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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, W 3064, 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 H₂O
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.

	Spread	Pour	B spr.	B pour
13	0 0	(14) (11)	1 0	0 0
40	41 49	40, 60	40 49	40 35
120	(109)		(115)	
240	223		223 (sic)	

Spread equivalent to blend!

	Spread	Pourplate
0	(196), 242	~~[illegible]~~ 44, 64
0 1/10	1, 0	0, 1
0 1/100	1, 0	0, 0

∴ pourplating 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 dilution of 13′ sample)

Margin notes: note linear increase of [illegible] — enter & label in this record! — [illegible] S.p. [illegible] (M Sm [illegible]) [illegible]

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1423B

19

REF:

	1	2	3	4	5	6	7	8	9	10
	t'	Blended Sr.P	SRP	Cal+	Ratio	Lac+/among Cal+		Lac+/among Cal-		
13	S	0	—	—						
		01	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		~~21/64~~			
		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 Cal ratio except:

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

② The Cal 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 Ta++.

④ Plate recombinants show Cal ratio about equal to 120' and << 240'

⑤ Plate recombination is negligible at $\frac{1}{10}$ and $\frac{1}{100}$ dilutions ($\frac{1}{100}$ was expt'l plating dilution.

⑥ No reliable effect of plating on increasing the Cal ratio, e.g. at 40' vs at 0'. ∴ no evidence of prolonged entry

⑦ High (except ?) prevalence of Lac+ among Cal+ crosses (due of alternate interr

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

Psmic:	#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 SAP selection.

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Comparison of pour plating and spreading
? prolongation of mating ?

1423B

June 13 1958 REF:

	1	2	3	4	5	6	7	8	9	10
	Not Blended				Lac+/among Cal+		Lac+/Cal−			
	t'	Sr. P	SRP	Cal+	Ratio					
undiluted = plate recount	0	S	196	9/Σ	.055	17/18	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			[illegible]				
	40	S	42	1/Σ	.022	1/1	2/2			
			49	1/Σ		1/1	[illegible]			
	40	P	40	0/9 1/83	.011	1/1	17/[illegible]			
			60							
	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	[illegible]			
	240	P	2[illegible]	14/100	.14	13/14	18/94			
			227							

Σ = replica test of surface plates to B Cal B, 5m. others individually stroked to B Cal. From these, only Cal+ tested for lac. Replica test letter for lac.

For further study:
variety of: (1) High Cal compared to 13' pour plate compared to 0'
(2) continued increase of Cal+ (reduction?)
(3) true function of lac ratio among Cal+. 0S and 240S both have
(4) other markers, especially 0 and 240. a rather lower lac ratio than, say, 240P.

