P.S. I forgot to say that Hfr behaves like F+ in relation to SM. Some of the Hfr prototrophs from the M selected S^rHfr X F- crosses were S^s. I am getting very involved with teaching and routine work and will not be able to do much more with K-12.

Department of Bacteriology.

9th.March,1953.

My dear Jim,

I have some news for you which certainly confirms the separateness of the TL-Az-Lac chromosome and also throws some light on Hfr behaviour though I am not clear yet as to what it all means. I will try to put it in a nutshell and supply details when next I see you. You remember that I got an Hfr strain arising spontaneously in a 58-161/sp/F+ culture,and was checking on its behaviour. Among other things I found that its fertility was not increased by UV(unlike that of the parent F+ strain) and that it formed an F- phenocopy to the same extent as the F+ strain. By crossing the F- phenocopy(Lac+Mal+S^r) with W677/F+ on SM-minimal agar I obtained prototrophs which were Lac+ Mal+Sr,in the formation of which I assumed Hfr had acted as acceptor so that these prototrophs should have been Hfr. When,however,I tested them against 58-161/F- they all behaved as F+. I then tested two Lac-Mal+S^r prototrophs obtained from the same cross against Y-10(TLB_1-Lac+) and both showed Hfr behaviour,though again only F+ behaviour was obtained against 58/F-. It looked as if Hfr behaviour was only associated with the TL→Lac chromosome,normal F+ behaviour being shown when M was selected for. To check this I put up two experiments:

1. A Lac+B_1- "Hfr" prototroph was crossed with an F- phenocopy of W-705(Lac-M-), a. on MA + M + Lac ... Lac selected for;
   b. on MA + Glcuose ... M selected for.
   Cross a. showed Hfr behaviour & cross b. normal F+ behaviour.
2. Auxotrophic 58/Hfr was crossed with W677 on 3 media:
   a. MA + B_1 ... TL selected for ..... Hfr behaviour.
   b. MA + TLB_1 + Lac ... Lac selected for ..... Hfr behaviour.
   c. MA alone ... B_1 selected for as well as TL ..... instead of the prototroph count being reduced to approx.1/10-1/20 as in the usual F+ X F- cross,it was reduced to 1/1000.

It therefore seems clear that Hfr is only associated with the TL-Lac chromosome and not with B_1-M. This fits the segregation data for Hfr X W677/F- crosses. I have now scored 300 prototrophs for Lac,Mal,Az,SM & B_1. The Lac & Az crossovers are normal,but there were no SM or B_1 crossovers at all,and only one Mal(and this might have been a mutation),as against the usual 2-10%.

I then crossed one of my Lac-Hfr prototrophs, before and after UV, on MA + B_1 + Lac with Y-10(Lac+TLB_1- ... TL selected for) and with 58/F-(Lac+M- ... M selected for). With TL

selection there was Hfr behaviour as before and no UV enhancement; with M selection there was F+ behaviour and a normal degree of UV enhancement. Moreover, treating the Hfr prototroph with F+ antiserum had no effect on the the Hfr recombination rate but markedly reduced recombination when M was selected.

And now comes the crux of the matter. As you know, in the Hfr X F- cross none of the prototrophs are either F+ or Hfr. I expected to find that, when selection was made for a chromosome showing normal F+ behaviour, the prototrophs would be F+. Thus the "bound form" of F+ in Hfr would be revealed though why it is not transduced by Hfr strains would still be a mystery. In fact, about 5-10% of prototrophs from two distinct Hfr X F- crosses, in which B_1 or M were selected for, were Hfr. An equivalent number of other prototrophs showed only a few prototroph colonies when mated with W677/F- or 58/F- —below the expected number for an F+ X F- cross; in these latter cases I have not been back to the initial prototroph culture to check it but I guess this was only a temporary F+ carriage (such as is occasionally found with filtrates) and that they would be F- on subculture. A second important point is this. As you know, when B_1 as well as TL are selected for in the usual $58/S^r/F+$ X W677/F- cross on MA alone, the number of SM & Mal crossovers is less than 10%. When the same cross is made using $58/S^r/Hfr$, selection being made for B_1 as well as TL, however, the following ratios were obtained:

Mal-S^s... 69%
Mal+S^s... 0
Mal-S^r... 13) = 31% S^r.
Mal+S^r... 18)

Lac crossovers = 29%...normal.
Only 30 prototrophs examined.

As I mentioned, in the same control cross where only TL were selected, there were no S^r prototrophs among 300 tested.
Again, in the cross "Lac-B_1-Hfr prototroph" X 58/F- on MA + B_1 + Lac (i.e. M selected), the following SM & B_1 ratios were obtained — 50 prototrophs tested:

S^sB_1+ ... 22%
S^sB_1- ... 40%
S^rB_1+ ... 8%)
S^rB_1- ...30%) = 38% S^r.

The prototroph Hfr parent was S^r, of course.

I think this work clearly shows the separateness of the TL-Az-Lac linkage group. I think it also lends weight to my vector theory. If F+ is a single cytoplasmic factor which determines ability to conjugate but can be itself transmitted by fusion without conjugation, then in Hfr strains why is it that recombination involving one or two linkage groups only occurs in 1/1000 cells that must be assumed to have conjugated normally with respect to another linkage group? One could postulate a different F+ agent for each linkage group, each with its own potentialities but this does not explain why a recombinant arising from the transmission of only one linkage group, or F- strains converted to F+ without recombination, are capable of showing recombination affecting all the linkage groups (perhaps this has'nt been adequately tested). It seems to me more plausible to suppose that Hfr is an altered F+ agent, in a new relationship with the cell such that every Hfr agent liberated is associated with the TL linkage group, but only a small proportion hitched to the other linkage groups (perhaps both together) —a proportion that can be increased by UV. I have decided to write up the whole story in detail for C.S.H. but will clearly have to present a much more condensed & dogmatic account to the meeting itself, if only for semantic reasons. I will shortly let you have a sketch of the paper. I hope to come to Cambridge about Mar.23 to have some electron microphotographs done. I am sending a copy of this to Delbruck & Cavalli. Yours,