155 Corona Ave
Pelham 65, N. Y.
Friday, April 14

Dear Joshua:

You have probably been rather eager to hear my further comments about pleiotropy, and I'm sorry that this is the first chance I've had to sit down and write to you.

The secondary fermentation affects I mentioned in my last letter were in our own mutant strains: tryptophaneless was lac⁻, mal⁻ (plus-minus on second day); histidineless was mal⁻, mannitol and galactose slow plus-minus; another with unidentified requirements was xylose plus-minus, and a cysteineless appeared to be negative for all fermentations until we found that its metabolism was somehow interfered with by the EMB medium.(we never solved it completely). Some were, unfortunately, already lac⁻, and we didn't test on other sugars at that time because plates weren't madeup.

Your evidence against pleiotropy is convincing in the case of maltose, and if maltose can behave that way, so can the other sugars. The pleiotropy idea would have explained most of the aberrant behavior of sugar markers insofar as there was an excess of negatives, because there might have been two or more mutations which singly, or in combination with other mutations, could prevent a ~~other~~ fermentation. The great excess of negatives among all my recombinants would be nicely explained by this, and it would seem also to account for the segregation bias you told me of this summer. It could account for some degree of "partial segregation" because a Lac v Mal- might be segregating for two different Mal- mutations. To account for more than 75% of Mal- you'd have to assume 3 or more loci, or else postulate some sort of repulsion between the two mutations. If Lac v Mal+ occurs ~~common,~~ though, pleiotropy could not explain it.

Bernie's suggestion of physiological side-effects is also worth considering, I think. He compares it with the mucoid character, which is very common among recombinants and among some mutants, especially on selected media. He thinks the mucoid trait depends on a physiological state that can be achieved by many different factors, and combination of factors, both genetic and environmental, Some of the non-fermenters, e.g. galactose in W-677, actually utilize the sugar very effectively without producing enough acid to be stained, or so his experiments seem to indicate at this stage, and the failure to stain might conceivably have a diversity of causes like the mucoid character. The only difference between this and pleiotropy, I think, is that it could depend on a variety of genetic differences between the parent strains, instead of on the known mutations.

For our quadruple stocks we are going to neglect fermentations, since they would so limit the number of mutations we could use, and since we can probably get plenty of drug-resistant and phage-resistant markers, in addition to the requirements, to mark the best-understood part of the chromosome. The crosses I'm doing now involving the descendants of W-677 should give enough informa-

tion on the fermentations, that is if they take a turn for the better. Of 6 times I have done the experiment, only 2 the 2nd and 3rd yielded complementaries. The main trouble seems to be incompleteness of the requirement for B_1. I have therefore decided to take your suggestion on selecting the complementaries by drug resistance. If I get the V_6^r mutation tomorrow, then next week I'll repeat the experiment with the cross

W1234 Ia^r by (B-)M-P-$S^rV_6^r$,

selecting for Az^rS^r complementaries.

Of the stocks you sent us, W760 was the most desirable because it did not duplicate the requirements of any of the doubles we were using. But the vial contained no growth; we tried incubating it further and even washing it out, without success. Why don't you use slants instead of stabs? I can't see that it makes very much difference, but Bernie's technicians get quite angry about it. By the time you could send us another sample of W760 now we'll certainly be working with triples.

You asked if I used dilute inocula for making the crosses. In the most successful experiment many of the colonies were picked from a plate with only 66 colonies on it, and it was my aim to get the numbers even lower than that. However, I have been using different stocks since then, and, perhaps because of different densities in the cultures, they seem to require different size inocula to give the same number of recombinants. The result is that my crosses since that last successful one have all given too few or too many prototrophs.

The short-cut methods we use for testing markers include a lot of details that it would be silly to list. But the main points are the following:

1. Eliminate all handling of tubes.
2. a. Pick two colonies at a time into a double-depression slide freshly flamed, containing a drop of water in each depression.
   b. test for auxotrophy on plates, each plate of the series omitting one of the growth factors.
3. Spot the cultures on the plates instead of streaking them.
4. Mark (preferably permanently) a grid on the back of each plate, in vertical rows of ten with a heavy line across the middle, so that the spots can be put on without looking to see where the last one was, and so that the number of each will correspond to its position on the plate. I spot 60 on one set of plates, breaking up the two end rows into 4 and 6 (first 5 above, second five below the heavy line), but if you can always get plenty of plates 50 or even 40 might be more efficient.

I mark the plates and lay them out in order, and then spot two colonies at a time on the whole set (one colony all the way thru, then the other, just moving my loop back and forth between the slide and the plates). after every two I dip the slide in alcohol, then water, then wipe it and flame it, but if you can get about 50 depression slides they could all be washed & dried together, flamed first.
Yours, Gordon