SE 1417

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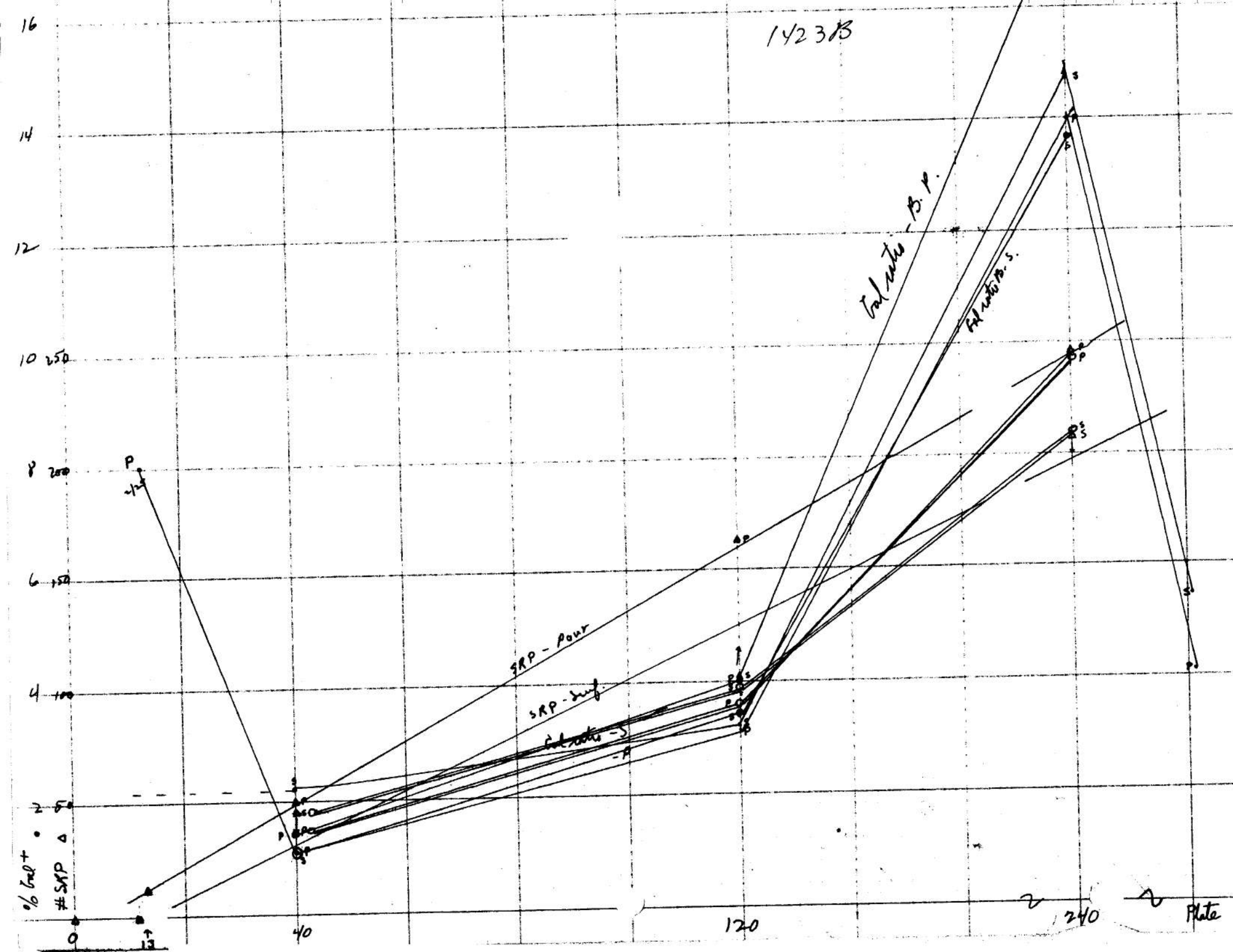
1423B

19 REF:

	1	2	3	7	8	9
	lac+ retro among Gal+.			Gal−		
0 B1	1/2	} 4/5	✓	15/48	} 28/95	
B2	3/3			13/47		+ 1 Gal± lac±
40 B1	1/1	1/1	✓	14/~~5~~ 49	17/72	✓
	0			3/23		
40 B	1/1	1/1	✓	9/49	} 13/66	✓
	0			4/17		
12 B	2/3	2/3		6/41	} 17/91	
	0			11/50		
120 B	3/3	(sci) 1 new +		10/47	} 21/94	✓
	3/3	6/6	✓	11/47		
240	8/8	} 13/14		10/50	} 18/94	
	5/6			18/44		
240 B	5/8	14/17	✓	15/39	} 28/79	✓
	9/9			13/40		

P.

S.


14238
16
14
12
10 250
8 200
6 150
4 100
2 50
0
13
40
120
240
Plate
% Engl +
# SRP Δ
SRP - pour
SRP - surf
Calculate - B. P.
Calculate - S.
- A

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Screen Hfrs.

1424A

June 20 1958

REF:

	available stocks:		$F^- = \sout{W3014}$ 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	~~4~~ +	++	+	++	
8	18	3887	0	±	0	++	
• 2	7	3888	±	++ heavy backg.	3	4	
• 4	11	3890	1	+±	0	0	

parents ORC pen assay, 20X chilled .1 + .1 + 4 ml. dispense 1 ml to each of 4 tubes. Time by transfer 0° → 37° → 0° (1 tube at each time: 0', 10', 20', 30'. 0' is undiluted (plate use.) 10-20-30 dilute 1:100 and plate .05 ml on M Gal B, sm (as on hand).

