PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 147, 72–79 (1974)

The Effect of DL-Methionine, Vitamin B_{12}, and Thyroid Powder on Metabolism of Formiminoglutamic Acid in Rats[1] (38283)

K. K. BATRA, K. U. BUEHRING, AND E. L. R. STOKSTAD

Department of Nutritional Sciences, University of California, Berkeley, California 94720

It has been reported in the literature that urinary excretion of formiminoglutamic acid (FIGLU) is increased in rats by vitamin B_{12} deficiency (1, 2) or by feeding thyroid powder (3). These results with thyroid may be explained on the basis that feeding thyroid powder induces vitamin B_{12} deficiency (4, 5) which, in turn, produces a secondary folic acid deficiency (2) and thus results in a rise in urinary excretion of FIGLU. Also, it is known that administration of methionine either to vitamin B_{12} deficient (1) or to thyroid-fed rats (3) results in a rapid decrease in the excretion of FIGLU in urine.

The addition of methionine to a vitamin B_{12}-deficient diet increases considerably the folic acid level of the liver (6), particularly the level of polyglutamate forms (7, 8). In studies with perfused rat liver, Buehring *et al.* (9) showed that the addition of methionine to the perfusion medium decreased the levels of 5-methyltetrahydrofolate and increased the levels of reduced forms of monoglutamates other than 5-methyl-tetrahydrofolate and also increased the polyglutamates. It was therefore of interest to look further into the relationship between vitamin B_{12}, thyroxine, and methionine and study the effect of these compounds on the breakdown of FIGLU in liver homogenates.

Materials and Methods. Diets. The composition of basal diet is as follows (g/kg): glucose monohydrate, 714; soy assay protein,[2] 200; mineral salt mixture (10), 35; water-soluble vitamins premixed in glucose monohydrate, 10; corn oil with vitamins A, D, and E, 40; and choline chloride, 1. The vitamins were supplied in the following amounts (per kg): vitamin A acetate, 15,000 IU; vitamin D (viosterol), 2000 D IU; DL-α-tocopherol acetate, 50 mg; biotin, 0.2 mg; thiamine HCl, 15 mg; pyridoxine-HCl, 15 mg; Ca-pantothenate, 50 mg; niacin–HCl, 50 mg; folic acid, 5 mg; menadione, 10 mg; riboflavin, 15 mg. When the basal diet was supplemented with DL-methionine, vitamin B_{12}, and thyroid powder,[3] the levels were 15 g/kg, 100 μg/kg and 3 g/kg, respectively.

Animals. Male weanling rats, three weeks old, of the Sprague–Dawley strain[4] were placed individually in metabolic cages in a temperature- and light-controlled room. Six rats were used in each group. Both food and water were given *ad lib.* Body weights were recorded and urine samples collected weekly. A 24-hr urine sample was collected from each animal, using two drops of concentrated HCl and 0.2 ml of toluene in the collection flask to avoid degradation of FIGLU and contamination by bacterial growth. The urinary and liver contents of FIGLU were assayed by the enzymatic method of Tabor and Wyngarden (11).

Materials. *dl*-tetrahydrofolic acid was synthesized by reduction of folic acid in glacial acetic acid with sodium dithionate (12). *dl*-5,10-Methenyltetrahydrofolic acid was prepared by acidifying 5-formyltetrahydrofolic acid (Leucovorin) (13), which was a generous gift from Lederle Laboratories.[5] *dl*- 5- Methyltetrahydrofolic acid was obtained by reduction of *dl*- 5,10-Methenyltetrahydrofolic acid with sodium borohydride (14).

Preparation of liver homogenates and determination of FIGLU breakdown in liver homoge-

[1] This study was supported in part by U. S. Public Health Service Grant No. AM-08171 from the National Institutes of Health.

[2] Nutritional Biochemical Corp., Cleveland, Ohio.

[3] Mann Research Laboratories, New York, NY.

[4] Simonson's Laboratories, Gilroy, CA.

