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19 June 13, 1958. REF: 1423 B

POURED AGAR AS A MEANS
OF DELAYING INTERRUPTION
AND AVOIDING PLATE RECOMB.

ORC W3870, W3064, spun, resusp in chilled water 20x.
♀ susp. had to be blended because of coarse agglutination.

0': plate recombination. 0.1 ml ♂ + 0.1 ml ♀ + 4 ml broth, in ice bath → .05 plated on DSmB1, surface and poured agar.

↓ 1/10 H_2O
1/10 → plated as above
↓ 1/10
1/100 → plated as above.

↓ incubated in waterbath and sampled at:
13', 40', 120', 240'.

0.1 ml samples diluted 1/100 in chilled water and a fraction blended. Unblended and blended fractions plated, .05 in DSmB1, on surface and poured.

note linear increase of prototrophs

enter slow on this expt!

Replicate [illegible] Sp [illegible] [illegible]

	Spread	Pour		B spr.	B pour
13	0 0	14, 11		1 0	0 0
40	42, 49	40, 60		40 49	40 35
120	107			115	
240	223			223 (sic)	

Spread equivalent to blend!

	Spread	Pour plate
0	196, 202	~~[illegible]~~ 44, 64
0 1/10	1, 0	0, 1
0 1/100	1, 0	0, 0

∴ pour plating gives some diminution in plate recombinants (possibly compensated by not breaking up nonspecific agglutinations) but not a great deal. This is not a serious problem with this particular cross. (cf 1/100 dilutions of 13' etc)

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1423B

19 REF:

1	2	3	4	5	6	7	8	9	10

Blended

t'	Sr.P	SRP	Gal+	Ratio	Lac+/among Gal+	Lac+/among Gal-
13	S	0	—	—		
		1	0/1 —	—		
13	P	0	—	—		
		0	—	—		
40	S	40	0/Σ	.011		
		49	0/Σ			
40	P	40	0/9	.014	1/1	13/66
		35	1/67			
120	S	115	5/Σ	.036	5/5	~~[illegible]~~
		81	2/Σ		5/5	
120	P	90	6/180	.033	6/6	21/94
		90				
240	S	223	33/Σ	.137	27/29	
		216	27/Σ			
240	P	237	16/94	.17	14/16	28/79
		256				

Conclusions: the 4 treatments (blend or not)·(pour vs. surface) are concordant for SRP and Gal ratio except:

(1): SRP, pour, unblended is consistently ≧, esp at 120 minutes.

(2) The Gal ratio of this class at 13' is exceptionally high

(3) at 13' only this class shows SRP – others presumably interrupted very nearly at "first entry" of TL++.

(4) Plate recombinants show Gal ratio about equal to 120' and << 240'

(5) Plate recombination is negligible at 1/10 and 1/100 dilutions (1/100 was exptl. plating dilution.

(6) No reliable effect of plating on increasing the Gal ratio, e.g. at 40' or at 0'. ∴ no evidence of prolonged entry

(7) High (expected) incidence of Lac+ among Gal+ moves idea of alternate entry

The following were also n.p. tested for Mal$^-$, Xyl$^-$, Mtl$^+$ and were all $^{--}$, $^+$ ...

Pseudo:	# tested
0	18
40	1
120	3
120B	5
240	20
240	8
240B	32

These markers are very low (>0?) even with prolonged entry, under conditions of SP solution.

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Comparison of pocer plating and spreading
? prolongation of mating ?

1423B

June 13 1958 REF:

1	2	3	4	5	6	7	8	9	10

Not Blended

t'	Sr.P	SRP	Gal+	Ratio	Lac+/among Gal+		Lac+/Gal−
0	S	196	9/Σ	.055	14/18	26/34	
		292	18/Σ		12/16		
0	P	44;75	0/21	.041			
		64;137	5/100		4/5		28/95
13	S	0	—	—			
		1	— 0/1	—			
13	P	11	2/25	.08	2/2		5/23
		14					
40	S	42	1/Σ	.022	1/1	2/2	
		49	1/Σ		1/1		
40	P	40	0/9 1/83	.011			
		60			1/1		17/72
120	S	107	3/Σ	.032	3/3	6/6	
		100	4/Σ		3/3		
120	P	157	3/95	.032	2/3		17/91
		178					
240	S	223	32/Σ	.147	23/31	38/51	
		192	29/Σ		15/20		
240	P	226	14/100	.14	13/14		18/94
		227					

undiluted = plate recomb.

Σ = replica test of surface plates to B Gal B, Sm. Others individually stroked to B Gal. From the same, only Gal+ tested for Lac. Replica test latter for Lac.

For further study:

reality of: (1) High Gal compared of 13' pour plate compared to 0'
(2) continued increase of Gal+ (reduction?)
(3) time function of Lac ratio among Gal+. 0S and 240S both have
(4) other markers, especially 0 and 240. a rather lower Lac ratio than, say, 240P.

