# BIOSYNTHESIS OF SERINE FROM GLYCINE

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The labile methyl groups of choline and methionine could go into formate (Sakami, 1949; Siekewitz and Greenberg, 1950). Besides these, a large number of other compounds are known to be precursors of formate and therefore of the β-carbon of serine (Weinhouse and Friedmann, 1952). Although the nature of the 1-C fragment active in glycine-serine conversion is still uncertain, formate and formaldehyde are both apparently closely related to it. It has been suggested that folic acid may serve as the cofactor for 1-C compound transfer reactions; some formyl derivatives of folic acid are more active in this respect. Buchanan and Schulman (1953) found that *Leuconostoc citrovorum* factor (CF) was acting in a specific manner in the incorporation of formate into position 2 of purines and postulated that CF could act in the form of a coenzyme for transmethylations in a manner similar to coenzyme A in acetylations. Lascelles, Cross and Woods (1954) showed that CF had no coenzyme function but is converted to an active form. Rauen and Jaenicke (1951) showed that $N^{10}$-formyl-tetrahydrofolic acid could take part in transformylations more directly in mass action fashion. CF is known to be interconvertible with $N^{10}$-formyl THFA (Silverman and Gardiner, 1956). In fact, Greenberg (1954) postulated the existence of an intermediate similar to anhydrocitrovorum factor (Cosulich *et al.*, 1952) in which $N^5$ and $N^{10}$ are linked with a 1-C bridge (Fig. 1). The active form of folic acid concerned in glycine-serine interconversion was postulated to be tetrahydrofolic acid (Blakley, 1954) which acts as a carrier of the hydroxymethyl group (Kisliuk and Sakami, 1955; Alexander and Greenberg, 1955). The enzyme system for PGA to CF conversion (Welsch and Nichol, 1950; Nichol, 1953; Doctor *et al.*, 1953, 1954; Mitbander and Sreenivasan, 1954) may have a function in the involvement of folic acid derivatives in glycine-serine interconversion. Thus, the β-carbon of serine is known to be incorporated into CF (Doctor and Awapara, 1956) and in a reverse manner it is possible for the formyl group of CF to be incorporated into serine, through formation of hydroxymethyl THFA (Kisliuk and Sakami, 1955; Alexander and Greenberg, 1955).

Studies on glycine-serine conversion and the effects of deficiencies of pyridoxine and folic acid were reported (Nadkarni and Sreenivasan, 1957 *a*,