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

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

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

Note – Esther says W2945 (Hfr_1) is high on all Galx except Gal_3!

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Recheck timing of Hfrs.
x W3119

1424B

26 Jun 1958 REF: A.

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

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

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

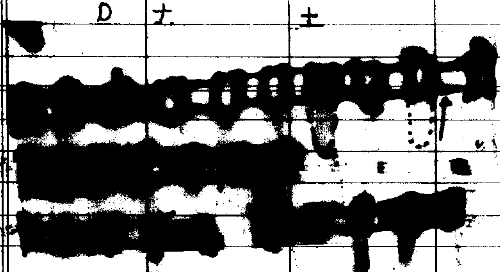
Note high "0" for W3945.

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 Hfr 6 and W3886 should be studied further.

Cross Brush tests on single colony isolates. All colonies homogeneous.
20
In 24 hours, W3945 gave very high response! (This is Alan's Hfr 6 and is donating for Gal)
(It is roughly complementary to Hfr 1.)

	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. Hfr 2 ~~+~~ is rather late for Gal.

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Hfr 4 (pulsed 10 m.)

1424C

June 27 1958

REF:

	1	2	3	4	5	6	7	8	9	10
1	W3752 = Hfr 4 $X^+ S^s$		x	W2961						
2										
3	ORC M, x .1:.1; 2ml D(0); ~~.5ml to 10ml test tubes~~						$t' = 0, 10, 22 \ldots$			
4										
5	at t = 10', add 10ml warm D(0) and plate samples from time to time									
6										
7	P = predilute (choices on the pulse). (dilute after ~10 seconds, mil. agitation).									
8										
9										
0	SRP DsmB,		MValsmB,	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	½ - ⅔						
	24 C					0				
5										
6	P29. Save for tests of other markers: B_1; Mal; Xyl; Lac; ara; Gal assumed 0!									
7										
8										
9										
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

? Does Hfr go to Gal end? Any markers very late? TL entry is at about 60 minutes! (Could test chromosomes 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   sent ✓ Please Thermofax 2-J.   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-I-T comparisons. But the 15' time for Hfr_1 was mistake + rejected.
t(T)=5' t(L)=7' t(P)=12-13'. Leucine-sensitivity?

-6 Repetition.

1402 - Pulsing

1. Various concentrations of cells. Redrawing. "0" is prediluted. 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". Someness 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. Overcame. 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 ~~Hfr~~ 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 Tc^+. But 5x increase of X^+ 20'-60'! Was under mis-conception of pulse in 140(2-F1.) 140(2-R may have been pulsed by saturation.

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

1403- 1 Manipulations. Hfr → F^+ N.C.

2 See protocol

3 How to plate s/ interruptions. See also 1423B

4 Do strains vary in stability to plating? See 1410-1. ~~Thanks~~

1404 F^+. Hfr. F^- Test for cross infection of F^- progeny on plates. So far mechanisms 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. Better experiment! Do protoplasts show delay in Cal (and Lac) or spontaneous interruptions and lysis before Cal? Cal unranied 0 to 60' See 1405-6

4.14 4. protoplast × protoplast. Based on auxotrophy of W3060. An interesting result: crosses on sucrose B, gave very high malemediates (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 shocked suspensions. No effect on enlarged scale. Good for interruption by lysis

-7 Osmotic shock on rods. No interruptions of rods 6-7. (rods only).

-8 repeat -4

1406. +Hfr Conditions of mating: age of cells.

4.16. 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. (because know 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 as contaminations

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 Cals. Low numbers: F^+ rev.
$Cal_2 < Cal_1$.

