Bruce Stocker: 10/4/52 (1)

It was a week ago that I wrote a longish letter, but I held it up from day to day for so many postscripts that I thought it better to start over. We had a very pleasant vacation in Canada and Maine, and hope you had an equally bon voyage. We returned to the lab to find everything in very good order-- I am especially grateful to you for leaving the Salmonella cultures and notes in such trim shape, as it permitted me to resume work without delay.

I am waiting for Spicer's arrival, expected momentarily, to make more definite plans for the Salmonella work. Before going ahead, it seemed important to consult you (last week would have been none too soon). It should not take long to clean up the loose ends for a paper on the transmotilization experiments if we can organise the work between us. Beyond this, I am hoping to extend the scope of transduction in Salmonella with diverse phages, and to prepare for a visit to Chamblee to set up an assembly line there, the logical place to manufacture Salmonella types. Spicer will probably emphasize the development of material and technique for the Salmonella groups, esp. C and E, not hitherto covered, and from some of the literature on phage specificities, it may be feasible to bridge these with the B and D. Kauffmann has reported an unusal paratyphi A with XII_2, which may help to bring the A group into the picture if necessary.

The situation that has occupied most of my attention the last couple of weeks has been the apparent linked transduction of i--H+ from typhimurium to SW-543 (-60), but I'll save this to the last.

The stability of 24 Gal+ transductions to SW-435 from PLT22/2 has been confirmed. The stability of transinduced genotypes may have nothing to do with the tracks, but it must be recalled that all of the track studies have involved recipient strains that have not been studied for any other character. (I am sure most of these points have come up before, but I don't have any record of our joint speculations). To be on this point, I have made a Gal- in SW-603 (SW-666) and a Xyl- in SW-541 (SW-665). Both of these mutants are ideal for transduction studies, giving clear papillae with a zero background frequency. SW-665 does seem to show a very efficient response to transduction. A careful comparison with LA-22 is in progress. SW-666 shows a good many plaques when plated with PLT22 (host range mutants?), but the transducti is fairly smooth nevertheless. The transinduction to + of both mutants is being studied for stability. No linkage of Gal to H+ has been noticed in transmotilizations of SW-666.

4 more spontaneous H+ have been picked up from SW-603, all b. I am inclined to agree that SW-572 was a fortuitous transinduction. But to avert the question, we can deal with FA's from a variety of other serotypes. This has been done with abony, dublin, san diego, enteritidis, to give in each case a mixture of b and the FA-type swarms. I am waiting for some additional sera to check the phasicity (mono- or di-) of the transinductions. We are both willing to bet they will all be strictly monophasic, as is SW-543. This may be of some use for antigens for practical serum production. FA (PLT-22) has also been produced from heidelberg and altendorf. A very few tested swarms were all b; some fresh platings with the urging of b- antiserum are in the incubator. A good many new serotypes are already represented in these experiments. I think it certainly is time to consider suggestions for replacing the place nameswith simplified diagnoses--unless the artefacts are to be dismissed as madison-1, Q.... Wilson & Miles (in Topley and Wilson) already supported this. I don't have much bearing on the phase of the FA-donor in relation to the phase of the output. SW-603 should be a good tests system for this question. My abony strain, which is almost all phase 1 to start, did give almost all b transmotilis I have a plate going now to switch its phase, and see what happens.

A few prelim. expts were started with the various FA's on typhimurium

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for both potential antigenicities are transduced. I hope you can pay some attention this also so that enough material can be studied to permit sound generalization. There is no question that the antigen substitution in these experiments is quantitatively very much less productive than the transmotilizations. There is a problem here—it is difficult to see how phenomic delay or things of that sort can be invoked. Possibly there is just entanglement in the agglutinated flagella, so that only the best situated cells have a chance to swim out. It should be possible to set up reconstruction experiments of sorts in which simple transmotilizations are made to occur in the midst of otherH-agglutinated cells. I have not intended to intrude on the track problem, which is your own very healthy baby, but I do have a small comparison on the influence of b or i-12 serum on the trahsmotilizations by typhimurium FA. This is needed to validate the use of b serum in the initial plating when the second type is desired.

Although the cultures are apparently lysed (with plaquing) by PLT22 on agar, I have had rather poor luck in growing PLT22 on typhi H901 or on stanley. However, some other phages are turing up that look more promising.

Re Chi, I have only just grown a batch on SW 592, as you recommended, and have not run many tests. With the original Chi, both typhi Watson and its i transinduction were resistant, H901 and its i deriv. are sensitive. I intend to compare the b and the donor type transmotilizations of SW-603, but don't expect that the response depends on the flagellar antigen as such. Have you done the adsorption experiments you were planning? It would certainly be very useful to have any fairly clearcut taxonomic criterion for a Salmonella H antigen.

