PPS. I seem to have lost the ? non transducing isolate from SL13

The Lister Institute of Preventive Medicine.

now numbered SW1048. Could you send it again when convenient?

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16th.March 1954.

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P.S. If this arrives in time please ~~[illegible]~~ quote anything you like at your meeting. Hope to hear what you are saying, in due course.

Dear John,

Many thanks for your (long-awaited) letter. Glad you liked our tape message, hope to hear your reply soon. I will deal with the mapping and micro-manipulation situation first. I see my letter of Dec.4 was ambiguous at one point, lysate of 543 produces single but no (detectable) double transductions of 28, whereas lysate of 543 H mutant evokes both types. All the other 5 possible interactions of the 3 strains have given double transduces, though in very small numbers in some cases as you would expect so that even using concentrated phage-treated cells and antibody-gelatin plates several attempts were necessary: however in this type of experiment where there is theoretically no possibility of mutation confusing the picture I think even a single "double" is conclusive. As to logical basis, the essential assumptions are (1) that only a single (continuous) fragment of imported gene structure is incorporated into (the progeny of) a treated cell, where it takes the place of a homologous section (2) that the genes judged allelic because they replace one another in transduction are arranged in the same order in the strains under study. Having made these assumptions one makes the further working assumption that the gene order is linear, not two-dimensional etc. On these 3 assumptions the interactions of, say, 543 and 553 in each direction to give doubles establishes the order F(543) - H_1 -(553), similarly the interaction of 28 and 553 establish F(28) - H_1 - F(553) and the action of a lysate of 28 in evoking doubles from543 excludes the orderF(543) - F(28) - H_1 and so established F(28) -F(543) - H_1- F(553). The working assumption of linearity now predicts that a lysate of 543 will never evoke doubles from 28 though it may evoke singles, while a lysate of 543 H mutant may (probably) evoke both singles and doubles; these predictions have been confirmed. As to quantitation, my findings on 543 and 553 are similar to yours: I do not think one can be very confident in converting "% of doubles" into "relative map-distance" since one can't compare one pair of

loci with another using the same donor and recipient strains. Factors such as "host modification" may perhaps affect the proportion of singles and doubles. However, the above assumptions and the inferred order do make one further type of testable prediction, not involving "map-distances", viz, that a lysate of an $F(1)^- - H^a - F(2)^+$ strain and of its H mutant $F(1)^+ - H_1^a - F(2)^+$ should evoke equal numbers of b swarms from an $F(1)^+ - H_1^b - F(2)^-$ recipient; I have tried this with lysates of 543 and 543H on 553 and found no increase in singles. There was no obvious increase in doubles either (about 1% of number of singles) but this is not surprising for one would be looking for differences between number of transfers of two-loci section and of sum of these and of three-loci section and the triples might be small fraction of the doubles. I have not yet tested the other interactions of this sort, but mean to soon, also to repeat the whole thing using fresh lysates from single plaques etc., to make sure there has been no "coincidences" 544 and 966 have both given doubles, their Fla- loci are distinct from each other and from those of 543, 28 and 553, and so should be mappable. However, so far we have not got them placed, though certain positions are excluded. Difficulties that may account for it are that in each case yield of doubles is very poor, some interactions give poor yield of singles anyway, and the fact that these are diphasic means one must start with a stock in latent phase 1. We will try again soon I hope.

I have had one apparent example of quantitative evidence of linkage. In trying to complete chess-board test for allelism we repeated various negative, or nearly so, combinations using lysate of H derivatives of donors in parallel. SW544 with lysate of SL 123 (=28H) gave more swarms than SW544 + lysate of S128. This was the first evidence for possible linkage of Fla (544) and H_1, since confirmed. However, SL123 was an H derivative obtained by transduction (FA.LT2), not by mutation, and I suspect that this may affect its efficacy as a donor, for in some H-linkage experiments a lysate of 28H got by transduction evoked more swarms (from SW966) than did a lysate of a 28H <u>mutant</u>.

