

# LONG ISLAND BIOLOGICAL ASSOCIATION

COLD SPRING HARBOR, LONG ISLAND

THE BIOLOGICAL LABORATORY

July 29, 1952

Dear Dr. Lederberg,

Thank you for your letter of July 18th; I am glad the suggestion concerning the use of blue tetrazolium was of help to you. Although we have not worked with it in Dr. Mudd's laboratory, Bismark brown is said to vitally stain certain Protozoal cells and cells of higher organisms and may be of use to you. Also, though it requires special microscopy. some fluorescent dyes have been used for vital staining and one of these has been applied with success to *E. coli* in which it stains the nucleus (recent number of *EXPERIENTIA*).

Your remarks on the differences in the staining pattern of triphenyl tetrazolium and blue tetrazolium were of interest to me. We, too, have noted differences in the superficial pattern. It is possible, however, to discern small secondary loci of reduction, almost comparable to BT staining, in K12 "overexposed" to TTC; these minute loci are difficult to see without the very best microscopic equipment. Also, double staining with BT and TTC in the same cells give BOTH colors localized in the pattern of the numerous small loci. Other agents (e.g. Nadi reagent, Janus Green B) give rather intermediate effects, as does neotetrazolium. Perhaps the combination of violet or neotetrazolium with blue tetrazolium would give you two differential agents which would be partitioned to the daughter cells. Also, shorter exposure to TTC might enable partition of the granule.

In all of the cases of these supra-vital stains, the greatest staining is in the unipolar locus (in K12 or bipolar in aerated *E. coli* B). The granules can be seen with the phase microscope in older cultures as distinct spherules. We have thought of several possible explanations for the rapid piling up of TTC at the primary, most active locus of reduction but, as yet, these thoughts are still conjecture.

Probably, in *E. coli* (as is evident in electron micrographs of transparent *Mycobacteria* and *Corynebacteria*) there is a heterogeneity or gradient of enzyme activity and particle size. That there are smaller cytoplasmic elements comprising much of the mass (and RNA) of the cytoplasm of the bacterial cell is quite probable. Such a heterogeneity of cytoplasmic components has its parallel in the studies of Novikoff's group, Brachet, Laird, etc. We are undertaking a program of physical fractionation which will allow correlation of fractions with specific cell structures; it is impossible to make any but the most arbitrary correlation from the fractionation work of TN Harris, Sevag & Smolens, Militzer & Georgi, Stone & Wilson, Stanier & Gunsalus & Schlachtmann and others.

As I mentioned above, there is evidence by other staining procedures (and some others not mentioned here) that the polar granules of the cell are by far the largest and enzymatically the most active, at least as regards terminal

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electron transport. We hope to use other cytochemical methods (e.g. beta-D-glucuronidase) to determine the localization of other enzymes in the adapted cell and, if appropriate, to follow cytochemically the process of adaptation. As yet, I know of no valid procedure for beta-galactosidase activity; if you are acquainted with any such technique that would be suited for cytochemical work, news of it would be most welcome.

Perhaps an independent technique not so greatly influenced by oxygen tension would aid us in another problem. That the larger polar granules of *E. coli* may segment rapidly in adaptation to anaerobiasis is indicated by preliminary experiments (1.1 granules in aerated cultures and 3.3 granules two minutes later under anaerobiasis). However, the study of the regulation of physiological processes by these organelles must await more appropriate methods. Phase contrast and our new TV attachment may aid us ~~here~~ in the study of "starved" cells. Recent electron micrographs by Mudd, Winterscheid, and Hillier show that the large granules of *Mycobacterium* spp. are composed of smaller spherules with a fibrous texture; Knaysi has observed the fusion and division of these large "granules" (actually aggregates of granules enclosed in a membrane) by phase microscopy (although he still maintains that they are nuclei). By triple staining methods, nuclei, mitochondria, and cell walls (and septae) are clearly demonstrable as distinct entities, at least to our current satisfaction. Actually, much of our time has been taken with the differentiation of the cytoplasmic granules from nuclei, a subject which is still debated by some (most notably Knaysi, Hoffmann & Bringmann). But now we feel it is time to use time and confirmation in other laboratories as weapons and go on to the more interesting problems mentioned above and below.

You will be interested in the study that is in progress in Dr. Lawrence Weed's laboratory (Army Med. Sch., Wash., D.C.). He has, so the grapevine explains, isolated stable anaerobic small colony variants of *E. coli* induced by  $Cu^{++}$  ions. Also, I do not recall at the moment whether or not I called your attention to the publications of Charlotte Cowell on induction of small colony variants with naphthoquinone derivatives (J.BACT., ca 1947-8).

Your mention of studies on resistant and dependent streptomycin mutants recalls some studies made in Dr. Mudd's laboratory. Cytologically, the mitochondrial granules are still present in the resistant cells. A second aspect enters into the mode of origin of the resistant cells (i.e. spontaneous mutants to streptomycin resistance OR spontaneous mutation to "a susceptibility to specific induction"). Replica-plating and BIOCHEMICAL characterization of the resistant, yet unexposed, clone could possibly adequately answer the above-stated question (especially from the eyes of the biochemist who is more difficult to persuade with genetic data).

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Apparently, different strains of *E. coli* vary with the demonstrable biochemical effects produced by streptomycin inhibition. Thus, experiments on B and K12 may not (and do not) reflect the same biochemical effects as the Merck group found for their other strains. Sevag and Rosanoff have characterized some of the biochemical properties of B and K12 resistant mutants and Rosenbloom some of the properties of B and B/r mutants (/s and /sd). The work of Sevag and Rosanoff is in manuscript and probably could be obtained from Dr. Sevag upon request (certainly there is more validity in their biochemical study than in the genetic study previously reported in J.BACT).

The contributions of bacterial cytology to microbial genetics have been disappointing in the past; the next few years, however, in my opinion will see a great reversal of this position. The excellent work of Dr. DeLamater on the cytology of *B. megaterium* needs genetic confirmation (which Dr. Yaverbaum is again picking up and will continue in an extra year, staying on with Dr. DeLamater). Your studies on K12 should be enriched by adequate cytological demonstrations, especially now that fertile  $F^+ \times F^-$  crosses and Het stocks make study feasible. However, one can only obtain a clear cytological picture of the nuclear processes in K12 in enough detail through the use of critical microscopic equipment and techniques. Dr. DeLamater has recently improved his microscopic set-up considerably and added several new twists to his staining procedure, with remarkable results. I hope he is keeping you informed of these advances, for they mean the difference between uncertainty and positive interpretation of the minute structures just at the limit of resolution (in K12). In progress in Dr. Mudd's laboratory is an investigation of induced K12 (and related situations, e.g. with lambda on W1485, induced W1485, incompetent K12, etc.).

Please excuse the rather irregular nature of this letter; I am at Cold Spring Harbor for the summer genetics course and have set down my thoughts and typed them out on a series of unfamiliar and somewhat ancient typewriters! I will be interested, as will Dr. Mudd and others in our group, in hearing how the applications of these new techniques progress.

Very truly yours,

*Philip E. Hartman*  
Mr. Philip E. Hartman