January 6, 1950.

Mr. Gordon Allen,
155 Corona Avenue,
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Dear Gordon:

There have been very few published accounts of recombination by other workers, and such as there are, may mention the experiments rather casually. However, there can be cited:

Haas, et al PNAS 34: 229, 1948
Cavalli and Heslot, Nature (current— I haven't seen it yet)
Newcombe Rec. Gen, Soc. Amer. 1949.

In addition, as you must know, there is unpublished work at Columbia (Tom Nelson), Chicago (Novick and Szilard), Indiana (Miss Kahn) and Cold Spring Harbor (Demerec), and New York (Allen).

The "Hfr" strain is from K-12 (58-161). Its present status is doubtful, as the effects may be due to syntrophic efficiency rather than sexual potency. I am inclined to accept, however, that it may be 100 - 1000 x as active as standard stocks, under optimum conditions. He wrote last month that in the cross S^r x Az^r (i.e., using inhibitor selection) that an augmentation of ~~a~~ 100x was found, and this should be more or less definite. I think the question is still open whether the Hfr effect is oppositional — i.e., in the direction of heterothallism.

As to outcrossing, Cavallis strain "123", which I have put down as W-1258, very definitely crosses with K-12. However, except for a rather complex nutrition (3 amino acids +? 1 vitamin, not yet run down), W-1258 does not greatly differ from K-12. I have an additional strain, W-1113 isolated from chicks, auxotroph mutants from which appear to cross with K-12 mutants. This strain differs in many respects from K-12, but the yields on crossing are very low. In addition, Norton Zinder has <u>one</u> experiment in which "<u>Salmonella coli</u>" (i.e., Vi antigen) has crossed with K-12. If all these are correct, the score is about 4/ 10 $\pm$. I would not completely dismiss the so far negative results, however, Zinder also has promising, but still not yet conclusive, evidence of recombination in several other Salmonella species. That is, occasional prototrophs, but not yet satisfactory recombination of unselected markers.

Concerning 4-strand crossing-over:

On EMS Lac or Mal, a small percentage of prototrophs are found which are obviously sectored (~~xxxxxx~~ diametrically), containing a Lac+ and a Lac- component. They occur too frequently, on dilute plates, I think to be entirely explainable as coincident colonies. The problem is to decide whether they are the issue of the same zygote! Since so many cells are obviously multinucleate, it would seem to be possible for a single fusion to result in a binucleate diploid cell. Alternatively, the zygote nucleus might exceptionally undergo one mitosis-- we know that about .1% of the prototrophs may be diploid even in non-Het crosses.

In some runs, I think there has been a significant correlation between the components of a "duplex" prototroph, suggesting interference in crossing-over, and supporting their origin from the same segregating nucleus. (And in turn, of course, 4-strands). However, these studies involved only Mal and Lac, and, as you know, I am deeply suspicious of anything that depends on Mal. The problem should be gone into more deeply, using a larger array of characters. Aother point that has to be controlled is the possiblity of reverse mutations on the plates. However, I think this is unlikely, but as far as possible, stable allels should be used. For example, (Exp. 636;10/25/49):

58-161 x W-677 on EMS Mal. 6 duplex colonies/ "several hundred" (heavy background)

The twins are reported in order, resp.

	Mal	Lac	Xyl	Mtl	T5
1	+ -	+ +	--	--	ss
2	+ -	- -	+ -	+ -	ss
3	+ -	+ +	- -	- -	ss
4	+ -	- -	- -	- 0	ss
5	+ -	+ -	+ -	+ -	ss
6	+ -	+ -	+ -	+ -	ss

The first 4 pairs are indubitably correlated re Lac and T5. I also have rather more extensive data of the same kind, but without Xyl and Mtl.

EMS Mal has been used here, because the largest number of duplex prototrophs is found on this medium. With Mal, containing as many as 20% of the + segregants may be duplex. I've just found the earlier data (45 twins) They are distributed (Lac and Mal):

	M-L-	M-L+
M+L-	19	5
M+L+	4	17

This would speak for rather intense coincidence of double crossing over, but you can see why reversion has to be eliminated, although I don't think it is at work here.

If you would be interested to take up this problem, I would be very pleased to send you any additionallstocks that might be useful (stable Lac-; multiple marker, etc.) I have been thinking of dabbling in it further myself, but I think it might be just the thing to divert you, and provide a distinct fact-grubbing problem (vs. shot in the dark) to push concurrently with the meningococci. It ought especially to be done with the Lac+- twins which occur at ca. 1% on EMS Lac B_1.

There is some other evidence for 4-strands from the diploids. Not infrequently, the diploids recevered show "double-reduction", e.g., are homozygous Lac- where the parents were different. This indicates a) crossing-over from a 4-strand system in the formation and recovery of the "diploids" -- i.e., they are not merely the persistent zygotes, or b) a complex compression of a series of fusion and reduction cycles. In either case, there is very little precedent! I have to look at the "spontaneous" diploids to see whether they may be doubly reduced.