[5] American Cyanamid Company, Pearl River, NY.

nates. Each rat was killed by decapitation, its liver removed and immediately homogenized with an equal volume of ice-cold 0.2 *M* phosphate buffer, pH 7.0, and 0.005 *M* in 2-mercaptoethanol. The incubation mixture for determination of FIGLU breakdown contained 2.0 ml of liver homogenate (1 g equiv. of liver) in 4 ml final volume. The substrates were added as indicated in the Results section. The reaction mixture was incubated at 37° for 1 hr. At time intervals of 15, 30, and 60 min, appropriate aliquots were removed and the reaction stopped by diluting each aliquot with 3 vol of 0.1 *N* HCl. The aliquots were either analyzed immediately for FIGLU or stored at 4° until analysis was completed. Before the determination of FIGLU, the samples were centrifuged and the pH adjusted to 7. Folic acid was measured by *L. casei* assay using the tissue preparation procedure of Bird *et al.* (15) to rapidly heat the liver tissue to minimize hydrolysis of polyglutamates. Total folic acid was determined after treatment with hog kidney conjugase (15).

Results. The effects of vitamin B_{12}, methionine, and thyroid powder on growth and FIGLU excretion are shown in Table I. The average body w ight of all the animals receiving thyroxine was 28% less than that of all the animals not receiving thyroxine. Methionine and vitamin B_{12} had no significant effect on growth under the conditions of this experiment.

The urinary excretion rates of FIGLU in the eight groups, given in Table I, show that vitamin B_{12} deficiency increased FIGLU excretion which was further increased by feeding thyroid powder. This is in agreement with previously published results (3). These results may be interpreted on the basis that thyroxine induces vitamin B_{12} deficiency which brings about a secondary folic acid deficiency, and as a result of this, the FIGLU metabolism is reduced and it is excreted in the urine. However, vitamin B_{12} at a level of 100 μg/kg of diet (3 times the requirement for normal growth) is not sufficient to reduce the FIGLU excretion to normal values, in groups which are limiting in methionine (groups 5 and 6, Table I). This suggests that methionine deficiency rather than vitamin B_{12} deficiency causes increased excretion of FIGLU. Since vitamin B_{12} is necessary for the synthesis of methionine, increased excretion of FIGLU in the urine of vitamin B_{12} -deficient animals could be explained as due to the resulting deficiency of methionine.

Results with liver homogenate. The content of FIGLU in liver and urine of various groups at different ages is compared in Table II. The liver content of FIGLU during the growth period of group 1 (-B_{12}, -Meth, -TP) and group 2 (-B_{12}, Meth, +TP) is quite similar and is much higher than that of the other groups. This corresponds with the urinary excretion data, except that the FIGLU excretion in group 2 (-B_{12}, -Meth, +TP) is higher than in group 1 (-B_{12}, -Meth, -TP), in contrast with the similar content of FIGLU in livers of groups 1 and 2. The feeding of methionine reduces the concentration of FIGLU in liver as well as the urinary excretion of this

TABLE I. Effect of DL-Methionine, Vitamin B_{12}, and Thyroxine on Urinary FIGLU Excretion and on the Growth Rate of Rats[a].

Group No.	Supplement per kg diet			Average body weight			FIGLU excretion		
	Vit. B_{12}	DL-Methionine	Thyroid powder	Initial	25 days[b]	46 days	11 days	18 days	46 days
	0.1 mg	15 g	3 g	g	g	g	μmoles/kg body wt/day		
1	−	−	−	66[6c]	186[4]	286[4]	213[6]	344[5]	186[4]
2	−	−	+	70[6]	159[4]	237[2]	865[5]	664[5]	617[2]
3	−	+	−	67[6]	209[4]	332[3]	0[6]	1[5]	0[3]
4	−	+	+	64[6]	146[3]	195[3]	114[6]	13[5]	4[3]
5	+	−	−	65[6]	217[4]	291[4]	149[6]	98[5]	2[4]
6	+	−	+	77[6]	157[4]	252[4]	164[6]	271[4]	8[4]
7	+	+	−	73[6]	220[4]	330[3]	2[6]	2[5]	0[3]
8	+	+	+	69[6]	117[4]	202[2]	0[6]	8[5]	0[2]

[a] Six animals started for each group.

[b] Days on experiment.

[c] Number animals surviving.

TABLE II. Comparison of FIGLU Content in Liver and Urine of Various Groups at Different Ages.

