THE METABOLISM OF SERINE IN FOLIC ACID DEFICIENCY

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In a study of choline biosynthesis in the rat, Stekol *et al.* (1952, 1953) indicated that folic acid may be involved in the synthesis of both the ethanolamine and methyl moieties of choline from serine. In earlier work from this laboratory (Fatterpaker, Marfatia and Sreenivasan, 1955), it was observed that the influence of folic acid on choline synthesis may be limited only to the extent to which it serves to provide the ethanolamine moiety of choline. Martin and Beiler (1947) had observed that 3-4-dihydroxy-phenylalanine (DOPA) decarboxylase was reversibly inhibited by folic acid displacing agents such as 7-methyl-folic acid; desoxypyridoxine which is a potent inhibitor of tyrosine decarboxylase was ineffective. It was reported earlier (Nadkarni and Sreenivasan, 1957) that pyridoxine deficiency had no apparent influence on serine decarboxylation. It is now shown that folic acid is involved in serine decarboxylation.

The role of folic acid in glycine-serine interconversion has recently been reported by various workers in the micro-organisms (Holland and Mienke, 1949; Lascelles, Cross and Woods, 1954) and in animals (Totter *et al.*, 1950; Plaut *et al.*, 1950; Elwyn and Sprinson, 1950; Blakley, 1954; Kisliuk and Sakami, 1955; Alexander and Greenberg, 1955; Arnstein, 1955). The work reported here on glycine-serine conversion is discussed in the light of the present available information. Serine deamination was also followed in relation to dietary folic acid.

Experimental

Weanling rats (Wistar) were kept on a purified diet of the following composition (percentages): Alcohol extracted casein 18; starch 60; sucrose (vitaminised) 10; salt mixture (U.S.P. IV) 4; sesame oil 6; shark liver oil 2; and succinyl sulphathiozole 2; vitamin supplements added to the diet (mg. per kg.) were: Thiamine HCl 6; pyridoxine HCl 6; riboflavin 10; calcium pantothenate 20; *p*-aminobenzoic acid 100; nicotinic acid 30; biotin 1·0; vitamin B_{12} 0·5; α-tocopherol 50; vitamin K 10; inositol 500; and choline chloride 500.

One group of animals received the same diet but with 5 mg./kg. diet folic acid added. Folic acid deficiency was inferred from depressed leucocyte count observable after 6–8 weeks. There was also weight loss and loss of hair.

The animals from the deficient and folic acid-fed groups were killed at the end of a 8-week-period by decapitation. Livers were quickly removed, chilled and homogenised in cold distilled water by means of a Potter-Elvehjem homogeniser to a 20% suspension which was used for enzyme studies: Serine decarboxylation, glycine to serine synthesis, serine deamination by methods already outlined (Nadkarni and Sreenivasan, 1957).

Liver folic acid.—Folic acid in autolysed samples was determined microbiologically with *S. fæcalis* R. (Teply and Elvehjem, 1945) using the assay medium of Mitbander and Sreenivasan (1955).

Liver choline.—Total choline in liver and blood and free choline in liver were determined by the method of Appleton *et al.* (1953).

All results are averages of determinations for not less than four different samples.

Results

The effects of folic acid deficiency on leucocyte count and liver levels of folic acid and nitrogen are given in Table I.

TABLE I

Folic acid deficiency on lecucocyte count and liver levels of folic acid and nitrogen

Group	Liver weight per 100 g. body weight	Leucocyte count	Folic acid in liver μg./g. fresh weight	Liver nitrogen	
				Fresh weight per cent.	Dry weight per cent.
− PGA	3·80±0·15	4020±520	2·87±0·03	3·45±0·30	11·01±1·30
+ PGA	3·56±0·20	10230±320	5·75±0·04	3·71±0·10	12·84±0·40

In preliminary experiments it was observed that *in vitro* addition of aminopterin inhibited serine decarboxylation. Thus the activity (μl. CO_2/ mg. N/30 minutes) in presence of 200 μg. of aminopterin was 1·60 as compared to 3·95 without added aminopterin. To ascertain if aminopterin inhibition is observable *in vivo*, rats (120 g. wt.) were given 25 μg. per day aminopterin intraperitoneally for 6 days. They were then sacrificed. The

results obtained for serine decarboxylase activity (μl. CO_2/mg. N/30 minutes) were $1 \cdot 89 \pm 0 \cdot 08$ as compared to $3 \cdot 61 \pm 0 \cdot 03$ for the control animals.

