June 18, 1947.

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Your recent paper in J. Bact. "Inhibition of methionine synthesis in E. coli by 2-Cl-PAB and SA" was very interesting to me, particularly in the light of the genetical problem which it seems to introduce: namely, the induction of heritable resistance by the drug to which the induced mutant is resistant. I would enjoy very much, sometime, the opportunity of repeating your experiments on a strain of E. coli in which genetic analysis is possible (see a paper in the next forthcoming J. Bact.) For this purpose, I would appreciate it if you could send me a sample of 2-Chloro-p-AminoBenzoic Acid, if that is convenient.

However, before I can wholeheartedly accept your conclusion that the inhibitor plays a direct role in the production of the adapted types, I would appreciate your opinion on an alternative interpretation. As I understand it, the crucial finding is that a) resistance is rather slowly developed in liquid medium, and b) a large proportion (10%) of the cells slowly develop heritable resistance when inoculated into agar medium. It is not clear from your paper whether a resistant culture, when plated into Cl-pab agar shows the same lag in the development of colonies from single cells, as in the ""selection"" of the resistant cells from the original sensitive population. If there is no such lag, the conclusion is of course inescapable that there are not cells in the original sensitive population comparable to

those which you found after the treatment. If this were so, then the only loophole left open is perhaps that there is some growth of the sensitive cells (perhaps to microcolonies of 10^3-10^4) which is not readily perceptible, but which provides a population large enough for an occasional spontaneous mutation to resistance, then selected for and forming the 10-25% yield of visible colonies. I hope you will not find obnoxious this kind of heckling, but in the light of the little genetic knowledge we have of bacteria the geneticist is still inclined to fight to the last ditch against directed claims of specific adaptive mutations.

For the slow manifestation of "resistant mutants" in liquid culture, I have but one additional thought to suggest, namely that there may be an interaction between the sensitive and resistant cells, such that in the presence of Cl-pab, the resistants are inhibited to a greater extent than they would be in the absence of the sensitives. This could be checked very easily by using artificial mixtures. This is not a purely hypothetical occurrence, since Dr. Ryan has been finding just such an interaction between histidineless and wild type E. coli, and he and I have described a similar sort of behavior in Neurospora heterocaryons. (Adaptation and reverse mutation of leucineless Neurospora- Proc. Nat. Acad. Sci.)

Finally, a reprint would be appreciated.

Yours sincerely,

Joshua Lederberg.