..... Current Caltimings in EML hands

-20 No evidence of early slow rise in titre. 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 occt. for the protoplasts 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.C. W3060 → F^+.

-6 } N.C. " " "

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

-8. Test for suppression. (Reconstruction).

-9 Repeat -1. No reblend recombinants.

-10 Repeat 2. Two stage transfer. Negative.

1415 – Need to evaluate notes Azide

General conclusion: no differential effect.

No definite verification of reversible inhibition.

–3 concentration too low, no effect. Azide .2% in Difco gelatole

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

–5 Azide + DNP – 3% inhibits mating. DNP – M/200. maybe reversible (no evidence new recruitment).

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

–7 Differential concentration lost on fx. Stratified fertility of crosses!

– Nothing mean Azide-resistance!

DNP^R — see record. – low degree of resistance. .2–.5 ml/2 cm loop of M/20 DNP see 1419.

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

240–260 mμ difference.

1417 – Tuning of Hfr 1. TL: ~20'

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

In Progress

–3 (12) – Time entry of Mal, Xyl...; effect of chloramphenicol

1418 – DNA → F^+ mating with F^- prototrophs.

1419 – DNP-resistance

1420 – Pulsation.

–B – periodate "pulse" } e.g. Hfr → F^+

–C " " }

–D. Effect of F^- cells. (High density!) Apparent interruptions

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

– E. Cal counted on Scalsys B. 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 to plates

D. – Other oxidants. Rather low yields

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

F. –

1422. RDE + Ca^{++} No yields: more titers

1423 Delay interruption c/ chloramphenicol.
A. 50r/ml. Maybe bactericidal; no postfusion. No evidence of any effect on Cal and Lac entry. Part II OK.
B. Pour plating to delay interruption. No interruption; no postirradiation

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 deposits for bulk of experiments of May - June 1958. Then 1426 - 1427.

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Analysis of Gal⁺ × Gal⁻ diploid
Relationship of two modes of segregation

Segregation of 1410F48.

[1410C] 1426 ←

1 July 1958 REF: 1410C1T(1-7)

1	2	3	4	5	6	7	8	now 9	10

7 Gal⁺ heterogenotes have been isolated from 1410C1. Label these, now 1426 A-G
Each derived 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⁻ . . Ara^v Gal^{+v} → Ara^v Gal^{+-}
C 4/6 ↘ Ara⁻ Gal^{+-}
D 3/6
E 1/3
F 5/9 . Ara⁻ Gal^{+v} presumed from consequences of earlier streaking.

The new labels (1426 (A-F)) now refer to the D(Ara) broths mentioned above. all are still Gal⁺ Ara^v

1 July: plate out A and B on B Ara.

2 July Streak out individual Ara^v on B Ara for examination of single, discrete segregants.
Replicate groups of 4 Ara⁻ from each Ara^v streak out → B Gal. See next page (1426 July 4)

also from first plating, Ara⁻ picked to B Gal and B Lac.

	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 Ara^v		+	−	14	+	v → Lac^v Ara^v

{ ?- restreak for purity.

Thus most Ara⁻ here are Lac⁻

↘ are these Lac^v really Ara⁻? On streak out, 1 was Ara⁻ Lac⁺, 3 were Ara^v Lac^v and had been simply misspecified 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 sites in same colonies. Much higher incidence of Gal⁻ Ara^v in several broth generations (2/13; 13/32).
Defer this problem for a more suitable marked diploid + prefer a Gal⁻ - hence 4490 etc!

analysis of a heterogenotic heterozygote:

$\frac{\quad\quad\quad\quad + }{ara\ TLP \quad\quad Gal_2\ S}$

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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 / B.32 Ara^v — Stock cultures were plated on B Ara. Single ara^v were streaked out on B Ara, and 4 Ara^- ~~picked~~ stroked 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 $Ar^v\ Gal^+$ (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 $Ara^v\ Gal^v$ → $v\ -$ → $-\ -$ 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 ~~genes~~ since all viable cells presumably have the same Gal factor.