Sc 1417

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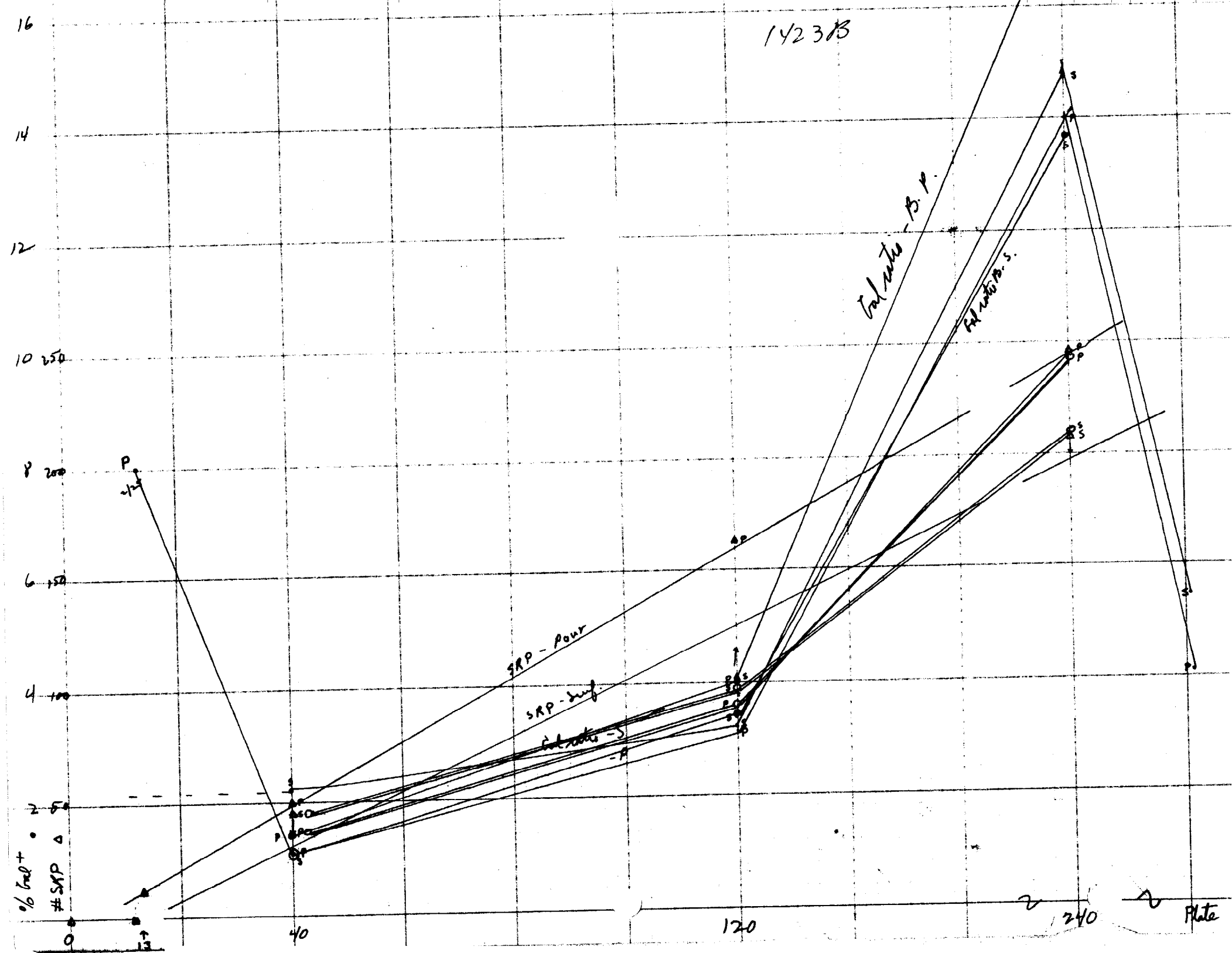
1423B

19 REF:

	1	2	3	4	5	6	7	8	9	10
	Lac+ ratios among Gal+.						Gal−			
O.P.	1/2	4/5 ✓					15/48	28/95		
P.	3/3						13/47		+ 1 Gal± Lac±	
40	1/1	1/1 ✓					14/~~50~~ 49	17/72 ✓		
	0						3/23			
40B	1/1	1/1 ✓					9/49	13/66 ✓		
	0						4/17			
120	2/3	2/3					6/41	17/91		
	0						11/50			
120B	3/3 (sci)	1 new +					10/47	21/94 ✓		
	3/3	6/6 ✓					11/47			
240	6/8	13/14					10/50	18/94		
	5/6						18/44			
240B	5/8	14/17 ✓					15/39	28/79 ✓		
	9/9						13/40			

S.



1423B
16
14
12
10 250
8 200
6 150
4 100
2 50
% Gal+ •
# SRP △
0
13
40
120
240
Plate
Gal ratio - B.P.
Gal ratio B.S.
SRP - Pour
SRP - Surf.
Gal ratio - S
- P

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Screen Hfrs.

1424A

June 20 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	available strains:			F⁻ = ~~W3044~~ W3991 × Hfr x.				(all are M⁻ S^s)		
	Hfr	W		0'	10'	20'	30'			
1	2	3870		+±	±	±	++			
3	8	3889		±	0	+	0			
5	13	3200		±	0	0	±			
6	15	3885		±	1	0	2			
• 7	16	3886		~~+~~ +	++	+	++			
8	18	3887		0	±	0	++			
• 2	7	3888		±	++ heavy background	3	4			
• 4	11	3890		1	+±	0	0			

parents ORC penassay, 20x chilled .1 + .1 + 4 ml. dispense 1 ml to each of 4 tubes. Time by transfer 0° → 37° → 0° (1 hr heat each time: 0', 10', 20', 30'. 0' is undiluted (plate ice.) 10-20-30 dilute 1:100 and plate .05 ml on M Cal B, sm (as on hand).

+ >10 ++ ~100 Recombinants as rather small papillae in most cases.

#11, 0 #16 and #7 appear most promising in this screening: note that both show a considerable decrease after 10 minutes. This might be due to the segregation of M⁻ in absence of streptomycin. Spreading and elimination of plate recombinants may have been important in this crude screening trial.

Purpose: for mapping Cal it would be best to have an Hfr that shows early entry of Cal. W3991 gives very weak Cal⁺. Try W3119

Note – Cistron says W2945 (Hfr 6) is high on all Cal x except Cal 3!

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Recheck timing of Hfrs.

x W3119

1424B

26 Jun 19 58 REF: A.

ORC, chilled, 20x ~~.05 + .0~~ ~~[illegible]~~ .1 + .1 + 5 ml pinassay. Time by diluting into cold water .1 ml/10. Plate .05 ml on MGal B, sm... ~~[illegible]~~.

