penicillin likewise caused a significant increase in the number of enterococci of the intestine during the first 14 weeks of the experiment. The administration of penicillin either orally or intravenously resulted in a marked increase in the number of penicillin resistant micro-organisms in the intestinal tract.

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Inhibition of Growth of C3H Mice by Ethionine and its Alleviation by Methionine or Choline.* (18727)

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In a recent communication we reported(1) that either optical isomer of ethionine (S-ethyl-homocysteine) inhibited the growth of albino rats which were maintained on a complete casein diet. The inhibition could be alleviated by either isomer of methionine or by choline, but not by cystine, homocystine, or cystathionine. The observation that choline was effective suggested that ethionine was interfering with the economy of the labile methyl group. Further studies employing ethionine labeled with C-14 in the ethyl group revealed that the ethyl group carbon was incorporated into the molecule of choline and creatine of rat tissues. This suggested the possible "transethylation" of ethionine(2). Similar conclusions were made in experiments employing S^{35}-ethionine(2). It appeared of interest to extend these observations to other species, such as the mouse.

Experimental. Weanling mice of C3H strain, inbred by brother-sister method, born and raised in the laboratory, were used. At the age of 23 days the males and females were separated and placed in separate cages, 3 mice per cage. A total of 66 mice were used. The composition of the basal diet was as follows: Labco casein 30; corn starch 36; sucrose 15; Crisco 10; inorganic salts (USP XII No. 2) 4; cod liver oil 5%. Per kg this diet was fortified with the following vitamins: thiamin hydrochloride 20; riboflavin 20; calcium pantothenate 50; pyridoxine hydrochloride 20; nicotinamide 20; folic acid 5; inositol 1000; p-amino-benzoic acid 1000; biotin 1.0; alpha-tocopherol 300 mg; and vit. B_{12} 150 μg. No choline was added to the basal diet. The preparation and analytical purity of ethionine, methionine, cystine, and homocystine used in the present study were previously described (1). The food and water were fed *ad libitum,* the mice were weighed twice a week, and the food consumption was recorded. At the end of the experimental period the livers of mice in each group were pooled, and their lipid content was determined gravimetrically(1). The supplements fed with the basal diet are indicated in Table I.

Results. Since the incorporation of 0.5% of DL-ethionine into the diet proved fatal to the growing mice within 24 to 48 hr after its ingestion, the concentration was reduced to 0.25%. As will be observed from the data in Table I, this amount retarded or inhibited the growth. Supplementation of this ethionine-containing diet with 0.25% of methionine per-

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1. Stekol, J. A., and Weiss, K., *J. Biol. Chem.*, 1949, v179, 1049.

2. Stekol, J. A., and Weiss, K., *J. Biol. Chem.*, 1950, v185, 577.

TABLE I. The Effect of Ethionine in Mice Fed a Complete Casein Diet.

Mice group‡	Initial wt, g	Final wt, g	Food per day, g	Gain per day, g	Days on diet	Wet liver lipids, %	Supplements, %
1 ♂	10.8	19.4	1.8	.41	21	4.7	0
2 ♀	10.8	16.8	2.3	.30	21	4.7	0
3* ♂	11.2	10.5	1.0	−.03	21	4.0	.25 DL-ethionine
4† ♀	10.6	12.5	1.0	.09	21	4.0	.25 "
5 ♂	11.7	17.5	1.6	.32	21	5.84	.25 " , .25 DL-methionine
6 ♀	12.3	17.2	1.6	.23	21	5.84	.25 " "
7 ♂	9.3	8.0	2.0	−.10	14		.25 " , .5 choline chloride
	8.0	15.0	2.0	.37	19		.25 " , 1. "
8 ♂	9.2	10.2	1.7	.05	22	5.71	.25 " , .25 L-cystine
9 ♂	10.4	18.0	1.9	.35	22	5.67	.25 " " .5 choline
10 ♂	9.6	8.7	1.8	−.06	14		.25 " , .3 homocystine
	8.7	14.8	2.0	.37	16		.25 " , 1. choline chloride
11 ♂	10.7	19.0	2.1	.40	21		.25 " "

* One mouse died on 19th day.
† Two mice died on 12th day.
‡ Each group consisted of 6 animals from the same litter of inbred mice. All values are average. Data on dead mice were discarded.

mitted resumption of growth, but not quite at normal rate. Addition of 0.5% of choline chloride in lieu of methionine was ineffective (unless fed together with 0.25% of cystine, as in Group 9), while 1.0% of choline chloride completely reversed the inhibition. Neither cystine nor homocystine was effective. No lipid accumulation in the livers of ethionine-fed mice of either sex was noted. Since the ethionine-fed mice showed a reduction in food consumption which, as is well known, might affect the lipid content of the livers, we hesitate to attach unequivocal significance to the lipid data.

Discussion. The growth data obtained on mice are similar to those reported for the rat (1) in that either methionine or choline alleviated the inhibition of growth by ethionine, but that neither cystine nor homocystine was effective. It appears significant that 0.25% of ethionine in a diet which contained 30% of casein (hence, ample methionine) produced such drastic effects on growth of mice, if ethionine is merely an "antimetabolite" of methionine. Of further interest is the fact that the addition of 0.25% of methionine to the 30% casein diet alleviated to a large measure the inhibition by the ethionine. Since choline also completely counteracted the inhibition induced by ethionine, the metabolic disturbance appears to be concerned with groupings common to methionine and choline, namely their labile methyl groups. The incorporation of C-14 or S^{35}-labeled ethionine into various metabolites and tissues of the rat(2) could be duplicated in the mouse(3). These considerations do not exclude the possibility of a direct or indirect incorporation of ethionine *per se* or of only its ethyl group into tissue proteins(1).

Summary. 0.25% of ethionine fed together with a complete 30% casein diet inhibited the growth of weanling C3H mice. The inhibition was alleviated by either choline or methionine, while neither cystine nor homocystine was effective. The observations are discussed in the light of active participation of ethionine or of its ethyl group in metabolic transformations in which methionine and choline methyl groups are normally involved.

3. Stekol, J. A., and Weiss, K., Unpublished data.

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