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

A

Gal^-: 2.4-sets pure Gal^- are pure Lac^-; $Gal^-\ Ara^v$ is $Lac^{\pm}$

Seod Gal^-: 3 all Lac^- $Gal^+\ Ara^-$: All but 3 are also Lac^-. These 3 are Lac^+Gal [ara]

~~and~~ two from a 2:2 split; 1 from a 1:3. ∴ The A-sets are

2 $Ara^v\ Gal^-\ Lac^{\pm}$: 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⁺lac⁺); 1 ara⁻gal⁻lac⁻; 1 ara⁻gal⁺lac⁻; 2 ara⁻gal⁺lac⁺
see below. one of these is ara⁺!

∴ again almost all ara⁻ are lac⁻.

These diploids are not optimal to study segregation in any more detail; wait for development of more suitable markers (V_1; V_6; Az) 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⁻ (among all descendants from ara⁺ colonies)

is A) 3⁺/41⁻ | corrected (see below) ~~2⁺/44⁻~~ 2⁺/41⁻

B) 2⁺/128⁻ | 1⁺/126⁻

→ But on recheck, The 4-set from A was ara⁺lac⁺: (1) ara⁻lac⁺; (2,3) 2 ara⁻lac⁻

B: ara⁻lac⁺; ara⁺lac⁺; ara⁻lac⁻Gal⁺; ara⁻lac⁻Gal⁻

(1) (2) (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 linkage in Gal.

1410X
2 July 58

Plans for further diploids:

1. additional markers should be segregating: V_1, V_6 and perhaps Az. Possibly leave 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? (Maynard B, + isolate!) — should cross to get me!

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

Plans for present diploids:

11 ~~#~~. Reexamine 2 stated pure lac^+, say F26 and F31. | G10, 3, 13
Reexamine the 2 $Gal^+ lac^V$ F5, F12
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 $Ara^- lac^V Gal^-$ autonoict 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 also? What to save from 1410H?

14 to C, examine for bp^h.

Also H plans.

* to D (and B,)!

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Segregation of 1410F48

1426 C

July 11 1958. REF:

F48 is an unusual diploid insofar as it rarely segregates, and hardly ever ara+. Pick 1 ara− from streaks of individual ara+ colonies and purify, s. colonies to broth, crossbrush (XB) with Hfr ara testers on Mara B, as follows:

{− · = − with} {(1 or 2 + (+ p.p.)}

	W4068 ara_3	W4069 ara_4 [~2]	Bara	Diagnosis
	+	−·		2
	−	++		3
	−	++		3
	−	++		3
	+	−·		2
	−	++		3
	+	−		2
	+	−		2
	+	−·		2
	+	−·		2
	+	−·		2
	+	−		2
	−	+		3
	−	++		3
	−	++		3
← SIC.	−	−·	?	2,3
	+	−·		2
	+	−·		2
	+	−·		2
	−	++		3
	+	−·		2
	+	−·		2
	−	++		3
	−	++		3
	−	+		3
	−	++		3
	−	++		3
	−	++		3
	−	++		3
	+	−·		2
	+	−·		2
	+	−·		2
	−	++		3
	+	−·		2
	+++	+++	+++, − +	
	+	−·		2
	−	++		3
	+	−		2
	+	−		2
	±	++ (±)	− +	3, (+ ~~2~~ mix
	+	−		2
	+	−		2
	+	−		2
	+	−		2
	+			

Testers:

		4068	4069	
	2979	++	+	
W4176	(K3)	−	++	
4177	(K4)	++	−	
4178	(K5)	−	−	
mem. no test		−	++	3
dromic ++ (more)		++	++	

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− 1426C2
3− 1426C3
putative 2−3− 1426C1

~~Repur~~ Repurify.

also 2 pure ara+, rep. (not this series but noted in these isolations.)