0, 5, 10, 20 minutes. Count Gal+

	0	5	10	15	~~20~~	00
A W3945 = Hfr6	~30	1	6	13		~10^4
B W3886	0	1	0	0		10^2
C 3888	0	0	8 (BGal)	0		15
D 3890	0	0	6 (BGal)	0		26.

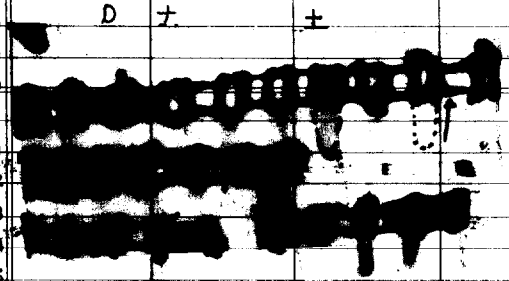
Note high "0" for W2945.

This is not a very satisfactory experiment, as it does not indicate any particular timing for the Gal's and the yields are very low. The high "0" may be real — possible significance? —; Gal-S linkage perhaps. A and B should be repeated to longer times.

Inconclusive but Hfr6 and W3886 should be studied further.

Cross Breach tests on 20 single colony isolates. All colonies homogeneous.
In 24 hours, W3945 gave very high response! (This is Alan's Hfr6 and is unique for Gal)
(It is roughly complementary to Hfr1).

	W3119 MGal	W3064 DsmB.
A	++++ (sic)	—
B	++	++
C	+	±
D	±	±



3945
3890
3888
3886
3886

No evidence of reversion to F+ among any of these (20 tests each)

2½ days

Main purposes: to validate a better Hfr timing system for Gal markers. Hfr2 is rather late for Gal.

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Hfr4 (pulsed 10 m.)

1424C

June 27 1958

REF:

W3752 = Hfr4 X+ S^s. x W296'

ORC Mix .1:.1: 2ml D(0); ~~.15ml to 10ml test tubes~~ t' = 0, 10, 22 ...

at t = 10', add 10ml warm D(0) and plate samples from time to time

P = predilutid (check on the pulse). (diluted after ~10 seconds, incl. agitation).

	SRP Dsm B1	M Gal sm B1	Gal	Mal all +	Xyl
00	~200	0			2?
10	0, 0				
22	1, 0				
30	0, 0				
35	0, 0				
40	2, 1	0			
50	6, 5	0			
60	27,	0	3		1
75	35, 46	0	8, 4		1, 2
P10	4				
P60	1, 1	0			
P75	2	0	1/2 - 2/3		
24	C				0

P29. Save for tests of other markers: B1; Mal; Xyl; Lac; ara; Gal assumed 0!

? Does Hfr4 go to Gal end? Any markers very late? TL entry is at about 60 minutes! (Could test chloramphenicol here simply by SRP count if desired) Gal ratio at this time must be very low, or 0.

Note: use of D(0) may have reduced yields.

notes send ✓ Please Thermofax A-L. June 30 1958

1401-2 Best pulse for T, L, V, Lac. Good test of interruption.

-3 P^+ selection; also delay in T, L. ? different F^- different pattern. These were one-hour cultures! Cannot be ascribed to non freshness. [discussion of lag in temperature equilibrium and initiation of male fertility

-4 Compared 3052 v. 3064 as F^-: no difference in time. Separately and a differential (Ara, $^-$) marker test

-5 Hfr_1 v. Hfr_2 × W3052. Preliminary. P-L-T comparisons. But the 15' time for Hfr_1 was irregular + rejected. $t(T) = 5'$ $t(L) = 7'$ $t(P) = 12-13'$. Leucine-sensitivity?

-6 Repetition.

1402 - Pulsing

1. Various concentrations of cells. See drawing. "0" is predilected. Unselected markers: dilution and incubation does not interrupt at 1:100 dilution, mating was only 10^{-1} less. No compliance with product.

2. Try to concentrate above "standard". Some increase noted but not product. 10× conc. → 4× recombinants.

5/14 3. Pulse in various media (various cultures). Buffers, penassay, predilution controls .... (many experiments were not properly controlled.) Hfr_1: very few recombinants - expt. n.g. Pulsing gave lower yields. Overcom. conditions are inhibitory. Cells were conc'd into penassay and respun. Too high conc. (?) makes this a poor test of optimum medium.

5/21 4. Repeat, keeping cells cold. Compared penassay BGA (buffer, glucose, asparagine) and ~~D(o)~~ etc. No great difference. But too many recombinants were observed even at 10^{-4} dilution for accurate pulsing. Clarification of ABCDE on record. No inference possible for BGA. penassay failure at 10^{-4}!

1402-5 Attempted repetition of pulse for step of TL^+. But 5x increase of X^+ 20'-60'. Was under misconception of pulse in 1402-1. 1402-1 may have been pulsed by saturation.

5/29. -6. Trial improvement by using BGA for entry after penassay mating. No evident effect on step 20'-40'.

1403- 1 Manipulations. Hfr → F^+ N.G.
2 See protocol
3 How to plate s/interruption. See also 1423B
4 Do stocks vary in stability to plating? See 1410-1. ~~There is~~

1404 F^+. Hfr. F^- Test for cross infection of F^- progeny on plates. So far inconclusive a/c too low fertility of F^+ and poor design of F testing.

1405 Protoplasts ♂ and ♀
1. Interruption by lysis of ♂ protoplasts. Preliminary
2. 4 combinations of (♂.♀)(p.B). Hfr → F^+ n.g.
4.14. 3 Repeat 1. Best experiment! Do protoplasts show delay in Gal (and Lac) or spontaneous interruption and lysis before Gal? Gal remained 0 to 60' See 1405-6
4.14 4. protoplast x protoplast. Based on auxotrophy of W3060. An interesting result: crosses on sucrose B, gave very high male markers (poor conditions of selection). Worry again about nutrition of W3060.