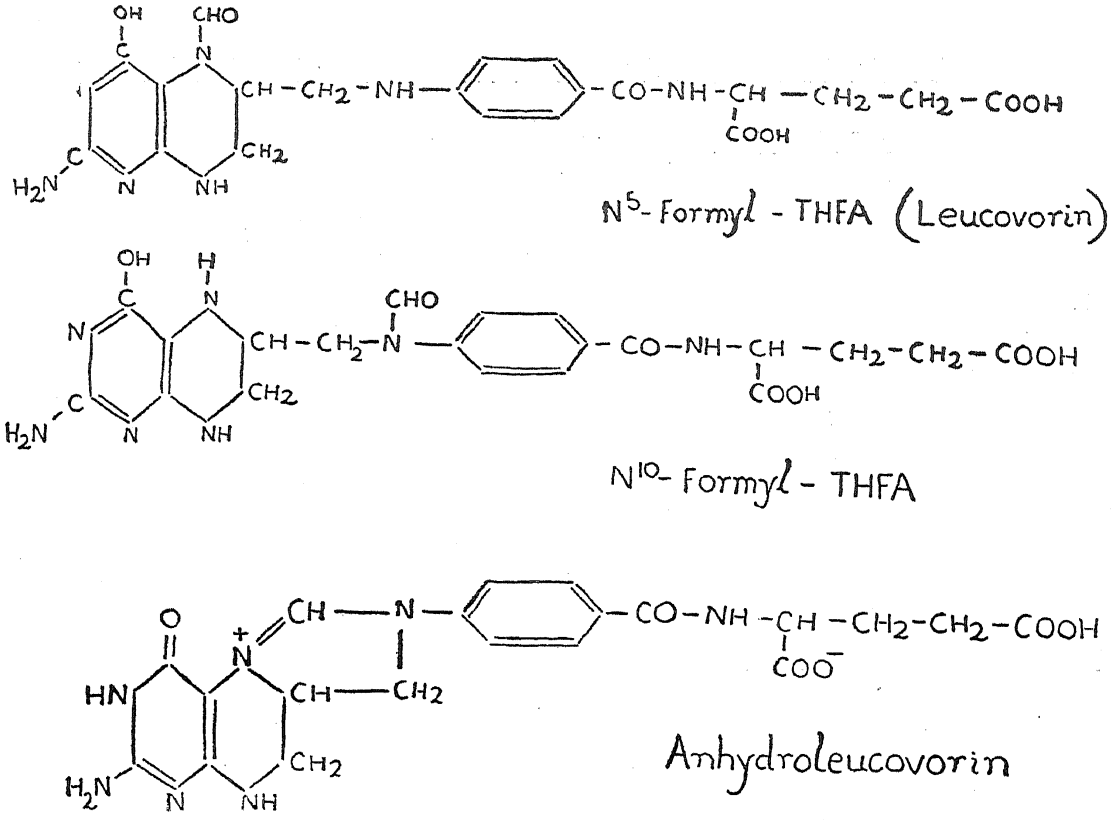


FIG. 1. Functional forms of Folic Acid.

1957 *b*). Additional observations on this system with particular reference to effects of (i) various formate precursors, (ii) leucovorin, and (iii) niacin are now reported.

Although formate was used as the source of the β-carbon atom in bio-synthesised serine it was of interest to study the effectiveness of formate precursors such as glycine, sarcosine, methionine, etc. Again, since the hydroxymethyl group of $CH_2OH$-THFA apparently acts as a shunt in the acceptance of the active 1-C fragment and in its donation to glycine for serine synthesis, some experiments were carried out using leucovorin ($N^5$-CHO-THFA) to provide the β-carbon of serine.

The postulated involvement of DPN in one or both the steps FA↔THFA (Kisliuk and Sakami, 1955) and $CH_2OH$-THFA to $N^5$-CHO-THFA (Alexander and Greenberg, 1955) would imply an effect of nicotinamide in glycine-serine conversion which has now been studied.

## EXPERIMENTAL AND RESULTS

Young adult rats (Wistar) weighing 150–200 gm. reared on a laboratory stock diet were sacrificed by decapitation and the liver quickly removed,

chilled and homogenised to a 20 per cent. suspension in ice-cold distilled water using Potter-Elvehjem all-glass homogeniser. Glycine to serine conversion was followed up as reported earlier (Nadkarni and Sreenivasan, 1957 *a*): 1·0 ml. of the liver homogenate was incubated with 0·5 ml. of 0·2 M glycine, 0·5 ml. of 0·2 M sodium formate, 0·4 ml. of 0·05 M sodium citrate, 0·1 ml. of 0·05 M sodium fumarate and 0·1 M phosphate buffer pH 7·0 in a total volume of 4 ml. at 37° C.; control substituting water for glycine was run alongside. At the end of the incubation period the mixture was deproteinised by heating on a boiling water-bath for 10 minutes. An aliquot of the supernatant after centrifugation was used for the estimation of serine by microbiological assay with *Lactobacillus casei* (Alexander *et al.*, 1953). The difference in serine values with and without added glycine represented net synthesis of serine.

It was observed that serine synthesis from glycine and formate was better aerobically than under anaerobic conditions. The experiments of this conversion were carried out in Warburg flasks, one set of flasks having air as gas phase, while in the other set nitrogen replaced air. The serine synthesised at the end of five hours incubation was ($\mu$ moles/mg. N) 1·068 and 0·398 respectively. Siekewitz and Greenberg (1949) had shown that $C^{14}$ formate was fixed into serine at a rapid rate in air.

*Effect of formate precursors.*—The effects of substituting for formate certain compounds known to be formate precursors were tried (Table I). These were added in concentrations equimolar to that of sodium formate.

Large differences between the various additions probably reflect differences in rates at which the 1-C fragment is donated by them for the glycine-serine conversion. Sarcosine aids serine formation maximally. Mackenzie (1950) had shown that the methyl group of sarcosine was derived exclusively from the $\beta$-carbon of serine and that the carboxyl and the $\alpha$-carbon of serine from their counterparts in the glycine moiety of sarcosine. Such an oxidative rearrangement could occur extensively in the presence of air. The high values for biosynthesised serine even without addition of glycine when sarcosine was used probably represent such a rearrangement. The increase in serine synthesis with added glycine may indicate the ability of sarcosine to donate its methyl group as formate to glycine in the net synthesis of serine. Sarcosine methyl could go into urinary formate (Mackenzie, 1955) and is twice as effective in this regard as its molar level of methanol. The methyl group of methionine is utilized only to a limited extent (Siekewitz and Greenberg, 1950; Alexander and Greenberg, 1955). Glycine itself appears to be least effective in the production of 1-C fragment for serine synthesis.

