Dear Bruce:

Got yours of the 3d. I want to reassure you that I'm still neck-deep with the b'y trails; in fact I've been able to do nothing else since the start of the year. After some fumbling again, I decided to concentrate on SW-534 —x SW543, which does give a remarkably high yield of profuse trails. Another advantage is the rarity of swarms, so one can safely economize on plates when planting single cells.

I think one just has to look closely at these trails to see that they are not unilineas, but they do give just the impression you suggest of one main track and numerous side branches. I have also had a few not very impressive successes in getting two or more trails from one clone; unimpressive, because most of these (side-)tracks are very weak. While this may weaken the accuracy, or at least the generality, of the idea that only pluri-catenate cells can form trails, they do not touch closely on the main point: is there a single primary branch in each catenate system. I have been hoping to be able to get at this by trail observations, planting sibs, etc., so far with rather poor look owing to inconsistencies in indidence of trails. But yesterday, e.g., I picked out 894 (sic) motile initials, diluted them in about .2 ml broth and plated this out in various ways. Pour plates look the most promising; the incidence of trails is about 1/8 (which is much higher than the incidence of strikingly pluricatenate clones, which is about 1/25) and their appearance under these conditionsnot at all unilinear. I have had indifferent luck imposing a definite chemotactic gradient, viz. with phenol or excess non-motiles, but shake tubes now may help. In the deep pour plates, there presumably will be no significant metabolite gradient, and the aerobic one should be minimized. One of these deep trails, by the way, counted to 125 colonies at 17 hours, with a nice size gradation of microcolonies to orient with respect to time. The distribution of smaller colonies made it quite clear, beyond the general appearance of these "clusters", that numerous side-trails are indeed formed; I think they are minimized under surface plating conditions because all the motile cells will be moving in response to a common gradient. But even these clusters do seem to show a "unilinear" trend; it will be devil of a job to pin this down as evidence of two hierarchies of unicatenation.

The trick in making so many isolations easy is just to concentrate the treated bacterial suspensions about 10x before setting up the trap drops. The motiles then come out in draves, partly, I think, from the tactic pressure as well as from there simply being more of them.

A few (incomplete) comments on yours. At (11), e.g., I think the problem is recognizing twin-trails under tactic orientation; the deep pours should help. I had misunderstood you at (12). I do have some doubts, by the way, about the randomness of distribution of few motile cells; plating out gives a direct count. Spreading on surface is essentially as efficient. 14): if you put a drop of motile Salmonella under oil about .3 mm. from a drop of .5% phenol and watch 20-40 mins: see what happens. (I picked this up accidentally while preparing some tests with motility in serum: the serum had some cresol preservative).

As to your PPS, I am only a little worried about the specificity of the srum inhibition of trails. I did not get it with i/ i—x543, or some similar thing. It should be shown that i or b will inhibit trails from i—x SW543, but not, say, a—x SW-543, and vice versa, and I have not done this. I don't think this necessarily proves that the H_1 and Fla_1 factors continue to be associated on a single fragment, only that the initial cell does receive both. This would be worth pursuing, however, as a possible distinction (hypothetically) between pluricatenate "F" cells, and unicatenates. Indeed if unicatenates of SW553 are more vigorously motile, this might explain whey they were less affec-

This is just an interim note. More. much more will follow.

Wearily,

[signature]

P.S. Who is spreading that bearded story of my visiting Israel? Probably the [illegible] — Sol Spiegelman — who is supposed to [illegible]. J.