June 3, 1952

Experiments on the Breeder

To date the principal difficulty in operating the "breeder" has been the occurrence of wall growth after about 3-4 days in synthetic medium (F medium without NH_4Cl + 1 gram/l Asparagine) and about 2 days in broth. The latter difficulty seems to be resolved by the use of 0.05% Tween 80 and coating the wall prior to use with General Electric Dri Film 9987. (Coating is accomplished by thorough washing and multiple rinsing with distilled water, drying at 100°C, coating thoroughly with dri-film, rinsing off excess with benzene and baking at 120°-150°C for $\frac{1}{2}$ hr.) This also seems to be successful in dealing with the wall growth in synthetic medium. It may also work in the absence of Tween 80, but this is yet to be demonstrated. The effects of Tween 80 on the growth of "B" are small since the growth rate in its presence is 33 minutes in broth and about 74 minutes in synthetic medium.

The mutation rate of B in synthetic medium to T5 and T6 resistance is about the same as that for B/1+ in the Chemostat but no extended runs have been made to demonstrate this conclusively. This tentative conclusion is based on a number of short runs where several mutant frequencies could be measured prior to the appearance of wall growth. This experiment has not been tried with the Dri-Film wall coating.

In broth, a growth tube coated with Dri-Film permitted an observation of the mutation rates to T5 and T6 resistance and showed 4.0 and 3.4 mutants/ 10^8 Bacteria/hr. respectively. This experiment also demonstrated a fall after about 70 generations and the mutant frequency began to rise again after about 155 generations from time zero. A reconstruction experiment with 10 single colony isolates ~~and~~ of each T5 and T6 resistant mutants in synthetic medium

showed less than 1% selection.

An experiment with synthetic medium and 150 mg/l Theophylline gave mutation rates to T5 and T6 resistance of 8.3 and 1.1 mutants/10^8 bacteria/hr. respectively in agreement within experimental error with the Chemostat results.