1405-5. Protoplast crosses. Repeat 1405-4. High residual viability. Still selecting on sucrose B_1. No conclusions

4.19.58 -6 Repeat -3. Effect of incubating shorland suspensions. No effect on enlarged scale. Good for interruption by lysis

-7 Osmotic shock on rods. No interruption of rods ♂-♀. (rods only).

-8 repeat -4

1406. 4.16. HB Conditions of mating: age of cells. No difference between fresh and old in yield; not obvious for timing either.

1407 EML Transfer of Lp^+

1408 -1 EML JL. Gal timing. -1 washout.

-2 Showed linkage of try-gal. (Luca knew of Hfr-try linkage).

3 Various Hfr's - intensity of linkage of Gal-try.

4 Gal timing. Hfr_2 Preliminary only.

5 Hfr_2. Close timing. Discarded a/c contamination

6 $Hfr_{4,13}$. Preliminary. Try enters at 15'

7 Hfr_{13} More accurate. " " " 13'. Exponential kinetics

8 } Hfr_2 Try-Gal mapping. ? Two modes of entry.

9 } Early rise, later more rapid.

10 Hfr_2 Gal_2. See disc. of two modes of entry.

11 Hfr_1: Gal timing. Very late entry.

12. Repeat: no effect

1408-13 Hfr_2 - compare two Gals. Low numbers: F^+ rev.
$Gal_2 < Gal_1$.

..... Current Galtimings in EML hands

-20 No evidence of early slow rise in try. Probably was due to growth of plate recomb.

1409-1 Enzymes on protoplasts.

-2 " " mating. (?? slight effect of lysozyme).

1410- Diploids send interpretative summary when ready.

1411- Colchicine no effect.

1412- Freezing. Parents OK.
Need to summarize and compare B1-B2.

1413-1 Reblending — differential F^-
See record. Plate Rec. probably acct. for the prototrophs seen.
But unselected markers showed interruption.

-2 Two stage transfer.

-3 Exhaust males by excess F^-? (to use them for 2' stage transfer to a new batch.) Required 20' to exhaust, which is too long to use most convenient available markers

-4 Increase recruitment s/entry. Temperature. 37° optimal. zero rate at 0°.

-5 } Crosses were altogether - N.G. W3060 → F^+.
-6 } N.G. " " "

-7 Check selection for $Lac^+ S^R$; $Gal^+ S^R$ on W2323. Blacson. Rather few!

-8. Test for suppression. (Reconstruction).

-9 Repeat -1. No reblend recombinants.

-10 Repeat 2. Two stage transfer. Negative.

1415 - Need to correlate notes Azide

General conclusion: no differential effect.

No definite verification of reversible inhibition.

-3 concentration too low, no effect. Azide .2% in D(asp.) acetate

-4 necessary conc. to inhibit. Some effect at 10^{-4} - 10^{-3}. Still no prevention of Lac^+ entry. Gal excluded. (slow action?)

-5 Azide + DNP - 3% inhibits mating. DNP - M/200.

maybe reversible (no evidence on new recruitment).

-6 R x S; S x R. Inconclusive. Graded effect. Counts too high in controls

-7 Differential concentration looked for. Stratified fertility of crosses!

- Nothing more on Azide-resistance.

DNP^R — see record. - low degree of resistance. .2-.5 ml/20 ml agar of M/20 DNP see 1419.

1416 - DNA leakage. - some leakage in every case.

240-260 mμ difference.

1417 - Timing of Hfr_1. TL: ~20'

-2 TL ~<20' Th very late ... 60' Cf 1401-5. Thus 35-40'.

In Progress

-3 (JL) - Time entry of Mal, Xyl...; effect of chloramphenicol

1418 - DNA → F^+ mating with F^- protoplasts.

1419 - DNP-resistance

1420 - Pulsation.

- B - periodate "pulse" } n.g. $Hfr \rightarrow F^+$
- C " "

- D. Effect of F^- cells. (high density!) Apparent interruption

- E " " (lower density). Confused?? Cells does not interrupt, pipetting controls does!

– E. Gal' counted on S Galsen B1. Good internal consistency – Luca repeat!

1421 – Periodate I.S.

C – will be finished by I.S. Collate protocols
– treatment of F^+; and effect on F infection etc. – look for plates

D. – Other oxidants. Rather low yields

E. – RDE. No Ca^{++} used see 1422
RDE may still be open question. Not yet used Eric French's.

F. –

1422. RDE + Ca^{++} No yields: inconclusive

1423 Delay interruption c/ chloramphenicol.
A. 50 v/ml. Maybe bactericidal; no proliferation. No evidence of any effect on Gal and Lac entry. Part II OK.
B. Pour plating to delay interruption. No interruption; no prolongation

1424

Yudkin's E. coli: to make protoplasts L2, double strength; 300 – 1000 u/ml pc.
Bursts more readily. (to prepare DNA from W3514 S^R).
→ W ___.

Experiments Still Pending July 1958.

See 1410 diploids for bulk of experiments of May - June 1958. Then 1426 - 1427.

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Analyses of Gal$^+$ × Gal$^-$ diploid
Relationship of two modes of segregation
Segregation of 1410F48.

[1410C] [
1426 ←

1 July 1958

REF: 1410C1T(1-7)

7 Gal$^+$ heterogenotes have been isolated from 1410C1. Label these now 1426 A–G

Each derives from an Ara^v colony picked to D(Ara B,) and retrospectively verified as Gal$^+$. From the same streaks, Gal$^-$ were picked to EMB Ara to verify (qualitative) independence of Gal and Ara segregation. Results: (of Gal$^-$ tested)

B 5/5 Ara$^-$
C 4/6
D 3/6
E 1/3
F 5/9.

Ara^v Gal$^{t\,v}$ → Ara^v Gal^{t-}
→ Ara$^-$ Gal^{t-}
→ Ara$^-$ Gal$^{t\,v}$ presumed from consequences of earlier streaking.