I think that is all on the linkage point. I have never been able to satisfy myself that trails are produced on agar containing antibody for intrinsic H antigen of recipient. Usually they have been certainly absent, occasionally I thought present but only in plates which had also produced swarms, so one could not be sure they were not "flares" or something of that sort. Your idea of looking for them by micro-manipulation experiments seems a good one. I agree one cannot predict whether or not a cell abortively transferred in H antigen as well as for motility would be immobilised by serum for the original H antigen. I suppose that it might have double H specificity, or either of the single ones, if there was any "position effect" of a linked Fla locus.

Micro-Manipulation experiments.

I have done only a few of these since I moved, and am not much further forward than I was. I am not sure I follow exactly what you have done, your results sound different to mine but I am not sure how much this is because of differences in interpretation. Anyway I will tell you both my experimental findings and how I interpret them, and brood again on your description to see if there is a real difference (which there might well be as we have used different material).

Material. Always SW 541 (or Sw 541 lysogenic) and lysate of LT2; mixed in broth at 37°, usually using log phase recipient culture, as I think it accelerates appearance of motile cells. Input ratio not standardised but always well over 1. I have not seen motile cells earlier than about 1 hour after mixing and as there may have been a generation or two during this time I cannot tell if there is segregation of motility in earliest divisions. In most experiments I incubate mixture at 37° for a couple of hours or so and then transfer to oil chamber (at room-temperature). To facilitate picking up motile cells I usually put a "trap" droplet of sterile broth next to the culture droplet and bring them together; one can then pick up scores of motiles if one wants to. In the earlier experiments I transferred single motiles to separate droplets, and re-examined in the morning. A variable proportion, usually small, do not multiply, and may lyse, and in the rest the rate of division is at first very irregular. In the morning, a proportion, about 1 in 10, of the droplets contain clones of motile cells; I have never got a "mixed" clone, i.e. both motile and non-motile sub-clones in progeny of a single motile cell, even when this has been picked as soon as motile cells were detectable. This is evidently different from what you have found with 666 as recipient.

The remainder of the droplets contain predominantly non-motile cells. From 10 to 50% contain no detectable motile cells. These I interpret as result of (i) abortive, some "motile" cells present but undetectable because stuck to an interface or wedged in a mass of non-motiles or (ii) abortive, but "trail" has ended, i.e. bearer of Fla + gene has died, or it has ceased to function or (iii) cell picked was sib or cousin of the Fla+ gene-bearer and motility was phenotypic only.

Rest of droplets contain "trail equivalents", that is large non-motile population with usually 1 to 8, rarely up to 20, motiles. When these are in turn isolated a high proportion, say 60%, given only non-motile progeny (so far as one can see). Sometimes a lot of them die, which is very annoying after one has gone to the trouble of transferring them to fresh pastures. Some again give "trail equivalents". At first I attributed the presence of several motile cells in the progeny of the original motile to phenotypic lag, but soon found that on occasion one might find more than one of the motile progeny in their turn producing "trail equivalents", i.e. "branching".

In ad hoc experiments on the same combination on gelatin agar at 37° and on semi-solid agar at 23°, I have yet to find indubitable branching though there have been suspicious appearances a few times. This suggested that cells motile in broth might not all be able to produce trails in agar; like you I found that early picked motiles transferred to gel-agar mostly produce single colonies only, or else swarms. However I did get a few trails. Have not done any more of this as it is an awkward operation on my ~~medium~~ machine. I still don't know for sure how much of the discrepancy results from occurrence of a majority of cells motile in broth but unable to move in agar, and how much from the difference in temperature.

To detect when "branching" occurred I have split up progeny of original single cells at various times, when progeny have numbered up to 20 or so; number of "trail equivalents" then obtained has varied from 1 to 5 or so, and for a long time I had no evidence that "branching" ever occurred after the fourth generation or so from time that the original cell was picked up; that is, even if the population of a drop inoculated with 1 cell isolated at the C.16-cell stage contained more than one motile cell amongst the predominant non-motiles, I never got more than one "trail equivalent" when I isolated the several motiles. I was therefore ~~induced~~ inclined to attribute the early "branching" to fragmentation into sub-units of a chromosome piece received in a "polytenic" state, or, what is perhaps indistinguishable, to the fragment being received in a "complete" state, e.g. with polypeptide "former", so as to be self-replicating, but with "former" rapidly decaying when not incorporated into chromosome, leaving "gene-oid" able to affect phenotype but not replicable.

