

Wind River Ranch - 1 -
Estes Park, COLOR.

NESTER.

6/24/61

Dear Josh,

FRI. morn.

In considering our discussion further on the penicillin studies, I think we overlooked the results of several previous experiments which throw a new light on the latest results. Both Bruce and I have consistently observed that the addition of penicillin at 120', immediately following DNA addition leads to no decrease in the absolute no. of transformants over what one obtains if no penicillin is added. Therefore, it seems to me that there cannot be both a cyclical competency and no effect of DNA on conferring penicillin resistance.

For the cells which have picked up DNA at ^{90-120'} (DNase then added) ~~120'~~, must be the same cells which are resistant at 150', and indeed resistant up to 270' after DNA addition. Since DNase is added at 120', ~~no new transformants~~ there can be no balance between killing of "old" transformants and the appearance of "new" transformants.

The original picture that Bruce and I
came up with was that competent cells
have lost the ability to multiply at the
time they reach maximal competence. These
some cells further do not begin to
multiply until about 4 hr. after DNA add'n.
I think the data now ^{indicates} ~~points to~~ that
cells maximally competent are resistant to
penicillin before DNA add'n (and 30 min.
before maximal competence, they are 10X as
sensitive compared to the whole population).
These competent cells can behave in 2
ways. If DNA is not added, they go through
cycles of competency, and coincidentally
penicillin sensitivity + resistance.
But, the integration or some related
phenomenon of DNA interaction with the
recipient cells ~~confers~~ ^{stabilizes} the penicillin
resistance, presumably by inhibiting cell
multiplication.

The most confusing area in this picture is why we haven't been able to demonstrate penicillin resistance of a portion of the entire population when transformants specifically haven't been studied (but DNA is added). I suspect that perhaps this negative finding could be explained by assuming a very heterogeneous recipient population, and specific requirements for penicillin resistance, presumably those necessary for transformation.

I think it might be worthwhile to try Spizerg's procedure for competency, thereby giving a different recipient population as judged by the ~~in terms of the~~ number of unlinked double transformants. ~~Perhaps~~ ^{Perhaps} resistance could be demonstrated under these conditions. Certainly we shall have to do more experiments to try and determine whether we can show it under

Conditions of penicillin killing > 99%.

It looks like another sun-free, sunny day at the park. We were planning on making a 10 mile hike, but the maxim of "Early to bed doesn't seem to hold for Indians and some women. So I doubt that we'll get more than a few miles.

Alan and I are both quite anxious to return, since there are many obvious experiments to do (and competitors who are trying to do them).

I was told by Kozinska that Herriot has stopped his annealing experiments. Apparently he was trying to get a third linked marker (or maybe unlinked) into his system and found that his 2nd linked marker system wasn't behaving anymore.

Regards to all,

Gene