The new labels (1426 (A–G)) now refer to the D(Ara) bottles mentioned above. all are still Gal$^+$ Ara^v

1 July: plate out A and B on BAra.

2 July: Streakout individual Ara^v on BAra for examination of single, discrete segregants.

Repick groups of 4 Ara$^-$ from each Ara^v streakout → BGal. see next page (July 4)

also from first plating, Ara$^-$ picked to BGal and Blac.

	G	L		G	L		G	L		G	L
A 1	+	−				B	−	−	8 B	+	−
2	+	−					+	+	9	−	−
3	+	−					+	−	10	−	−
4	+	−					+	v	11	−	−
5	+	v	→ Lac^v Ara^v				+	−	12	−	−
6	+	−					+	−	13	+	v → Lac$^+$ Ara$^-$
7	+	v	→ Lac^v Ara^v				+	−	14	+	v → Lac^v Ara^v

? particles for purity.

Thus most Ara$^-$ lines are Lac$^-$.

Are these Lac^v really Ara$^-$? As streakout, 1 was Ara$^-$ Lac$^+$, 3 were Ara^v Lac^v and had been simply suspected as Ara$^-$. There may be some position effect as some Ara^v have microscopic central spots.

Conclusions (two pp.) Segregation of heterozygote + exogenote occur independently, as if

Data are not sufficient to establish statistical independence. Among Ara$^-$ incidence of Gal$^-$ was 3/44 and 12/84 respectively in segregation of single Ara^v colonies. No data for the Ara^v sibs in same colonies. Much higher incidence of Gal$^-$ Ara^v in several bottom generations (2/13; 13/32).
Defer this discussion to a more suitable method: diploid + prototroph a Gal$^-$ hemozygote!

Analysis of a heterogenotic heterozygote:

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$\frac{\quad\quad}{\text{Ara TLP}}$ $\frac{+}{\text{Gal}_2\ \text{S}}$

1426.

July 4 1958. REF:

Analysis of A and B. Is there linkage of the Gal factors to Lac or Ara?

A.13 Ara^v Stock cultures were plated on B Ara. Single Ara^v were streaked out on B Ara, and
B.32 Ara^v 4 Ara$^-$ ~~picked~~ streaked from each streakout to B Gal, together with mass brush.

A: 2/13 Ara^v were pure Gal$^-$ (already segregated). Of the other 11, there was one 3$^+$:1$^-$ and one 2$^+$:2$^-$ split among the 4-sets of Ara$^-$. ∴ Total incidence of Gal$^-$ among Ara$^-$ (recently derived from Ara^v) was 3/44.

B. 11/32 Ara^v were pure Gal$^-$. Where 4-set derived from Ara^v Gal$^+$ (21 sets), the following splits:

4$^+$ 0$^-$	3$^+$ 1$^-$	2$^+$ 2$^-$	1$^+$ 3$^-$	0$^+$ 4$^-$
10	10	1	0	0

and total incidence of $^-$ here is 12/84. This suggests that B is somewhat less stable a heterogenote than A (or that A gives significant Gal$^+$ segregants) but much of this may be selection. It is evident that Ara^v Gal^v $\nearrow^{v-}\searrow$ $\nearrow\ \ \searrow$ $\searrow\ \ \nearrow$ $\searrow^{-+}\nearrow$ -- at least qualitatively independently. Replica plate to Lac to determine if there is any linkage of Gal$^+$ to Lac (viz. interaction in segregation) when both have segregated. This tells little about linkage of the exogenote to Gal ~~[illegible]~~ since all viable cells presumably have the same Gal factor.

Replica plate to EMB Lac. ~~Among the Gal~~

Gal$^-$: 2 A 4-sets pure Gal$^-$ are pure Lac$^-$; Gal$^-$ Ara^v is Lac$^\pm$

Isol Gal$^-$: 3 all Lac$^-$ Gal$^+$ Ara$^-$: All but 3 are also Lac$^-$. These 3 are Lac$^+$ Gal$^-$ [Ara$^-$] ~~are~~ two from a 2:2 split; 1 from a 1:3. ∴ The A-sets are

2 Ara^v Gal$^-$ Lac$^+$: 4 sets Ara$^-$ Gal$^-$ Lac$^-$

1 Ara^v Gal^v Lac^v : 3 Ara$^-$ Gal$^+$ Lac$^-$: 1 Ara$^-$ Gal$^+$ Lac$^+$
1 " : 2 Ara$^-$ Gal$^+$ Lac$^-$: 2 Ara$^-$ Gal$^+$ Lac$^+$

7 Ara^v Gal^v Lac^v : 4 Ara$^-$ Gal$^+$ Lac$^-$: 0 ——

(over)

B sets: All but 1 4-set are pure Lac^- This one has following elements
($Gal^+ Ara^v Lac^+$); 1 $Ara^- Gal^- Lac^-$; 1 $Ara^- Gal^+ Lac^-$; 2 $Ara^- Gal^+ Lac^+$
see below. one of these is Ara^v!

∴ again almost all Ara^- are Lac^-.

These diploids are not optimal to study segregations in any more detail; wait for development of more suitable markers (V_1; V_6; A z) But it might be worthwhile saving a set of sibling Lac^+ and Lac^-, Ara^- for typing for Ara. Spot these on B ara.

∴ Lac ratio among Ara^- (summing all colonies from Ara^v colonies)
corrected (see below)

is A) $3^+/41^-$ | ~~$2^+/41^-$~~ $2^+/41^-$

B) $2^+/128^-$ | $1^+/126^-$

But on recheck, The 4-set from A was $Ara^v Lac^+$: $Ara^- Lac^+$ (1); $2 Ara^- Lac^-$ (2, 3)

B: $Ara^- Lac^+$ (1); $Ara^v Lac^+$ (2); $Ara^- Lac^- Gal^+$, $Ara^- Lac^- Gal^-$ (3)

See 1410K for typing:

The two Lac^+ are Ara_3^-

The four Lac^- are Ara_2^-.

