STUDIES ON THE ACCUMULATION OF 4-AMINO-5-IMIDAZOLE CARBOXAMIDE IN ESCHERICHIA COLI

H. R. ALIMCHANDANI AND A. SREENIVASAN
Department of Chemical Technology, University of Bombay, Bombay, India

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When cultures of Escherichia coli are partially inhibited by sulfonamides they accumulate a diazotizable and nonacetylable amine in the medium (Fox, 1942). The amine was isolated by Stetten and Fox (1945) and characterized by Shive et al. (1947) as 4-amino-5-imidazole carboxamide. The latter authors suggested that the amine was a precursor of purines as it only required the addition of a one carbon fragment for completion of the purine skeleton. Its transformation to purines is mediated by p-aminobenzoic acid (PABA) and sulfonamides interfere in this conversion.

The nature of this amine for purine synthesis in E. coli has since been amply confirmed. Some purine requiring mutants of this organism accumulate it (Gots, 1950). Ben-Ishai et al. (1951) have reported that certain other purine auxotrophs of E. coli can utilize the amine for growth, though higher concentrations of it are needed.

Woolley and Pringle (1950) observed that the amine accumulated during growth inhibition of E. coli by aminopterin, suggesting that PABA acted through folic acid (FA) in the conversion of the amine to purine. The implication of FA in purine synthesis has been suggested by other lines of investigation. Purines have been shown to be active in decreasing or replacing the FA requirement for growth (Mitchell and Snell, 1941; Snell and Mitchell, 1941; Stokstad, 1941; Stokes, 1944; Krueger and Peterson, 1945) and in overcoming growth inhibition of a variety of microorganisms caused by FA analogue antagonists (Rogers and Shive, 1948).

Vitamin B12 has also been implicated in purine biosynthesis. Shive (1950) has shown that in the presence of vitamin B12 high concentrations of sulfanilamide are required to inhibit the synthesis of purines. Purine auxotrophs of E. coli utilize the amine for growth better in the presence of vitamin B12 (Ben-Ishai et al., 1951).

The involvement of vitamin B12 and FA in purine synthesis discussed above suggested a study of the effects of pteroylglutamic acid (PGA), leucovorin (5-formyl, 5-6-7-8-tetrahydrofolic acid; LV) and vitamin B12 on amine accumulation under cultural conditions of restricted growth in E. coli.

Methionine has also been included in the experiments for a study of its effects in view of the replaceability (Davis and Mingioli, 1950) of methionine and B12 for growth of certain induced mutants of E. coli.

METHODS

Escherichia coli (MacLeod strain) and the mutant strain of E. coli requiring vitamin B12 (NCIB 8134) were both maintained by fortnightly transfer in peptone yeast extract agar slants (Alimchandani and Sreenivasan, 1957a, 1957b).

The basal medium employed for the studies reported here was that of Green and Sevag (1946) supplemented with glycine which is known to stimulate the production of the amine (Ravel et al., 1948). In case of the mutant, vitamin B12 or methionine were used additionally as supplements. The pH of the medium was adjusted to 7.

An aqueous solution of 10 mg per cent of sulfadiazine (SD) was prepared and pH adjusted to 7.

To 5 ml of double strength basal medium in test tubes the other additions were made and the volume adjusted to 10 ml. The tubes were capped and sterilized for 15 min at 121 C. A 40-fold dilution of a 24 hr growth in the basal medium was used to inoculate the tubes dropwise. Growth was measured in a Klett-Summerson photoelectric colorimeter at 660 μμ. The amine was estimated by the method of Bratton and Marshall (1939) after acetylation of interfering amines with acetic anhydride (Stetten and Fox, 1945). The concentrations of the amine were expressed in terms of galvanometer readings in the Klett-Summerson photoelectric colorimeter at 540 μμ.

RESULTS

The effects of PGA, LV, vitamin B12 and methionine on arylamine accumulation by E. coli
in the presence of varying concentrations of SD are reported in Table 1.

It may be seen that LV or PGA do not reverse growth inhibition by SD nor have they any effect on amine accumulation. Both vitamin B₁₂ and methionine influence growth inhibition as well as amine formation in SD bacteriostasis. Reversal of growth inhibition is more pronounced with vitamin B₁₂ than with methionine. The reverse is the case with amine formation. This latter is also brought about when amine accumulation per unit growth is plotted against SD concentration (figure 1). When amine accumulation per unit growth is plotted against growth (figure 2) it is found to be more with methionine than with vitamin B₁₂. It should be borne in mind that to get a certain inhibition varying concentrations of SD are required in the presence of the different metabolites.

The above experiments indicated an association of vitamin B₁₂ and methionine in the transformation of the amine to purine. To elucidate this, experiments were undertaken using the E. coli mutant requiring B₁₂ or methionine, as well as their antagonists with the wild strain.