Dietary deficiency of folic acid also depressed serine decarboxylase activity (Table II). Glycine to serine conversion was also reduced in the deficient animals. Serine deamination was unaffected.

TABLE II

Folic acid deficiency and serine decarboxylation, glycine to serine synthesis and serine deamination

Group	Serine decarboxylation QCO_2 μl. CO_2/mg. N/ 30 minutes	Glycine to serine conversion μ moles of serine per mg. N	Serine deamination μ moles of NH_3 per mg. N
− PGA	$2 \cdot 52 \pm 0 \cdot 10$	$0 \cdot 72 \pm 0 \cdot 14$	$3 \cdot 67 \pm 0 \cdot 10$
+ PGA	$3 \cdot 68 \pm 0 \cdot 05$	$1 \cdot 36 \pm 0 \cdot 12$	$3 \cdot 89 \pm 0 \cdot 02$

The effects *in vitro* and *in vivo* of folic acid on serine decarboxylation and on glycine to serine conversion are shown in Table III. Folic acid-deficient rats were used in all these experiments. Folic acid was given (200 μg. per rat) intraperitoneally 24 hours prior to sacrifice. *In vitro* folic acid was added to the system in a final concentration of 60 μg. per ml.

TABLE III

Folic acid and serine decarboxylation and glycine to serine conversion

System	*In vitro*		*In vivo* 200 μg. PGA/rat
	No addition	60 μg./ml. PGA	
	μl. CO_2 per mg. N per 30 minutes		
Serine decarboxylation	2·74	3·68	3·94
	μ moles of serine per mg. N		
Glycine to serine synthesis	0·68	0·71	1·14

Folic acid administration *in vivo* nearly restores the impairment in the activities of the two systems in folic acid deficiency. *In vitro* effect is observable to some extent in serine decarboxylation.

Choline contents of blood and liver are shown in Table IV. It may be seen that in folic acid deficiency the levels for free and total choline of liver as well as for blood choline are less. An influence of the vitamin on phospholipid levels of liver was reported by Fatterpaker, Marfatia and Sreenivasan (1955 *e*).

TABLE IV

Choline levels of blood and liver

Group	Liver choline		Blood choline (Total)
	Free	Total	
	mg. per g. fresh weight		mg. per ml.
− PGA	0·183±0·007	5·34±0·04	0·624±0·021
+ PGA	0·243±0·003	6·10±0·02	0·816±0·030

DISCUSSION

Earlier work from this laboratory (Fatterpaker, Marfatia and Sreenivasan, 1955) had suggested that the influence of folic acid on methyl metabolism may be confined only to the formation of the ethanolamine moiety of choline. The present observations have shown more directly that folic acid aids in the conversion of serine to ethanolamine.

Stetten (1941) and Arnstein (1951) had shown that glycine serves as a precursor of the ethanolamine moiety of choline. Glycine is first converted to serine which is then decarboxylated to ethanolamine. The involvement of folic acid in the conversion of glycine to serine has been variously reported (*vide infra*) and is confirmed by the present studies. However, the vitamin is also concerned in the further metabolism of serine to ethanolamine.

The function of folic acid in the biosynthesis of ethanolamine is further borne out by the data on choline levels of liver and blood. The somewhat more marked lowering of free choline as compared to the bound choline would suggest a more direct influence of the vitamin on newly formed choline.

SUMMARY

Aminopterin inhibits serine decarboxylation in rat liver homogenates.

Dietary deficiency of folic acid in rats also reduces serine decarboxylase activity.

Folic acid does not influence serine deamination, but glycine to serine synthesis is impaired in a dietary deficiency of the vitamin.

In folic acid deficiency liver and blood levels of choline are also lowered.

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