TABLE I

*Effect of 1-C precursors on glycine to serine conversion*

| 1-C precursor | Without glycine | With glycine added | Net synthesis of serine from glycine |
|---|---|---|---|
| | $\mu$ moles of serine/mg. N | | |
| Sodium formate .. | 0·63 | 1·65 | 1·02 |
| 1-Methionine .. | 0·57 | 1·14 | 0·57 |
| Methanol .. .. | 0·35 | 0·88 | 0·53 |
| Glycine .. .. | 0·51 | 1·01 | 0·50 |
| 1-Histidine .. .. | 0·59 | 1·37 | 0·78 |
| Betaine .. .. | 0·63 | 1·33 | 0·70 |
| Sarcosine .. .. | 1·08 | 2·22 | 1·14 |

The system contained 66·6 $\mu$ moles of glycine, 66·6 $\mu$ moles of the 1-C precursor, 0·06 $\mu$ moles of sodium citrate, 0·002 $\mu$ moles of sodium fumarate, 1·0 ml. of rat liver homogenate (equivalent to 6 mg. N) and 0·1 M phosphate buffer pH 7·0 in total volume of 4·0 ml. and was incubated for 5 hours. The reaction was stopped by heating on a boiling water-bath for 10 minutes. Serine formed was estimated by microbiological assay with *L. casei* (Alexander *et al.*, 1953).

In the other experiments reported below, sodium formate was used as the source of the 1-C fragment.

The optimum pH for conversion of glycine to serine was 7·0 (Table II).

TABLE II

*Effect of pH on glycine to serine conversion*

| pH M (0·1 phosphate buffer) | $\mu$ moles of serine/mg. N |
|---|---|
| 5·0 | 0·66 |
| 6·0 | 0·79 |
| 7·0 | 1·06 |
| 7·4 | 0·95 |
| 8·0 | 0·69 |

Phosphate buffer was more effective than the other buffers studied at this pH (Table III).

TABLE III

*Effect of buffers on glycine-serine conversion*

| Buffer pH 7·0 (0·1 M) | $\mu$ moles of serine/mg. N |
|---|---|
| Phosphate .. .. | 1·12 |
| THAM .. .. | 0·67 |
| Bicarbonate .. .. | 0·45 |

*Effect of leucovorin.*—The effect of folic acid deficiency on glycine to serine conversion was reported (Nadkarni and Sreenivasan, 1957 *b*). In several experiments, it was observed that addition of folic acid (PGA) *in vitro* had little effect in restoring activity of the system in preparation from folic acid-deficient animals indicating that under conditions of these experiments PGA was not converted to the active form.

If citrovorum factor is a participant in glycine-serine conversion *via* hydroxymethyl-THFA, the transfer of this hydroxymethyl group to the $\beta$-carbon of serine under conditions when $CH_2OH$-THFA is not reformed, would leave the dehydroxymethylated form which is only 2·5 per cent. as active as leucovorin (Broquist *et al.*, 1951) for *L. citrovorum*. In the absence of any neogenesis of 1-C unit, therefore, a stoichiometric relationship may be expected to hold between CF disappearance and serine formation. Such a relationship has been shown in the case of $N^{10}$-formyl-THFA (Rauen and Jaenicke, 1951). In the present experiments the glycine-serine system used was studied without any added formate with leucovorin added as the 1-C donor. The system was studied anaerobically to prevent oxidative degradation of leucovorin. At the end of 5 hours incubation at 37° C., the reaction was stopped and serine determined microbiologically with *L. casei* (Alexander *et al.*, 1953). Unreacted leucovorin in the mixture was determined with *L. citrovorum* (Sauberlich and Baumann, 1948) (Table IV).

It may be seen that in the absence of glycine, addition of leucovorin had little effect on serine synthesis. However, when glycine is added, endogenous serine synthesis without added leucovorin is more, the values increasing with added leucovorin. While no stoichiometric relationship is observable

TABLE IV

*Effect of addition* in vitro *of leucovorin*

| | Without glycine | | With glycine | |
|---|---|---|---|---|
| | Without leucovorin | With leucovorin | Without leucovorin | With leucovorin |
| Serine formed ($\mu$ moles) | 3·04 | 3·12 | 5·89 | 7·56 |
| Residual leucovorin ($\mu$ moles) | * | 0·210 | * | 0·169 |

The system contained: 66·6 $\mu$ moles of glycine; 0·06 $\mu$ moles of sodium citrate, 0·002 $\mu$ moles sodium fumarate; 0·217 $\mu$ moles leucovorin, 1·0 ml. liver homogenate (equivalent to 6 mg. N) and 0·1 M phosphate buffer pH 7·0. Total volume 4·0 ml. The flasks were flushed with $N_2$ gas and incubated for 5 hours.