A genetic test of the "linkage" hypothesis for the SW-543 situation occurred to me (again?) and is under way. With the transduction of so many other H antigens, we must reject the notion of their latent retention in SW-543, or we would have to explain why any FA does not give the full range of types. To take typhimurium FA setio by way of example, it can be suggested that SW-543 is H- X^b, the b is a one factor mutation or transduction, resp., H+ X^b, and the i as a two factor transduction, H+ X^i. Then, H and X would have to be linked, of course, since there co-transduction occurs very much more frequently than with Gal, or as between any markers so far in typhimur. But there is an alternative hypothesis that while b is H+ X^b, as above, the i is also a one factor transduction, H- X^i. This would mean that the H- of SW-543 is a special inhibitor of that particular X^b (or that this X^b is inactive without H+, perhaps owing to an incipient separation of functions). However, other alleles X^i, X^{ehx} etc. (and possibly other $X^{b's}$) function effectively with H-. This is no stranger than the effect that the residual genotype of typhi or of SW-543 seems to have on the strict monophasic stability of i (if this general interpretation is correct). The test for this is the competence of FA from the i transinduction itself. If it can transmit either b or i activity, one has a good argument on general grounds that the transinduction was dual in the first place. The experiment is under way.

We were discussing the use of FA for unmasking O forms. I don't think this exception is fatal. In the first place, we can use FA from SW-603, and simply not reserve transmotilizations based on this locus. Secondly, one can always be suspicious of the recovery of the FA type, and review such cases with FA from another type. This seems not so difficult as the prolonged, repeated selections for spontaneous reversion that may well be unsuccessful. If only as a pretense for collecting additional materi I think we should keep this recommendation.

For a complete study of O-forms it would, of course, be valuable to have an accumulation of mutants in a single organism. Typhimurium LT-1 may be a satisfactory choice, as it seems to be nicely susceptible to Chi, and can be closely tied in with the previous work. I believe we also have several marked derivatives of it also. I suppose the one mutant we should especially seek would be H+ X^o of the above scheme. (Perhaps we already have X- H^o in gallinarum? --This is another longstanding problem, I get FA from gallinarum pullorum, but on this I have no experiments in progress, due to a lack of any new ideas).

A [illegible] overlooked in their proper sequence UV [illegible] effect in stimulating motility in SW-603. I had once (a long time ago) [illegible] see whether mutagens would accelerate phase variation, but I am a little [illegible] of the precision of the selective technique for picking out rare phase variations. On the other hand, I don't see too much point in studying the effects of UV etc. on a very frequent mutation. You may have noticed "transinduction", used a little sloppily here. This is just an experiment to avert the grammatical incongruity of referring to the transduced gene as well as the trans(in)duced recipient cell. I am not at all happy about the terminology either here or in transformism. Refers to "autogenic" and "allogenic" seem to be shortsighted, as they simply reflect how many character differences there are between the donor and recipient. It would be very useful to have a self-explanatory shorthand for the FA donor and recipient and their genotypes. Transduction itself is a ~~gunk~~ word with a common (albeit rare) denotation, the act of conveying over, and we would not have to apologize for replacing it with a better now that we have at least some idea of what is going on. The same for FA. Unfortunately, some microbial-genetic-philologists disagree with my feelings about words to the extent of impending polemics in MGB, but I do not propose to get into a public embroil over it.

P.S. Oct. 5 Although the results of the serial transduction experiment (i.e. whether the i transinductions transmit both b and i) I am going to resist the temptation to hold this letter up any longer. ~~XXXX~~ "Hx" antiserum does seem to inhibit transduction, e.g. in the Gal- test system, and this is presumably involved in the antigen substitution experiments. The inhibition takes place although the cells and phage have already presumably equilibrated, but this will have to be checked. As the typhimurium serum seems also to inhibit the transduction of SW-603 by altendorf (c,1,2), I doubt if the flagellar component is involved, but this will have to be studied further with absorbed sera. At any rate, this has to be cleaned up before the phenotypes of track-formers can be tested.

Another pointbthat may be causing some confusion is that spont. H+ (b) from SW-603 do seem to be giving another phase, not yet identified. The same showed up in transmotilizations of SW-603 by S. abony, in which some colonies appeared to be b; ? where ? was not 1,2 or enx as would have been expected. It is possible that SW-543 is misclassified as paratyphi B, and that the second phase will make it wien or some other similar type that I don't have typing sera for.

The lab. is more or less humming with other work as well. Tom Nelson is already quite busy with kinetic studies on Hfr crosses; not much you don't already know on Gal-duction. Esther's peculiar unstable coli strains, which ~~shrew~~ variants simultaneously susceptible to lambda, T1, and T2, probably represent garden-variety BR dissociation. [The classification of these phages as probably rough-specific seems to have been overlooked, but gives some rationale to the resistance displayed by most of the wg varieties.]

I shall be waiting to hear from you soon, but will write myself if anything very pertinant comes up first.

Sincerely,

Joshua Lederberg

P.S. Esther will write you further on other matters.

Joshua