However, I have a single instance of late multiple-branching which I think shows that there must sometimes be late, irregular, replication ~~and~~ division, for otherwise one would need to postulate that the initial fragment was very polytenic. This experiment became rather complex in the course of time, howevér, I will try to give essentials only. A motile cell was picked; later it had produced 8 progeny, all motile, which were separated. 3 of these gave only non-motiles, 4 gave the ordinary "trail equivalents" with no evidence of "branching". The 8th. cell (No.1e) produced about 14 progeny including 3 motiles which were isolated; the residual 11 cells later produced several more motile cells, four of which gave "trail equivalents" when isolated; 2 of the 3 motiles isolated from 1 e produced ordinary "trail equivalents", (So far 10 trails), the third, (cell 99a) gave offspring which were separated at 9-cell stage; 4 gave ordinary trail equivalents, 2 gave non-motiles only, 2 died, the 9th. (cell 98a) was exceptional, for after overnight incubation, it had produced a large non-motile population plus estimated 50 motiles. 42 of these motiles were isolated, and produced one exceptional (cell 93b), at least 22 trail-equivalents, the rest non-motiles or lysed. 93b produced after overnight incubation non-motile population plus estimated 100 motiles, of which I isolated at least 70, from which I got some 30 "trail equivalents" but no more "exceptionals" Experiment broke down at this time; very tiring performance. I interpret that there are T trail producer cells, segregating T and normal (i.e. parental Fla-)

Asheshov is now here: Felix moves here next month. I send you, by sea mail, some more reprint cards from US readers.

each time they divide, i.e. the original hypothesis, (plus some phenotypic lag) and E cells which produce in their progeny a high proportion of T cells, and very few, perhaps one, E cell (perhaps an E and a T at each cell division) and which perhaps finally decay into T cells, so that original motile cells are E cells but usually decay in a very few generations to T. For explanation of why, in my material, the decision as to production of motile clone or trail is apparently already taken at time first motile cells appear I rather lean on the speculation that foreign fragment is incorporated into replica chromosome either including Fla locus in continuity, to give swarm, or if ~~some~~ replication is already well advanced, with part in continuity and a section excluded, forming side-branch, including Fla locus. I don't know if this can be fixed up on W-C hypothesis of DNA, nor do I see how to account for T and E cells. I suppose it is possible that T cells are motile only in broth, and that trails are path of E cells.

There are various technical difficulties I have not solved in micromanipulation, in particular, when I put a motile cell in a droplet of fresh broth it usually gets stuck to one or other interface within a few minutes. I have tried adding albumin, or Tween, neither helps.

We have various other things in progress. I am having all the non-allelic Fla-strains screened for possible linkage of Fla and S^R loci; we have had "doubles" from SW541 and SW578 but probably from co-incidence, we are looking into this. I hope to do the same with an $A_Z{}^R$ marker but having trouble getting correct Azide concentration. Also looking into colicine resistance and resistance to virulent phage (BF23) from Frederiq as markers whose transfer to wild-type might be screened for. Thats about all I think.

Yours sincerely,

B.Stocker.

I shall send a carbon of this to Norton also.
I hope your meeting goes well. I intend to try the 543-28-553 story on a Genetical Soc. meeting here next month, partly to see how it sounds. Might also perhaps send note to MGB on it. I take it I may quote your earlier results on 543 × 553 interactions if this should prove appropriate. If we can get consistent results for mapping of 544 and 966 loci also I suppose the time will have come to consider publication. As it was your idea originally to use the H₁-linked Fla loci for mapping, would you be interested in idea of joint paper, incorporating your results also? In any event I think that soon, if we can improve the degree of certainty in a few very infertile combinations, there should be a note from here on the dozen or so non-allelic Fla loci and 3 Pal. loci now demonstrated. We must confer on numbering again. My love to Esther. How is house? yrs Bruce