	Supplement/kg diet								
	Vit. B_{12}	Met.	TP[a]	13 Days		24 Days		32 Days	
Group No.	0.1 mg	15 g	3 g	Urine[b]	Liver[c]	Urine[b]	Liver[c]	Urine[b]	Liver[c]
1	−	−	−	304	6.0	248	5.2	134	6.8
2	−	−	+	1741	6.8	1192	6.8	654	8.3
3	−	+	−	7	0.01	0	0	0	0
4	−	+	+	200	0.04	10	0.12	0	0
5	+	−	−	115	0.4	27	0	27	1.1
6	+	−	+	230	0.3	82	1.8	194	2.4
7	+	+	−	5	0.08	0	0.24	0	0
8	+	+	+	5	0	4	0.23	1	0

[a] Thyroid powder.
[b] Urine (μmoles FIGLU/kg body wt/day).
[c] Liver (μmoles FIGLU/g liver).

compound.

The rates of FIGLU breakdown by liver homogenate of eight dietary groups with the addition of *dl*-tetrahydrofolic acid or *dl*-5,10-Methenyltetrahydrofolic acid to the assay mixture are compared in Table III. These results show that the rate of FIGLU breakdown is greatly increased in all groups where methionine was added to the diet. With no additional methionine in the diet, vitamin B_{12} has a small but consistent stimulatory effect on the breakdown of FIGLU (compare rat number 1 vs 5 and 2 vs 6), but with methionine added to the diet, vitamin B_{12} has no effect on FIGLU breakdown (compare rat numbers 3 vs 7 and 4 vs 8). Feeding thyroid powder in combination with dietary vitamin B_{12} or methionine, or with and without both dietary vitamin B_{12} and methionine, has an inhibitory effect on the breakdown of FIGLU in liver homogenate. This inhibitory effect of dietary thyroxine is also noticed whether or not tetrahydrofolic acid or 5,10- Methenyltetrahydrofolic acid is added to the liver homogenate of various dietary groups (compare rat numbers 1 vs 2, 3 vs 4, 5 vs 6, and 7 vs 8).

The addition of 0.4 μmoles *dl*-tetrahydrofolic acid per g of liver homogenate results in an increased rate of FIGLU breakdown in all of the dietary groups. *dl*- 5,10- Methenyltetrahydrofolic acid is as effective as *dl*- tetrahydrofolic acid in this respect, which shows that conversion of *dl*-5,10-methenyltetrahydrofolic acid to tetrahydrofolic acid is not the rate-limiting step. However, even when tetrahydrofolic acid or 5,10- Methenyltetrahydrofolic acid is added to the liver homogenate in amounts 5–10 times higher than the total amount of folic acid usually found in rat liver (0.04 μmole or 17 μg/g), the overall rate of FIGLU breakdown is not the same in all dietary groups. Thus the rate of FIGLU degradation is *ca.* 75% higher in the homogenates from rats receiving methionine (rats 3 and 4) than in those without methionine (rats 1 and 2) even in the presence of added tetrahydrofolate. The increase in FIGLU breakdown due to the addition of tetrahydrofolic acid is approximately 1.5 μmoles/g of liver/hr and is about the same in all dietary groups.

Table IV compares the effect of *dl*- tetrahydrofolic acid and *dl*- 5,10- Methenyltetrahydrofolic acid at different concentrations on breakdown of FIGLU by liver homogenate. The results are similar to those given in Table III in the following three features: (a) *dl*- 5,10- Methenyltetrahydrofolic acid is as effective as *dl*- tetrahydrofolic acid in metabolizing FIGLU, (b) supplementation with methionine in the diet increases the rate of FIGLU breakdown without the addition of reduced folates to the liver homogenate, and (c) at low concentrations of *dl*-tetrahydrofolic acid or *dl*- 5,10- Methenyltetrahydrofolic acid, FIGLU breakdown was higher in the liver of animals which received methionine. The two rates become comparable only when tetrahydrofolic acid or 5,10- Methenyltetrahydrofolic acid are provided in the reaction mixtures in amounts stoichiometric with that of FIGLU present.

The total liver folate values of the dietary methionine supplemented group (rat numbers 2

TABLE III. Effect of Vitamin B_{12}, DL-Methionine, and Thyroid Powder on Degradation of FIGLU in Liver Homogenates[a].

