YALE UNIVERSITY
OSBORN BOTANICAL LABORATORY
NEW HAVEN, CONNECTICUT

Dec 19, 1947

Dear Josh:

1st to answer some accumulated questions:

1) Easter vacation will be O.K. for your oral -
2) The thesis copying is almost done, and I'll let you know what it cost as soon as I know. The copies will be bound with the petty cash acct. you have with Hinchcliffe. Incidentally - Marge Reaume is the typist!
3) We are rechecking Y 142, 141, 143. and will send Y 134 and 137.
4) The proteus story is somewhat improved - but entirely unsatisfactory for analysis of large bodies - a few "mutants" were derived from N-mustard followed by malachite green and total picking. These show consistent lags on minimal, and with group supplements other than the specific group (H.C. - or rit - or yna.) to which the response may be real - It remains for Carl to test individual component supplementation. With these strains, to pick up these effects, the inoculum must be very dilute, and readings made at short growth intervals - 17 - 24 hours - Since no comparable tests were made without Mal. Green - there's as yet no evidence on polyploidy or the role of malachite green - if any -!
5) Ed Adelberg is now getting reproducible assays, and consistent yields of his definitely ether soluble isoleucine precursor - So we're optimistic on this front.
6) I think I told you Dave's precursor is OH-anthranilic - a note has been sent to PNAS to appear with Mitchells & Nyc's. Also, one of the summers mutants appears to use OH anth but

not tryptophane or kynurenine - and may pile up kynurenine! Dave & I still aren't convinced of the normality of the tryptophane-nicotinic relations. Of course the rat may be quite different, ~~but~~ since it cannot use kynurenine or OH anthranilic, although tryptophane seems to be OK. (Bo. & Krehl unpubl.)

7) I think I also told you the folicless isn't, but is a C_2 less - requiring both acetate and ethanol.

8) Desoxycholate (at pH 6) gave lots of clear-cut small colonies of 58-161 cells by detection, appearing only after layering. However, all of over 200 picked & tested (Lucio) were not mutants, or exceedingly unstable. OK at 24h, but complete growth at 48 in minimal. By contrast, almost 100% of small colonies of the same strain treated with tris-N-mustard were stable mutants. Incidentally - I heard, not from Evelyn Witkin directly - that desoxycholate and NaCl - both increase reversion of a K-12 biochemical - How about it? - I'm writing her.

9) Lucio & I did a recombination of Y144 (acetyl-valine resistant [AV^R] 58-161) with Y53 - and got good segregation ratios suggesting AV is 15 units to the right of Th. The reciprocal should be done - as well as looking for alleles - We're now starting on enzyme investigations.

10) Your lactoseless evidence sounds fine -
As far as to the number of enzymes involved
I haven't any ideas at the moment except
a) that of isolating comparable enzymes from the genetically different types
b) mixed enzyme preps of the 2 strains should be + if 2 enzymes are concerned.
c) possible consideration of permeability as a factor — (Deere 1939 J. Bact.)

How do you isolate an inactive enzyme?

11) C_1 mutants - if any - behave unsatisfactorily - comparable to a) Proteus or
b) Desoxycholate on 58-161

Have you tried to get any?
I still haven't had time to try transformations but hope to this Xmas vacation.

Finally:
12) Bill for Genetics is O.K.'d.

13) Had a Xmas card from Carl Lindegren - he's moving to Southern Ill. Univ. at Carbondale, Ill.!

Regards to Esther - & best wishes to you both
yours
Ed