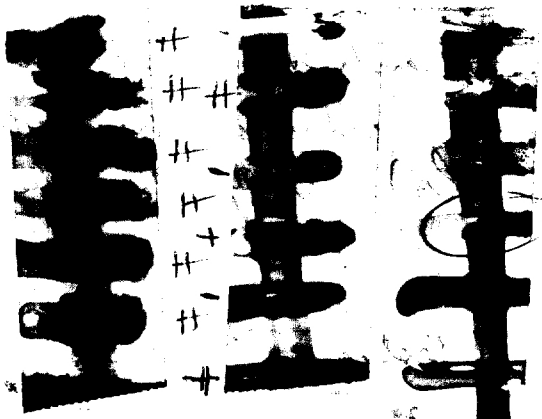
~~The so called~~ C1 might be infertile or F+, etc., and should be checked, say, with ara+ Hfr.

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

July 15, 1958.

Retested on C1-2-3

(W4163 only now available as Hfr, Aua2



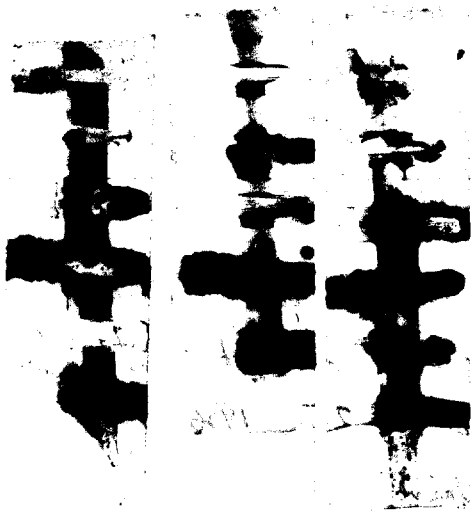
confirmed. as ++ with W4062

C1 — aua3 aua3 ← confirmed.
C2 — aua2
C3 — aua3
~~29-79~~ aua2
4176 aua3
4177 aua4
~~10H10A~~ auxo

4062 aua5 Hfr1
4068 aua3 Hfr2
4067 aua4 Hfr2

Note 2979 does have higher rate of + than W4274!

7/19.



W4274
1426C1 ∴ aua2⁻ aua3⁻ (no recomb. even tho with aua4 but V.S. ?)
1426C2 aua2 } same as C2, C3
1426C3 aua3 }
10H10A* aua1 (rather reversible)
W2979 aua2 ✓
W4176 aua3 ✓

W4163 Hfr1, aua2
W4068 aua3 Hfr2
W4069 aua4

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 orddr 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 φ.

