November 2, 1952

Dear Bruce:

Although your reply to my last letter may well be in the mails, afew things have come up that seem to warrant your immediate attention.

The behavior of the 1,2 phase from SW-534--SW-588 was very perplexing to me. If 534 came directly from 533, as stated in the stock list, and 533 = Edwards' diphasic S. paratyphi B #3, selection by Chi phage had a remarkable set of effects: a) picked out phase 2 (not incredible by chance); b) fixed the strain in this phase and c) so altered it as to allow its transmission to and manifestation in SW-543 transinductions. In my recent experience with #3 (= SW-703 by present numbering), its second phase readily varies back to b, and is not transducible to SW-543. This made me suspicious of the purported pedigree. You will recall that we had not systematically numbered immigrant serotypes until about the time of your arrival (I should long ago have set them up in our uniform list, as we discussed). My notes on the origin of SW-534 mention only "paratyphi B, non-motile, selected by Chi"-- I might very well have taken the culture now designated as SW-546 (or better, SW-857 = Edwards 157). As SW-546 does appear to be monophasic 1,2 it is a more logical candidate for the paternity of SW-534, if this can be cast in doubt by any means. (In addition, FA (SW-546) -x SW-543 gave 1,2.) Some further tests seem to bear this out. SW-703 differes from SW-546 and SW-588, and the latter are similar in the following: fermentation of rhamnose and inositol, intensity on EMB mannitol, and susceptibility to Chi phage (Boulgakov original), and to the phage carried by SW-543.** I am checking with Norton to see if his notes carry any additional gleanings, and with Edwards on the history of SW-857, but this revision is fairly certain. If so, the 1,2 phase carried by it is quite unique in its transducibility to SW-543,* and the notion that monophasicity has to do with an inability to manifest the second phase phenotype in certain genotypic backgrounds is still tenable, with this as an isolated exception.

* confirmed

** tends but no warrant.

A second point that surprised me considerably is the presence of a b (?) antigen in SW-543 (=603 = 666) line. Its manifestation is rather irregular, but indubitable. Immotility is not impaired-- the most reactive suspension was an inoculum in Penassay from a stationary blob in motility agar, and which did not budge when reinoculated to motility agar. I could not convince myself of a trace of active motility by microscopic observation, but there is always some uncertainty about this. Have you ever done a flagellar stain on this one? I'll send a culture to Leifson, and have some electron micrographs taken here. In view of our genetic results on the separability of antigen and flagellum loci, we might perhaps expect the production of H antigen independently of the latter. Don't Pijper and some others believe it to persist on the cell surface, anyhow? Until an antigenic analysis is done, I can't tell whether the complete b antigen is present-- or whether the whole thing is some sort of artefact.

The pedigree of progeny tests I outlined previously is almost completed: I will review the relevant section on the reverse sheet. I have still to complete the test of a back-X to SW-666 of an f-1 i; the difficulty was the resistance to PLT-22 of most of these, but I have one going now that I know to be sensitive. In fact, it is rather remarkable that these serial transductions should be possible, and the pedigree already shows some lines with several steps in which the transinductions remained susceptible to the transducing phage. This means

that the penetration of the phage (with delivery of its contents) may be followed either by lysis, or by lysogenicity (?), or by the recurrence of sensitive. Many of the transinduced swarms are, of course, self-plaqued. One speculation is that the phage itself may attach to a different nucleus than its FA does. I will have to check further on the establishment of lysogenicity for PLT-22 in SW-666. I have the impression that the i transinductions may be more regularly resistant (lysogenic?) to PLT-22 (in all the above discussion, adapted to SW-666) while the b's are sensitive, but will have to check further.

