zones act by interfering with mineral metabolism nor that altered mineral metabolism influences resistance of mice to vaccinia virus. The activity of the methoximes, in which metal chelation would be blocked, also points in this direction, although the possibility of *in vivo* demethylation cannot be ruled out. While it is possible to pick out a structural unit, $=N—C—C=NNHCSNH_2$, which is common to most of the active derivatives, a few active thiosemicarbazones outside the scope of this unit have been found, *e.g.*, benzaldehyde T.S., and a few compounds which possess this unit are inactive, *e.g.*, cyclohexanedione phenylhydrazone T.S. Modifications of the sulfur as in the semicarbazones and S-methyl derivatives result in complete inactivation.

The antibiotic, Netropsin, resembles the thiosemicarbazones in that it protects mice inoculated intracerebrally with vaccinia virus but fails to prevent the development of lesions at the site of virus inoculated into the skin of rabbits(5).

Summary. 1. Serial passage of vaccinia virus intracerebrally in mice treated with isatin thiosemicarbazone results in a gradual diminution in the amount of virus demonstrable in the brain. 2. Treatment with isatin thiosemicarbazone fails to protect rabbits inoculated intracerebrally with vaccinia virus. 3. Aliphatic oxime thiosemicarbazones may be as effective as benzene or heterocyclic derivatives as chemoprophylactic agents against vaccinia virus in mice.

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Effect of Diet on Rat Liver Betaine Transmethylase.* (20691)

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The methylation of homocysteine by betaine in the presence of rat liver preparations *in vitro* was first demonstrated by Borsook and Dubnoff(1). The activity of this enzyme system, called betaine transmethylase, has been shown to be reduced when animals are subjected to a deficiency of folic acid(2) or vit. B_{12}(3). The present experiments were designed to investigate further the effects of diet on the enzyme system.

Methods. Weanling Sprague-Dawley rats were used. The basal diet contained alpha protein,† 18 g; cod liver oil, 3 g; Crisco (hydrogenated vegetable fat), 10 g; salt mix (4), 2 g; sucrose, 66.4 g; inositol, 0.1 g; choline chloride, 0.1 g; DL-methionine, 0.43 g; α-tocopherol acetate, 25 mg; thiamine hydrochloride, 0.5 mg; riboflavin, 0.5 mg; folic acid, 0.5 mg; pyridoxine, 0.5 mg; nicotinic acid, 2 mg; calcium pantothenate, 1 mg; 2-methyl-1,4-naphthoquinone, 25 γ; biotin, 5 γ; vitamin B_{12}, 5 γ. The alpha protein was washed exhaustively with hot water, 95% ethyl alcohol, then dried and ground before incorporation into the diets. This basal diet was fed without supplement to some animals.

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† Alpha protein is an isolated soy bean protein obtained from the Glidden Company, Chicago, Ill.

TABLE I. Methionine Formation by Livers of Rats Fed Various Diets.

Diet	Series	No. rats	Avg daily wt gain, g	Avg methionine formed, γ
C*	1	8	—	715 ± 113†
B	"	6	—	3 ± 3
C	2	6	4.1 ± .4†	662 ± 117
B	"	6	1.4 ± .1	36 ± 40
B + 10% casein	"	6	2.2 ± .1	646 ± 232
B + complete amino acid mix	"	4	2.2 ± .2	553 ± 153
B + liver extract‡	"	4	2.1 ± .4	65 ± 39
B + 1% yeast extract§	"	4	2.7 ± .3	68 ± 45
B + valine, leucine, isoleucine	3	2	—	0
B + cystine, threonine, phenylalanine, tryptophan	"	2	—	332 ± 172
B + lysine, histidine, arginine, methionine	"	2	—	33 ± 33
B	4	4	1.9 ± .3	28 ± 28
B + complete amino acid mix	"	4	2.3 ± .3	1056 ± 146
B + cystine, threonine, phenylalanine, tryptophan	"	5	3.2 ± .1	406 ± 94
B + cystine	"	6	1.9 ± .3	120 ± 89
B + threonine	"	4	3.9 ± .2	188 ± 37
B + phenylalanine	"	4	1.8 ± .2	48 ± 31
B + tryptophan	"	4	1.0 ± .1	620 ± 278

* C = Chow; B = Basal.
† Stand. error.
‡ 1:20 extract of liver, Nutritional Biochemicals, Cleveland, Ohio.
§ Yeast extract powder, Nutritional Biochemicals.