* Endogenous leucovorin was only 0·001 $\mu$ moles.

between net serine synthesis and disappearance of leucovorin, the results sufficiently demonstrate the participation of leucovorin in the system.

*Effect of niacin deficiency.*—Weanilng rats were fed a diet containing (percentages): Casein 10, sucrose 45, starch 40, salt mixture 3, sesame oil 2. The vitamin supplements were (mg./kg. diet): thiamine HCl 6, ribcflavin 10, pyridoxine 6, calcium pantothenate 30, biotin 1·0, folic acid 5, vitamin $B_{12}$ 0·5, vitamin K 10, $\alpha$-tocopherol 10, choline chloride 500, inositol 500 and vitamin A 1500 I.U. (in sesame oil). This nicotinic acid-deficient diet was similar to that of Krehl *et al.* (1946). Control rats were given the same diet supplemented with 10 mg./kg. diet of nicotinic acid. Deficiency symptoms appeared in six weeks when the rats from the deficient and contro-groups were sacrificed for study of activities of the system in the liver for glycine to serine conversion. Details of procedure were as outlined earlier (Table V).

Glycine to serine conversion was reduced by about 40 per cent. as a result of niacin deficiency. *In vitro* addition of niacin or of DPN to the system had little effect in restoring the activity.

The *in vitro* conversion of PGA to CF by liver homogenates of niacin-deficient and control normal animals was also studied. The system contained in a final volume of 5·0 ml., 200 $\mu$g. PGA, 10 mg. serine, 20 mg. ascoıbic acid, 3·0 ml. of 20 per cent. liver homogenate and phosphate buffer

TABLE V

*Effect of niacin deficiency on glycine to serine conversion*

| | Expt. 1 | Expt. 2 | Mean |
|---|---|---|---|
| | $\mu$ moles of serine/mg. N | | |
| Normal (Control) .. .. .. .. | 1·12 | 0·83 | 0·97 |
| Niacin deficient .. .. .. .. | 0·63 | 0·55 | 0·59 |
| Niacin deficient + DPN *in vitro* (0·1 $\mu$ moles) .. | 0·60 | 0·56 | 0·58 |
| Niacin deficient + *in vitro* niacine (200 $\mu$g.) .. | 0·60 | 0·55 | 0·57 |

pH 6·4 (0·03 M). The reaction was carried under nitrogen for 2 hours at 37°, stopped by steaming and CF formed estimated with *L. citrovorum* (Sauberlich and Baumann, 1948). It was observed that in niacin deficiency PGA to CF conversion was reduced by about 50 per cent. CF formed ($\mu$g./gm. fresh weight of liver) was $1·275 \pm 0·285$ in niacin-deficient rats as compared $2·700 \pm 0·140$ in niacin-fed animals (J. M. Noronha, this laboratory).

## DISCUSSION

Formate precursors vary in their effectiveness in contributing to the biosynthesis of serine from glycine. These results are in agreement with various earlier observations on the relative rates of formation of formate or of the labile methyl group from such precursors (Sreenivasan, 1955).

The stimulation in glycine to serine system by CF is also demonstrated. There is, however, no stoichiometric relationship between the serine formed and leucovorin used up. The fact that the net synthesis of serine is far more than the disappearance of leucovorin rules out the possibility that leucovorin exclusively contributes to the $\beta$-carbon of serine; alternately other mechanisms may be operative under the conditions of these experiments for regeneration of CF-active compounds.

The lowered ability of the rat liver enzymes to effect glycine to serine conversion *in vitro* as a result of niacin deficiency lends support to the view that DPN-associated enzymes are involved in the reduction of folic acid to its enzymically active forms (Nichol, 1954; Kisliuk and Sakami, 1955; Alexander and Greenberg, 1955).

## SUMMARY

(1) Various formate precursors studied, aid in the conversion of glycine to serine *in vitro* by rat liver enzymes to different degrees.

(2) The participation of CF in the system has been demonstrated from *in vitro* studies on glycine conversion to serine anaerobically and in the absence of added formate or its precursors.

(3) In a dietary deficiency of niacin, there is impairment in the activity of the glycine to serine synthesizing enzyme.

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