Rat No.	Dietary treatment			Body wt.(g)	Urinary FIGLU	FIGLU in liver	FIGLU added to liver homogenate	μmoles FIGLU degraded per g of liver homogenate per hr[b]		
								Folates added to homogenate		
	B_{12}	Meth	TP	24 days	μmoles/day/kg body wt	(μmole/g)	(μmole/g)	none	*dl*-H_4-Folic acid 0.4 μmole	*dl*-5,10-Methenyl-H_4-folic acid 0.8 μmole
1	−	−	−	203	248	5.8	0.0	0.5	2.4	2.9
2	−	−	+	180	1192	7.1	0.0	0.3	1.9	2.6
3	−	+	−	210	0	0.0	6.0	3.0	4.5	4.8
4	−	+	+	161	10	0.0	6.0	1.3	3.8	3.8
5	+	−	−	212	27	0.0	6.0	1.5	4.0	4.3
6	+	−	+	188	82	1.8	6.0	1.4	3.1	3.7
7	+	+	−	240	0	0.2	6.0	2.9	4.5	4.6
8	+	+	+	162	5	0.0	6.0	1.6	2.7	3.5

[a] Eight rats, one from each of the eight dietary groups, after having been kept for 24 days on experimental diets, were used in this experiment. Liver homogenate from each rat was prepared as described in Methods.

[b] The assay mixture for determination of rates of FIGLU breakdown contained in a final volume of 4 ml: 2.0 ml of liver homogenate (ca. 1.0 g of liver); 50 μmoles phosphate buffer, pH 7.0; 2.5 μmoles 2-mercaptoethanol and in rats 3–8, 6.0 μmoles FIGLU. Since the liver FIGLU content of rats 1 and 2 was already high (5.8 and 7.1 μmoles FIGLU per g of liver), no FIGLU was added to the assay mixtures of these two dietary groups. As described in the table, the assay contained 0.4 μmoles *dl*-H_4-folic acid or 0.8 μmoles *dl*-5,10-methenyl-H_4 folic acid. The procedure for FIGLU determination is described in the text.

TABLE IV. Effect of *dl*-H_4-Folic Acid and *dl*-5,10-Methenyl-H_4-Folic Acid at Various Concentrations on Breakdown of FIGLU in Liver Homogenates[a].

Rat No.	Dietary treatment		Days on Diet	Body Wt. (g)	Urinary FIGLU μmoles day/kg of body wt.	Liver[b] Folate per g		FIGLU in liver (μmoles/g)	Total FIGLU in liver homogenate[c] (μmoles/g)	Form of folate added to liver homogenate	μmoles FIGLU degraded per g liver in 1 hr				
											μmoles Folate added/g liver homogenate				
	B_{12}	Meth				μg	μmoles				0.0	0.16	0.80	4.0	16.0
1	−	−	63	336	393	7.4	.017	7.2	11.2	*dl*-H_4-folic acid	1.0	1.7	6.3	8.3	11.2
2	−	+	66	380	0	10.0	.025	0	10.0	*dl*-H_4-folic acid	6.1	6.5	9.4	9.9	10.0
3	−	+	76	352	0	24.0	.054	0	10.0	*dl*-5,10-methenyl-H_4-folic acid	3.0	4.9	6.7	9.4	10.0

[a] The liver homogenate was prepared as described in Methods. 4 ml of incubation mixture contained 2 ml of liver homogenate (1 g of liver) and *dl*-H_4-folic acid or *dl*-5,10-methenyl-H_4-folic acid at concentrations of 0.16, 0.8, 4.0, and 16.0 μmoles. To the incubation mixtures of the $-B_{12}$, +Meth dietary group (rat numbers 2 and 3) were added 10 μmoles FIGLU. 4.0 μmoles FIGLU was added to the homogenate of rat number 1 ($-B_{12}$, −Meth) since the livers of these animals usually contain considerable FIGLU.

[b] Total folic acid after conjugase treatment.

[c] Difference between FIGLU in the homogenate and that present in the original liver tissue represents FIGLU added to homogenate.

and 3) are considerably higher than that of the methionine-deficient rat (rat number 1). This is consistent with the low values of FIGLU in the urine and liver of these animals.

The effect of methionine and homocysteine *in vitro*, either singularly or in combination with *dl*-tetrahydrofolic acid, *dl*- 5- Methyltetrahydrofolic, acid or *dl*- 5,10- Methenyltetrahydrofolic acid on FIGLU breakdown in liver homogenates of two dietary groups, is reported in Table V.