Other groups received the basal diet with a supplement of the essential amino acids and cystine. This amino acid supplement, referred to as complete amino acid mix in Table I, consisted of the following expressed as g per 100 g of diet: DL-valine, 1.74; DL-leucine, 2.09; DL-isoleucine, 1.39; L-cystine, 0.26; DL-threonine, 1.22; DL-phenylalanine, 1.04; L-tryptophan, 0.35; L-lysine monohydrochloride, 1.30; DL-histidine, 0.83; L-arginine monohydrochloride, 0.43; DL-methionine, 0.52. Other groups of animals were given the basal diet with various combinations of the above amino acids and with single amino acid supplements. In all these diets the amino acids were added to the diet at the same level at which they were added in the complete amino acid mix. Control animals were given a diet of a commercial laboratory chow.

The diets were fed for 2 weeks, then the animals were killed and the liver homogenates assayed for betaine transmethylase activity by the method previously described(2). Final substrate concentrations were: DL-homocysteine monohydrochloride, 64 mg %; and betaine, 128 mg %. The results are reported as μg of methionine formed per g of liver during the 3-hour incubation period.

The *results* presented in Table I show that livers from rats fed the basal diet exhibited no demonstrable betaine transmethylase activity. Supplementation of this diet with 10% casein or with the complete amino acid mix resulted in betaine transmethylase activities comparable to chow-fed rats. In contrast, yeast extract or liver extract was ineffective in restoring the enzyme activity. These results then indicated that the absence of betaine transmethylase in livers of the rats receiving the basal diet was due to an inadequate supply of amino acids. The remaining experiments were designed to determine which amino acids of the complete amino acid mix were active in producing the enzyme. The amino acids in the complete mix were arbitrarily divided into 3 groups and fed to rats. Only the combination containing cystine, threonine, phenylalanine, and tryptophan was effective. Livers from rats receiving these amino acids exhibited considerable betaine transmethylase activity but not as high an enzyme titer as was observed in livers from animals receiving the complete amino acid

mix.

This observation was repeated in series 4 and in addition each of the 4 amino acids was added separately to the basal diet. Of the single amino acid supplements tested only tryptophan produced an appreciable betaine transmethylase activity.

The growth data given in Table I are of considerable interest. The basal diet permitted approximately half-normal growth. Supplementation of the basal diet with 10% casein, complete amino acid mix, yeast, or liver extract improved the growth rate. Supplementation of the basal diet with threonine resulted in growth equal to that obtained with rats fed chow. With respect to growth, methionine is the first limiting amino acid in alpha protein(5) and the present experiments indicate that threonine is the second limiting amino acid.

It is apparent from the data in Table I that there is no correlation between the effects of the various supplements on growth and on betaine transmethylase activity. Thus tryptophan supplementation resulted in considerable enzyme activity but actually depressed growth. Yeast extract improved growth considerably but was ineffective in stimulating the synthesis of the betaine transmethylase enzyme.

These observations suggest that the amino acid pattern required for the synthesis of this particular liver enzyme must be considerably different from the pattern required for growth of the animal. Also, the observation that some of the diets permitted good growth but allowed negligible synthesis of betaine transmethylase suggests that when rats are fed methionine the enzyme is not vital to the animal's economy. Although the observation periods in the present experiments were of short duration, in other experiments rats have been fed the basal diet for 5 months and continued to grow at a rate of approximately 2 g per day throughout this period.

Summary. 1. Rat liver betaine transmethylase varies strikingly with the amino acid composition of the diet. 2. The pattern of amino acids required for synthesis of this enzyme is considerably different from the pattern required for growth of the animal. 3. When rats are given methionine, betaine transmethylase does not appear to be necessary for growth.

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Effect of Antigonadotrophic Serum on Testes of A-Strain Mice Treated with Estrogen.* (20692)

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The development of interstitial cell tumors in A-strain mice after prolonged estrogen treatment has been demonstrated repeatedly(1-4). Gardner(5) has suggested that these tumors arise as the result of enhanced secretion of interstitial cell stimulating hormone (ICSH) by the pituitary gland under the influence of low doses of estrogen rather than from a direct effect of estrogen upon the testis. The present report presents the results of an attempt to test this hypothesis by nullifying the presumed excessive ICSH secretion with antigonadotrophic serum.

Materials and method. For the production

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