July 28, 1948.

Dear Ed:

Since your visit, something very important has eventuated: namely, the finding of a heterozygote in K-12. As I told you then, I was cleaning up some details on the inheritance of phage resistance. V_{1c} is a mutation which is resistant to T1 and T1h (although there is some unexplained reaction, other than widespread lysis) and sensitive to T5. To test its allelism with V_1, I set up the cross W-416 x Y-64, or:

$$\frac{B\text{-}M\text{-} \qquad V_{1c}{}^{r}}{B_1{}^{+} \quad Lac_1{}^{-} \quad V_1{}^{r} \quad T\text{-} \quad L\text{-}}$$

in the usual way Somewhat over 200 prototrophs were tested; they segregated nicely for resistance to T5 (V_1 r/s) but there was only a single T1-sensitive prototroph. These tests were done, as routinely, on synthetic Lac EMB, and the exceptional culture was Lac+. Since the identification of the V_{1c} locus as distinct from V_1 rested on this lone culture, I set out to purify it and check--and it's a lucky thing! When streaked out on EMB Lac (complete), the prototroph (call it W-465), three kinds of colonies were noted: typical Lac-, Lac+, and an essentially + culture with numerous - streaks and sectors. The - and + cultures were both phage-resistant (T1); the "mosaic" type was sensitive on EMB-synth. On complete, it continued to segregate out + and - resistants. After a few passages of W-465 on minimal agar, it was plated out on complete, and individual + and - segregants studied in detail. To make a long story short, W-465 is repeatedly throwing off a very high proportion of recombination types, of all classes, and including multiple-requiring types such as B-M-T-L- which would be impossible to obtain by the earlier methods. A broth culture of W-465 will contain as many segregants as heterozygotes, and these segregants are of all the classes expected on the basis of the linkage relationships. Naturally, W-465 has to be preserved on minimal agar, and at that I don't know how long I can keep it. At present, it is still possible that W-465 is a synkaryon with karyogamy at least half the time, but the recombination frequencies seem too high even for this. I don't know what may be responsible for this exceptional delay in reduction of the zygote; previous data indicate that no more than .1% of the prototrophs can be heterozygotic, and probably much less. Now that I know what to look for, however, I am setting up crosses where the multiple dominant is the rarest crossover class, and may be able to repeat it, perhaps also by using segregants from 465. Aside from the obvious conclusions, it is interesting to note that phage-resistance is recessive. This supports the feeling I have always had that the "delayed effect" is a matter of segregation, probably of nuclei. Single-cell work, and probably some cytology, are under way, and of course I'll let you know what develops.

Best regards,