The addition of methionine in the absence of added tetrahydrofolate produced a small decrease in FIGLU breakdown in the -B_{12}-Meth homogenate, which is in contrast to its *in vivo* effect in promoting FIGLU degradation when added to the diet. Homocysteine, in contrast to methionine, had a small effect in increasing FIGLU breakdown. *dl*-5-Methyltetrahydrofolic acid was less effective than *dl*-tetrahydrofolic acid in promoting FIGLU breakdown, which is in accord with the "methyl trap" theory. This indicates that its conversion to tetrahydrofolate is a rate-limiting reaction.

Discussion. The marked decrease in urinary FIGLU excretion and the increase of total folates in liver by supplementation of methionine to a vitamin B_{12}-deficient diet, which has been reported (7), agrees with results presented here. Consistent with these results, we find that FIGLU content of liver is decreased and the rate of FIGLU breakdown is increased in liver homogenates of rats whose diets have been supplemented with methionine. Since the addition of tetrahydrofolic acid to liver homogenate results in an increased rate of FIGLU metabolism in all dietary groups studied, this shows that the enzyme formimino-tetrahydrofolic-transferase is not limiting. However, maximum rate of FIGLU breakdown is attained only when tetrahydrofolic acid is added to the assay mixtures

TABLE V. Effect of Various Compounds on Breakdown of FIGLU in Liver Homogenates[a,b].

	Dietary treatment (μmoles of FIGLU per g of liver)					
	$-B_{12}-$Meth			$-B_{12}+$Meth		
Compounds added to homogenate	0-time	1 hr	ΔFIGLU	0-time	1 hr	ΔFIGLU
Control	14.5	13.7	0.8	10.0	7.1	2.9
L-Methionine	14.5	14.1	0.4	10.0	7.1	2.9
DL-Homocysteine	14.5	13.4	1.1	10.0	7.1	2.3
dl-H_4-folic acid	14.5	7.1	7.4	10.0	1.7	8.3
dl-H_4-folic acid + L-methionine	14.5	7.0	7.5	10.0	1.1	8.9
dl-H_4-folic acid + DL-homocysteine	14.5	7.6	6.9	10.0	1.6	8.4
dl-5-CH_3-H_4-folic acid	14.5	8.7	5.8	10.0	2.5	7.5
dl-5-CH_3-H_4-folic acid + L-methionine	14.5	9.2	5.3	10.0	3.6	6.4
dl-5-CH_3-H_4-folic acid + DL-homocysteine	14.5	8.1	6.4	10.0	2.6	7.4
dl-5,10-Methenyl-H_4-folic acid	14.5	6.2	8.3	10.0	2.3	7.7
dl-5,10-Methenyl-H_4-folic acid + L-methionine	14.5	6.5	8.0	10.0	2.4	7.6
dl-5,10-Methenyl-H_4-folic acid + DL-homocysteine	14.5	7.3	7.2	10.0	2.9	7.1
Initial FIGLU/g of liver: μmoles	6.5			0		
FIGLU added to homogenate (per g/liver): μmoles	8.0			10		

[a] The liver homogenate was prepared as described in Methods and homogenate of two livers from each dietary group was pooled. Four milliliters of incubation mixture contained 2 ml of pooled liver homogenate (1 g of liver), 10 μmoles L-methionine, 20 μmoles DL-homocysteine, 2 μmoles each of *dl*-H_4-folic acid, *dl*-5-CH_3-H_4-folic acid, and *dl*-5,10-methenyl-H_4-folic acid. In incubation mixtures of dietary group ($-B_{12}$, +Meth), 10 μmoles FIGLU was added but in those of group ($-B_{12}$, −Meth) only 8 μmoles FIGLU was added because this group already contained 6.5 μmoles FIGLU per 2 ml of pooled liver homogenate.

[b] A companion bar graph for Table V is shown in Fig. 1.