This further confirms the structure of this diploid as $\frac{Ara_2^- Lac^-}{Ara_3^- Lac^+}$.

no special bearing on Gal.

1410X
2 July 58

Plans for further diploids:

1. additional markers should be segregating: V_1, V_6 and perhaps Az. Possibly have P free to segregate? These will help rapid screening of heterozygosity.
2. Include possibility of selecting for Gal^V? (Introduce Gal^- into a parent).
3. Time more precisely: perhaps pulse. Measure lac and Gal concurrently
4. Select some Hfr diploids? (May need B_1^+ isolate!) — should cross to get one!

5. Set up for Ara cistron preliminaries
6. Effect of streptomycin in disturbing appearance of Gal etc.?

Plans for present diploids:

11 ~~7~~. Reexamine 2 stated pure lac^+, say F26 and F31.
Reexamine the 2 $Gal^+ lac^V$ F5, F12 | G10, 3, 13
Examine zygosity of Gal, then lac in C2, F21, F22, F23, 27, 28, 30; G1, 2, 8 21, 24 * *
Defer linkage + segregation studies. Complete F37, 38. Trace F30

12. Get an Ara^- lac^V Gal^- auxotroph to use as F^- for secondary nondisjunction: Or use Ara^V lac^-, selecting lac^+? This is messy with Gal^-, and lac not balanced. In any case, polarity of lac^+ would encourage formation of lac^+ prototrophs.

13. Other Ara solns? What to save from 1410H?

14 In G, examine for bp^h.

See also H plans.

* to D(any B_1)!

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Segregation of 1410F48

1426 C

July 11 1958.

REF:

F48 is an unusual diploid inasfar as it rarely segregates, and hardly ever ara^+. Pick 1 ara^- from streaks of individual ara^{+v} colonies and purify, s. colonies to broth, cross bush (KB) with Hfr_2 ara testers as follows:

Mara B, $\left\{ \begin{matrix} - \cdot 2 - \text{with} \\ \{1 \text{ or } 2 + 7 \text{ hyp. } 1^2\} \end{matrix} \right\}$

	W4068 ara_3	W4069 ara_4 [~ 2]	Bara	Diagnosis
1	+	−		2
2	−	++		3
3	−	++		3
4	−	++		3
5	+	−		2
6	−	++		3
7	+	−		2
8	+	−		2
9	+	−		2
0	+	−		2
	+	−		2
	+	−		2
	−	+		3
	−	++		3
	−	++		3
← 1C.	−	−		? 2,3
5	+	−		2
7	+	−		2
8	+	−		2
9	−	++		3
0	+	−		2
	+	−		2
1	−	++		3
2	−	++		3
3	−	+		3
4	−	++		3
5	−	++		3
6	−	++		3
7	−	++		3
8	+	−		2
9	+	−		2
0	+	−		2
	−	++		3
1	+	−		2
2	+++	+++	+++, − +	
3	+	−		2
4	−	++		3
5	+	−		2
6	+	−		2
7	±	++ (±)	− +	3, (+ ~~[illegible]~~
8	+	−		2
9	+	−		2
0	+	−		2
	+	−		2

Testers:

	4068	4069	
2979	++	+	
W4176 (K3)	−	++	
4177 (K4)	+++	−	
4178 (K5)	−	−	
mermaekert	−	++	3
dsoma, mar	+++		
	+++	+++	

Score: W4069 serves pro tem as tester for ara_2 pending isolation of a better one.

2^-:	24	
3^-:	18	
possible 2^-3^-:	1	

The ara^+ are mis-picks.

The 2^-3^- should be confirmed.

Save a 2^- 1426 C2
3^- 1426 C3
putative 2^-3^- 1426 C1

~~Repeat~~ Repeat.

Also 2 pure ara^+, sep. (not this series but noted in these isolations.)

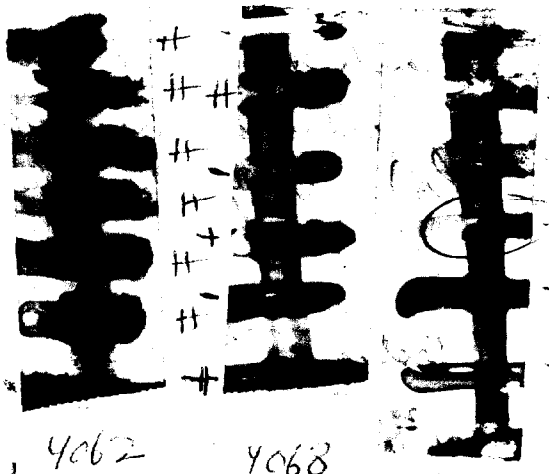
~~The so called~~ C1 might be infertile or F^+, etc., and should be checked, say, with ara^+ Hfr.

Needed for automation of bacterial technique a nutrient tape. (Cf. Rapini's ideas). This would be a marked advance over the copying to tape illustrated on this page.

July 15, 1958.

Reversals on C1-2-3

(W4163 only now available as Hfr, Ara2



- C1 ara2 ara3 ← confirmed. is # with W4062
- C2 ara2
C3 ara3
- 2979 ara2
4176 ara3
- 4177 ara4
~~10H10A~~ ~~ara~~

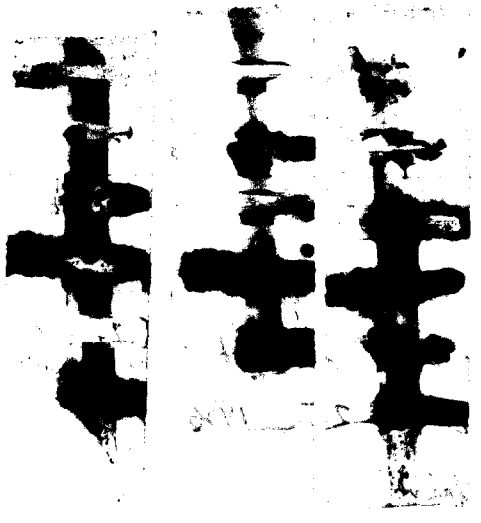
4062 ara5 Hfr1

4068 ara3 Hfr2

4067 ara4 Hfr2

Note 2979 does have higher rate of + than 1426C3!

