Some Observations on the Role of Folic Acid in Utilization of Homocystine by the Rat[1]

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Rats bred in this laboratory on our preexperimental diet, which has been described in detail (*1, 4*), have been shown to grow on a diet containing homocystine as the sole source of sulfur-containing amino acids, without known sources of labile methyl donors. The importance of the preexperimental diet for this phenomenon has been emphasized (*1*) and the possibility of stored factors involved in the utilization of homocystine suggested.

In subsequent studies, Sulfasuxidine was administered to rats under the same experimental conditions, on the assumption that the possible source of labile methyl donors could have originated from intestinal bacterial synthesis. This drug is known to be an inhibitor of intestinal synthesis of biotin and folic acid. The ability of the rats to utilize homocystine seemed to have been lost in conjunction with the destruction of the intestinal flora responsible for synthesis of folic acid, for growth could not be reestablished by dietary supplements of extra biotin and of folic acid concentrate or ryzamin-B (*2, 5*). This fact suggested that the bacteria synthesize a factor involved in the utilization of homocystine. However, the ability of the rats to utilize homocystine for growth could be reestablished with continued feeding of the sulfonamide by further supplementation of the diet with Liver Extract Lilly (LEL). These results could be interpreted as indicating that LEL furnishes to the rats factors made unavailable by the Sulfasuxidine. Since crystalline folic acid had become available, it seemed desirable to add it to the vitamin supplement to ensure adequate replacement of at least two vitamins involved, biotin and folic acid. The present report is concerned with the role of folic acid in the promotion of growth of rats on a labile methyl donor-free diet, containing homocystine as the sole sulfur amino acid, in the presence and absence of Sulfasuxidine.

The experimental procedure has been outlined in earlier publications (*3–5*). Table 1 gives a summary of the results obtained. The rats fed the Sulfasuxidine diet had received the basal diet approximately 50 days before the initial period reported in the table. Rats 34–37, littermates, show the best response to folic acid obtained under these conditions. The initial weight of the rats was 90.5, 80.5, 69.0, and 73.0 g, respectively; the average gain per day was 1.2, 0.9, 1.4, and 1.5 g. The growth response varies, however, in different individuals, and data for rat 62 are presented as an example of those animals that do not respond to folic acid even though growth is elicited in them by LEL. Still other rats, not reported here, showed intermediate degrees of growth.

The rats which did not receive the sulfa drug had been fed the basal diet approximately 34 days before the initial period reported in the table. When taken from the colony diet, at 36 days of age, the animals were depleted on the basal diet, without homocystine, for 14 days. Then on addition of homocystine they were allowed to regain their initial weight before the 26-day period reported; this took about 20 days. However, the rats received the extra biotin and folic acid from the beginning of the experimental period. The initial weight of the rats was 118.5, 119.0, 98.0, and 103.0 g, respectively; the average gain per day was 1.5, 1.7, 1.0, and 1.7 g.

TABLE 1

EFFECT OF FOLIC ACID ON THE GROWTH OF RATS FED A LABILE METHYL-FREE DIET CONTAINING HOMOCYSTINE WITH AND WITHOUT ADDITION OF SULFASUXIDINE

Rat Nos. ♀	Diet	Days on diet	Food intake per day in g	Initial weight in g	Total gain in g	Gain per day in g
	(With 2% Sulfasuxidine)					
	Basal*	12	2.9	78.3	−3.3	−0.3
34–37†	Basal + Ry‡ + Bi§	12	2.5	75.0	0.3	0.0
Avg (4)	Basal + Ry + Bi + Fo‖	28	4.2	75.3	34.9	1.3
	Basal + Ry + Bi	18	4.0	110.1	6.0	0.3
	Basal	12	1.9	63.0	−3.0	−0.3
	Basal + Ry + Bi	10	2.0	60.0	−2.5	0.3
62	Basal + Ry + Bi + Fo	16	2.1	57.5	0.5	0.0
	Basal + Ry + Bi + Fo + LEL¶	24	3.8	58.0	30.0	1.3
	Basal + Ry + Bi + Fo	8	4.0	88.0	1.0	0.1
	(Without Sulfasuxidine)					
88–91 Avg (4)	Basal + Bi + Fo	26	5.4	109.6	37.9	1.5

* The basal diet consisted of a 17% amino acid mixture (*5*) and 0.83% homocystine. When Sulfasuxidine was fed it was added to the basal diet, which was given *ad libitum*. Rats 88–91 were restricted to 6 g of the diet for the first 16 days and ate *ad libitum* for the next 10 days. The standard dose of B vitamins was 500 γ each of nicotinic acid, *p*-aminobenzoic acid, and inositol, 200 γ of calcium pantothenate, 40 γ each of thiamin hydrochloride, riboflavin, and pyridoxine hydrochloride, and 0.25 γ biotin (*5*). The ryzamin-B, extra biotin, folic acid, and LEL were added to the standard vitamin mixture and fed daily. DL-Homocystine (100 ± 0.5% by disulfide determination) was prepared in this laboratory by du Vigneaud's method modified by Brand (*6, 7*).

