NOTES

METHIONINE MADE AN ESSENTIAL GROWTH FACTOR BY CULTIVATION OF E. COLI IN THE PRESENCE OF METHIONINE AND SULFANILAMIDE

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The strain of *Escherichia coli* chosen for our work was No. 6522, American Type Culture Collection, because it grows well in mediun SG, composed of inorganic salts and glucose (Kohn and Harris, 1941). This characteristic has remained stable for several years, and was not lost when a subculture was transferred daily for several months in medium SG containing (*a*) 1 per cent proteose-peptone, (*b*) amino-acid purine mixture (see below), or (*c*) sulfanilamide gradually increased to 200 mg per cent during the course of a year. When, however, cultivation occurred in the presence of both (*b*) and (*c*), methionine became an essential growth factor, as shown in the following protocol, designed to demonstrate this fact.

EXPERIMENT

The bacteria were grown in medium SG made up to contain *l*-methionine and xanthine at 1×1010^{-5} M, and glycine and *dl*-serine at 4×10^{-5} M, with and without sulfanilamide. The initial sulfanilamide concentration was 2×10^{-3} M, which was gradully increased to 2×10^{-2} M during the course of thirty transfers. The transfers averaged 0.001 ml into 5 ml of medium. Without sulfanilamide the bacteria remained stable throughout, but by the tenth transfer those in sulfanilamide no longer grew in medium SG. The latter, tested in the various amino acid-purine combinations following the thirtieth transfer, were found to grow only in the presence of methionine. Since then the strain has been transferred 20 times in medium SG containing 2×10^{-5} M *dl*-methionine (but no sulfanilamide), and the methionine requirement still remains absolute.

DISCUSSION

Methionine in *E. coli* is a specific antagonist to the sulfonamides (Bliss and Long, 1941; Harris and Kohn, 1941), and the action of other secondary antagonists such as xanthine, serine, and glycine, is dependent upon its presence (Kohn and Harris, 1942). For a variety of reasons, we have argued that the sulfonamides inhibit anabolic reactions, and that of these, the synthesis of methionine is perhaps the first to be affected. The present experiment is consistent with and supports this general line of reasoning.

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When it is recalled that cultivation in sulfanilamide alone (or in methionine alone) does not change the methionine requirement, the present finding may appear somewhat puzzling. In a general way, the explanation is as follows. Resistance to sulfanilamide developed in a methionine-free medium must involve *inter alia* metabolic adjustments to protect methionine synthesis. Such adjustments need not be made in methionine-containing media.

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