7/19:



W4274

1426C1 ∴ ara2⁻ ara3⁻ (no recomb. seen here with ara4 but v.s. *

1426C2 ara2 } same as C2, C3

1426C3 ara3 }

10H10A ara1 (rather reversible)

W2979 ara2 ✓

W4176 ara3 ✓

W4163 Hfr1, ara2

W4068 ara3 Hfr2

W4069 ara4

JULY 20, 1958. terminal notes for resumption of work in the fall

It is now clear that Hfr_2 crosses tend to give both Lac^v and Lac^- hemizygotes. It is probably not profitable to attempt to categorize more of the Lac^- from the 1410 series; it may be worthwhile doing a time sequence experiment for the production of diploids, though it is reasonably clear that the occurrence of Lac^+ is related to the production of Lac^v, while the Lac^- are hemizygous, in supp ort of the progressive entry hypothesis. But the nonoccurrence of Gal^v (compared to Gal^+ is enigmatic, and probably some more Gal^v or $^+$ should be looked for

However, in order to distinguish Gal^+ hemizgous from homozygous, it will be necessary to use complementary Gal mutants, allowing either Gal' or Gal^o to be analysed by reversion. This will also afford the opportunity of using Gal/Gal selection for Gal heterozygosity.

Therefore the main programs are:.

1. Time sequence on heterozygote isolations, to complete that picture. Include segregation of Az, V1 and V6 as now available.
2. Gal x Gal selections of diploids a) are there any Galv; b)Ara2,4 pos.eff.

3. Complementary to 2: crosses of diploid x haploid

4. May be worthwhile to look for automictic derivatives of Lac^v diploids as a Lac^- homozygotes would probably be a preferable ϱ.

5. Time exogenote entry in crosses of Hfr heterogenotes.

Probably first item will be the review the accumulated Gal^- stocks for identity and complementarity to Gal_2.

other sugars for diploid selection: Rad?

Retesting of Cavalli Ara⁻ strains With F⁻ Ara's x Hfr Ara's as Controls

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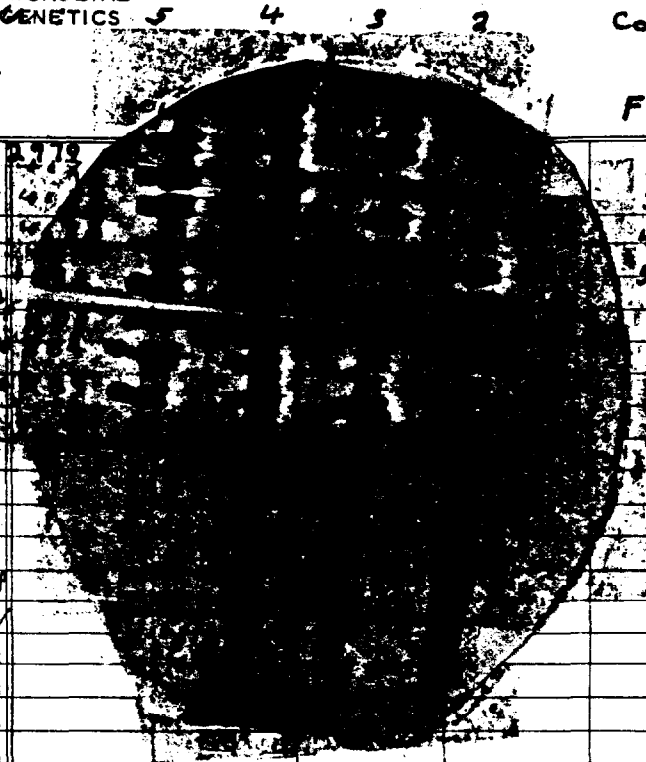
14/28

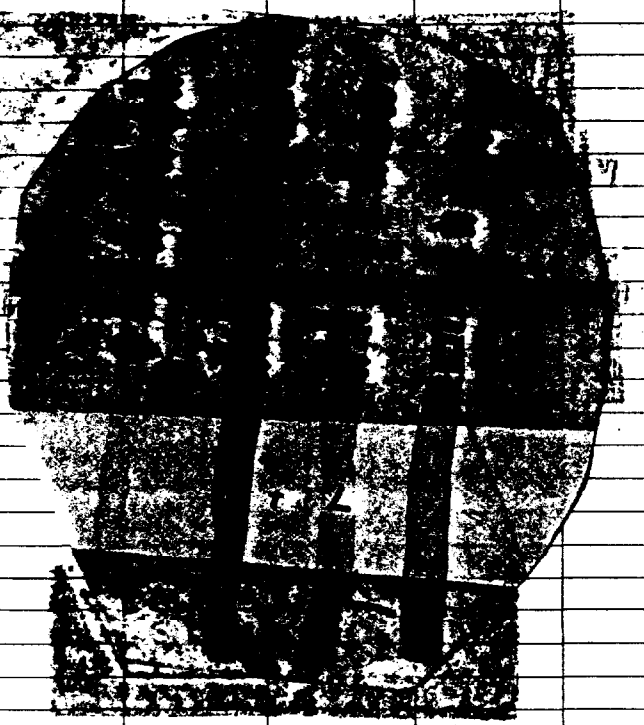
9/6/58

Hfr 5 4 3 2

F⁻

Cavalli strains





Medium = EM Ara B1 c/s indicated supplements.

