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My dear Lederberg,

Thank you for your letter and for your strains, which arrived in excellent conditions. Since when I wrote you, the small capacity of my laboratory has been entirely absorbed by the new strains, sh that I have nothing to add concerning mapping work. However, I am giving in an appendix data concerning W 836 crosses. I forgot to tell you in my last letter, concerning mapping work, that I mapped some time ago an azide-resistant mutant, which was localized between V_1 and TL. A chloromycetin-resistant mutant showed to be roughly in the same region (but in the latter case, selection was by successive subculturings and more than one locus or step may be involved). While chloromycetin resistance work is being continued (selection by successive transfers shows a nearly perfectly continuous increase of resistance!) I have discontinued azide-resistance, because it seemed to me that there is too little a gap between sensitiveness and resistance . Chloromycetin resistance was so far useless for selection of recombinants according to your streptomycin-azide method.

Re W 1113 strain, I had little experience with it, since crossings to K12 always yielded very few or no prototrophs. I have never tested them with sugars, so that I could not tell you about them much more than that. I dropped work with W 1113 because I found so little antigenic difference between it and K-12. If you are interested in a confirmation, I shall repeat these crosses, which appeared to me to give some, although scanty, results.

I am trying again, however.

The new strains have been rather deceptive. Finding now marked antigenic difference between first interfertile strains known at that time, we set up a patient search of fertile strains among coli-strains known to be antigenically different. Eventually, two were found (marked by Kauffmann O-antigens 3 and 5) that seemed to give consistently miriads of prototrophs, when crossed at high titres. On dilution, a smaller number of "prototrophs" appeared, but these colonies, which I should call "pseudoprototrophs", were always small, not greater than 1 mm in diameter, and grew slowly and badly, or not at all, on transplantation to fresh minimal medium.

Marking with sugars has confirmed suspects, that no true recombination is probably taking place among them. At present, two two-steps biochemical mutants are available for each of the two strains 3 and 5 ; pseudoprototrophs are formed in the cross within coli 3, not within coli 5 ; and in three out of the four possible crosses between 3 and 5, with these strains. What these pseudoprototrophs are, if recombination will be entirely excluded, I could not say ; I have been thinking of unstable heterokarions, although the mode of division of coli seems to prevent the possibility of formation of heterokarions having a minimum of stability?. Association with perhaps partial back mutation seems then the only alternative. I hope to be able to decide soon between extracellular or intracellular syntrophism. Controls of the strains are satisfactory, of course.

Although deceptive from the recombinational point of view, at least so far, these "crosses" have been found exciting from the antigenic point of view. For instance, five out of six pseudoprototrophs better than the others were found to have - and keep after six successive platings on complete - the antigenic reactivity of both parental strains. Decision between recombination, cytoplasmic inheritance, or extracellular transformation partly depends on the decision about the nature of these "prototrophs". I hope you will not mind receiving information of a research which is still at such an early stage . It will help me to know if you have any experience of such pseudoprototrophs. I have an impression that some of the smaller prototrophs in K12 crosses may be of the same type.

I found an early nitrogen mustard resistant mutant, in K12 which is incapable of crossing, to be non-motile. Unfortunately, decisions on motility are not the easiest, in coli, and flagella staining not very satisfactory.

Yours sincerely

Luigi Gallo.

SUMMARY OF OUTCROSSES TO W 836

a) Cross W 705 x W 836

Lac	V_1	Gal	Mal	Xyl	No. prototrophs	Exp.	c.o. (additional to c.o. between M and MlyT)
-	r	-	+	+	313	314.0	none
+	s	+	+	+	69	68.4	I
+	s	-	+	+	3	7.97	II
-	s	-	+	+	15	12.8	III
-	r	+	+	+	3	1.7	I, II
+	r	+	+	+	1	2.8	I, III
+	r	-	+	+	4	.3	II, III
-	s	+	+	+	0	.01	I, II, III
					408		

Of 408 prototrophs, 162 from plates supplemented with tryptohane; none was Tr-. Expectations calc. on basis of order: M-MlyT-Gal-Lac-V_1,
I II III

b) Cross W 705 x W 677

Of 108 prototrophs, all Gal- ; 24 Mal+, 14 Xyl+.

c) Cross W 677 x W 836

Lac	V_1	Gal	No. of prototrophs no addition	No. of prototrophs with B_1	c.o.
+	s	+	25	8	I
+	s	-	52	25	II
-	s	-	70	69	III
-	r	-	31	79	IV
-	s	+	6	1	I, II, III
-	r	+	1	0	I, II, IV
+	r	+	0	0	I, III, IV
+	r	-	1	0	II, III, IV
			186	182 of which 18 are B_1+	

Among all B_1+ in cross with B_1, 11 are Lac+V_1^s.

C.o. regions given assuming order : MlyT B_1- MlyTr- Gal - Lac - V_1 - LT
I or II III IV

Other possible order : B_1GalMlyTLacV_1LT, then strong/negative interference between Gal-MlyTr and MlyTr-Lac.
Data available for Mal and Mtl show linkage, not complete, with Gal.

Orders proposed : W 705 and W 836 : M-MlyTr-Gal-Lac-V_1
W 677 : B_1Gal(Mal etc.)MlyTr-M-Lac-V_1-LT %

The major difficulty encountered in assuming the same order, i, e, M-Gal-Lac-V_1-LT for all the three strains is in the comparison of frequencies of c.o. for the same regions in different crosses. For instance, M-Gal is greatly exaggerated in one instance and depressed in the other. Also, there always is negative interference between B_1-M and M-Lac in any cross where such regions are marked. It could be explained by double c.o. within the inversion loop.

Double c.o. in the inversion loop could also explain partial linkage of Mal, Gal, Mtl, Xyl etc.