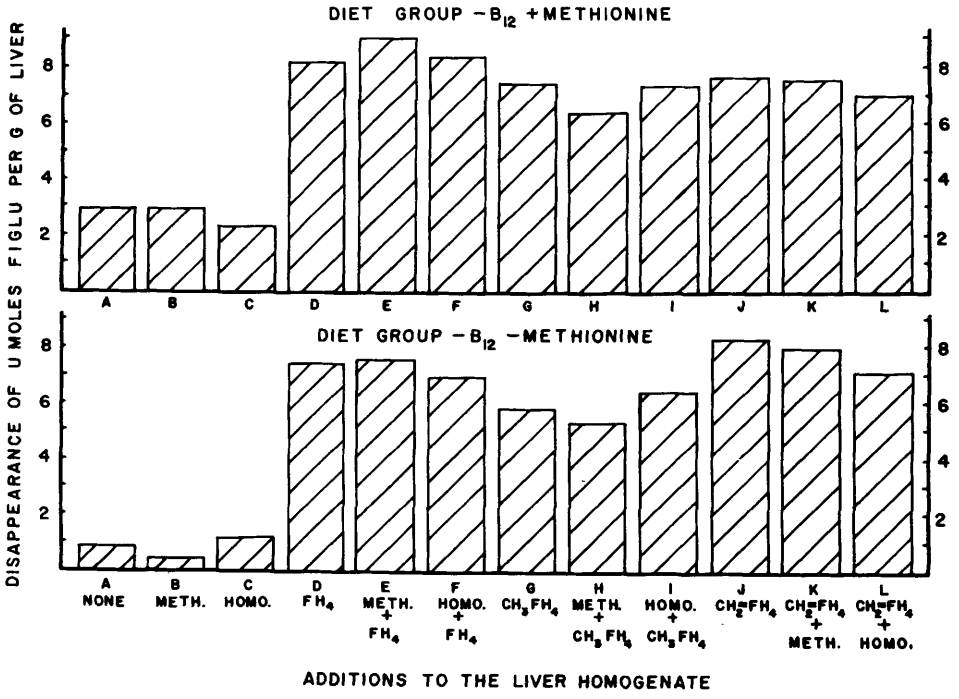

Fig. 1.

	A	B	C	D	E	F	G	H	I	J	K	L
Meth	–	+	–	–	+	–	–	+	–	–	+	–
Homo	–	–	+	–	–	+	–	–	+	–	–	+
FH_4	–	–	–	+	+	+	–	–	–	–	–	–
CH_3FH_4	–	–	–	–	–	–	+	+	+	–	–	–
$CH_2{=}FH_4$	–	–	–	–	–	–	–	–	–	+	+	+

in amounts stoichiometric with the FIGLU concentration. 5,10-Methenyltetrahydrofolic acid at the same concentration is equally effective in this respect, which indicates that conversion of 5,10-Methenyltetrahydrofolic acid to tetrahydrofolic acid is not the rate-limiting step. This shows that the rate of reaction of tetrayhdrofolate with FIGLU is the most rapid one and that the subsequent series of reactions involving breakdown of formiminotetrahydrofolate to regenerate tetrahydrofolate are rate limiting. It also appears that the folates originally present in liver are more active in facilitating the degradation of FIGLU than added tetrahydrofolate. Thus in Table III, 0.8 μmole of *dl*-tetrahydrofolate must be added to the homogenate of a -B_{12}-Meth animal (rat number 1) to give the same rate of FIGLU breakdown as produced by the B_{12}+Meth homogenate (rat number 3) which contained no added folate. The liver folate content of these animals is usually between 0.03 and 0.05 μmole/g. Similarly, in Table IV it was necessary to add 0.8 μmoles of *dl*- tetrahydrofolate to give the same rate of FIGLU breakdown in the -B_{12}-Meth homogenate as produced by the unsupplemented homogenate of the -B_{12}+Meth animal which contained only 0.025 μmole of liver folate. This apparent greater functional activity of the natural folate present in the methionine-fed animals may be due to their presence as polyglutamates which are present in larger proportion in methionine- or vitamin B_{12}-fed animals (6–9). However, a change in 50% in the relative proportion of polyglutamates does not seem adequate to explain the sixfold difference in the rate of FIGLU breakdown between the -B_{12}-Meth homogenate (1 μmole) FIGLU/g/hr) and the -B_{12}+Meth homogenate (6.1 μmole FIGLU/g/hr). The corresponding liver folate values were 0.017 and 0.025 μmoles/g, respectively. Although the livers of methionine-fed animals contain 50 – 100% more