Histidine affects Hfr transfer into W4283.

Others are unaffected.

SUMMARY: (A) In these tests, Ara⁻ W4178 as F⁻ gave no Ara⁺ with either Hfr2 tester. cf 1410 H - where only ± yields were seen. Should have a control for Ara⁺ segregation. Need a prototrophic Ara⁺ Hfr2.

(B) W4284-5 are distinct from all others; relatively "low" with W4163, perhaps a/c this is Hfr1.

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See EML notes and JSt C work book.

E 50

Dec. 1 1958

♂ = W4358 ⇒ 4362

♀ = W4265

REF:

Below = Ara V's from streaks from original ⊗ plates (from EM-Ara-Bi)

	1	2	3	4	5	6	7	8	9	10
		2 day		2 day		1 day	2 day	1 day	2 day	
		Ara	Lac			Gal		Xyl		
♂ = W4358	1 - 5	V	- (or weak V?)			—	—	—	—	
	- 7	V	- and V (weak)			—	—	—	—	
	- 10	V	V and - (weak)			—	—	—	—	
	- 14	V	V and -		probably V from pap	+	+	—	—	
	- 15	V	V and -			—	— 2 colony types	—	— 2 colony types	
	- 17	V	-, highly pap *	noV	possibly V from pap	+	+	—		
	- 18	V	- sl. pap. after 4 days			+	+	—	—	
	- 20	V	V? (weak?)			—	—	—	—	
♂ = W4359	2 - 2	V	V and -			—	—	—	—	
	7	+	-							
	9	V	-			—	—	—	—	
	11	V (segregates poorly)	V and - (weak)		too young	—	—	—	—	
	12	V	- (and weak ~~*~~?)		possibly V from pap	—	—	—	—	
	16	V	-			—	—	—	—	
segregate but no V col.	← 24	V ⊛	highly pap		possibly noV from pap	+	+	—	—	
	← 28	V ⊛	-			—	—	—	—	
	34	V	highly pap *		possibly no V from pap	+	+	—	—	
	38	V	- (and V?) no			—	—	—	—	
♂ = 4360	3 - 5	V	-, sl pap (or V?)			—	—	—	—	
	10	V ⊛	- or weak			—	—	—		
	11	V	V and -		V from pap	— (also ≈ tiny col)	—	—		
segregate but no V colonies	14	V	-			(large col segregating something) —	—	—		
	16	V	V and -		V from pap	+	+	—		
	← 18	V ⊛	-			—	—	—		
	22	—	-							
	23	V	V and -		possibly V from pap	—	2 colony types	—		

test all # 5's

~~5 5 [illegible]~~

X ~~[illegible]~~ HFr tests

Get Gal ~~[illegible]~~ 0265

transduction

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E 50 cont.

Dec. 1 1958 REF:

	1	2	3	4	5	6	7	8	9	10
		2 day Ara	2 day Lac			1 day Gal		1 day Xyl		
	4-1	V	-, highly pap *		prob Gal + No V from pap	+ +		- -		
	3	V	V and - (weak)		probably V from pap	- -		- -		
	6	V	V and -		prob Gal +	+ +		- -		
	12	V	-, highly pap *		probably V in pap	+	+	- -		
♂ = 4361	13	V	-			-	-	- -		
	14	V	-			-	-	- -		
	17	V	- and V (weak)		V from pap	- -		- -		
	19	V	- and V (weak)		probably V from pap	- -		- -		
	22	-	- (V?)		too young	#				
	23	V	-			- -		- -		
	30	V	- (and V?)		possibly V from pap	- -		- -		
	34	V	- and V (weak)		V from pap.	- -		- -		
	35	V *	-			- -		- -		
segregate but no V colonies	36	V	- and V? (weak)		probably V from pap	- -		- -		
	*41	V	-		V from pap.	- -		- -		
	5-5	V	-		sl. pap. after 4 days	+	+	-	-	
as above	*11	V	-, sl pap *		?	-	-	-	-	
liquid not SC	*13	V	-			-	-	-	-	
	14	V	-			-	-	-	-	
♂ = 4362	15	V	-, sl pap *		too young	-	-	-	-	
	20	V	-		sl. pap. after 4 days	+	+	-	-	
	23	V	-		too young	-	-	-	-	
	27	V	-, some pap *		= Lac V	-	-	-	-	
	12	+	-			-	-	-	-	
	16	V	-		sl. pap.	-	-	-	-	
	34	V	-		(see circled col.)	-	-	-	-	

* = "+"s from #'s possibly not V but simply mixed → restreaked and proved full +.

* = ara "+" is weak

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E 50

19 REF:

	1	2	3	4	5	6	7	8	9	10
1	More # 5's									
2										
3		Ara	Lac	2 days			Gal		Xyl	
4										
5	5-52	+	–	sl. pap.			–	ditto	–	–
6										–
7	53	–	–	1 Rev.			+		–	
8										
See 9	✓57	V	– 2 col types		tard weak ↓ = pap		– 2 col. types		– 2 col. types	ditto
0										
50R	60	V	–	–			–		–	–
1										
2	61	V V	–	–			–	–		
3										
4	72	– –	–	–			–	–		
5										
6	77	+ +	–	–			+	+		
7										
8										
9										
0										

Ø.

50C = straight Lac^r is from pap. streaks on Lac + Ara

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E 50

= Parents on Sugars

Dec. 2 1958

REF:

	1	2	3	4	5	6	7	8	9	10
		1 day 2 day								
		Ara		Gal		Lac		Xyl		
	♀									
	W4265	–	–	– –		– –		–	–	
	♂									
	1	–	–	+ +	all strong	–	blue-, sl pap	+	+	all strong
	2	–	–	+ +		–	bright orange	+	+	
	3	–	–	+ +		–	blue –	+	+	
	4	–	–	+ +		–	– very sl pap	+	+	
	5	–	–	+ +		–	blue –	+	+	