† Rats 34–37 were put back on the folic acid supplement in approximately 30 days and grew at practically the same rate for nearly 30 additional days, when the growth curves leveled off. When killed after 220 days on the synthetic diet, they weighed 181, 168, 166, and 153 g, respectively. On autopsy their livers did not appear fatty and weighed 7, 6, 8, and 6 g. Microscopic examination showed moderate fatty infiltration of the liver and some tubular degeneration of the kidneys.

‡ Ry signifies 62.5 mg of ryzamin-B unfortified (Wellcome Research Laboratories).

§ Bi signifies 2 γ of biotin. Rats 88–91 received 1 γ of biotin.

‖ Fo signifies 20 γ of folic acid (Folvite Lederle).

¶ LEL signifies 60.0 mg of Liver Extract Lilly.

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Under these conditions the rate of growth is quite consistent; the presence of folic acid seems to prevent the acute reversible drops in weight observed in previous experiments (*4*). Of 12 rats studied on this diet, only one rat, number 90, showed a slight drop in weight with food consumption dropping to zero for one day. This rat also showed an early leveling-off of her growth curve on the 15th day of the reported period; the growth of the other rats did not level off until after the 26-day period.

The above experiments indicate that rats do not always lose their ability to utilize homocystine, under the conditions prevailing in these experiments, even though their intestinal folic acid synthesis has been checked by the sulfa drug, since addition of large doses of crystalline folic acid enables some rats to continue growth. The fact that only some rats respond to folic acid indicates that it is not the only factor involved. These animals retain a latent capacity for the utilization of homocystine, which seems to depend for its manifestation on a fairly high level of folic acid and is not destroyed by the sulfa drug. The nature of the variability of the growth response becomes apparent when we realize that other vitamin B factors are probably involved, the most conspicuous one being B_{12}. These factors may be stored in the animals in varying amounts from preexperimental diets and may therefore be depleted in single animals at different periods of the experiment. Work is now under way to elucidate these problems.

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Separation of the Ionic Species of Lysine by Means of Partition Chromatography[1]

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It is a cardinal principle in chromatography that for each compound there is only one position on the chromatogram; this locus is unique in the sense that all of the substance is contained within that position, but not in the sense that entirely different substances may not also occupy that position. As a corollary, it is believed that the appearance of two or more loci of substance indicate the presence of two or more different compounds. It is the purpose here to cite exceptions to this corollary, viz., that the presence of more than one locus of concentration may still be identified with a single substance.

We have found that paper partition chromatography of lysine[3] in a saturated phenol > water system may result in a plurality of spots. If from a relatively large volume of aqueous solution of lysine, small aliquots are taken for partition chromatographs, the number and position of these spots will depend upon the pH of the aqueous solution of the lysine (see Fig. 1). The relative intensities will be in rough accord with the distribution of ionic species for the different pH's as calculated from ioniza-

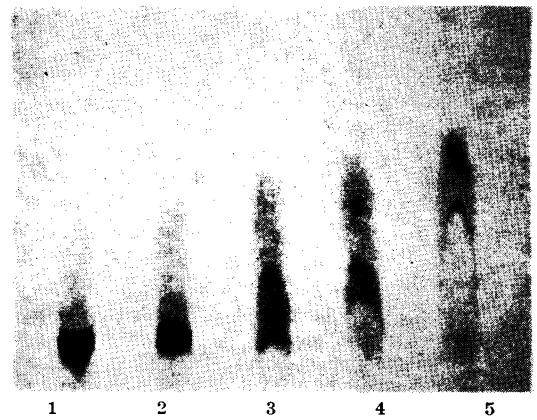

FIG. 1. Ninhydrin-developed spots of lysine aliquoted at various pH's: 1 at 2.20, 2 at 8.45, 3 at 9.50, 4 at 10.62, and 5 at 12.15.

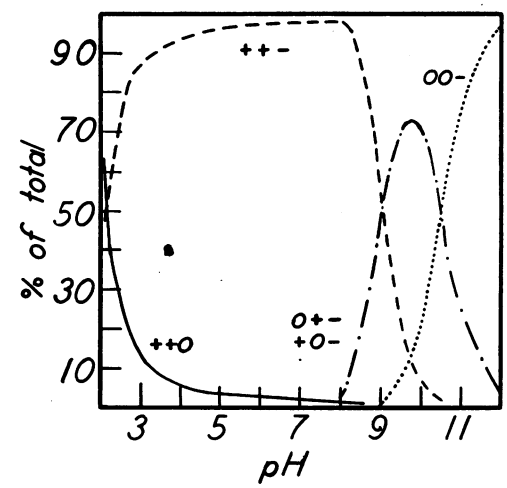

FIG. 2. The distribution of ionic species of lysine at various pH's.

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[3] The lysine was purified by two recrystallizations, once as the picrate and again as the dihydrochloride. This procedure was followed for three different sources of material, all of which gave the same results: synthetic DL-lysine (monohydrochloride), naturally-occurring L-lysine (monohydrochloride), and lysine regenerated from the hydrolysis of aminohomopiperidone, and precipitated as the dihydrochloride. Melting points were in agreement with values in the literature (*1*).