folic acid, the activity of the methionine-fed animals in metabolising FIGLU is far greater than can be accounted for on the basis of their folate content. The folate in the methionine supplemented livers is either metabolically more active or else the methionine modifies the rate of some folate-dependent reactions. Kutzbach *et al.* 7) reported that *S*- adenosylmethionine inhibits the reduction of 5,10- Methylenetetrahydrofolate to 5- Methyltetrahydrofolate. Buehring *et al.* (9) in a study with perfused rat livers observed that the addition of methionine to the perfusate greatly reduced the proportion of 5-Methyltetrahydrofolate and increased the proportion of tetrahydrofolate. The addition of methionine also increased the breakdown of FIGLU. This evidence supports the view that methionine which can be converted to *S*- adenosylmethionine (SAM) functions in increasing the breakdown of FIGLU by inhibiting the formation of 5- methyltetrahydrofolate.

In view of the action of methionine added to the perfusate in facilitating FIGLU breakdown (9), it is interesting to note the ineffectiveness of methionine added to homogenates. This could be ascribed to a failure in the conversion of methionine to *S*-adenosylmethionine. However, the effect of either ATP plus methionine or *S*-adenosylmethionine on the breakdown of FIGLU in liver homogenate was no different than that of methionine alone. However, previous studies with liver homogenate showed that tetrahydrofolate was not converted to 5-methyltetrahydrofolate under the usual assay conditions.[6] This could explain the ineffectiveness of either methionine or SAM in increasing the rate of FIGLU breakdown, since it is known that the target enzyme of SAM is the 5,10-Methylenetetrahydrofolate reductase (EC 1.1.1.68) (7).

Summary. The effect of methionine and folic acid on metabolism of FIGLU was studied in liver homogenate of rats fed diets which were either deficient or supplemented with vitamin B_{12}, thyroid powder, and methionine. The increased levels of FIGLU in urine and liver caused by either vitamin B_{12} deficiency or by feeding thyroid powder and absence of FIGLU accumulation in urine and liver by administration of methionine in diet confirmed the previously reported results. The addition of methionine either singly or in combination with tetrahydrofolic acid or with 5,10- methenyltetrahydrofolic acid to the liver homogenate of vitamin B_{12}-deficient and/or thyroid-fed rats did not affect the rate of FIGLU breakdown in liver homogenate.

[6] Amanda Vidal (personal communication), Dept. of Nutritional Sciences, Univ. of California, Berkeley.

1. Brown, D. D., Silva, O. L., Gardiner, R. C., and Silverman, M., J. Biol. Chem. **235,** 2058 (1960).

2. Stokstad, E. L. R., Webb, R. E., and Shah, E., J. Nutrition **88,** 225 (1966).

3. Stokstad, E. L. R., Webb, R. E., and Shah, E., Proc. Soc. Exp. Biol. Med. **123,** 752 (1966).

4. Lewis, U. J., Tappan, D. V., Register, U. D., and Elvehjem, C. A., Proc. Soc. Exp. Biol. Med. **74,** 568 (1950).

5. Betheil, J. J., and Lardy, H. A., J. Nutrition **37,** 495 (1949).

6. Thenen, S. W., and Stokstad, E. L. R., J. Nutrition **103,** 363

7. Kutzbach, C., Galloway, E., and Stokstad, E. L. R., Proc. Soc. Exp. Biol. Med. **124,** 801 (1967).

8. Jeejeebhoy, K. N. Pathare, S. M., and Noronha, J. M., Blood **26,** 354 (1965).

9. Buehring, K. U., Batra, K. K., and Stokstad, E. L. R., Biochim. Biophys. Acta **279,** 498 (1972).

10. Williams, M. A., Chu, L. C., McIntosch, D. J., and Hincenbergs, I., J. Nutrition **94,** 377 (1968).

11. Tabor, H., and Wyngarden, L., J. Clin. Invest. **37,** 824 (1958).

12. Davis, L., Anal. Biochem. **26,** 459 (1968).

13. Rabinowitz, J. C., *in* "Methods in Enzymology," Vol. V, p. 814. Academic Press, New York (1962).

14. Chanarin, I., and Perry, J., Biochem. J. **105,** 633 (1967).

15. Bird, O. D., McGlohan, V. M., and Waitkus, J. W., Anal. Biochem. **12,** 18 (1965).

Received January 12, 1974. P.S.E.B.M., 1974, Vol. 147.