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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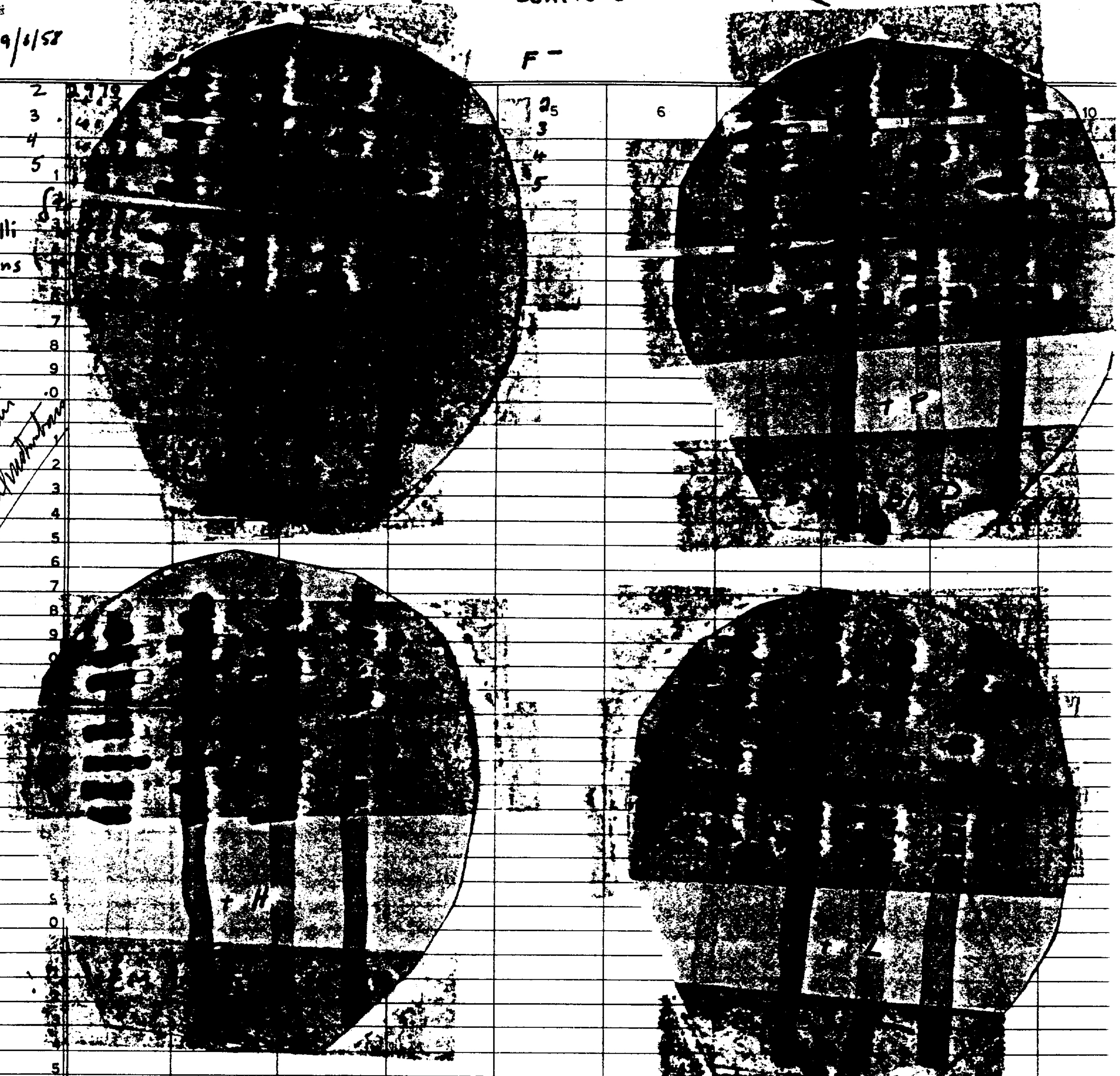
14/28

9/1/58

Hfr 5 4 3 2

F⁻

Cavalli strains



Medium = EM Ara B1 c/s indicated supplements.

Histidine affects Hfr transfer into W 4283.

Others are unaffected.

SUMMARY: (A) In these tests, W4178 (Ara₅) as F⁻ gave no Ara⁺ with either Hfr₂ tester of 1410 H - where only ± yields were seen. Should have a control for Ara⁺ segregation. Need auxotrophic 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 notiz and JStC work book.

E 50

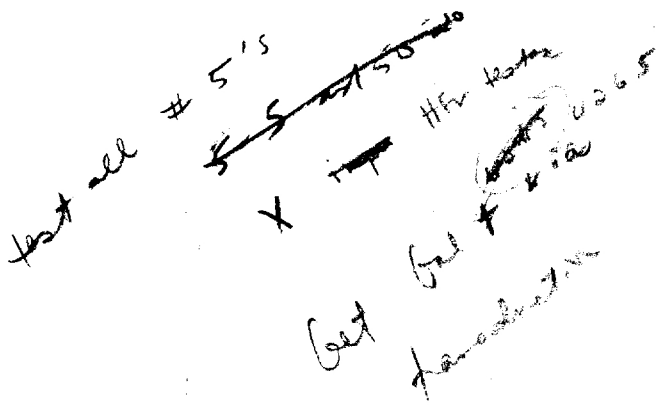
♂ = W4358 ⇒ 4362

Dec. 1 1958

♀ = W4265

REF:

