

UNIVERSITY of PENNSYLVANIA

PHILADELPHIA 4

The School of Medicine

June 25, 1952

DEPARTMENT OF MICROBIOLOGY

Dear Dr. Lederberg,

Dr. Mudd has referred your letter of June 22nd, requesting information about certain of the tetrazolium derivatives, to me. We have used several tetrazolium salts, only one of which does not have a red tinge:

Blue tetrazolium (BT). 2,3'-Dianisole bis-4,4' (3,5-diphenyl) Tetrazolium Chloride. Obtained from Dajac Laboratories Div., Monomer-Polymer, Inc., 511 Lancaster Street, Leominster, Mass. As of November, 1951, their prices were: \$5.50 for 1 gram, \$42.50 for 10 grams, and \$90.00 for 25 grams.

BT, in my experiences with *E. coli* strains B and K-12, is not as good a supravital stain as triphenyltetrazolium chloride (TTC). However, it may well be useful to you since, besides giving good contrast in color, superficial appearances of the staining pattern are also slightly different. It is reduced more slowly than TTC and neotetrazolium. In our experiments, optimal reduction with BT is achieved in *E. coli* at the same concentration as with TTC. Some properties of the various tetrazols have been described recently (Shelton & W.C. Schneider 1952 ANAT.REC. 112:61).

We have attempted to follow the partition of the reductive centers to daughter cells by microscopic examination of *E. coli* growing on medium containing small concentrations of TTC under anaerobic conditions and have failed to note division of the granules once stained although considerable growth took place in the microculture. Apparently, there is a very critical concentration of TTC which will allow adequate reduction for microscopic examination without inhibiting cell division. On the other hand, reduction of TTC in considerable amounts does not seem to impair motility for a considerable period, at least in complex medium. We have attempted to select non-reducing mutants of *E. coli* B and K-12 by transferring in increasing concentrations of TTC; unfortunately, the clones which were isolated in these preliminary studies were not stable; the same was true in other exploratory experiments, using acriflavine. Perhaps you might have techniques which would allow you to isolate stable non-reducing strains (interesting in themselves) and, thus, be able to retain the label in one of the parent strains (and products of fusions) by growth of the mixed types in or on small concentrations of TTC.

I do not know what the effect of S and R variation has on the rate of recombination in K-12; however, perhaps, as one of the "lables" a heavily capsulated mutant could be used. Darkfield, phase or slightly-oblique lighting in the light microscope could then be used to quickly differentiate cells with capsules and those with TTC granules. However, besides the possibility of the prevention of fusions by the capsule, the TTC-labeled cells may give mucoid growth (Szybalski 1951 MICRO.GEN.BULL. 5:10), thus hindering clear demarkation between the two types.

Very truly yours,

Best wishes
Stuart Mudd

Philip E. Hartman
Philip E. Hartman