A few more experiments on the efficiency of transduction of Gal+ and H+ to SW-666. Unfortunately, there is by no means a linear response of Gal+ to increasin FA, while, as you know, H+ seems to go up pretty well within the testable range. One curiosity that out to be checked: with the dame combination (FA-703 -x 666) and dilution, at 30° there were 3 swarms, no tracks; at 37° 3 tracks, no swarms, At any rate, the effect of environmental factors such as temperature on the TvsS choice will eventually have to be studied (I regard this as your territory, and this as only a casual excursion, may I add). In some of these experiments, there were some well isolated swarms, with very well developed flares (the track clusters of some hundreds of microcolobies), and I thought surely to be able to recover the postulated O. About 60 tests all told, all H (and antigenically uniform; as expected, each swarm is pure)! Together with your results, I just don't think the flare microcolonies are O, and another explanation will be needed. Perhaps it is just that the newly formed H's are relatively weak and unexercised; a close look at the tracks, and the time relations computed from expected division times shows they are not moving at the final high rate, either. The full development of motility might be either a phenomic process, or accumulation of polygenic modifier but I don't see that the flares can represent a segregation of clear cut O's. This still leaves the tracks, but if they don't have to be correlated with flares they may be cells which, as previously postulated, adsorb H+ phage, but in which the entering H+ factor never does get into the chromosome, whether or not the cell is lysed. For the analysis of the flares, it would obviously be desirable to have a clear cut selective technique. I've been playing with some angles on this-- especially Archer's method or some variants. It was in the course of reconstruction experiments with SW-603--666 and SW-618 that the b-agglutinability of the former showed up, and of course the rather negative results I had been getting are indecisive. SW-543 is, unfortunately, essentially resistant to Chi phage, but I did some reconstructions with SW-588 (Gal+H+) and SW-666 (Gal-H-). Starting with about 100:1 +:-, the survivors of Chi on agar are about 1:1. (This does not necessarily mean 1 Qautant/100 originally +, as the action of Chi is by no means immediate.) Unless the selection is much sharper in liquid medium, which it may be, the method will be probably too messy to be of much help with the flares. (But I don't see how the microcolonies could have been passed over in 60 tests anyhow!) Attempts to dilute out the H+ preferentially bytheir spreadin into non-nutrient soft agar were unsuccessful.

Perhaps the most interesting developments are some rather sketchy facts on which to hang a theory of phase variation. Abony and typhimurium (LT-2) have made a staisfactory combination, although it would have been amusing to have had different somatic groups as well, In both our experiments, barring SW-546..., the FA from phase 2 has shown no trace of the phase 1 component, e.g. in transduction to SW-543 or to typhi. This holds as well for enx and for 1,2 of the present material. However, FA(b:enx)--x i,12 gave b:1,2 (selection by i,12 serum), and b:enx —X 1,12 gave i:enx. That is, only one phase is transduced, the other is latent or residual in the transducee. This excludes the idea that the phases are simply alternative alleles, and suggests that there are two loci, one for specific alleles, the other for non-specific. This would fit very well also with the patterns of phase variation in the group. The paradox is that the alterna-

tive phase seems to be latent in the cells as transducees, but not as trans-inducers. One can now either make very special assumptions about transduction in general, or about the genic mechanism of phase variation. On the latter, we infer that a "cytoplasmic state" determination of phase is excluded. Nor can we accept my old "switch factor" hypothesis, as the factor should be separable in transduction from the loci it controls. I am left with a general notion of differential,(and mutually exclusive as between the to loci) gene states: i.e., the activator (or inactivator) of the locus which is expressed phenotypically in the antigenic phase is inseparable from it. On a particulate basis, this is analogous to McClintocks Dis factor in corn, but we could just as well think of it in physiological, albeit self-perpetuating, states for which there are innumerable possibilities--E.G. Huskins lateral reduplication. If transduction can use the cytoplasm, we could even drag in reduplicated plasmagenes of the kind that are fairly closely dependent on the locus. The transductions from O-forms fortunately relieve the antigens themselves of this genetic burden-- but all the more reason for making sure about the complete absence of H antigen from them.

Before long, I will have to submit abstracts to the International Congresses. I have a formal invitation from the Genetics Congress (Lake Como) and have reason to expect one another from the Microbiologists (I was already asked to speak, of all things, on actinomycetes!). I assume you are going to both Congresses yourself. I think it would be less complicated to avoid joint authorships, although we should consult with each other to economize on time. The Genetics paper will be a 20-30 minute affair, and probably a rather general review of genetic mechanisms in bacteria in general (that is to say Coli and Salmonella!) This should not conflict with your presentation of the work with motility transductions which might as well be under your sole authorship. I don't know yet whether Norton is travelling also, rather expect not unless he gets a windfall (we can say the same, for that matter!)

With the completion of the progeny tests, very close to hand, I don't see what remains now to be done that should postpone writing up this work. What explicit points are now evident that need to be included? I hope you can find enough time to do this during the next few weeks-- if it would help I would be willing (but not eager) to go ahead on the basis of the outline that you wrote up before leaving here. In any event, the authorship Stocker-Lederberg-Zinder should need no further discussion, nor, as I would imagine to be your preference, its preparation for the Journal of General Microbiology. If you put together all the rest of it, I will collect my data on the progeny tests and add them to it; a chart would probably be indispensable.

Sincerely

Joshua Lederberg