	1	2	3	6	7	8	9	10
	Below = Ara V's from streaks from original ⊗ plates (from EM-Ara-Bi)							
		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 * possibly noV from pap	+ +		—		
	18	V	- sl. pap. after 4 days	+ +		— —		
	20	V	V? (weak?)	— —		— —		
	2	V	V and -	— —		— —		
♂ W4359	7	+	-					
	9	V	-	— —		— —		
	11	V (segregates poorly)	V and - (weak)	— —		— —		
	12	V	- (and weak V?) possibly V from pap	— —		— —		
	16	V	-	— —		— —		
segregate but no Vcol.	24	V *	highly pap possibly noV from pap	+ +		— —		
	28	V *	-	— —		— —		
	34	V	highly pap * possibly no V from pap	+ +		— —		
	38	V	- (and V?) pro	— —		— —		
♂ W4360	3-5	V	-, sl pap (or V?)	— —		— —		
	10	V *	- or weak	— —		—		
segregate	11	V	V and - V from pap	— (also 2 tiny col) — (large col segregating some thin)		—		
	14	V	-	— —		—		
	16	V	V and - V from pap	+ +		—		
	18	V *	-	— —		—		
	22	—	-					
	23	V	V and - possibly V from pap	— 2 colony types		—		



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E 50
cont.

Dec. 1 19 58 REF:

	1	2	3	4	5	6	7	8	9	10
		2 day Ara	Lac 2 day			Gal 1 day		Xyl 1 day		
1										
2	4- 1	V	-, highly pap *		prob Gal+ No V from pap	+	+	-	-	
3										
4	3	V	V and - (weak)		probably V from pap	-	-	-	- (*)	= "+"s from #'s possibly not V but simply mixed → restreaked and proved full +.
5										
6	6	V	V and +		prob Gal +	+	+	-	-	
7										
8	12	V	-, highly pap *		probably V in pap	+	+	-	-	
9										
0	6' 13	V	-			-	-	-	-	
1	14	V	-			-	-	-	-	
2										
3	17	V	- and V (weak)		V from pap	-	-	-	-	
4										
5	19	V	- and V (weak)		probably V from pap	-	-	-	-	
6										
7	22	-	- (V?)		too young	///				
8										
9	23	V	-			-	-	-	-	
0										
	30	V	- (and V?)		possibly V from pap	-	-	-	-	
1										
2	34	V	- and V (weak)		V from pap.	-	-	-	-	
3										
4	35	V (*)	-			-	-	-	-	
5										
6	36	V	- and V? (weak)		probably V from pap	-	-	-	-	
7	*41	V	-		V from pap.	-	-	-	-	
8										
9										
0	5- 5	V	-		sl. pap. after 4 days	+	+	-	-	
1	*11	V	-, sl pap *		?	-	-	-	-	
2										
3	*13	V	-			-	-	-	-	
4										
5	14	V	-			-	-	-	-	
6										
7	15	V	-, sl pap *		too young	-	-	-	-	
8										
9	20	V	-		sl. pap. after 4 days	+	+	-	-	
0										
	28	V	-		too young	-	-	-	-	
1										
2	27	V	-, some pap *		= Lac V	-	-	-	-	
3										
4	12	+	-			-	-	-	-	
5										
6	16	V	- sl. pap			-	-	-	-	
7										
8	34	V	- (see circled col.)			-	-	-	-	
9										

segregate but no V colonies

as above

liquid not SC

36 hr

* - and "+" is weak

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E 50

19 REF:

	1	2	3	4	5	6	7	8	9	10
	More # 5's									
		Gra	Lac	2 days			Gal		Xyl	
	5-52	+	−	sl. papp.			−	~~white~~	−	−
	53	−	−	1 Rev.			+		−	−
See 50R	57	V	− 2 col types		hard weak ↓ papp		− 2 col types		− 2 col types	~~white~~
	60	V	−	−			−		−	−
	61	V V	−	−			−	−		
	72	− −	−	−			−	−		
	77	+ +	−	−			+	+		

50C = straight Lac⁻ 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 −	+	+	