A PABA mutant grown in suboptimal amounts of vitamin B₁₂ has been reported (Gots and Chu, 1952) to accumulate the amine. If vitamin B₁₂ or methionine were involved in a common pathway directly then the amine should be expected to accumulate when the B₁₂ auxotroph is grown in suboptimal concentrations of B₁₂ or methionine. However, with the B₁₂ auxotroph of E. coli it was observed that there was no amine accumulation whatsoever.

It was also ascertained that cell suspensions of the mutant (0.2 mg dry wt/ml) when incubated in 5 ml of the basal medium for 12 hr without any addition of vitamin B₁₂ or methionine did not accumulate any amine.

Ethionine was studied as an antagonist of methionine (Jensen et al., 1951; Levine and Tarver, 1951; Levine and Fopeano, 1953) and a B₁₂ oxidation product as an antagonist of B₁₂ (Beiler et al., 1951; Rege and Sreenivasan, 1954). In the latter case it was ensured that, contrary to the report of Hendlin and Wall (1954), the oxidation product exerted an inhibition independently of any sodium chloride concentration (Alimchandani

![Figure 1](image_url)

**Figure 1.** Influence of vitamin B₁₂ (20 µg/10 ml) and methionine (0.5 mg/10 ml) upon arylamine accumulation per unit growth of *Escherichia coli* (MacLeod strain) in the presence of increasing levels of sulfadiazine.
and Sreenivasan, 1957a, 1957b). Using E. coli it was observed that both these inhibited growth though no amine accumulated.

DISCUSSION

While increasing concentrations of SD result in the expected increase in inhibition of growth of E. coli, amine accumulation in general bears no relation to growth. Further, PGA or LV has no effect on either growth inhibition or amine accumulation in SD bacteriostasis. Both vitamin B₁₂ and methionine in the concentrations used overcome to a considerable extent growth inhibition by SD; vitamin B₁₂ is more effective in this respect than methionine. While there seems no relationship between growth and arylamine formation, the latter is less in the presence of vitamin B₁₂ than with methionine. This superiority of vitamin B₁₂ over methionine is also seen when amine formation per unit growth is plotted against SD concentration. The nearly linear relationship obtained in all cases between SD concentration and amine accumulation per unit cell mass suggests that SD blocks the conversion of the amine to purine in proportion to its concentration. Purine formation from the amine would itself appear to be favored more by vitamin B₁₂ than by methionine. Bergmann et al. (1952) had also observed a similar effect of vitamin B₁₂. However, they could not get depression of amine formation with methionine unless catalytic amounts of PABA were also present. These authors explained that methionine served as “methyl” donor. Methionine activity could also be explained on the basis that it spares the PABA required for its synthesis. The effect of vitamin B₁₂ may be explained as due to its potentiating action on PABA-associated enzymes (Shive, 1950).

In view of the fact that the concentration of SD required to bring about the same degree of inhibition varies with methionine and vitamin B₁₂ it was of interest to compare amine accumulation per unit growth with growth itself. When this is done (figure 2) it is seen that amine accumulation is least in the control set and is increased by methionine and to a less extent by vitamin B₁₂. It would seem that in the absence of methionine or vitamin B₁₂ the growth inhibition is primarily due to obstruction in methionine synthesis while in the presence of methionine the inhibition is solely due to a block in the conversion of the amine to purine. As in the latter case, purine synthesis is the main reaction blocked, amine accumulation should be higher for the same degree of growth inhibition (figure 2).

In presence of vitamin B₁₂, the amine accumulation per unit growth is increased but not to the same extent as with methionine. This may be because vitamin B₁₂ potentiates methionine synthesis to a greater extent than that of purines.

From the foregoing, it would seem that vitamin B₁₂ and methionine are not involved directly in the metabolism of the amine. This view is further borne out by the results obtained with the B₁₂ auxotroph of E. coli and with the methionine and B₁₂ antagonists using the wild strain. There is a possibility that in these cases reaction(s) other than purine formation is the limiting one.

SUMMARY

The effects of pteroylglutamic acid (PGA), leucovorin (LV), vitamin B₁₂ and methionine on the accumulation of 4-amino-5-imidazole carboxamide by Escherichia coli (MacLeod strain) during sulfadiazine (SD) bacteriostasis were studied.
PGA and LV were ineffective in reversing SD growth inhibition or in influencing amine accumulation; vitamin B₁₂ and methionine at identical concentrations depressed the amine accumulation per unit growth to different degrees, vitamin B₁₂ being more effective than methionine.

Amine accumulation when expressed as a variable against growth was more in the presence of methionine and least in the control set without added vitamin B₁₂ or methionine, vitamin B₁₂ effect being intermediate.

These observations are interpreted to mean that the primary effects of vitamin B₁₂ or methionine are on growth and other aspects of cell metabolism that are interfered with by sulfonamides. Their involvement in amine to purine conversion is only indirect.

Ethionine and a vitamin B₁₂ oxidation product depressed growth but no amine accumulation was observed.

The inability of an E. coli mutant to accumulate the amine under conditions of deficiency of vitamin B₁₂ or methionine also suggested that these two metabolites are involved only indirectly in purine synthesis.

REFERENCES


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